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 substances, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of general issues brought to the attention of the Committee. The next part of the report, of particular relevance to manufacturers and national regulatory authorities, outlines the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines; and an Amendment document to the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) were adopted on the recommendation of the Committee.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: antibiotics; biotherapeutics other than blood products; blood products and related substances; cellular and gene therapies; in vitro diagnostics; and vaccines and related substances.

A series of annexes are then presented which include an updated list of all WHO Recommendations, Guidelines and other documents on biological substances used in medicine (Annex 1). The above two WHO documents adopted for publication on the advice of the Committee are then presented as part of this report (Annexes 2 and 3). Finally, all additions and discontinuations made during the 2019 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 4. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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

To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO's books contribute to achieving the Organization's principal objective – the attainment by all people of the highest possible level of health.

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

For further information, please contact: WHO Press, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (tel. +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int; order on line: www.who.int/bookorders).

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WHO Expert Committee on Biological Standardization

Seventieth report

This report contains the views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.
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WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological substances used in medicine

**Annex 2**

Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines

**Annex 3**

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

Amendment to Annex 3 of WHO Technical Report Series, No. 993

**Annex 4**

Biological substances: WHO International Standards, Reference Reagents and Reference Panels
WHO Expert Committee on Biological Standardization
21 to 25 October 2019

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International Society of Blood Transfusion
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Abbreviations

ABRF  African Blood Regulators Forum
AMH   anti-Müllerian hormone
AmpR  ampicillin-resistance
B2M   Beta-2 microglobulin
BRN   WHO Blood Regulators Network
BRP   biological reference preparation
CAG   Containment Advisory Group
CBER  Center for Biologics Evaluation and Research
CEPI  Coalition for Epidemic Preparedness Innovations
CFU   colony forming units
CI    confidence interval
CRP   collaborative registration procedure
cVDPV circulating vaccine-derived poliovirus
CV    coefficient of variation
DNA   deoxyribonucleic acid
ECSPP WHO Expert Committee on Specifications for Pharmaceutical Preparations
EDQM  European Directorate for the Quality of Medicines & HealthCare
EGFR  epidermal growth factor receptor
ELISA enzyme-linked immunosorbent assay
EMA   European Medicines Agency
EML   WHO Model List of Essential Medicines
ERD   enhanced respiratory disease
EUAL  WHO emergency use assessment and listing (procedure)
EV71  enterovirus A71
FDA   US Food and Drug Administration
FMT   faecal microbiota transplantation
FSH   follicle-stimulating hormone
FXIII  blood coagulation factor XIII
GAPIII  WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use
GBS  group B streptococcus
GBT  WHO Global Benchmarking Tool
GCV  geometric coefficient of variation
GMP  good manufacturing practice(s)
GPW 13  WHO 13th General Programme of Work
GVAP  Global Vaccine Action Plan
HA  haemagglutinin
HAE  hereditary angioedema
hCG  human chorionic gonadotrophin
HCV  hepatitis C virus
HFMD  hand, foot and mouth disease
HIV  human immunodeficiency virus
HK  high molecular weight kininogen
hPSC  human pluripotent stem cell
HPV  human papillomavirus
IABS  International Alliance for Biological Standardization
ICDRA  International Conference of Drug Regulatory Authorities
IFN-α 2b  Interferon alpha-2b
IFPMA  International Federation of Pharmaceutical Manufacturers & Associations
IFU  instructions for use
INN  international nonproprietary name(s)
iPSC  induced pluripotent stem cell
IPV  inactivated poliomyelitis vaccine
ISBT  International Society of Blood Transfusion
ISCT  International Society of Cellular Therapies
<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>IU</td>
<td>International Unit(s)</td>
</tr>
<tr>
<td>IVD</td>
<td>in vitro diagnostic</td>
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<tr>
<td>Lf</td>
<td>limit of flocculation</td>
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<tr>
<td>LMIC</td>
<td>low- and middle-income countries</td>
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<tr>
<td>LV</td>
<td>lentiviral vector(s)</td>
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<td>MAPREC</td>
<td>mutant analysis by polymerase chain reaction and restriction enzyme cleavage</td>
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<tr>
<td>MenC</td>
<td>meningococcal serogroup C</td>
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<tr>
<td>MenW</td>
<td>meningococcal serogroup W</td>
</tr>
<tr>
<td>MenY</td>
<td>meningococcal serogroup Y</td>
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<tr>
<td>MSC</td>
<td>mesenchymal stromal cell</td>
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<tr>
<td>NAC</td>
<td>National Authority for Containment</td>
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<tr>
<td>NAT</td>
<td>nucleic acid amplification technique</td>
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<td>NCL</td>
<td>national control laboratory</td>
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<tr>
<td>NGS</td>
<td>next-generation sequencing</td>
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<tr>
<td>NIBSC</td>
<td>National Institute for Biological Standards and Control</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
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<tr>
<td>OPV</td>
<td>oral poliomyelitis vaccine</td>
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<tr>
<td>PAI-1</td>
<td>plasminogen-activator inhibitor 1</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PDMP</td>
<td>plasma-derived medicinal product</td>
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<tr>
<td>PDVAC</td>
<td>WHO Product Development for Vaccines Advisory Committee</td>
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<td>PEI</td>
<td>Paul-Ehrlich-Institut</td>
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<td>Ph. Eur.</td>
<td>European Pharmacopoeia</td>
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<tr>
<td>PIC/S</td>
<td>Pharmaceutical Inspection Co-operation Scheme</td>
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<tr>
<td>PK</td>
<td>prekallikrein</td>
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<tr>
<td>PKA</td>
<td>prekallikrein activator</td>
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<td>PT</td>
<td>pertussis toxin</td>
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<tr>
<td>Q fever</td>
<td>query fever</td>
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<tr>
<td>qNMR</td>
<td>quantitative nuclear magnetic resonance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>RBC</td>
<td>red blood cell</td>
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<tr>
<td>RCL</td>
<td>replication-competent lentivirus</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rPT</td>
<td>recombinant pertussis toxin</td>
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<tr>
<td>RSV</td>
<td>respiratory syncytial virus</td>
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<td>RVF</td>
<td>Rift Valley fever</td>
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<tr>
<td>SAGE</td>
<td>Strategic Advisory Group of Experts (on Immunization)</td>
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<tr>
<td>SBP</td>
<td>similar biotherapeutic product</td>
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<tr>
<td>sIPV</td>
<td>Sabin inactivated poliomyelitis vaccine</td>
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<tr>
<td>SK</td>
<td>streptokinase</td>
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<tr>
<td>SRA</td>
<td>stringent regulatory authority</td>
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<tr>
<td>TIg</td>
<td>tetanus immunoglobulin</td>
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<tr>
<td>TNF-α</td>
<td>tumour necrosis factor-alpha</td>
</tr>
<tr>
<td>UHC</td>
<td>universal health coverage</td>
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<td>WHOCC</td>
<td>WHO collaborating centre</td>
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<tr>
<td>WHO-NNB</td>
<td>WHO National Control Laboratory Network for Biologicals</td>
</tr>
<tr>
<td>WLA</td>
<td>WHO-listed authority</td>
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1. Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 21 to 25 October 2019. The meeting was opened on behalf of the Director-General of WHO by Dr Mariângela Batista Galvão Simão, Assistant Director-General, Access to Medicines and Health Products. Dr Simão welcomed the Committee, meeting participants and observers.

Dr Simão noted how the new global agenda, as articulated in the sustainable development goals, places a strong emphasis on equity and human rights in health. This provides WHO, and in particular the WHO Department of Essential Medicines and Health Products, with an opportunity to build upon the progress made to bring about access to quality essential medicines and health products for all. Dr Simão then gave a brief overview of the WHO 13th General Programme of Work (GPW 13) approved by the 2018 World Health Assembly, and of the subsequent transformational activities that will guide the activities of the three tiers of WHO, namely its country offices regional offices and headquarters. GPW 13 focuses on the three strategic priorities of advancing universal health coverage (UHC), addressing health emergencies and promoting healthier populations. The associated WHO “triple billion” targets aim to ensure that by 2023 one billion more people benefit from the gains made in each of these strategic priority areas. The transformation of WHO is intended to facilitate delivery of these targets, including through the creation of a new science division.

Dr Simão concluded by noting that the WHO Expert Committee on Biological Standardization was one of the longest serving WHO expert committees, having this year reached its milestone seventieth meeting. Topics of direct relevance to the work of the Committee discussed at the 2019 World Health Assembly included antimicrobial resistance, falsified medicinal products, and access to medicines and vaccines. Dr Simão also highlighted the importance of the upcoming discussion by the Committee of similar biotherapeutic products (SBPs), and expressed her thanks for all of its efforts in these and other areas.

Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Simão for her opening remarks. Dr Knezevic reminded meeting participants that, as a specialized agency of the United Nations, WHO serves as the directing and coordinating authority for international public health matters on behalf of its 194 Member States. WHO is therefore responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends. The setting of norms and standards and promoting their implementation is an affirmed core function of WHO with direct relevance to the work of the Committee. Dr Knezevic also highlighted that this was the seventieth meeting of the
Committee and provided details of its working arrangements. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would participate in the meeting from 21 to 24 October 2019. This section of the meeting would end following the morning session of 24 October, with the chairs and rapporteurs meeting in the afternoon to draft an early version of the Committee report. An open information-sharing session involving all participants, including non-state actors, would be held on 21 October. Final decisions on the adoption of written standards and the establishment of measurement standards would be made by the members of the Committee during a closed session on 25 October.

Dr Knezevic then moved on to the election of meeting officials. In the absence of dissent, Professor Klaus Cichutek was elected as Chair and Dr Ian Feavers as Rapporteur for the plenary sessions and for the vaccines and biotherapeutics track. Dr Harvey Klein was elected as Chair and Dr Clare Morris and Dr Jens Reinhardt as Rapporteurs for the blood products and in vitro diagnostics track. Dr Knezevic then presented the declarations of interests that had been made by the members of the Committee and by WHO temporary advisers and participants. After evaluation, WHO had concluded that none of the declarations made constituted a significant conflict of interest and that the individuals concerned would be allowed to participate fully in the meeting.

Following participant introductions, the Committee adopted the proposed agenda and timetable (WHO/BS/2019.2379).
2. General

2.1 Current directions

2.1.1 Strategic directions in the regulation of medicines and other health technologies: WHO priorities

Ms Emer Cooke, Director of Regulation and Prequalification, presented an overview of the strategic directions of WHO in the regulation of medicines and other health technologies, with a particular focus on biologicals. Following a broad overview of the overarching mission and strategic priorities of WHO, Ms Cooke outlined the WHO 5-year action plan to improve the quality and safety of health products. This action plan had been aligned with GPW 13 and comprised four strategic priorities: (a) strengthening country and regional regulatory systems in line with the drive towards UHC; (b) increasing regulatory preparedness for public health emergencies; (c) strengthening and expanding the WHO prequalification and product risk assessment processes; and (d) increasing the impact of WHO regulatory supportive activities. Ms Cooke then highlighted a number of specific activity areas underpinning each of the four strategic priorities.

In the area of strengthening country and regional regulatory systems, publication of the updated WHO Global Benchmarking Tool (version VI) was seen as a key step, along with the imminent operationalization of the WHO-listed authority (WLA) concept to promote reliance. Collaborative registration procedures have now been established to facilitate assessment and accelerate the national registration of prequalified products, or to accelerate the registration of health products already approved by a stringent regulatory authority (SRA). Although this approach was well established for medicines there was a need to improve its application to accelerating vaccine access, and efforts were now under way to determine how best to take this forward. In the strategic priority area of increasing regulatory preparedness for public health emergencies, key WHO activities include the further implementation of the WHO Blueprint for Research and Development: Responding to Public Health Emergencies of International Concern (R&D Blueprint), the development of associated WHO written and measurement standards, the WHO emergency use assessment and listing (EUAL) procedure, safety monitoring activities, communication and coordination, and regulatory systems strengthening. Ms Cooke highlighted that a roadmap for the revision of WHO emergency use procedures was in the final stages of development and was one of a wide range of activities under way to address the considerable challenges in this area. The specific example of collaborative efforts to accelerate access to Ebola vaccines was given to illustrate their potential benefits and highlight some of the challenges faced. Ms Cooke then outlined a number of recent achievements in strengthening and expanding...
the WHO prequalification and product risk assessment processes in the areas of diagnostics, medicines, vaccines and vector control. To date, a clear emphasis had been placed on vaccines, with many other product classes not currently within the scope of WHO prequalification. One prequalification pilot for insulin SBPs had now been launched and there was demand to further extend the concept into new in vitro diagnostics (IVDs) and other classes of medicines. One key element in this area will be collaboration in the harnessing of new technologies, including with the newly created WHO Science Division. Ms Cooke concluded by outlining recent efforts aimed at increasing the impact of WHO regulatory supportive activities. Strong and efficient regulatory systems rely upon concepts such as reliance, work-sharing and international collaboration and a rich portfolio of concepts, tools, networks and enablers now existed. Opportunities had been identified to systematically improve and streamline WHO processes, strengthen collaboration and increase awareness and implementation of WHO written standards.

The Committee welcomed the strategic priorities outlined in the context of its own work. Discussion took place on the benefits of streamlining processes to avoid duplication of effort, and on the ways in which reliance could reduce the overall regulatory burden without lowering standards. On the question of whether there was a risk that reliance might lead to reduced regulatory capacity-building in low- and middle-income countries (LMIC) it was clarified that WHO was committed to regulatory strengthening balanced against the gains of efficient working practices. In closing, it was suggested that a representative of the new WHO Science Division be invited to provide an overview of its activities and to set out the ways in which these would align with the work of the Committee.

2.1.2 Vaccines and biotherapeutics: recent and planned activities in biological standardization

Dr Knezevic reported on recent and planned WHO activities in the area of standardization and regulatory evaluation of vaccines and biotherapeutics. At the present meeting, new WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines, and an addendum to the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) were to be considered for adoption (see sections 3.5.1 and 3.5.2 respectively). In addition, written standards scheduled for completion in 2020 included revised guidance on DNA vaccines and on typhoid conjugate vaccines, new recommendations on EV71 vaccines, and an amendment to the current WHO Guidelines on the safe production and quality control of poliomyelitis vaccines. Dr Knezevic noted that in line with past demands, more guidance had been developed for vaccines than for biotherapeutics. Related activities included addressing a request made to revise the 2009 WHO Guidelines
on evaluation of similar biotherapeutic products (SBPs), the preparation of a white paper on state-of-the-art cellular and gene therapies, and the revision of the 2004 WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards.

Dr Knezevic then reported on a number of recent workshops conducted by WHO to support implementation of its written standards, which are viewed as important tools for promoting regulatory convergence and for improving the expertise of national regulatory authorities (NRAs). During the course of 2017–2019, numerous such workshops had been held covering a wide range of WHO written standards, including those on good manufacturing practices (GMP), typhoid conjugate vaccines and biotherapeutics including SBPs. In 2019, two implementation workshops had been held on the topics of post-approval changes for vaccines and post-approval changes for biotherapeutics. Dr Knezevic emphasized that not all of WHO written standards required workshops to support their implementation.

In addition to the new or revised WHO written standards being proposed for adoption in 2019, four new or replacement measurement standards for vaccines and related substances (see section 9.1) and two for biotherapeutics (see section 5.1) were being proposed for establishment. Such reference materials continue to be essential in the development, licensing and ongoing lot release of biological medicines. Dr Knezevic emphasized the importance of measurement standards in facilitating the use of scientific evidence through the standardization of assays, refinement of quality control tests and the setting of product specifications. Dr Knezevic suggested that more could be done to promote the use of such reference materials and cited the example of an editorial which had been placed in *Vaccine* to encourage uptake of the First WHO International Standard for antiserum to respiratory syncytial virus, and of its assigned unitage, among a broader target audience.

An overview was then provided of a range of opportunities and challenges presented by the shift towards regulatory convergence, and of the role of WHO in promoting such convergence. Examples were given of a number of successful collaborations between WHO and the International Alliance for Biological Standardization (IABS), and opportunities outlined for collaboration with other bodies in the provision of training. Dr Knezevic also highlighted a proposal scheduled for consideration by the Committee at its current meeting on the further implementation of the 3Rs principles (Replacement, Reduction, Refinement) regarding the use of animals in research with relevance to this area.

During ensuing discussion of the benefits of more broadly disseminating information on the availability of WHO reference materials it was noted that the *Vaccine* editorial had increased the level of enquiries concerning the standard in question. The Committee further noted that publication of the outputs of written standards implementation workshops aimed at a broader audience also
encouraged the use of the associated measurement standards. To date, most such publications have related to biotherapeutics and SBPs, and improvements were needed in the area of vaccines. The example was given of a very successful Biologicals special issue on the stability of vaccines. The Committee welcomed the collaboration between WHO and IABS, emphasized the benefits of such collaboration and encouraged broadening of the range of activities undertaken.

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

Dr Yuyun Maryuningsih reported on recent and planned WHO activities in the area of blood products and in vitro diagnostics. As part of the transformation of WHO the responsibilities of the group dealing with blood products and related biologicals would be expanded to include blood transfusion safety, organ and tissue transplantation and snakebite envenoming.

The Committee was then provided with an update on the history and activities of the African Blood Regulators Forum (ABRF). Although NRAs are encouraged by World Health Assembly resolutions to ensure that the quality, safety and efficacy of blood products meet internationally recognized standards, this is challenging for many African countries. Recent efforts made by WHO and partner organizations had resulted in the establishment of the ABRF under the umbrella of the African Medicines Regulatory Harmonization initiative. Consisting of a network of regulators and allied stakeholders, the ABRF works to promote cooperation and coordination in blood product regulation across Africa to increase access to quality-assured, safe and affordable blood products. Dr Maryuningsih went on to provide a detailed overview of the Terms of Reference of the ABRF and of its Strategic Plan and 2020 Work Plan, the latter of which will be implemented by four working subgroups.

In the area of snakebite envenoming, the Committee was updated on the establishment and intended activities of the WHO Collaborating Centre (WHOCC) for venom and antivenom. At present, antivenom products are largely unregulated and of uncertain quality. The WHOCC will assist WHO by providing technical support for the development of international standards for snake venoms and antivenoms for snakes in the WHO Western Pacific, South-East Asian and African regions, and for improved disease surveillance related to snakebite envenoming in these regions. Areas of collaboration with cross-cutting WHO programmes will include the control and monitoring of venom and antivenom products with the WHO Prequalification Unit, and the implementation of the WHO Snakebite Roadmap developed by the WHO Department of Control of Neglected Tropical Diseases.

An update was then provided on progress made in the development of a multi-criteria decision analysis tool intended to guide regulatory decision-making on the safety of the blood supply in the context of emerging infections.
Following the recommendations of a 2017 WHO Expert Consultation, an Excel prototype tool had now been developed and subjected to testing. This testing had identified the need for an accompanying user guide, which was subsequently produced in 2019. The next phase of development will seek expert inputs prior to publication of the user guide on the WHO website and the conducting of demonstration and training events.

The Committee was then updated on the purpose and outcomes of a range of relevant meetings that had been held during 2018–2019. The meetings had included: (a) the 5th APEC Life Science Innovation Forum – Blood Safety Policy Forum; (b) an ECDC consultation on the role of pathogen-reduction technologies as a blood safety intervention during outbreaks of infectious diseases when laboratory blood screening is unavailable; (c) a WHO – International Society of Blood Transfusion (ISBT) meeting to explore how ISBT might support the activities of the WHO Action Framework for Blood Products; and (d) a Pharmaceutical Inspection Co-operation Scheme (PIC/S) Expert Circle meeting on human blood, tissues, cells and advanced therapy medicinal products.

Dr Maryuningsih concluded by reporting on the numerous issues discussed during two recent virtual meetings of the WHO network of collaborating centres for blood products and in vitro diagnostics. Among the topics discussed and agreed upon were the list of 11 measurement standards in these two areas to be submitted to the Committee for consideration for establishment at its current meeting (see sections 6.1 and 8.1), along with 13 project proposals for endorsement (see sections 6.2 and 8.2).

The Committee noted that WHO had undertaken a number of initiatives with African regulatory authorities in the past and enquired whether sufficient resources were available to support such a large and important programme. Clarification was provided that the role of WHO was to coordinate the efforts being made which were largely dependent upon the resources available to WHO regional offices, academic institutions and other partners.

2.2 Reports

2.2.1 Report from the WHO Blood Regulators Network

Dr Anneliese Hilger, Chair of the WHO Blood Regulators Network (BRN), reminded the Committee that the network had been established in 2006 following recognition by the Committee of the need to establish a global network of regulatory agencies with expertise in the fields of blood, blood products, risk assessment and IVDs. BRN currently comprised seven leading regulatory authorities with extensive experience in these fields. The objectives of BRN were to promote the science-based convergence of regulatory policy, foster international consensus on regulatory approaches, provide scientific
assessments of current and emerging threats and propose solutions to specific issues, particularly in relation to emerging public health challenges.

Dr Hilger then provided an overview of recent BRN activities and highlighted its ongoing collaboration with WHO regional offices to strengthen stakeholder engagement. In addition, the WHO assessment criteria for national blood regulatory systems, prepared by BRN and adopted by the Committee in 2011, had been integrated into the WHO Global Benchmarking Tool (GBT). BRN position papers had also been produced on hepatitis E viruses in blood and on pathogen-reduced cryoprecipitate. Dr Hilger concluded by reviewing the topics scheduled for discussion at this year’s face-to-face meeting of BRN members.

The Committee noted the importance of the work carried out by BRN and received assurance that, taken together, the membership of the network provided a sufficiently comprehensive overview of blood-related issues to ensure that the topics focused on were of global relevance and aligned with the aims of the Committee, particularly in relation to the adoption of WHO guidance in the area of blood regulation. It was further noted that during the Ebola outbreak, BRN had played an important role through the prompt and timely issuing of recommendations.

2.2.2 Report from the WHO network of collaborating centres on standardization and regulatory evaluation of vaccines – proposal for implementation of 3Rs principles

Dr Richard Isbrucker presented a proposal to systematically review the animal testing requirements and procedures set out in WHO written standards. Significant issues currently exist in relation to animal testing for in-process, batch-release and stability-testing purposes. Such testing is time consuming, expensive and labour intensive, and leads to significant delays. In addition, it is typically highly variable and increases the risk of failure of otherwise acceptable product batches. Poor repeatability between manufacturer and control laboratories can further delay vaccine batch release. There is also a lack of harmonization in animal-testing requirements across regulatory jurisdictions.

The purpose of the proposed review would be to determine how much and which animal testing should be included in WHO documents for biologicals and vaccines. An assessment would also be made of whether relevant 3Rs strategies are currently available that have not been considered within existing WHO documents. The review process would seek to determine if a WHO strategy for the adoption of 3Rs principles would be useful to NRAs, national control laboratories (NCLs) and manufacturers, and would investigate barriers to the adoption of 3Rs principles.

The review would be conducted in two stages. Stage 1 would be led by the National Centre for the 3Rs (NC3R) in the United Kingdom. This scientific
organization would provide staff and partial funding, seek external funding, establish a broad working group and organize meetings and conferences to seek expert inputs. This process will avoid any perceptions of bias that might have arisen due to WHO reviewing its own documents. Stage 1 is estimated to take 3 years. Stage 2 would then be an implementation phase dependent on the findings of Stage 1 and would be coordinated by WHO over a period of 2–3 years. The management scope of the project was still under review but will be limited for practical reasons. Dr Isbrucker concluded by clarifying those aspects that would lie within the scope of the review and those which would not.

The Committee queried whether this review would bring about an extensive revision of existing WHO documents, which would not be practical. It was suggested that documents could be updated as they came up for revision and that a general document setting out the available 3Rs approaches would be helpful. The Committee indicated its approval for this project, supported the involvement of an external, impartial body to conduct the first stage and agreed that WHO should seek external funding to support the work.

2.3 Feedback from custodian laboratories
2.3.1 Developments and scientific issues identified by custodians of WHO biological reference preparations

The Committee was informed of recent developments and issues identified by the following custodians of WHO biological reference preparations.

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

Dr Christian Schneider informed the Committee that NIBSC currently holds 373 different WHO measurement standards, of which more than 90% are regularly ordered. During the period 2018–2019, such standards were distributed to more than 1400 recipients in 80 countries. A review of the uptake of WHO standards established since 2010 revealed that the newer standards were no more likely to be high-use standards than older standards, and there was no clear link between the type of standard and rate of use. Dr Schneider did note however that the two monoclonal antibody standards established so far by the Committee – rituximab and infliximab – appeared to be in high demand relative to other standards, especially in Asia. This highlights the importance of such measurement standards and of the SBP field in general. A new measurement standard for adalimumab, a humanized monoclonal antibody that targets TNF-α and which will facilitate the harmonization of product bioactivity, was being proposed for establishment this year (see section 5.1.2), with a further four monoclonal antibody standards currently in the NIBSC work programme.
Dr Schneider further noted that should all of the proposed new projects being submitted to the Committee this year be endorsed, there would then be 98 active NIBSC projects under way to develop new or replacement WHO standards, reflecting the ongoing high level of commitment to the work of WHO in this area. Dr Schneider then drew the attention of the Committee to the calibration of WHO measurement standards and the implications of this for companion diagnostics. Among the proposals to be submitted by NIBSC to the Committee this year was the establishment of a WHO international reference reagent for anti-Müllerian hormone (AMH) for the calibration of immunoassays used to calculate the required dose of recombinant follicle-stimulating hormone in therapeutic products. Concerns had been raised that the introduction of an AMH standard in International Units (IU) could impact upon the previously agreed mass unit dosing for such products (see section 8.1.3). As this was not a unique situation, NIBSC felt that this was an issue which should be brought to the attention of the Committee, specifically in the context of the proposed revision of the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards. Dr Schneider then concluded by highlighting several areas of focus arising from the NIBSC Science Strategy 2019–2019 which has been developed as a blueprint for the future of the Institute in a changing scientific, political and economic environment.

The Committee acknowledged both the importance of standardization efforts in the development of future SBPs, especially monoclonal antibodies, and the workload associated with such efforts. Dr Schneider emphasized that NIBSC aimed to ensure a level playing field by harmonizing assays and would continue to prioritize SBP standard development based on the entry of new products to the market as patents on innovator products expired.

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

Dr Michael Wierer reminded 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 (EML). These international standards are indispensable in the calibration of regional and in-house standards. No issues had been identified in relation to these standards since the previous meeting of the Committee in 2018. Since EDQM took over the responsibility for these standards from NIBSC in 2006, nine replacement standards had been established. This year, a proposal was being submitted to the Committee for the establishment of the Third WHO International Standard for amphotericin B to replace dwindling stocks of the current international standard. Amphotericin B
is used in the treatment of serious fungal infections and leishmaniasis, and is on the EML.

Dr Wierer then updated the Committee on the activities of the EDQM biological standardisation programme, the goals of which were: (a) to establish European Pharmacopoeia biological reference preparations; (b) to standardize test methods for the quality control of biologicals; (c) to further the broad application of the 3Rs concept to replace, reduce and refine the use of animals in research testing; and (d) to promote international harmonization in the field of biologicals through collaboration with WHO and non-European partners. Current projects of particular interest to the Committee included ongoing work on the replacement of current in vivo assays used for the potency testing of rabies vaccines with a standardized enzyme-linked immunosorbent assay (ELISA). A quantitative direct sandwich ELISA method had been chosen in a small collaborative study coordinated by the European Partnership for Alternative Approaches to Animal Testing. The assay uses two well-characterized monoclonal antibodies that bind conformational epitopes of the virus glycoprotein known to induce immune protection, and had been shown to detect most rabies virus strains used in the production of vaccines. The assay can also discriminate between sub-potent and compliant vaccine batches. Dr Wierer concluded by outlining the next steps for the project which were scheduled for 2020.

During discussion, the Committee enquired whether the assay could be used with alternative monoclonal antibodies and was assured that the assay was indeed open to further development along such lines. The difficulties associated with validating such an assay by means of correlation with the in vivo challenge assay were also discussed.

\textit{Paul-Ehrlich-Institut (PEI), Langen, Germany}

Dr Micha Nübling presented an update on a number of measurement standards projects currently under way at PEI. A project to develop an anti-Chikungunya virus reference material had been endorsed by the Committee in 2016 and was progressing successfully. Large volumes of candidate materials had been obtained, with the lead candidate material performing well during an international external quality assessment. Materials had now been filled for evaluation in an upcoming international collaborative study that will include a range of antibody-binding and virus-neutralization assays. In addition, serum had now been collected and characterized as part of the development of an anti-hepatitis E virus genotype reference panel. Finally, the outcomes of a collaborative study report on the proposed First WHO Repository of red blood cell transfusion relevant bacterial reference strains would be presented to the Committee during the current meeting for its consideration (see section 6.1.6).

Dr Nübling went on to highlight the key importance of commutability in the harmonization of assays (see section 3.1.1). While it was acknowledged
that commutability should be assessed as part of WHO collaborative studies, this was in reality challenging and only limited data on commutability were available. During previous discussions on this topic it had been proposed that external quality assessment studies that included clinical samples should be leveraged to also include international reference materials to assess their commutability. The contribution made by PEI to the development of recent and upcoming WHO written standards was then outlined. This included PEI involvement in the development of the WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus which was scheduled for adoption at the current meeting (see section 3.5.1). PEI was also involved in the ongoing development of WHO written standards on DNA vaccines and on EV71 vaccines. PEI had also provided support to a WHO implementation workshop on the procedures and data requirements for changes to approved vaccines, and to a training workshop to support the updating of the National guideline on surveillance of adverse events following immunization in Armenia. PEI also continued to make important contributions to the WHO prequalification and regulatory capacity building programmes, and to provide support for the integration of the WHO assessment criteria for national blood regulatory systems into the WHO GBT.

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

Dr Celia Witten provided the Committee with an overview of recent CBER activities in the areas of vaccines and IVDs. To support the development of new vaccine technologies, CBER had participated in an international multicentre study to evaluate next-generation sequencing (NGS) detection of potential adventitious agents in vaccines. A workshop with the National Institute of Standards and Technology had also been co-organized to discuss the standards and reference materials available for NGS detection of viral adventitious agents in biologicals and biomanufacturing. In addition, five reference preparations for use in the detection of viruses by NGS had been prepared. CBER had also co-organized and participated in collaborative studies on the use of NGS to monitor the consistency of manufacture of oral poliomyelitis vaccine (OPV) and Sabin inactivated poliomyelitis vaccine (sIPV). CBER had also participated in a series of workshops and other initiatives on potency testing of sIPV and conventional IPV, and on the development of alternative potency assays for influenza vaccines, including an alternative assay to the currently used single radial immunodiffusion assay. As a WHO essential regulatory laboratory, CBER participates in the calibration of influenza reference reagents and prepares candidate vaccine viruses for both seasonal and pandemic influenza vaccines.

CBER had also developed a further 18 reference reagents for blood group genotyping that covered several polymorphisms not present in the current WHO reference reagent collection for blood group genotyping. These reference
reagents were scheduled for consideration for establishment by the Committee during its current meeting (see section 6.1.5). An update was also provided on the progress of three projects to develop IVD reference materials for infectious diseases, namely: (a) a reference reagent panel for *Babesia microti* antibody; (b) a *B. microti* genetic reference reagent; and (c) a joint project with NIBSC to expand the current First WHO International Reference Panel for HIV-1 circulating recombinant forms RNA for NAT-based assays.

Dr Witten concluded by outlining the rate of distribution of the current WHO blood coagulation factor standards for thrombin, factor VIII and factor IX. The lack of stakeholder interest in the Fourth WHO International Standard for blood coagulation factor IX (concentrate) and establishment of the subsequent Fifth WHO International Standard for blood coagulation factor IX (concentrate) in 2015 prompted a brief discussion of the practice of distributing previous WHO standards once a replacement was established. The Committee received assurances that this would not be done. With regard to the proposed additional collection of reference reagents for blood type genotyping, the Committee considered the coverage to be offered by such an expanded panel of reagents and whether there would be a need for further reagents. The need for consistency and clarity concerning the terminology to be used for both panels and their individual constituents would require further discussion when the panel was proposed for establishment later in the meeting (see section 6.1.5).

2.4 **Cross-cutting activities of other WHO committees and groups**

2.4.1 **Update from the WHO Expert Committee on Specifications for Pharmaceutical Preparations**

Dr Sabine Kopp updated the Committee on the recent activities of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP) which had held its 54th meeting in Geneva on 14–18 October 2019. At this meeting, more than 150 background documents had been reviewed. The ECSPP agenda had included several cross-cutting topics of interest to the Committee, including the content and use of the EML, antimicrobial resistance, regulatory systems strengthening and WHO prequalification, as well as an update on the International Meeting of World Pharmacopoeias which had been co-hosted by WHO. Current ECSPP joint projects with external agencies include collaborations with the International Atomic Energy Agency on updating GMP guidance for radiopharmaceuticals, and with the United Nations Population Fund on three new guidance texts on the prequalification of contraceptive devices. Within WHO, the ECSPP was working closely with the Prequalification Team on various inspection and regulatory strengthening issues.
ECSPP activities of particular interest to the Committee included the revision of GMP guidance for sterile pharmaceutical products which had been initiated in 2017. This collaboration between the European Union, the European Medicines Agency (EMA), PIC/S and WHO aims to align global standards in this area. The revised guidance includes new sections on utilities, environmental aspects and process monitoring, and introduces the principles of quality risk management to allow for the inclusion of new technologies and innovative processes. Following public consultation and several rounds of revision, a consolidated document is expected to be produced by mid-October 2019 for approval by the EMA Inspection Working Group. A further round of public consultation is then planned for 2020 with the ECSPP emphasizing the need for its worldwide applicability.

Another activity of particular interest to the Committee was a proposal to remove the test for undue toxicity from The International Pharmacopoeia, including removal of all references to the test in the monographs on kanamycin acid sulphate and kanamycin monosulphate. This move follows the 2018 recommendation by the Committee to delete the innocuity tests for undue toxicity for biologicals and vaccines, and does not compromise the quality and safety of biological medicines.

Dr Kopp concluded by outlining the transition from microbiological to chromatographic methods for the assessment of small-molecule single-component antibiotics, which has now been largely completed. Dr Kopp noted that in the past year new evidence had emerged on the risk of using capreomycin-containing medicines to treat multidrug-resistant tuberculosis, resulting in their removal from the EML and invitations for expression of interests in prequalification. However, it has proved impossible to establish a correlation between mass concentration and the microbiological activity of capreomycin. Despite these challenges, the ECSPP had recommended retaining the monographs on capreomycin sulphate and capreomycin for injection in The International Pharmacopoeia for now as prequalified capreomycin-containing medicines remained in circulation.

2.4.2 Requests made to the 69th WHO International Nonproprietary Names Consultation in relation to cell-based advanced therapies

Dr Raffaella Balocco provided the Committee with an overview of a number of recent requests made for international nonproprietary names (INN) in the area of cell-based therapies. This was considered to be an important issue in preparing for novel cell-based advanced therapies and it was noted that regulators were generally not in good agreement. As further therapies were now being developed, the reaching of agreement could prove to be crucial for the harmonization of nomenclature in this area. Thirteen new requests had
been submitted in 2019, including six for CAR T-cells, three for cell-based gene therapy and four for other cell-based therapies. These requests were now being considered alongside eight other outstanding requests, one request for an amendment to the cell therapy lifileucel and two publication issues.

The Committee noted that this was a rapidly developing area and requested clarification of how evolving applications would be addressed. It was clear that INN did not change and were used for the product and not the process used in treatment. Applicants were expected to have a well-developed product before submitting an application for naming. The Committee was informed that WHO had recently launched training courses on INN issues.

2.4.3 Update on the WHO R&D Blueprint for action to prevent epidemics
Dr Marie-Pierre Preziosi updated the Committee on the WHO R&D Blueprint for action to prevent epidemics. The overarching vision of this blueprint was to reach the point where diagnostics, medicines and vaccines would be available to prevent and respond to epidemics across the world. This vision would be achieved by coordinating and accelerating global research efforts to: (a) target the key diseases threatening humanity; (b) rapidly develop appropriate diagnostics, medicines and vaccines; and (c) promptly respond to outbreaks before they became epidemics. Dr Preziosi presented the list of blueprint priority diseases. In addition to well-known priority viral pathogens, the list also includes “Disease X” to reflect the fact that a serious international epidemic could be caused by a pathogen not currently known to cause human disease. The currently reported geographical distribution of each priority disease was highlighted and the iterative delivery process being used to make progress outlined. Dr Preziosi then summarized the progress made in evaluating the baseline situation and developing appropriate roadmaps and target product profiles for each of the priority pathogens. Dr Preziosi concluded by highlighting the online WHO dashboard which will serve as a single reporting interface for conveying at-a-glance information on the vision appropriate to each pathogen and on the progress made towards achieving the associated strategic and landmark goals.

The Committee discussed the importance of establishing appropriate reference standards for these diseases in a timely manner and the challenges this would present.

2.4.4 Update on the activities of the Coalition for Epidemic Preparedness Innovations
Dr Johan Holst updated the Committee on the status of the standards portfolio of the Coalition for Epidemic Preparedness Innovations (CEPI). Launched 2017, the role of CEPI was to fund the development, licensure, production and delivery of vaccine stockpiles. By offering a 5-year funding package CEPI works
to advance the most promising vaccine candidates to an investigative stockpile of 100 000 doses, ready for deployment during an outbreak. Organized into small disease-specific taskforces, CEPI has initially focused on five priority pathogens – MERS coronavirus, Lassa virus, Nipah virus, chikungunya virus and Rift Valley fever virus. CEPI also considers “Disease X” with an emphasis on vaccine development platforms with the potential to develop a vaccine within 8 weeks. CEPI thus has a similar mission to the WHO R&D Blueprint but with a primary focus on vaccine development.

As assay development and standardization are a critical part of the enabling science that underpins vaccine development, it is envisaged that timely standard development will accelerate vaccine development and use. The status of CEPI activities intended to support standardization of vaccines and other biologicals was briefly reviewed and the vital importance of its relationship to the work of NIBSC and the Committee highlighted. Clarification was provided that the current CEPI reference materials were considered to be working standards until they were established by the Committee, and were made available to vaccine developers and others who were prepared to be open and to share their knowledge.

The Committee expressed its support for this important work and indicated that it was prepared to offer CEPI advice when required. It was clear that CEPI funding had accelerated the production of critical measurement standards essential for the development of vaccines against emerging infectious diseases. Discussion then took place of the experiences gained during the response to Ebola, specifically the finding that passive antibodies may often be the first line of defence. Dr Holst indicated that CEPI would also be willing to support the development of promising novel antibody therapies. During further discussion on the proposed approach to “Disease X” several Committee members expressed reservations concerning the likelihood of any vaccine being developed within 8 weeks. Dr Holst acknowledged that this was a challenging target but, at a minimum, the establishment of a sound safety database might support the use of a vaccine during a disease outbreak. CEPI would assemble groups of experts to develop efficient trial designs working closely with the most-affected countries; however, it was also acknowledged that vaccine effectiveness data may have to be acquired over the course of more than one outbreak.

2.4.5 Update on the WHO pilot procedure for the prequalification of biotherapeutic products and SBPs

Dr Guido Pante provided the Committee with an update on the WHO pilot procedure for the prequalification of rituximab and trastuzumab, and their corresponding SBPs. These molecules had been among the first monoclonal antibody therapies to be listed in the EML, and revised WHO Guidelines on
the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology had been adopted in 2013. As a result, a number of SRAs now have extensive experience in evaluating such biotherapeutic products and their corresponding SBPs. In December 2018, an orientation session had been held for experts on biotherapeutic products and SBPs. In addition, pilot-specific procedures and guidelines had now been developed and published on the WHO website, along with associated pilot documents and templates for use during the procedure.

The two envisaged pathways for prequalification were either full assessment (in cases where a rituximab or trastuzumab SBP has been registered by a non-SRA) or an abridged assessment that would rely upon inspections and dossier approval by an SRA. For the full assessment pathway, the reference biotherapeutic product must have been approved by an SRA and the SBP itself must have been registered by the non-SRA and be marketed in the country of registration. A non-SRA carrying out a full assessment should have a thorough understanding of the production process and quality control of the product, and should perform a comprehensive assessment of the product dossier as set out in WHO guidelines on the evaluation of SBPs and on the evaluation of monoclonal antibodies as SBPs. In areas such as manufacturing site and clinical testing site inspections, random sample testing, risk management planning and handling of complaints and recalls reported to WHO the non-SRA should meet the same standards expected of an SRA. In the case of the abridged assessment pathway, prequalification would rely upon inspections of manufacturing and clinical sites conducted by the SRA, and upon verification that the product proposed for prequalification is identical to that of the approved product.

Dr Pante informed the Committee that a total of 23 dossiers had been received – nine for the full assessment pathway and 14 for the abridged pathway. Of the nine dossiers for full assessment, four had been withdrawn by the applicant prior to a pre-submission meeting and five were deemed to be significantly deficient following a pre-submission meeting. In contrast, good progress had been reported for the 14 dossiers subject to an abridged assessment, with most being close to prequalification.

The Committee applauded the work of the WHO Prequalification Team on this pilot study, noting its importance for many countries and manufacturers, and its potential to improve access to this class of SBP. The Committee was informed that it was difficult to generalize regarding the length of time taken to complete assessments by either pathway as this largely depended upon the applicant’s response time. Further discussion then took place on the specific requirements of LMIC and on the need to adapt risk management plans accordingly. The WHO Prequalification Team typically required an addendum to the application that takes into account any additional risks in LMIC and noted that as the risk management plans were country specific this must ultimately be
the responsibility of the importing country. It was further noted that as LMIC typically do not have unified health care systems, the risk management plans would also potentially vary for different groups within a country.

2.4.6 Update on the WHO Model List of Essential Medicines

Dr Nicola Magrini provided the Committee with an update on the EML and EML for Children (EMLc) with a particular focus on biological medicines. In 2019, the list had received 65 applications resulting in the addition of 28 new medicines to the EML, 23 to the EMLc and 16 new formulations. New indications for 26 already-listed medicines were made and nine medicines and four formulations deleted. A total of 21 applications, involving 31 medicines, were rejected.

Dr Magrini reported on work to develop the EML Access, Watch, Reserve (AWaRe) classification database for antibiotics and antibiotic stewardship. The EML Access group consists of a core set of 19 antibiotics, generally characterized by their narrow spectrum and limited risk of selecting for microbial resistance. These antibiotics represent the first- or second-line choice for the empirical treatment of priority clinical infections and should be prioritized for use over EML Watch and EML Reserve antibiotics. EML Access antibiotics should be made available everywhere in appropriate quantity, dose and formulation.

EML Watch antibiotics have a higher potential to drive bacterial resistance, while EML Reserve antibiotics are antibiotics of last resort, with proven activity against high-priority pathogens, and should only be used when all other group alternatives have failed. The development of the AWaRe database represented a significant commitment for the EML.

The Committee was also briefed on the decisions made during 2019 in the areas of cancer medicines, reproductive and perinatal health, mental health and antimicrobial agents used against human immunodeficiency virus (HIV), tuberculosis, malaria and hepatitis C. Dr Magrini noted that a number of existing and newly added essential medicines were highly priced and associated with significant budgetary impacts on health systems. In recent years there had also been a steady increase in the number of biological medicines added to the EML, while the development of cellular and gene therapies was being closely monitored. In 2019, WHO established a multi-disciplinary Expert Advisory Committee to examine the scientific, ethical, social and legal challenges associated with human genome editing and there are plans to evaluate such therapies for EML inclusion based on the magnitude and relevance of the benefits they offer.

During discussion, the role of the EML as a model list for reimbursement in LMIC was clarified. Since 2002, cost was no longer a consideration in adding new medicines to the list. It was noted that since its inception the EML had listed
unaffordable drugs, while many cost-effective drugs remained unaffordable in many countries. Nevertheless, the message that a medicine was considered to be essential was thought to drive countries towards adoption. In this respect, the Committee wondered if the impact of the list had been analysed. Dr Magrini indicated that studies had reportedly shown that the list stimulates implementation and may speed up the approval of medicines. However, although a searchable database had now been developed, a lack of frequent updating of national reimbursement lists remained a significant limitation. Further discussion on the prospective addition of cellular and gene therapies to the EML led to a suggestion that this was an area in which EML and the Committee might work more closely in the future.

2.4.7 Update on WHO regulatory systems strengthening activities

Mr Mike Ward reminded the Committee of the 2014 resolution WHA67.20 which had recognized the importance of strong regulatory systems in maintaining well-functioning health care systems. He then updated the Committee on the progress made by the WHO Regulatory Systems Strengthening programme, the principal objectives of which were: (a) to build regulatory capacity in WHO Member States; and (b) to promote regulatory cooperation, convergence and transparency through networking, work-sharing and reliance.

The Committee was updated on the development and implementation of the WHO GBT version VI used for the evaluation of national regulatory systems. A total of 76 countries (mainly LMIC) had been benchmarked against GBT indicators between 2016 and September 2019. Although the absolute figure remained relatively low, the number of countries with a stable functional regulatory system (that is, meeting GBT Target Maturity Level 3) was increasing.

In the area of regulatory harmonization and convergence substantial progress had being made in expanding the scope of the AMRH initiative in terms of both the range of product types covered and the regulatory activities addressed. In addition, the Association of South-East Asian Nations Joint Assessment Coordination Group had, with WHO technical support, finalized the assessment of one product with further products scheduled for joint assessment. As part of accelerating access to quality-assured medical products, 456 medicines had been registered under the WHO collaborative registration procedure (CRP), and a pilot CRP initiated for the assessment and accelerated registration of WHO prequalified IVDs.

WHO was also working with partners to develop a global competency framework for regulators to support the training and professional development of regulatory staff. The framework was currently being piloted in several countries to support appropriate training to meet current and future local needs. Mr Ward also reported on a recent international consultative meeting held in Geneva
on replacing the concept of “stringent regulatory authorities” with “WHO-listed authorities” (WLAs). It was envisaged that, following the development of operational guidance, the WLA Framework would be operational from 2021.

Mr Ward then reported on the progress being made in the development of draft WHO Guidelines on good regulatory practices. This foundational document is intended to be relevant to all regulators, irrespective of resources and regulatory system. Work on the Guidelines had begun in 2014 and following a process of updating and international consultation the document was scheduled to be considered by the ECSPP for adoption in 2020. The Committee was also informed that a WHO Guideline on the implementation of quality management systems for NRAs had been recommended for adoption by ECSPP in 2019. An update was then provided on the work of the WHO National Control Laboratory Network for Biologicals (WHO-NNB) which had been established to promote best practices and build regulatory capacity by reducing duplicative lot release activities in vaccine-importing countries through reliance on the test data evaluated by the NCLs of vaccine-producing countries, thereby promoting global access to prequalified vaccines. Mr Ward concluded with a brief overview of the history, role and objectives of the International Conference of Drug Regulatory Authorities (ICDRA) which after more than 30 years of existence continues to be an important tool in efforts by WHO and medicines regulatory authorities to harmonize regulation and improve the safety, efficacy and quality of medicines.

During discussion, the Committee expressed some concern that a reduction in lot release testing under the WHO-NNB initiative could lead to a loss of national expertise and hence a reduction in capacity among NCLs. In response, it was acknowledged that this was a potential problem across most areas of regulatory strengthening but that this had to be balanced against NCLs gaining access to data and technologies that would otherwise be unavailable to them.

2.4.8 Update from the WHO Product Development for Vaccines Advisory Committee

The Committee was provided with the report of the 5th meeting of the WHO Product Development for Vaccines Advisory Committee (PDVAC) for its consideration. The work of PDVAC focused on 10 prioritized pathogen areas and on new pathogens for which candidate vaccines or therapeutic antibodies were in clinical development. The Committee was informed that progress in relation to HIV had been slow but two large efficacy trials of candidate vaccines for use in adults were due to report by 2021. A broadly neutralizing monoclonal antibody had also been developed. WHO preferred product characteristics for tuberculosis vaccines had been finalized and several candidate vaccines have progressed through the pipeline since 2017. Significant progress was also reported in the
preparations being made to test the first-ever malaria vaccine in pilot studies in Ghana, Kenya and Malawi. These pilot studies will collect evidence on the safety, programmatic feasibility and likely impact of the vaccine.

Given the continuing threat of influenza epidemics, the development of a universal influenza vaccine continues to be another major R&D focus for PDVAC. It has been recognized that the current preferred product characteristics need to be revised so as to be more appropriate for young people, and PDVAC had raised the issue of whether current guidelines in this area needed to be revised. New technologies offer novel development options for influenza vaccines and candidate vaccines based on a number of such technologies have been developed.

Maternal immunization also remains a high priority particularly as current vaccination programmes do not provide neonates with protection against potentially vaccine-preventable infections such as those caused by RSV and group B streptococcus (GBS). The RSV vaccine pipeline is robust with a diverse range of vaccine and monoclonal antibody candidates in clinical development. The adoption of WHO guidelines to support RSV vaccine development was scheduled for consideration by the Committee at its current meeting (see section 3.5.1). In addition, WHO preferred product characteristics and a technical R&D roadmap were now available for GBS vaccine development and a GBS Assay Standardization Group had now been established.

In the area of enteric infections, a number of Shigella vaccines were also in clinical development with the most advanced candidates aiming to elicit immune responses to the O-antigen. WHO had held a workshop to review the potential of controlled human infection model studies to accelerate the clinical development of these and other vaccines, while the development of serological reference materials for the standardization of immunoassays was also progressing. Although the leading enterotoxigenic E. coli candidate vaccine was making good progress in clinical development and had been shown to be safe and immunogenic, recent burden of disease estimates suggest that the associated mortality rates are declining, creating uncertainty about the value proposition of such vaccines. Although downgraded as a priority area overall, PDVAC will continue to support the development of the most promising candidate.

Other PDVAC priority areas include the development of Group A streptococcal vaccines and herpes simplex virus vaccines. PDVAC also recognized the importance of vaccines in reducing levels of antimicrobial resistance, and in this respect the prospective development of candidate gonococcal vaccines was being followed with interest.

The Committee noted the content of the report and its relevance in helping to guide its own priorities for measurement standards in the short term and for written standards in the near future. Consideration would also be given to the development of guidance on specific monoclonal antibody products, especially where vaccine development had not progressed as rapidly as expected.
2.4.9 Report of the October meeting of the Strategic Advisory Group of Experts on Immunization

Dr Susan Wang reported on the meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization held in October 2019, with a specific focus on: (a) measles and rubella vaccines; (b) human papillomavirus (HPV) vaccine supply; (c) the Global Vaccine Action Plan (GVAP) review and related Immunization Agenda 2030; (d) the quality and use of immunization and surveillance data; and (e) poliomyelitis vaccine issues.

Regarding rubella vaccines, SAGE had confirmed that no changes were needed to the current WHO recommendations relating to their performance. Achieving high coverage through the routine immunization of all infants with rubella-containing vaccine should be the primary focus of national immunization programmes, and the introduction of such vaccines should be accompanied by wide age-range and gender-neutral catch-up campaigns. At the 2017 World Health Assembly, the WHO Director-General had been requested to report back in 2020 on the feasibility of measles and rubella eradication. After detailed consideration, SAGE had concluded that eradication would not be feasible in the short to medium term and that substantial strengthening of primary health care systems for routine immunization delivery would be required before an eradication target could be considered.

SAGE also reaffirmed its support for the WHO recommendation to vaccinate girls aged 9–14 years with HPV vaccine using a two-dose schedule. However, it also expressed concern that current HPV vaccine supply issues could result in a failure to introduce or sustain HPV vaccination programmes in some countries, especially those with a high burden of cervical cancer. SAGE called for a dialogue on global access to HPV vaccine and recommended that all countries temporarily put on hold the implementation of HPV vaccination strategies that are gender neutral and/or target multi-age cohorts until all countries had equitable access to HPV vaccine. The Committee was informed that current supply problems could persist for up to 2 more years with manufacturers planning to ramp up supply.

Dr Wang informed the Committee that SAGE had welcomed the high-level recommendations and lessons learnt arising from the report of the GVAP review. SAGE had noted both the achievements and shortcomings of GVAP and the Decade of Vaccines and had endorsed the proposed Immunization Agenda 2030 which outlines an immunization vision and strategy for the next 10 years. On the related issue of the quality and use of immunization and surveillance data, SAGE had considered the current knowledge gaps, including gaps in vaccine-preventable disease surveillance data, and had endorsed a number of recommendations to address these.

SAGE had also been provided with a broadly positive update on the current Ebola situation in the Democratic Republic of the Congo. Since 2014,
the majority of SAGE recommendations had been implemented and shown to be effective. The rVSV-ZEBOV-GP Ebola vaccine had recently been licensed and evaluation of a second Ebola vaccine was anticipated. The SAGE Ebola Working Group was now developing criteria for the implementation of an Ebola vaccine dose reduction scheme should future vaccine supplies become limited.

The Committee was informed that SAGE had expressed serious concerns about the overall state of polio eradication efforts, especially the upsurge in wild poliovirus cases in Afghanistan and Pakistan, and the inability of the polio eradication programme to effectively control outbreaks of circulating vaccine-derived polioviruses (cVDPVs) in Africa and Asia. Moreover, there was a high risk of supply shortages over the next 6 months of the monovalent OPV (mOPV) needed to respond to cVDPV outbreaks. SAGE also recognized the problem of countering such outbreaks with mOPV for type 2 (mOPV2) which could seed further circulation of vaccine-derived polioviruses in the absence of good mucosal immunity.

During discussion, the Committee enquired about the use of off-label schedules to reduce the number of required doses of HPV vaccine but was informed that the consortium evaluating the potential use of a single dose of HPV vaccine was not expected to report for around 2 years. The possibility of reducing the number of doses of HPV vaccine used in immunocompromised and HIV patients was discussed but dismissed by the Committee. The Committee further enquired about the availability of mOPV2 beyond 6 months and was assured that this was a short-term issue, with sufficient bulk vaccine available.

2.4.10  Report of progress made in integrating blood products into the WHO Global Benchmarking Tool

Dr Alireza Khadem reported on the progress being made towards the integration of blood product assessment criteria into the WHO GBT. Dr Khadem reminded the Committee that there were currently many different assessment tools used to collect information from regulatory authorities and affiliated institutions. In 2013, WHO had taken the strategic decision to work towards a GBT through unification of its own tools and convergence with those of other agencies. The aim of the GBT was to align the policy, scope and methodology of all the various benchmarking programmes to provide greater consistency in standards and approach. The initiative is expected to improve outcomes and impact, reduce the unnecessary burden of assessments, costs and duplication for WHO Member States, WHO and partner agencies involved in regulatory system strengthening, and contribute to improved resource mobilization.

The integration of blood product assessment criteria into the WHO GBT had resulted in the development of “GBT Plus”, the use of which had been successfully piloted by PEI in the WHO African Region during 2018.
A revised version had subsequently been subjected to a further round of public consultation and review by BRN and WHO. It was intended that following its presentation to the Committee and the BRN that GBT Plus would be published before the end of 2019.

The Committee enquired whether GBT Plus linked to the relevant WHO written standards and was informed that it did. Moreover, if a particular WHO written standard did not exist, the tool linked to other international guidance. Although the development of GBT Plus had not yet resulted in a need for new WHO guidance, this might change. It was noted that the tool might therefore potentially help inform the future work of the Committee in this area.

### 2.4.11 Update on the WHO snakebite envenoming strategy

Dr David Williams provided the Committee with an update on the WHO snakebite envenoming strategy. It is estimated that there are between 1.8 and 2.7 million cases per year, resulting in around 400 000 permanent disabilities and 100 000 deaths. The aim of the strategy was to reduce these figures by 50% by 2030 thereby helping to achieve UHC and meet sustainable development goals. The strategy is underpinned by the following four concurrent approaches: (a) empowering and engaging communities; (b) ensuring safe and effective treatments; (c) strengthening health systems; and (d) building partnerships, coordination and resources.

The victims of snakebite envenoming are typically young, healthy and productive members of the community until bitten. Frequently they are heads of families who make a significant contribution to family and community life. The WHO strategy therefore emphasizes community engagement in improving education on the risks of snakebites, their avoidance and appropriate health-seeking behaviour, including through the integration of snakebite awareness into public health programmes. Additionally, many parts of sub-Saharan Africa, and South and South-East Asia lack safe, effective and affordable antivenom products. Ensuring access to such products and to good medical care, including rehabilitation, is thus another key element of the strategy. As health systems in the most-affected regions lack the resources and infrastructure required to manage snakebite effectively, improving snakebite treatment will also require strengthening of the entire health care system. This will involve the putting in place of improved disease surveillance and reporting systems, strong regulatory and policy frameworks, and well-trained staff working in well-equipped facilities. Finally, the strategy aims to encourage partnership and coordination initiatives to alleviate the often prohibitive costs of antivenom and associated treatments. Stockpiling and centralized distribution are viewed as effective ways of reducing supply costs and ensuring the availability of quality-assured and effective antivenoms.
Dr Williams then updated the Committee on the sub-Saharan African antivenom assessment programme, which was examining the effectiveness and suitability of antivenoms for use in sub-Saharan Africa. A number of manufacturers had now undergone GMP inspections that had highlighted an urgent need for funding and technical support. Of nine initial applications to the programme, four products had been rejected, one had been accepted and four were still under assessment following GMP inspections.

The Committee raised the issue of the use of monoclonal antibody products for the treatment of snakebite. It was informed that developments in this area had been slow. In addition, as most people did not know what type of snake had bitten them, polyclonal treatments were likely to be more appropriate and more cost effective. It was acknowledged that the diversity of snake venoms was a challenge but this is partially mitigated by the focus being placed on working at a regional level as this limits the range of snake species to be covered. The Committee noted that there was a need for reference materials for both the venoms and the polyclonal products.

2.4.12 Development of reference materials for *Plasmodium vivax* antigen

Dr Maryuningsih informed the Committee that the need for a WHO international standard for *Plasmodium vivax* antigen for the control of diagnostic test kits had been raised at the sixth meeting of the WHO network of collaborating centres for blood products and in vitro diagnostics in 2018 and in discussions between the network WHOCCs and the WHO Prequalification Team. It was noted that the development of international reference materials for IVD assays to facilitate the calibration of quantitative IVDs was a Global Fund priority and that NIBSC had a *P. vivax* antigen reference material in development.

The Committee was reminded that *P. vivax* did not usually occur at high titre in natural infection making the development of nucleic acid and antigen reference materials challenging. In response, a number of potential strategies had been identified including the pooling of clinical material to provide *P. vivax* material for antigen and serum standards, and the collecting of clinical material from controlled human infection or, dependent upon the available infrastructure, from wider geographical areas. Other potential approaches include the use of in vitro culture of *P. vivax* or the use of a genetically modified malaria parasite.

It is intended that the *P. vivax* reference material would be used by various WHO units in the prequalification of malaria rapid diagnostic tests, in lot verification as a form of post-marketing surveillance and in external quality control in the context of safety and vigilance. For illustration, Dr Maryuningsih outlined a number of *P. falciparum* antigen preparations currently being developed for use as reference materials. One of these materials, although not
yet calibrated against the WHO international standard, is used for WHO prequalification assessment while cultured *P. falciparum* is used for the lot verification of *P. falciparum* rapid diagnostic tests.

Dr Maryuningsih concluded by outlining the current challenges in developing *P. vivax* reference materials, including difficulty in sourcing *P. vivax* material for developing secondary reference materials, the lack of financial return on investment in this area and the need to identify an appropriate custodian laboratory. The Committee discussed both the ongoing developments in relation to *P. falciparum* funded by PATH and FIND, as well as the work to develop a *P. vivax* standard antigen at NIBSC.
3. International Recommendations, Guidelines and other matters related to the manufacture, quality control and evaluation of biological substances

3.1 General

3.1.1 Assessment of commutability in international collaborative studies

Commutability is an important property of a reference material and is defined as “the equivalence of the mathematical relationships among the results of different measurement procedures targeting the same measurand for a reference material and for representative samples of the type intended to be measured”. As several of the standards being proposed for establishment in 2019 had been subjected to an extensive evaluation of commutability, the Committee was provided with an overview the current approach of NIBSC to assessing this attribute.

The aim of a commutability assessment is to demonstrate that the bias in the results obtained for a given standard is equivalent to that of the patient samples. In this context, bias is defined for each individual result as the difference from the true value for the sample. As this value is unknown in most studies, the consensus mean of the study results is used for each sample. The approach taken by NIBSC is to assess the “difference in bias” observed between candidate standards and patient samples. As part of the collaborative study design, a range of “patient” samples and dilutions of the proposed standard(s) are tested by participating laboratories. For illustration purposes, the Committee was presented with a detailed hypothetical example of a commutability assessment by an individual laboratory.

One of the advantages of the difference in bias approach is that a single commutability conclusion on the proposed standard is obtained for each collaborative study participant. In addition, commutability criteria can be derived statistically using large amounts of study data. There are, however, a number of potential challenges also associated with this approach, including: (a) commutability will likely not be observed for all participants; (b) different outcomes may be observed for participants performing the same assay; and (c) commutability may only be apparent over a limited range of concentrations. It was noted that this last issue could be addressed by stating in the IFU the concentration range over which a standard would be considered commutable.

The Committee accepted that the current approach was best practice and that different approaches were rarely used. Following discussion of how well collaborative study participants were informed about the concept of commutability, the Committee recommended that a section specifically on this be incorporated into the previously proposed revision of the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards.
3.2  Biotherapeutics other than blood products

3.2.1  Revision of the WHO Guidelines on evaluation of similar biotherapeutic products (SBPs)

The WHO Guidelines on evaluation of similar biotherapeutic products (SBPs) were developed following exhaustive international public and stakeholder consultations. These Guidelines set out the principles for the development and evaluation of SBPs and were recommended for adoption by the Committee in 2009. Since then, the Guidelines have served well as a basis for the development of national regulatory frameworks for the licensure of such products. WHO has subsequently provided a range of further guidelines and other resources in this area, and is developing a prequalification process for SBPs to facilitate access to products of assured quality in countries with limited regulatory capability.

In 2014, the World Health Assembly adopted resolution WHA67.21 on access to biotherapeutic products, including SBPs, which included a request that WHO update the 2009 Guidelines “taking into account the technological advances for the characterization of biotherapeutic products and considering national regulatory needs and capacities”. In response, WHO convened an informal consultation in 2015 to review the 2009 Guidelines, which involved NRAs, NCLs, associations of manufacturers and individual manufacturers, representing both developed and developing countries. It was concluded that revision was not required at that time as the principles set out in the Guidelines remained applicable for the evaluation of SBPs and were consistent with guidance used by regulators worldwide.

Subsequently, in an open letter addressed to the WHO Director-General, the Third World Network reiterated the request to revise the 2009 Guidelines. Following discussion of the arguments made in support of revision during a virtual meeting held with the signatories of the letter in October 2019, participants were invited, in their capacity as individual experts, to clarify the case being made for revision at the current meeting of the Committee. On behalf of the invited experts, Professor Huub Schellekens presented the case for reappraisal of the regulatory approach to SBPs, focusing in particular on the application of physical and chemical analyses to ensure their quality. Professor Schellekens argued for the removal of the requirement for clinical studies to assess efficacy and for their replacement with in vitro assays. In response, the Committee felt there was sufficient flexibility in the current Guidelines to allow for variation in the quality assessment and scale of clinical data packages required for different products. It also noted that the guidance provided was largely consistent with the approach taken by SRAs worldwide. The Committee also discussed the challenges of ensuring the quality of SBPs and concluded that although modern analytical methods were likely to reveal differences between
products, there was, in the absence of clinical studies, currently no way of knowing which differences would be important for product safety and efficacy.

Following discussion, a second letter was addressed to the Chair of the Committee during the current meeting, proposing that section 10 of these WHO Guidelines, on the clinical evaluation of SBPs, be reviewed and an independent expert consultation organized to discuss in depth the major issues raised, particularly the requirement for clinical trials. The Committee considered that the hypothesis that quality data alone would be sufficient to ensure the safety and efficacy of these products was not supported by the information provided. It was noted that numerous SBPs have been approved in accordance with the current 2009 WHO Guidelines. However, in order to increase access to medicines, a reduced clinical data package may in certain circumstances be acceptable but only where there was clear evidence in support of such an approach. The Committee reiterated that case-by-case flexibility, in terms of clinical considerations, was already provided for in the current WHO documents and that all current WHO guidance in this area is consistent with national and international regulatory guidance.

The Chair of the Committee communicated its conclusions to the WHO Assistant Director-General, Access to Medicines and Health Products, who recognized the challenge of improving the accessibility of SBPs in both developing and developed countries, but noted that WHO should base its standards and norms for the quality, safety and efficacy of biological medicines on the scientific evidence established by the Committee.

3.2.2 Update on the standardization of similar biotherapeutic products

The Committee was updated on the work of WHO in the area of standardization of SBPs, starting with a brief review of the purpose of the 2009 WHO Guidelines on evaluation of similar biotherapeutic products (SBPs) which had been adopted following exhaustive international public consultation. The scope of the Guidelines had been restricted to well-established and well-characterized recombinant proteins, and excluded vaccines and plasma-derived products, as recommended by the Committee in 2008. Since 2009, WHO has been committed to working towards global harmonization of the terminology and regulatory frameworks used for SBPs. Several implementation workshops have been held at both global and regional level covering a range of topics, including clinical study design, quality assessment and assessment of the immunogenicity of monoclonal antibodies.

In 2019, a survey had been conducted to assess the progress made during the 10 years since the WHO Guidelines had been adopted. The survey focused on determining the ways in which the regulatory landscape for SBPs had changed in 21 countries and on identifying areas where Member States required further support. Survey results indicated that the WHO Guidelines
have made an important contribution to the development of regulatory frameworks for SBPs in many countries, resulting in increased regulatory convergence. Several SBPs had now been approved in all participating countries, with monoclonal antibodies dominating the candidate products currently in development. In some countries, locally produced SBPs may come to dominate in the future. With regard to terminology, it was found that the terms “biosimilar”, “similar biotherapeutic product” and “similar biological medicinal product” are used interchangeably, with “biosimilar” being the most common. The term “biogeneric” has largely been abandoned.

The survey also highlighted a number of problem areas, with some countries providing evidence of the difficulty in distinguishing between SBPs and non-innovator products that have not been produced according to the WHO guidance. In addition, it was noted that non-innovator products approved prior to the development of an appropriate national regulatory framework for approving biotherapeutics and SBPs may now need to be reassessed by NRAs. In some countries, the inappropriate or misleading use of terminology continues to cause confusion and the inappropriate labelling of some products as biosimilars may undermine confidence in the use of genuine SBPs. Although some attempt has been made to resolve the problem by using terminology that corresponds to the type of evaluation a product has undergone, it remains an issue requiring further attention.

The Committee discussed the challenges of regulatory convergence in this area given the availability of EMA, US Food and Drug Administration (FDA) and WHO guidelines. The Committee observed that some countries had adopted EMA guidelines prior to the adoption of the WHO Guidelines. While noting the success of this programme of WHO activities over the last 10 years, the Committee also acknowledged the challenge of ensuring the retrospective compliance of older products.

### Blood products and related substances

#### 3.3.1 WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023

The Committee was briefed on the progress made in the development of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023. The aim of this document is to provide a strategic direction to global efforts to address the present barriers to safe blood. The framework thus addresses resolution WHA63.12 on the availability, safety and quality of blood products, and is aligned with WHO GPW 13. It is intended to drive the implementation and funding of national, regional and international strategies to ensure safe blood as an integral element in achieving the sustainable development goals. Following a development
process that began in 2018 and that culminated in extensive stakeholder engagement and public consultation in 2019, the document is scheduled for publication in 2020.

The document sets out the importance of blood transfusion and plasma-based products in health care, recognizing that the establishment and strengthening of national blood systems has been slow in many regions. It describes the critical challenges to be addressed and proposes six strategic objectives to ensure that national blood systems are well-managed, properly regulated and sustainable. Activities arising from the framework are expected to include the dissemination of existing WHO guidelines and other documents, the development of new WHO guidelines, and the mobilization of technical assistance to Member States as required.

During discussion, the Committee drew a distinction between ensuring a sustainable supply of safe blood for transfusion and managing access to plasma-derived medicinal products (PDMPs) but agreed that this distinction did not need to be reflected in the framework document as it does not cover the manufacture of PDMPs. The Committee also discussed the impact of including blood products in the EML. Although the Committee had been instrumental in ensuring that blood products were included in the EML, there was little evidence of this having had an impact. In reality, both the supply and regulation of blood and blood products remained problematic in many developing countries, despite a number of World Health Assembly resolutions. The Committee recommended that WHO review the earlier Achilles project, which had successfully facilitated the development of blood services in some countries, in order to help focus on those activities likely to have an impact in this area.

### 3.4 Cellular and gene therapies

#### 3.4.1 Update on the standardization of cellular and gene therapy products

The Committee was updated on the work of WHO in the area of standardization of cellular and gene therapy products. A holistic approach was being taken which involved a number of appropriate WHO teams and units. Key activity areas include nomenclature schemes for cellular and gene therapy products, the donation and management of medical products of human origin, access to innovative technologies, regulation and bioethics. Of particular relevance to the work of the Committee was the proposed development of a white paper on the standardization of cellular and gene therapy products to address previous recommendations made by ICDRA. The purpose of the document would be to facilitate global convergence among regulators from both high-income countries and LMIC while also providing the conceptual basis of future WHO guidance in this area. It is envisaged that the white paper would be a relatively short document covering the fundamental high-level principles. The scope of
the document would include the provision of definitions, identifying the key elements of a conceptual framework, and setting out the considerations for quality, safety, efficacy and post-market surveillance.

The Committee welcomed the development of the white paper as a first step in the standardization of cellular and gene therapy products, and supported the need for an overarching conceptual document to inform regulators in LMIC of the elements of a regulatory system for such products. While noting the ongoing development of measurement standards and the need to keep the involved WHOCCs informed of expectations, the Committee acknowledged that the focus of the document would primarily be on promoting the development of WHO written standards. The Committee recommended that the document should provide clear definitions and should distinguish between somatic cell and germ line gene editing. The scope of the document should also include products based on autologous cell therapies.

3.5 Vaccines and related substances

3.5.1 Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines

Human respiratory syncytial virus (RSV) is a globally prevalent cause of lower respiratory tract infection in all age groups, especially in populations at high risk. In infants and young children, the first infection may cause severe bronchiolitis that can occasionally prove fatal. RSV is also increasingly recognized as an important pathogen in older adults. In the absence of safe and effective antiviral agents to treat RSV infection, there is an unmet need for RSV vaccines. In recent years, increased understanding of the biology of RSV and associated technological advances have resulted in the clinical development of multiple candidate vaccines, some of which may receive regulatory approval in the near future. There is therefore a recognized need for harmonized technical expectations to guide and facilitate the international development and assessment of candidate RSV vaccines.

In response to this need, WHO convened a series of consultations with experts from academic institutes, industry, regulatory authorities and other stakeholders to review and discuss all aspects of RSV vaccine development. WHO then established a group of experts to prepare the draft WHO Guidelines on the quality, safety and efficacy of human RSV vaccines. Following further expert consultations and several rounds of public consultation over the course of 2018–2019, WHO organized a meeting of all stakeholders to review the final draft of the document prior to its submission to the Committee.

The scope of the Guidelines encompasses the leading technologies currently used to develop prophylactic RSV vaccines at the clinical development stage. These include live-attenuated vaccines (including those based on
genetically modified organisms, such as chimeric virus vaccines), vaccines produced using recombinant viral and other vectored systems, and protein-based vaccines (including subunit and nanoparticle formulations with and without adjuvants).

The Committee was informed that the development of these Guidelines had been funded as part of the Bill & Melinda Gates Foundation project to facilitate RSV vaccine development, licensure and prequalification. This project also includes the development of RSV international measurement standards and the standardization of RSV assays to ensure the global harmonization of RSV vaccine development.

During discussion, the advice of the Committee was sought on whether candidate RSV vaccines with properties similar to formalin inactivated RSV vaccines and intended for use in RSV-naive infants should be tested in nonclinical studies to assess the risk of enhanced respiratory disease (ERD) as a prerequisite to clinical testing in such infants. The Committee, whilst acknowledging the limitations of nonclinical models in predicting the risk of ERD, agreed that an assessment of this risk was essential before clinical testing in RSV-naive infants. The Committee agreed that the risk of ERD was low in older age groups, who were likely to have experienced natural RSV infection. After much discussion on whether such a risk assessment should be limited to specific classes of vaccine, the Committee concluded that the Guidelines should take a precautionary approach rather than explicitly excluding any candidate vaccine from being assessed for its potential to cause ERD. The Committee accepted that, based on current evidence, such investigations may not be needed for live-attenuated RSV vaccines, but noted that sponsors and NRAs were best placed to consider the latest data during establishment of the nonclinical programme. The Committee concurred with the view of the drafting group that the Guidelines should not indicate a preference for any particular animal model for this testing. During further discussion, the Committee, noting the upcoming proposal (see section 9.1.3) to extend the use of the First WHO International Standard for antiserum to respiratory syncytial virus, also requested that section C of the Guidelines include a statement on the assessment of RSV subtype-specific efficacy.

The Committee reviewed a number of comments that had been received during public consultation and, after making a number of changes to the text, recommended that the document WHO/BS/2019.2355 be adopted and annexed to its report (Annex 2).

3.5.2 Amendment to the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

The WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) were adopted by the Committee in 2014. These Recommendations included guidance on the use of several assays that
required the use of live poliovirus, and were produced based on limited data and experience with Sabin inactivated poliomyelitis vaccines (sIPV). In addition, there were no specific biocontainment requirements for IPV manufacturing at that time. Subsequently, the third revision of the *WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use* (GAPIII) was adopted by the World Health Assembly in 2015 to provide guidance on the implementation of biosafety and biosecurity measures at facilities handling poliovirus in the post-eradication era. In addition, the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines were adopted by the Committee in 2018. These Guidelines align the biocontainment requirements for the production of both sIPV and IPV derived from wild-type strains with GMP and GAPIII requirements. As few laboratories have the high-containment facilities required by GAPIII, global capacity for performing assays using live poliovirus, particularly type-2, is currently limited. The proposed amendment to the 2014 Recommendations would help to remove a potential bottleneck in the global supply of urgently needed sIPV caused by the shortage of high-containment facilities that meet GAPIII requirements.

To address the range of issues identified, the amendments being proposed included: (a) modification of the definitions of “virus sub-master seed lot” and “virus working seed lot” to cover all poliovirus strains; (b) updating of information on the WHO international standards now available to support quality control testing; (c) modification of the requirements for confirming the genetic stability of attenuated vaccine seeds and monovalent virus pools to provide more flexibility to vaccine developers; (d) inclusion of additional cell substrates for use in the test for effective inactivation; (e) removal of the general safety (innocuity) test in line with the decision made by the Committee in 2018 to discontinue the inclusion of this test in all WHO Recommendations, Guidelines and other guidance documents for biological products; and (f) updating of the recommendation for the evaluation of sIPV immunogenicity in nonclinical and clinical studies to offer more flexibility and thus facilitate the development and licensure of new vaccines. The Committee was provided with an overview of the discussion and review process which had led to the development of the proposed amendments and was informed that this had included two rounds of public consultation.

The Committee reviewed the proposed amendments as well as the comments raised during public consultation. Given its previous decision to omit the general safety (innocuity) test from all future WHO documents on vaccines and other biological products, the Committee agreed with the removal of this test from the Recommendations, and with the addition of text highlighting that the test was no longer required. The Committee further agreed that the amendment should provide more information on the use of the WHO
international standards established in 2018 and on the assignment of a new Sabin D-antigen Unit. The Committee noted the other proposed amendments and requested that the proposed text on developments in molecular analysis be modified to better reflect that consensus nucleotide sequencing did not provide a measure of attenuation but was intended to confirm consistency of vaccine production.

The Committee then discussed at length a proposed amendment concerning the use of heterologous challenge strains in the measurement of vaccine-induced immunity. The Committee was informed that a number of objections had been made to the approach set out in the proposed amendments. After carefully considering the arguments put forward, the responses of the drafting group and the published evidence, the Committee took the firm view that in the case of sIPV the use of heterologous, wild-type challenge strains provided a more rigorous test of vaccine efficacy and breadth of protection, while avoiding the introduction of unjustified bias resulting from the sole use of homologous strains. The Committee further noted that the use of an appropriate comparator vaccine was essential for interpreting the results of non-inferiority studies and recommended that the currently licensed wild-type IPV vaccines be used as comparator in such studies as they had been shown to be efficacious against paralytic poliomyelitis both in clinical studies and over 60 years of use.

During discussion of biocontainment issues, a query was made regarding the availability of the S19 strains recommended in the proposed amendments as heterologous alternatives to wild-type virus strains. The Committee expressed interest in the proposal made by the drafting group to establish such materials as WHO reagents for worldwide distribution. In light of comments received during public consultation, the Committee also supported the further proposal to hold implementation workshops to help manufacturers and regulators understand key elements of the current set of WHO Recommendations, amendments and Guidelines on poliomyelitis vaccines, thus promoting harmonization in their interpretation.

After further discussion, the Committee recommended that the document WHO/BS/2019.2354 be adopted and annexed to its report (Annex 3).

3.5.3 Revision of the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines

The Committee was reminded that following the second round of public consultation on the recently adopted WHO Guidelines for the safe production and quality control of poliomyelitis vaccines, comments had been received from the WHO Department of Polio Operations and Research regarding the following three key issues: (a) whether personnel should shower on leaving containment; (b) the requirement for dedicated poliovirus laboratories; and (c) the application
of a risk-based approach to handling certain samples (for example, for water and environmental monitoring). Specifically, a request had been made to align the guidance provided in these three areas with current GAPIII and Containment Advisory Group (CAG) decisions. At its previous meeting, the Committee had agreed that WHO documents should not deliver conflicting messages and that the Guidelines should be modified with respect to these key issues prior to adoption. The requested modifications were subsequently made and web links provided to the ongoing discussions of GAPIII and CAG. Following publication of the WHO Guidelines in 2019, complaints were received from the manufacturer Sanofi and from the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) to the effect that the shift towards strict adherence to the GAPIII and CAG decisions was not scientifically justified, had not been subjected to public consultation, and would adversely impact upon productivity, potentially leading to vaccine supply problems.

In response to these concerns, the Guidelines drafting group participated in discussion of the three issues at the 4th CAG meeting in July 2019. Based on current CAG recommendations, the interpretation of the implementation of routine showering-out is left to the discretion of the National Authorities for Containment (NACs). As with any proposed alternative measure of compliance, this would follow the submission of a documented and detailed risk assessment for consideration by the NAC. Any subsequent changes in associated circumstances must be reported to the NAC and where necessary a new risk assessment submitted. It was concluded that the text of the 2018 draft Guidelines had been appropriate.

On the issue of dedicated versus non-dedicated quality control laboratories for poliovirus, it was concluded that quality control laboratories not located within the containment perimeter used for production may be non-dedicated (that is, multi-pathogen) provided all processes, personnel and requirements adhere to GAPIII. Standalone quality control laboratories, however, are expected to be poliovirus-dedicated facilities. This is already similar to the language used in the 2018 draft.

On the issue of handling samples outside the containment facility, it was again concluded that the guidance provided in the 2018 draft was similar. Subject to an appropriate risk assessment, samples from within the containment perimeter may be tested outside GAPIII containment requirements provided all processes, personnel and requirements adhere to GAPIII.

The Committee supported the moves to amend the current WHO Guidelines for the safe production and quality control of poliomyelitis vaccine, recommending that only a number of minor changes be made to the text of the 2018 draft, thus reverting to the language originally used. The Committee further recommended that there should be a round of public consultation on the amendments, restricted to the three key issues in question.
International collaborative study to investigate the utility of NGS as a molecular test of virus stocks used in the manufacture of oral poliomyelitis vaccines

Although OPV has been the preferred vaccine throughout the WHO global poliovirus eradication initiative, the Sabin poliovirus strains used to produce it are genetically unstable and have been shown to lose their attenuating mutations and revert to a neurovirulent phenotype during passage in vivo and in vitro. It is therefore important that maintenance of the attenuated phenotype of OPV strains is carefully monitored during vaccine production. Batches of live-attenuated OPV are currently tested for molecular consistency as part of the batch release process using the mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) test. This test targets nucleotide 472 located in the 5’ non-coding region in type 3 OPV (OPV3) and measures the level of reversion from nucleotide uracil to cytosine, which is directly associated with increased neurovirulence in animal models. The MAPREC test requires specific reagents, is technically demanding and expertise in the assay is difficult to acquire and maintain. For this reason, the availability of high-throughput NGS technologies that allow for quantification of nucleotide polymorphisms in viral genomes is being explored as an alternative approach.

The Committee was updated on the outcome of a collaborative study in which 11 study samples had been analysed by eight laboratories using their own in-house NGS method. The results obtained for 472-C measurements using each of the NGS methods were then compared against those obtained using MAPREC. Intra-assay, intra-laboratory and inter-laboratory variability of for NGS 472-C estimates across samples and laboratories were very low, resulting in excellent agreement between laboratories. No differences in mean NGS estimates or levels of variability were detected when comparing in-house and NIBSC calculations. A high degree of correlation between percentage 472-C results by MAPREC and NGS was observed in all laboratories, with an overall Pearson correlation coefficient of $r = 0.996$. However, complete equivalence in the MAPREC and NGS results was not observed following Deming regression analysis, which means that analysis methods and validity criteria for future NGS tests will require careful consideration.

Based on the long experience with MAPREC and the results of this collaborative study, a test format and criteria for test validity and pass/fail decisions were proposed. NGS estimates of sequence composition at nucleotide 2493, with known polymorphism among OPV3 lots, also exhibited low assay variability and excellent agreement between laboratories. The overall conclusion reached was that NGS could be used as an alternative to MAPREC for measuring 472-C content in OPV3 seeds and bulks. The high consistency of NGS data and remarkable agreement between laboratories suggests that NGS analysis could be
used to establish whole-genome profiles of OPV seeds and lots that could then be used as a high-resolution quality control test for vaccine production consistency. Such tests would be very beneficial for the quality assessment of next generation poliomyelitis vaccines and could be adapted for other live-attenuated viral vaccines in the future.

Acknowledging the rapid technological advances now being made in nucleotide sequence analysis, the Committee agreed that NGS could be used as an alternative to MAPREC for OPV3, providing an appropriate test format and analytical process to establish assay validity and pass/fail decisions were agreed with the NRA. The Committee noted that the next phase of the study, to assess NGS as an alternative to MAPREC for OPV1 and OPV2, was under way, and looked forward to receiving an update in due course.
4. International reference materials – antibiotics

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

4.1 WHO International Standards and Reference Reagents – antibiotics

4.1.1 Third WHO International Standard for amphotericin B

Amphotericin B is a mixture of antifungal polyenes produced by the growth of certain strains of Strepotomyces nodosus or obtained by other means. It induces membrane permeability by forming complexes with ergosterol located in fungal membranes, leading to intracellular leakage and subsequent fungal cell death. The drug is used in the treatment of serious fungal infections (aspergillosis, blastomycosis, candidiasis, coccidioidomycosis and cryptococcosis) and leishmaniasis. Amphotericin B is on the WHO Model List of Essential Medicines required for a basic health system.

The Second WHO International Standard for amphotericin B was established in 2007 with an assigned potency of 944 IU/mg. As stocks of this international standard were now dwindling, EDQM as the responsible custodian for the production, establishment and storage of WHO international standards for antibiotics had taken appropriate steps to develop a replacement material. As with the previous international standard, the candidate material (EDQM code ISA_70339) proposed for use as the Third WHO International Standard for amphotericin B was produced at EDQM by powder filling. An accelerated stability study was carried out by EDQM on vials stored in different climatic chambers at 25 °C, 40 °C and 50 °C for different time periods (1, 3 and 6 months). Stability was then assessed using both liquid chromatography and microbiological assays. The results indicated that the vials of the proposed international standard did not exhibit any significant reduction in microbiological potency upon storage at 25 °C and 40 °C for up to 6 months. A change in the composition of the material at 50 °C detected by liquid chromatography was found to have impacted upon the potency of the material, with a loss of activity of about 9% noted. It was therefore concluded that the stability of the material at the customary storage temperature of −20 °C would be satisfactory.

The outcome of an international collaborative study to establish the Third WHO International Standard for amphotericin B, involving 16 laboratories in 14 countries, was presented to the Committee. The potency of the candidate material ISA_70339 was estimated based on the results of microbiological assays using sensitive microorganisms. To ensure continuity between consecutive batches of the international standard, the Second WHO International Standard for amphotericin B was also included in the study. The combined data from
15 of the 16 participating laboratories indicated a potency of 953 IU/mg for the candidate material ISA_70339 (95% confidence interval (CI) = 98.3–101.7%; inter-laboratory geometric coefficient of variation (GCV) = 3.2%).

The Committee considered the report of the study (WHO/BS/2019.2375) and recommended that the candidate material ISA_70339 be established as the Third WHO International Standard for amphotericin B with an assigned unitage of 953 IU/mg.
5. International reference materials – biotherapeutics other than blood products

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

5.1 WHO International Standards and Reference Reagents – biotherapeutics other than blood products

5.1.1 First WHO International Standard for darbepoetin

Darbepoetin is an engineered form of erythropoietin and, like erythropoietin, is prescribed to treat anaemia caused by chronic renal failure and chemotherapy. There is currently a single licensed darbepoetin product available worldwide, for which the patent expired in 2016 in Europe and is due to expire in 2019 in Japan and 2024 in the USA. It is anticipated that this will lead to the emergence of SBPs. There are currently no monographs for darbepoetin products in any national or international pharmacopoeia. At its 2015 meeting, the Committee endorsed a proposal to develop a First WHO International Standard for darbepoetin. The establishment of such an international standard would allow for the use of a single consistent reference preparation for assessing biological activity by in vitro bioassay during the life-cycle of darbepoetin products, thus facilitating the worldwide harmonization of such bioassay measurements.

A preparation of darbepoetin was formulated, dispensed into ampoules and lyophilized to produce a candidate material (NIBSC code 17/204). The suitability of this material to serve as an international standard was then evaluated in an international collaborative study involving 10 laboratories in seven countries. Using in vitro bioassays based on a broad range of cell lines and readouts, all laboratories returned data for independent statistical analysis. The results of the study indicated that the candidate preparation would be suitable to serve as an international standard for darbepoetin. In addition, an accelerated thermal degradation study was conducted which found no significant loss of potency in samples stored at elevated temperatures for over 16 months, indicating that the material would be very stable at −20 °C.

The Committee considered the report of the study (WHO/BS/2019.2356), along with the results of stability testing, and recommended that the candidate material 17/204 be established as the First WHO International Standard for darbepoetin with an assigned unitage of 100 000 IU/ampoule. It was noted that this international standard was intended to support in vitro bioassay assay characterization, calibration and validation based on its defined IU of bioactivity. The international standard was not intended to be used to revise product labelling or to change therapeutic dosing requirements. Nor was the international standard intended to define the specific activity of darbepoetin products or to serve as a reference product for biosimilarity determination.
5.1.2 First WHO International Standard for adalimumab

The recognition almost 30 years ago that tumour necrosis factor-alpha (TNF-α) is one of the key mediators in the pathogenesis of autoimmune and inflammatory disorders prompted the intense development of molecules targeting this pleiotropic cytokine. Since then, five different TNF-α antagonists have been approved by the FDA and EMA. These medicines have revolutionized treatment and have had a significant positive impact on the quality of life of patients with inflammatory, dermatological and autoimmune diseases. However, despite its acclaimed clinical success as one of these antagonists, adalimumab is also associated with a number of safety and efficacy issues and there is therefore a need to rationalize treatment strategies, including through the implementation of therapeutic drug monitoring.

An international collaborative study was conducted, involving 26 laboratories in 13 countries, to evaluate the suitability of two candidate materials (NIBSC codes 17/236 and 18/124) to serve as the First WHO International Standard for adalimumab for the in vitro bioassay of adalimumab products. In addition, a second such study, involving 16 laboratories from eight countries, was also conducted to assess the suitability of the candidate material 17/236 for use in clinical monitoring assays. For the bioassay study, the two candidate preparations were formulated and lyophilized prior to evaluation using a range of in vitro cell-based bioassays and binding assays. An additional lyophilized preparation to assess assay sensitivity was also included. Study results indicated that the candidate preparation 17/236 was suitable for use as an international standard for adalimumab bioactivity. The use of this standard reduced the observed variability in potency estimates for the tested preparations when compared to estimates expressed relative to in-house reference standards. This candidate material also proved to be stable in accelerated degradation studies.

In the separate study conducted to evaluate the suitability of the candidate material 17/236 for use in clinical monitoring assays, the candidate standard, along with a panel of human serum samples spiked with different levels of adalimumab, were also assessed using a range of methods. Excellent agreement was observed in estimates of adalimumab content in the spiked samples regardless of the method or standard (candidate material 17/236 or in-house) used. Inter-laboratory variability was also similar regardless of the standard used. It was therefore concluded that candidate material 17/236 would be suitable for use in the calibration of in-house/kit standards for therapeutic drug monitoring tests thus facilitating harmonization in clinical practice.

The Committee considered the combined report of the two studies (WHO/BS/2019.2365) and recommended that the candidate material 17/236 be established as the First WHO International Standard for adalimumab, with assigned values for the in vitro bioassay of adalimumab as follows: (a) 500 IU/ampoule of TNF-α neutralizing activity; (b) 500 IU/ampoule of ADCC activity;
(c) 500 IU/ampoule of CDC activity; and (d) 500 IU/ampoule of binding activity. The Committee also recommended that this same candidate material be established as the international standard for therapeutic drug monitoring with an assumed mass of 50 µg/ampoule to help standardize assays used to inform clinical decisions and treatment strategies. It was noted that this international standard was intended to support in vitro bioassay characterization, calibration and validation based on its defined IU of bioactivity. In addition to its role in the calibration of secondary standards, it will also serve to harmonize bioactivity assessments across adalimumab products thus ensuring access to products of consistent quality and effectiveness. The international standard was not intended to be used to revise product labelling or to change therapeutic dosing requirements. Nor was the international standard intended to define the specific activity of adalimumab products or to serve as a reference product for biosimilarity determination.

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

5.2.1 Proposed Sixth WHO International Standard for chorionic gonadotrophin (human)

This international standard is used by manufacturers of therapeutic human chorionic gonadotrophin (hCG) used in the treatment of infertility. The standard allows for the assigning of a dose in IU to therapeutic preparations of hCG by bioassay. Measurement of serum and urinary hCG by immunoassay is also used for the diagnosis of pregnancy and other clinical conditions, and manufacturers of such diagnostic assays may also wish to calibrate their immunoassays against the proposed reference preparation. The Committee was informed that stocks of the Fifth WHO International Standard for chorionic gonadotrophin (human) established in 2009 are now being depleted, and at the current rate of dispatch will last until 2020. As a result, there is a need to prepare a replacement international standard. A portion of the batch of native hCG used to prepare the current international standard was still available for the preparation of the replacement standard, and a trial had been conducted to ensure that the material was still suitable. It was intended that a second study would be conducted to assess the suitability of the candidate material using immunoassay. The study will include the current international standard to allow for calibration in IU/ampoule along with the First WHO Reference Reagent for immunoassay of intact hCG to allow for calibration in nmol/ampoule. Patient samples containing a range of hCG concentrations will also be included in the study, though as hCG exists in multiple forms in serum, assay specificity must be known if commutability is to be fully assessed. It was also noted that most manufacturers have not yet recalibrated their immunoassays against the current international standard.
The Committee recognized the clear need for this international standard and after due consideration endorsed the proposal (WHO/BS/2019.2378) to develop a Sixth WHO International Standard for chorionic gonadotrophin (human).

5.2.2 Proposed Third WHO International Standard for follicle-stimulating hormone (human, recombinant)

The Committee was informed that stocks of the Second WHO International Standard for follicle-stimulating hormone (human, recombinant) are now being depleted and will likely be exhausted in 2020. As a result, there is a need to prepare a replacement international standard. The users of this standard include manufacturers of therapeutic follicle-stimulating hormone (FSH) used in the treatment of infertility. The standard allows for the assigning of a dose in IU to such therapeutic preparations by bioassay. Recombinant FSH products dosed in IU against this international standard are marketed and used worldwide, with licensed SBPs also now available. Inaccurate dosing of these products can result in either decreased effectiveness in follicular maturation or ovarian hyperstimulation. It was noted that increasing demand for lower-cost fertility treatment will increase the number of providers thus highlighting the continued need for effective standardization.

The current international standard consists of purified material obtained from one manufacturer who had agreed to provide material for the replacement standard. Participants in the proposed collaborative study were likely to be manufacturers or contract research organizations that perform FSH bioassays on behalf of manufacturers. The candidate preparation will be calibrated against the current international standard by in vitro and in vivo FSH bioassays. Study data will then be sent to NIBSC for statistical analysis and consensus reached on an assigned unitage.

After due consideration, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a Third WHO International Standard for follicle-stimulating hormone (human, recombinant).

5.2.3 Proposed First WHO International Standard for ustekinumab

Pro-inflammatory cytokines such as interleukin-12 and interleukin-23 have been linked to the mechanisms of several autoimmune diseases, such as plaque psoriasis, psoriatic arthritis and Crohn’s disease. Ustekinumab is a monoclonal antibody that binds to the shared P40 subunit of interleukin-12 and interleukin-23, preventing them from binding to their cell surface receptor thus limiting T-cell activation, and is approved for the treatment of the above conditions. The patent for ustekinumab treatment of psoriasis is due to expire in 2024 and non-innovator products (including SBPs) are now under development.
There is therefore a need for an ustekinumab international standard to facilitate global harmonization of the potency of ustekinumab products through the calibration of bioassays. One manufacturer has donated material for this project, and a pilot lyophilization will be conducted to identify suitable formulations for the proposed standard prior to the full definitive fill. The potency standard will be used by ustekinumab manufacturers and regulatory authorities. Although usage is difficult to predict, uptake of the first WHO international standards for the monoclonal antibodies rituximab and infliximab has been brisk.

It is intended that a stable candidate preparation of ustekinumab will be produced for use in a multicentre international collaborative study on the calibration of in vitro bioassays. As the majority of bioassays currently measure the neutralization of interleukin-12 by ustekinumab, the First WHO Reference Reagent for interleukin-12 (human, lyophilized) will be provided as a common source of interleukin-12. Other bioassay approaches used include immunoassays and biophysical binding assays.

Following discussion of the current approach used for the standardization of the innovator product, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for ustekinumab, while emphasizing the importance of consulting with SBP manufacturers on the overall aims of this project.

5.2.4 Proposed Third WHO International Standard for interferon alpha-2b

Interferon alpha-2b (IFN-α 2b) products are approved worldwide, predominantly for the treatment of chronic hepatitis B and hepatitis C but also for various cancers including hairy cell leukaemia, chronic myelogenous leukaemia, multiple myeloma, follicular lymphoma, carcinoid tumour and malignant melanoma. The proposed Third WHO International Standard for interferon alpha-2b is intended to replace the current international standard, stocks of which are nearing exhaustion. As the primary biological reference standard for IFN-α 2b bioactivity, the material is used for the calibration of secondary bioactivity standards, as well as by manufacturers and control laboratories for the calibration of in vitro bioactivity assays for IFN-α 2b products and for the monitoring of marketed products.

Sourcing new material is likely to be challenging as there are few manufacturers of this outdated therapeutic, and pegylated IFNs have now been developed which are dosed in mass units. Nevertheless, a freeze-dried preparation of IFN-α 2b lyophilized in 1995 and previously included in the WHO international collaborative study on human interferon alpha could serve as a replacement standard, provided this is supported by stability data. The preparation is an *E. coli* expressed clinical-grade protein, similar to that used for the current international standard. The Committee was informed of a
number of potential study approaches under consideration, including the use of original study data supported by the results of a reporter gene bioassay. Should the lyophilized preparation prove to be unstable, a new preparation would be sourced and lyophilized for the collaborative study, with the proposed timeline for the project having to be extended.

Discussion centred on the acceptability of using data from the original collaborative study, supported by the results of the in-house assay, to establish an international standard. Discussion also took place on the reporter gene assay to be used and on the stability of the candidate material, which initial data had suggested would be stable. After due consideration, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a Third WHO International Standard for interferon alpha-2b.

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

6.1 WHO International Standards and Reference Reagents – blood products and related substances

6.1.1 Third WHO International Standard for prekallikrein activator

Prekallikrein activator (PKA) is an impurity present in preparations of therapeutic blood products such as albumin. The level of PKA in such preparations is controlled using a WHO international standard and the European Pharmacopoeia (Ph. Eur.) biological reference preparation (BRP) for PKA in albumin. As stocks of both of these materials were almost depleted, replacement international standards were required.

An international collaborative study involving 26 laboratories in 16 countries had been jointly coordinated by NIBSC and EDQM to establish a Third WHO International Standard for prekallikrein activator and the Ph. Eur. BRP for PKA in albumin batch 7. Independently sourced lyophilized 20% albumin candidate preparations were calibrated against the current WHO international standard for PKA for their suitability to serve as the replacement standards, along with an additional reserve preparation. The Ph. Eur. BRP for PKA in albumin batch 6 was also included to evaluate the continuity of the consecutive BRP batches. The centrally calculated overall Huber’s mean based on the results from laboratories with at least two valid assays was 29.6 IU/ampoule for both the candidate material (NIBSC code 16/364) proposed for use as the Third WHO International Standard for prekallikrein activator and for the proposed reserve preparation (NIBSC code 16/370). The corresponding values for the current Ph. Eur. BRP for PKA in albumin batch 6 and the candidate Ph. Eur. BRP for PKA in albumin batch 7 were 38.4 and 37.0 IU/vial respectively. Intra-laboratory variation expressed as the coefficient of variation (CV) was in the range 1.4–16.6%, with inter-laboratory variation expressed as CV based on Huber’s means in the range 4.4–5.4%. Stability studies indicated no significant loss of activity when stored at −20 °C for more than 2 years.

The Committee considered the report of the study (WHO/BS/2019.2357) and recommended that the candidate material 16/364 be established as the Third WHO International Standard for prekallikrein activator, with an assigned unitage of 30 IU/ampoule. It was further recommended that candidate material 16/370 be kept as a prospective future replacement WHO international standard. The Committee was informed that the proposed Ph. Eur. BRP for PKA in albumin batch 7 had been independently established by the European Pharmacopoeia Commission with an assigned unitage of 37 IU/vial.
6.1.2 Fourth WHO International Standard for streptokinase

Streptokinase (SK) was introduced as a therapy for acute myocardial infarction over 50 years ago, and is still used in developing countries as a cheap and effective treatment. Its worldwide importance as a thrombolytic is highlighted by its inclusion in the WHO Model List of Essential Medicines. As with all thrombolytics, the narrow therapeutic window for SK requires balancing effective thrombolysis against increasing risk of major bleeding. The current Third WHO International Standard for streptokinase is used worldwide to calibrate therapeutic SK products in order to ensure accurate potency labelling and dosing.

The Committee was reminded that the First WHO International Standard for streptokinase was established in 1964 following trials confirming the effectiveness of SK as a thrombolytic. This low-purity preparation also included a potency assignment for contaminating streptodornase. Subsequent WHO international standards had then been produced using high-purity SK preparations to better reflect the preparations used clinically. As stocks of the current Third WHO International Standard for streptokinase were now low, a replacement was required. Two candidate materials (NIBSC codes 16/356 and 16/358) had been donated by manufacturers and formulated, filled and freeze-dried into sealed glass ampoules. An international collaborative study involving 15 laboratories in nine countries was conducted to assign potency values to the candidate materials relative to the current international standard. A fourth material (NIBSC code 88/824) used in the previous two international collaborative studies to establish the second and third international standards was also included.

All of the participant laboratories returned results, using chromogenic and/or fibrinolytic methods. The assays results from each laboratory were then used to calculate laboratory geometric mean potencies, which were combined to give overall geometric mean potencies for each method. Very good agreement was observed between the methods, with the results obtained from a total of 69 independent assays being combined to give overall potencies of 909.1 IU/ampoule for candidate material 16/356 and 1012.7 IU/ampoule for candidate material 16/358 (inter-laboratory GCV = 8.2% and 7.2% respectively). The potency obtained for the study material 88/824 was the same as that obtained in 2000 during the establishing of the current international standard, suggesting excellent long-term stability of the material and good continuity of the unit. Accelerated degradation studies indicated that both candidate materials were very stable, in line with previous findings for streptokinase standards. It was clarified that 8000 ampoules of the candidate material 16/358 would be available.

The Committee considered the report of the study (WHO/BS/2019.2364) and recommended that the candidate material 16/358, which in the
A collaborative study had resulted in slightly lower inter-laboratory variation and better agreement between assay methods, be established as the Fourth WHO International Standard for streptokinase with a unitage of 1013 IU/ampoule.

6.1.3 **Second WHO International Standard for anti-tetanus immunoglobulin (human)**

The First WHO International Standard for anti-tetanus immunoglobulin (human) is used for the calibration of in vitro and in vivo assays for testing the potency of therapeutic preparations of tetanus immunoglobulin (TIg). This international standard was also established by the European Pharmacopoeia Commission as the Ph. Eur. BRP for human tetanus immunoglobulin batch 1 for testing toxin-neutralizing capacity in mice and for potency measurement by immunoassay. The current WHO international standard is also used for the calibration of serological assays used to measure tetanus antibody levels in human serum, and a number of commercial tetanus ELISA kits are available that report results in IU. The current international standard was prepared from a batch of human TIg and was established in 1992 as a replacement for the previous international standard of equine origin. Due to diminishing stocks, a project had been initiated to prepare and establish a replacement WHO international standard and Ph. Eur. BRP.

Bulk liquid TIg, provided by a European manufacturer, was used to prepare a candidate material (NIBSC code 13/240) which was freeze-dried and calibrated in an international collaborative study coordinated by NIBSC and EDQM, and involving 20 laboratories in 15 countries. Study results indicated that there was good agreement between laboratories for the potency estimates obtained for the candidate material relative to the current reference preparations. The study also demonstrated the suitability of the candidate material for use in Pharmacopoeia assays used for potency testing of TIg products, with good agreement observed in the potency estimates obtained using the different assay methods included in the study. Accelerated degradation studies performed over a period of 4 years suggested that the freeze-dried candidate material would be very stable. The results of a commutability study suggested that the candidate material is commutable with patient samples across a range of tetanus immunoassays.

The Committee considered the report of the study (WHO/BS/2019.2367), agreed that this was a straightforward replacement of an existing standard, and recommended that the candidate material 13/240 be established as the Second WHO International Standard for anti-tetanus immunoglobulin (human) with an assigned unitage of 45 IU/ampoule. The Committee was informed that the same proposal would be made to the European Pharmacopoeia Commission with regard to the establishment of the replacement Ph. Eur. BRP.
6.1.4  First WHO International Standard for blood coagulation factor XIII-B subunit antigen (total, plasma) via assignment of additional analyte to the current First WHO International Standard for blood coagulation factor XIII (plasma)

The current First WHO International Standard for blood coagulation factor XIII (plasma) was established in 2004 with an assigned potency for activity of 0.91 IU/ampoule and an assigned potency for antigen (A2B2 complex) of 0.93 IU/ampoule. This standard is used to measure the potency (functional activity and antigen value) of blood coagulation factor XIII (FXIII) in patient plasma during the diagnosis of FXIII deficiencies, and to evaluate FXIII therapeutic concentrates. FXIII-B subunit measurements are however required for the diagnosis and characterization of the type of FXIII deficiency. Furthermore, therapy for FXIII-A deficiency with recombinant FXIII-A relies on available FXIII-B. The proposal to assign a value for total FXIII-B subunit antigen to the First WHO International Standard for blood coagulation factor XIII (plasma) was reviewed and endorsed by the Committee in 2016. Furthermore, following a feasibility study carried out in 2018, this proposal was also agreed to during the ISTH/SSC Factor XIII and Fibrinogen Subcommittee meeting in July 2018.

An international collaborative study involving seven laboratories in six countries had therefore been undertaken to assign a value for total FXIII-B subunit antigen to the current WHO international standard relative to locally collected normal plasma pools. Laboratories were instructed to use a validated method for the assessment of total FXIII-B subunit antigen potency using the ELISA reagents provided. All laboratories used the recommended ELISA method with one laboratory additionally using an in-house method. The establishment of an IU for the new analyte followed the same approach that had been used to assign the FXIII activity and A2B2 antigen values, relying on the consensus mean from assays relative to local normal plasma pools that were each arbitrarily assigned a value of 1.00 unit/mL. Nine datasets were received, representing 37 assays in total, and providing a total of 35 valid estimates for the new assignment. Analysis of the results produced an overall mean value of 0.98 units/mL (95% CI = 0.86–1.11; inter-laboratory GCV = 18.3%).

During discussion it was clarified that the size of the plasma pools used by the different participating laboratories ranged from 16 to 28 donors per pool. The Committee considered the report of the study (WHO/BS/2019.2370) and recommended that the current First WHO International Standard for blood coagulation factor XIII (plasma) be assigned a total FXIII-B subunit antigen unitage of 0.98 IU/ampoule.
Almost 390 antigens organized into 36 blood group systems are recognized by the ISBT and the genetic background for most of these blood group antigens is known. As a result, blood group genotyping methods for donor/recipient matching are becoming widely used to overcome serological typing limitations encountered by immunohaematology laboratories. Red blood cell (RBC) antigen matching between donors and recipients by means of DNA-based typing is an effective strategy for identifying antigen-negative donor units for the prevention of alloimmunization and for transfusion into chronically transfused alloimmunized patients, such as those with sickle cell disease and thalassaemia. In addition, molecular typing: (a) predicts the correct RBC antigen of patients with a positive direct antiglobulin test; (b) identifies fetuses at risk of haemolytic disease of the newborn by predicting fetal RhD phenotype and other blood group antigens; (c) resolves serological typing discrepancies when informative serological reagents are unavailable; and (d) aids in the identification of Rh variants. Moreover, large-scale blood group genotyping of blood donor populations allows for the identification of individuals with both rare and multiple antigen-negative phenotypes, thus assuring better transfusion outcomes for alloimmunized patients.

The molecular methods used for blood group genotyping vary from conventional polymerase chain reaction (PCR) to mass-scale genotyping. Commercially available blood group genotyping platforms differ in format, allele content, processing steps, results-interpretation algorithms, cost and regulatory status. The preferred method of a laboratory is influenced by cost, throughput capabilities, turnaround time and coverage of specific genotypes. The clinical use of any genotyping assay requires validation of test methods, which can be achieved by using reference reagents. However, there is a paucity of validated reference reagents for blood group antigen genotypes. Genotyping kit manufacturers, genotyping laboratories and proficiency schemes often resort to the use of diverse clinical materials as reference materials. Reference reagents are therefore limited in volume, not widely available and sometimes poorly characterized, potentially compromising the quality of results and patient care.

In 2011, the Committee had recommended the establishment of the WHO international reference reagent collection for blood group genotyping, which consisted of four reference reagents with limited coverage of blood group alleles in six blood group systems.

The Committee was informed that an international collaborative study involving 28 laboratories in 13 countries had been conducted under the auspices of the FDA to assess the suitability of an additional set of reference reagents for blood group genotyping. This set consisted of 18 genomic DNA lyophilized
samples generated from genotyped and phenotyped blood donors, covering 17 blood group systems, and had been produced using protocols similar to those used to produce the existing WHO reference reagent collection. The candidate materials were characterized using multiple sequencing platforms. Although there was some overlap in allele coverage with the current WHO collection, the overlapping alleles were extensively characterized using a variety of advanced techniques not available in 2011. It had been agreed between NIBSC and CBER that redundancy in the collections would be acceptable and would add value for the user since different technologies had been used in the corresponding studies. Moreover, it had also been agreed that NIBSC and CBER would maintain the separate sets of reagents, with the availability of the other set cross-referenced in the IFU. It was further clarified that as some assays are designed to predict phenotypes rather than detect genotypes, the proposed new reference reagent set would be validated for the detection of 38 genotypes or predicted phenotypes. Addition of the new set would expand the total collection to 22 reagents which would be available to assay manufacturers and researchers for the development and validation of assay kits, and to genotyping laboratories for qualitative test evaluation and monitoring of assay performance.

During discussion, the issue of how to name the two coexisting sets to best facilitate ordering by users was raised. As the proposed new set was not a replacement it could not become a second international reference reagent collection, and nor would it be an addendum to the current collection as both could be ordered independently. Moreover, the two collections differed in terms of composition and data provision with each having different IFU. Since the two collections were not being merged, but coexisting, it was suggested that the new set could be labelled as “Set B” and considered to be an extension of the current collection (“Set A”).

The Committee took into account the naming, labelling and distribution issues raised and following consideration of the study report (WHO/BS/2019.2371) recommended that the new reference reagents be established in order to extend the existing WHO international reference reagent collection for blood group genotyping. It further recommended that the existing WHO collection be named “Set A” and the extension materials “Set B” and requested that the two WHO custodian laboratories work together on the labelling of the individual reagents to ensure consistency and clarity in the WHO catalogue.

6.1.6 **First WHO Repository of red blood cell transfusion relevant bacterial reference strains**

The transmission of bacteria by blood products remains one of the major risks in blood transfusion. A number of strategies and methods for addressing this risk have been implemented or are under development. These approaches require the use of appropriate bacterial reference strains to produce reliable test results
for detecting, or confirming the elimination of, potential bacterial contaminants in blood components. Validation of these new techniques requires the use of both blood and its components as a matrix and microorganisms representing typical contaminants. However, due to the antimicrobial activity exerted by blood compounds (due to the presence of antibodies, leukocytes or complement factors) such microorganisms have to meet certain requirements. Bacterial isolates generally exhibit different growth patterns in blood, which is also affected by donor variability. Thus, the artificial inoculation of blood components with uncharacterized bacteria can lead to false results depending upon the method used. Transfusion-relevant bacterial reference strains are therefore provided as frozen suspensions with a known cell count to be used in the assessment of microbiological methods or testing strategies for improving blood safety.

In 2010, the Committee recommended the inclusion of four bacterial strains in the First WHO Repository of platelet transfusion relevant bacterial reference strains. In 2015, this repository was expanded following the addition of 10 further strains. However, due to the different storage conditions required for platelets compared to RBCs, the majority of these reference strains are not suitable for use with RBCs.

Two international collaborative studies involving 15 laboratories in 10 countries had therefore been coordinated by PEI in cooperation with members of the ISBT Working Party on Transfusion-Transmitted Infectious Diseases Subgroup on Bacteria. The following six strains had met previous selection criteria and were tested for their ability to grow in RBCs: *Listeria monocytogenes* (PEI code PEI-A-199); *Serratia liquefaciens* (PEI code PEI-A-184); *Yersinia enterocolitica* (PEI code PEI-A-105); *Yersinia enterocolitica* (PEI code PEI-A-176); and two strains used in the platelet repository, *Pseudomonas fluorescens* (PEI code PEI-B-P-77) and *Serratia marcescens* (PEI code PEI-B-P-56). During the study, these strains were intentionally inoculated into RBC units at low concentration (approximately 10–25 colony forming units (CFU)/platelet bag) and the total number of bacterial counts determined over time. To take account of donor variability and different RBC compositions, the studies were conducted in 10 countries.

With the exception of *S. marcescens*, all tested strains showed good or excellent growth in RBCs. A distinction could also be made with respect to the growth kinetics of *L. monocytogenes* which exhibited slow but steady growth during the testing period of 42 days (reaching up to $10^6$ CFU/mL) while the remaining four strains exhibited faster growth, reaching a stationary phase after 21–28 days (up to $10^9$ CFU/mL). However, all of these five strains grew consistently in RBCs and were therefore proposed as the First WHO Repository of red blood cell transfusion relevant bacterial reference strains.

During extensive discussion of the proposal, the Committee was informed that there were no plans to further expand the bacterial reference
repository or to replace the *S. marcescens* isolate selected for the study, though this could be reconsidered should evidence emerge indicating its necessity. Several other *S. marcescens* isolates had been tested during the preparatory phase of the collaborative studies and one isolate implicated in a transfusion incident tested for growth in RBCs. None of these isolates had exhibited more-promising growth characteristics. The Committee noted that although the reference strains would be issued with a description of how to dilute the isolates, no general instructions for their use and microbiological handling was included. It was therefore suggested that some instruction should be included to facilitate standardized handling. In addition, the use of colour changes as an indicator of bacterial contamination was considered to be insufficiently sensitive, as the effect was only seen at very high bacterial concentration, and could be confused with the discoloration caused by haemoglobin degradation. The Committee considered the report of the study (WHO/BS/2019.2377) and recommended that the five candidate materials PEI-A-199, PEI-A-184, PEI-A-105, PEI-A-176 and PEI-B-P-77 be established as the First WHO Repository of red blood cell transfusion relevant bacterial reference strains.

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

6.2.1 Proposed Second WHO International Standard for plasminogen-activator inhibitor 1 (plasma, human)

Plasminogen-activator inhibitor 1 (PAI-1) is a serpin inhibitor of tissue-type plasminogen activator and urokinase-type plasminogen activator, which are key activators of the plasminogen-plasmin system responsible for the dissolution of fibrin blood clots. PAI-1 is synthesized as an active inhibitor but spontaneously converts to an inactive (latent) form which does not interact with target proteases. Elevated levels of PAI-1 are associated with cardiovascular disease and post-operative thrombosis risk. PAI-1 levels may also correlate with prognosis in certain forms of cancer, metabolic syndrome, obesity and aging. Loss of PAI-1 is associated with bleeding.

The Committee was informed that the long-term stability of the First WHO International Standard for plasminogen-activator inhibitor 1, which is now almost 30 years old, had been queried. NIBSC is planning to investigate this issue and would like to take the opportunity to calibrate a replacement international standard. This proposal has been based, in part, on the availability of better sources of active PAI-1, including recombinant PAI-1 (rPAI-1) expressed in *E. coli*, and on the development of new methods (bioimmunoassays) for PAI-1 activity determination. Anticipated users include manufacturers of PAI-activity kits. Although not used in large amounts, the current international standard is distributed worldwide and is of global importance. Candidate material
(recombinant PAI-1 expressed in *E. coli*) has been obtained and will be added to plasma prior to filling and freeze-drying. It is anticipated that between 4000 and 5000 ampoules of the replacement international standard would be available. A collaborative study will compare the activity of the candidate material relative to the current international standard using the range of methods currently in use, which can broadly be divided into chromogenic assays and bioimmunoassays.

The Committee enquired if any differences were expected in the activity of rPAI-1 compared with native PAI-1 and was assured that differences in post-translational modifications do not influence PAI-1 activity. Discussion also took place on the stability of PAI-1 and, given the apparent loss of activity of the current standard, whether a comparative study for stability was planned that included the current standard as reference. The Committee endorsed the proposal (WHO/BS/2019.2378) to develop a Second WHO International Standard for plasminogen-activator inhibitor 1 (plasma, human) and recommended that only the stability of the new material be assessed.

6.2.2 Proposed Third WHO International Standard for thrombin

Thrombin catalyses the conversion of fibrinogen to fibrin, which forms an insoluble scaffold that holds blood clots together to minimize blood loss following blood vessel injury. The WHO international standard is used worldwide by manufacturers to calibrate the thrombin and fibrinogen components of fibrin sealant (fibrin “glue”) kits, which can be used as a topical haemostat, sealant or adhesive in surgical procedures. Recently, standalone recombinant thrombin products have been approved as a topical haemostat for minor bleeding in surgery. In addition, suppliers of thrombin reagents for clinical and research laboratories use the current WHO international standard to label the potency of their products, while alpha-thrombin (the type used in the international standard) is also used to measure the biological activity of hirudin-based anticoagulant pharmaceutical products.

The Second WHO International Standard for thrombin was established in 2003 and unified the WHO/NIH standards with a common unit. Sales of this standard have been in the region of 290 ampoules per year for the past 10 years. As stocks are now running low, a replacement is required. The source material used will be purified alpha-thrombin derived from human plasma. The material used for the current international standard has performed very well and the same material will be sought for the replacement standard. The candidate material will be calibrated relative to the Second WHO International Standard for thrombin in an international collaborative study using chromogenic and fibrin-based methods. A number of industry, academic and regulatory laboratories will be invited to take part in the study, which will also include a preparation used in a previous collaborative study.
Following an assurance that the multicentre study would involve a wide range of laboratories, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a Third WHO International Standard for thrombin.

6.2.3 Proposed First WHO International Reference Reagent for platelet flow cytometry

Platelet transfusions are used to prevent or treat bleeding in people with either a low platelet count or poor platelet function. There are currently only limited reference materials that can aid in test validation and quality control, training and education of operators, and the evaluation of modified platelet products. The clinical community and academic field have now highlighted the need for physical platelet standards that can support the flow cytometry characterization of platelet-infusion products.

The main product parameters that the standard would need to establish by flow cytometry would be size and granularity, expression of CD41+ and CD42+, viability, and contamination with nucleated cells such as megakaryocytes, which are used in the in vitro production of platelets. The proposed reagents would be run prior to the platelet products to ascertain the correct set-up of the flow cytometry assay and to confirm that the assay will reliably evaluate these parameters in a given sample (either platelets derived in vitro or platelets generated from donation). The standard would also help to identify technical issues and would allow for the comparison of data between laboratories.

It is proposed that candidate material, sourced from pooled platelet donations from NHS Blood and Transplant, will be assessed in a collaborative study involving clinical and academic laboratories that perform platelet flow cytometry on products intended for transfusion, or for use in platelet-transfusion research. An external scientific expert will be included in the project team to help ensure that the design and intended use of the reagent will be beneficial to the platelet community.

The Committee was assured that the flow cytometry assays to be used would be suitable for the characterization of both existing and new platelet products and clarification was given that the proposed reference reagent would not be designed to test the function of the platelets in coagulation. The Committee then endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Reference Reagent for platelet flow cytometry.

6.2.4 Proposed First WHO International Standard for plasma prekallikrein and high molecular weight kininogen

Prekallikrein (PK) and high molecular weight kininogen (HK) are part of the contact activation pathway of blood coagulation in which PK is activated by FXIIa into kallikrein. In turn, kallikrein continues the FXII activation loop
while also initiating the intrinsic clotting cascade via FXI. Activated PK also cleaves HK to liberate the pro-inflammatory peptide bradykinin. The excessive production of bradykinin is implicated in hereditary angioedema (HAE), a disease characterized by severe bouts of swelling. If the swelling develops in areas such as the respiratory tract, it can be life threatening. HAE type I and type II patients often have low levels, or a defective form, of the C1 inhibitor of plasma kallikrein. However, in the case of the recently discovered HAE type III, patients exhibit normal levels of C1 inhibitor, and so conventional treatments are not effective. In addition to the two currently licensed drugs, a number of other products are now in development. The overall incidence of HAE is estimated to be around 1 in 50 000 people. Deficiencies in PK and HK also exist, with affected individuals exhibiting prolonged clotting times in laboratory tests.

A number of in-house and commercial assays are available for the measurement of PK. The cleavage of and subsequent reduction of HK levels can also be used to measure inhibition of PK activity. However, there is currently no international standard for either PK or HK, with clinical laboratories using their own normal plasma pools or (non-standardized) commercially available plasma pools in laboratory tests. An international standard for PK and HK activity would serve to standardize the laboratory measurement of these analytes and thus help monitor the effectiveness of treatment. Anticipated users of the proposed standard include clinical laboratories and laboratories investigating PK inhibition for HAE treatment. The candidate material will be sourced from human plasma from the national blood service, with approximately 5000 ampoules expected to be produced. An international collaborative study will be conducted, involving 10–20 laboratories with experience in laboratory assays for PK and HK. The candidate material will be calibrated relative to local normal human plasma pools, based on 1 unit of activity per 1 mL plasma. The inclusion of common samples to demonstrate closer agreement with the use of the standard is also anticipated. Moreover, an antigen assignment may be considered if enough laboratories can perform the relevant assays.

The Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for plasma prekallikrein and high molecular weight kininogen.

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

7.1 WHO International Standards and Reference Reagents – cellular and gene therapies

7.1.1 First WHO International Reference Panel for lentiviral vector copy number and First WHO International Reference Reagent for lentiviral vector integration site analysis

Gene therapy is a rapidly evolving field, with lentiviral vectors (LV) commonly used to deliver the functional therapeutic gene. The number of LV integrated into cells is an important indicator of clinical efficacy; however, LV integration is also a safety concern as it can potentially cause insertional mutagenesis. The development of WHO reference materials for lentiviral vector copy number and integration site analysis would be a crucial step towards ensuring delivery of safe and efficacious doses of LV products to patients, and facilitating patient follow-up worldwide.

The Committee was informed that four candidate materials (NIBSC codes 18/142, 18/126, 18/132 and 18/144) had now been evaluated for their suitability to serve as WHO reference materials for LV integration analysis. The materials comprise human genomic DNA containing zero, low or high levels of integrated LV nucleic acid. LV copy number per cell was determined during an international collaborative study using three principal methods (TaqMan qPCR, SYBRGreen qPCR and digital PCR), along with supplementary sequencing-based methods. A total of 32 laboratories in 15 countries participated in the study. It is intended that three of the candidate materials (NIBSC codes 18/142, 18/126 and 18/132) will constitute a First WHO International Reference Panel for lentiviral vector copy number (NIBSC code 19/158). End users will then be able to generate a genomic DNA standard curve from candidate material 18/132 using 18/142 as the diluent and including 18/126 as a positive control (to confirm comparable LV copies/cell to the consensus value for the sample 18/126) in order to validate the assay for unknown sample quantitation. Candidate material 18/144 would then serve as a qualitative First WHO International Reference Reagent for lentiviral vector integration site analysis to support the confident detection of 10 defined integration sites in order to validate the integration-site study protocols used. Stability studies at eight months indicated that all the candidate materials would be stable during long-term storage at −20 °C.

The Committee questioned why the LV copy number assigned per cell was not a whole number. It was clarified that as the number of insertional events per cell was not predictable and the PCR methods were not totally accurate,
the results would be heterogeneous. Following discussion, the Committee considered the report of the study (WHO/BS/2019.2373) and recommended that three of the candidate materials should be established as the First WHO International Reference Panel for lentiviral vector copy number, with assigned values of 0 LV copies/cell (candidate material 18/142), 1.42 LV copies/cell for the low-value material (18/126) and 8.76 LV copies/cell for the high-value material (18/132). The Committee further recommended that the candidate material 18/144 be established as the First WHO International Reference Reagent for lentiviral vector integration site analysis, with 10 defined integration sites.

7.2 Proposed new projects and updates – cellular and gene therapies

7.2.1 Proposed First WHO International Standard for replication-competent lentivirus

Gene therapy protocols involving replication-competent lentivirus (RCL) are rapidly being developed, with seven products already on the market and 138 LV-based and 58 CAR-T cell clinical trials currently ongoing. RCL can be generated by homologous recombination during LV production and the risk of pathogenicity is high due to their HIV origins. The FDA recommends a tolerable limit of < 1 RCL/ human dose and that the level of RCL should be assessed in master cell banks, vector harvest and ex vivo cells (for example, CAR-T cells). Patients are required to be monitored for RCL for 15 years.

The principal aim of the proposal is to develop a primary DNA standard for the limit of detection of RCL as determined by PCR. It is expected that the reference standard will: (a) facilitate the approval of novel products by regulatory authorities; (b) serve as a calibrant for assays and secondary standards (for example, genomic DNA, plasmids, synthetic DNA); and (c) support the development of novel products based on LV independently of product or disease. Anticipated users include manufacturers assessing the safety of their vectors and genetically modified cells, and hospital diagnostic laboratories responsible for following-up on the clinical safety of patients, and where necessary ensuring timely clinical intervention.

The Committee recognized the importance of RCL testing, and after due consideration endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for replication-competent lentivirus.

7.2.2 Proposed First WHO International Reference Reagent for pluripotent stem cell identity for flow cytometry

Human pluripotent stem cells (hPSCs) are currently being used as a starting material for the generation of other cell types (for example, neurons) for evaluation in clinical trials. At present, no approved products or treatments are
available, though over 100 clinical trials involving hPSCs are now under way. Differentiation protocols depend upon highly characterized starting populations of hPSCs while final products must also be checked to ensure the absence of undifferentiated cells. Although both of the above can be assessed using flow cytometry, no corresponding reference material is currently available.

The proposed reference reagent is intended to be used for flow cytometry identity assessment of products, and will facilitate the validation and verification of the identity of hPSC populations used in research and clinical applications. The reference reagent will allow for assessment of batch-to-batch consistency, validation of equipment and identification of contaminants in final products, while also aiding in the harmonization of cellular and gene therapy products.

The human induced pluripotent stem cell (iPSC) line NIBSC8 will be used as the starting material. A master bank will be generated, with each batch derived from this master bank to ensure a potentially unlimited supply. Three vials of fixed material will be sent for testing to each of the laboratories participating in the proposed collaborative study. Each laboratory will be asked to test the reagent, via flow cytometry, for both positive and negative stem cell markers. Laboratories will be asked to use their own in-house procedures and to report back using a provided template.

After due consideration, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Reference Reagent for pluripotent stem cell identity for flow cytometry, but recommended that the project should be renamed to clarify that both iPSCs and embryonic stem cells will be included in the study.

7.2.3 Update on the proposed First WHO International Reference Reagent for mesenchymal stromal cell identity for flow cytometry

Mesenchymal stromal cells (MSCs) – also called mesenchymal stem cells or multipotent stromal cells – are a type of multipotent adult stem-like cell possessing unique regenerative and immunomodulatory abilities that have propelled them into the cellular therapy spotlight. Currently, patients were being recruited for over 100 clinical trials involving either MSCs or MSC-derived products. In 2006, the International Society of Cellular Therapies (ISCT) published the minimal criteria for the definition of MSCs. However, a recent survey had highlighted considerable inconsistency in the characterization and definition of MSC populations. This is further compounded by the inherent variation seen in flow cytometry due to the biological variability of cells, limited stability of samples and different requirements for cytometer set-up and data interpretation. Together, these factors have made it difficult to compare different MSC-based products both within and between laboratories.

A candidate material (NIBSC code 15/270) was prepared from the human iPSC line NIBSC8 and assessed in an international collaborative
pilot study involving 15 laboratories in nine countries. Three ampoules were provided to each of the participating laboratories, who were asked to perform their own in-house method for flow cytometry, using their preferred antibodies and protocols for MSC analysis. The laboratories were deliberately not provided with protocols or standard operating procedures as the aim of the study was to determine the suitability of the candidate material under local conditions. Only instructions on how to reconstitute the MSC vials were provided. Each laboratory reported back on the percentage of cells exhibiting each of the MSC markers (positive or negative) that were tested for.

The candidate material performed well in all conditions, with generally good agreement observed between laboratories. The mean values from the study fell very close to the ranges for percentage expression for each of the ISCT-recommended markers for MSCs and it was possible to generate a range for each marker that was ± 2SD. The reagent is not intended to be a replacement for the ISCT values but rather a tool to help researchers validate their equipment and results. In addition, the pilot study allowed for the identification of a number of issues relating to the development and establishment of novel reference reagents for cellular and gene therapy products. For the study reference reagent batch, it was proposed that positive markers CD105, CD73, CD90 and CD44 should ideally be close to the mean and not outside the 2SD range for each individual value. For negative markers, it was proposed that individually these should not be scored > 5% (negative cocktail > 6%) with higher values potentially indicating an assay outside of specification.

Following discussion of the challenges associated with the production of MSCs that express the correct markers, the Committee agreed that this “proof of principle” trial had successfully highlighted both the potential benefits and key issues in developing novel reference materials for advanced therapies. The Committee accepted the proposed specifications for the markers and after due consideration endorsed the proposal (WHO/BS/2091.2376) to further pursue the development of a First WHO International Reference Reagent for mesenchymal stromal cell identity for flow cytometry.
8. International reference materials – in vitro diagnostics

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

8.1 WHO International Standards and Reference Reagents – in vitro diagnostics

8.1.1 Sixth WHO International Standard for hepatitis C virus RNA for NAT-based assays

Hepatitis C virus (HCV) infections remain a major global public health problem, with around 1.4% of the world’s population (approximately 100 million individuals) being infected. The virus is predominantly transmitted parentally, through intravenous drug use or unsafe medical procedures and contaminated blood. The detection and quantification of HCV by nucleic acid amplification technique (NAT)-based assays is an important tool in the prevention and management of HCV infections. Continued demand for the current Fifth WHO International Standard for hepatitis C virus RNA for NAT-based assays had now resulted in its near depletion.

The Committee was informed that two candidate materials had been prepared and evaluated for their suitability to serve as the proposed Sixth WHO International Standard for hepatitis C virus RNA for NAT-based assays. Both candidate materials (NIBSC codes 18/184 and 18/198) had been prepared from the same HCV RNA-positive plasma donation diluted in pooled human plasma and lyophilized in separate runs. The candidate materials were then evaluated alongside the current WHO international standard, two inactivated cell cultured HCV samples and three HCV-positive plasma samples comprising different HCV genotypes in an international collaborative study involving 19 laboratories in 12 countries. A range of HCV NAT-based assays were used in the evaluation, the majority of which were commercial quantitative assays based on real-time PCR technology.

Overall mean estimates for the potencies of candidate materials 18/184 and 18/198 obtained using both the qualitative and quantitative assays included in the study were 5.48 and 5.46 log_{10} IU/mL respectively. Inter-laboratory variation for all assays was generally lower across the study samples than had been observed in the previous collaborative study to establish the current international standard. HCV RNA measurements obtained from qualitative assays were higher than those from quantitative assays. Data from accelerated thermal degradation studies suggested that there were differences in the stability of the candidate materials, with only 18/184 considered to be demonstrably stable during prolonged storage at −20 °C. Additional data on the short-term stability of both candidates would be needed before any established replacement could be shipped at ambient temperature.
The Committee considered the report of the study (WHO/BS/2019.2358) and recommended that candidate material 18/184 be established as the Sixth WHO International Standard for hepatitis C virus RNA for NAT-based assays, with an assigned unitage of \(2.57 \times 10^5\) IU/vial (equivalent to \(5.41 \log_{10}\) IU/vial).

**8.1.2 First WHO international standards for human papillomavirus DNA for low-risk types 6 and 11, and high-risk types 31, 33, 45, 52 and 58 for NAT-based assays**

Assays for the detection and genotyping of human papillomavirus (HPV) DNA in clinical samples are the primary tools used to estimate the burden of HPV disease and assess the impact of vaccination programmes. The need for international reference materials for the standardization and control of HPV NAT-based assays is well recognized. Anticipated users of the WHO international standards for HPV DNA include: (a) public health and research laboratories conducting cervical cancer screening and follow-up testing, epidemiological studies and follow-up studies of vaccines; (b) organizers of proficiency studies; and (c) diagnostic kit manufacturers.

An international collaborative study had been conducted to evaluate a number of candidate materials for their suitability to serve as WHO international standards for HPV DNA for NAT-based assays, covering a range of low-risk and high-risk HPV types. The candidate materials (type 6: NIBSC code 14/256; type 11: 14/100; type 31: 14/258; type 33: 14/260; type 45: 14/104; type 52: 14/262; and type 58: 14/264) were prepared from bulk preparations of cloned plasmids containing full-length HPV genomes. Using an approach similar to that used to establish the current WHO international standards for HPV types 16 and 18 DNA, the candidate materials were formulated in a background of purified human genomic DNA and then filled and freeze-dried in separate procedures. The collaborative study consisted of two parts involving 14 laboratories in 11 countries.

In Part 1 of the study, the unitage (in IU) of each candidate material was determined by calibration against the First WHO International Standard for HPV type 16 DNA, using real-time PCR for the plasmid-encoded ampicillin-resistance (AmpR) gene \(bla\). The mean potency for each candidate, estimated across eight laboratories, ranged from 7.21 to 7.52 \(\log_{10}\) IU/mL. Although these estimates were above the formulation target of \(7 \log_{10}\) IU/mL their accuracy was supported by the correct result obtained for the First WHO International Standard for HPV type 18 DNA, which was included as a monitoring sample. In Part 2 of the study, a range of semi-quantitative and qualitative HPV assays were used to evaluate the candidate materials, with 12 laboratories returning 17 datasets for the detection and/or genotyping of HPV DNA. The genotyping assessment confirmed that each candidate material was monospecific for its designated HPV genotype. Although higher inter-laboratory variability was
observed in the potencies estimated by end-point dilutions compared to the estimates determined by quantitative AmpR assays, the end-point estimates obtained and their variability fell within the ranges observed in the previous study to establish the current WHO international standards for HPV types 16 and 18 DNA. In addition, with the exception of only one dataset, the candidate materials were correctly detected and genotyped across the three independent assays used. Accelerated degradation studies indicated that the proposed standards would be stable during long-term storage at −20 °C and during shipment at ambient temperatures.

The Committee discussed the limitations of assessing the commutability of HPV standards and the requirement for an effective standard to perform in much the same way as the clinical material. Because the proposed international standards are cell-free formulations of purified DNA, the material cannot be used to assess sample-processing steps such as centrifugation, DNA extraction or purification. The Committee acknowledged the challenges associated with producing standards that were similar to clinical material and concurred with the expert views expressed that plasmid-based international standards for HPV DNA genotypes could only be used in the standardization of the amplification steps of NAT-based assays. The Committee indicated that this should be made clear in the text of the instructions for use (IFU) associated with these standards. Discussion also took place on the challenges associated with the choice of matrix for HPV international standards for use in NAT-based assays.

The Committee considered the report of the study (WHO/BS/2019.2360) and recommended that the candidate materials 14/256, 14/100, 14/258, 14/260, 14/104, 14/262 and 14/264 be established respectively as the First WHO international standards for human papillomavirus DNA for low-risk types 6 and 11, and high-risk types 31, 33, 45, 52 and 58 for NAT-based assays, with corresponding assigned unitages of:

- HPV type 6 = $1 \times 10^7$ IU/ampoule (equivalent to $7.0 \log_{10}$ IU/ampoule);
- HPV type 11 = $1 \times 10^7$ IU/ampoule (equivalent to $7.0 \log_{10}$ IU/ampoule);
- HPV type 31 = $1.6 \times 10^7$ IU/ampoule (equivalent to $7.2 \log_{10}$ IU/ampoule);
- HPV type 33 = $1.6 \times 10^7$ IU/ampoule (equivalent to $7.2 \log_{10}$ IU/ampoule);
- HPV type 45 = $1 \times 10^7$ IU/ampoule (equivalent to $7.0 \log_{10}$ IU/ampoule);
- HPV type 52 = $7.9 \times 10^6$ IU/ampoule (equivalent to $6.9 \log_{10}$ IU/ampoule); and
- HPV type 58 = 7.9 \times 10^6 \text{ IU/ampoule} (equivalent to 6.9 \log_{10} \text{ IU/ampoule}).

8.1.3 **First WHO International Reference Reagent for anti-Müllerian hormone (human, recombinant)**

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a homodimeric glycoprotein expressed by the Sertoli cells of the testes and the granulosa cells of the ovary. Measurement of AMH in serum or plasma is used to assess ovarian reserve, the likely response to ovarian stimulation, menopausal status and in paediatric medicine, Sertoli cell function. Currently, immunoassays report in mass units (ng/mL) or in molar units (pmol) based on the apparent molecular weight of the glycosylated dimer using SDS-PAGE. The traceability of the calibration of current assays is unclear and an SI-traceable reference method is not available for accurately assigning a mass value to the protein. In 2014, the Committee recognized the need for an international standard for AMH to be used for the calibration of immunoassays to measure AMH in human serum and plasma.

A candidate material (NIBSC code 16/190) was evaluated by immunoassay in an international collaborative study involving seven laboratories in four countries and generating 21 datasets from 19 different immunoassays. The geometric mean of all laboratory estimates obtained using valid assays for the AMH content of the candidate standard was 511 ng/ampoule (95% CI = 426–612; n = 16; inter-assay GCV = 42%) with a robust mean of 489 ng/ampoule. Thermally accelerated degradation studies indicated that the candidate material would be sufficiently stable to serve as an international reference material. The study also included an assessment of the impact of the proposed reference material on the routine measurement of AMH in patient samples. All laboratories contributed data during the collaborative study through the concomitant measurement of the AMH immunoreactivity of seventeen human serum and five human plasma samples. Commutability of the candidate material 16/190 with patient samples was assessed at six nominal AMH concentrations. Of the 16 valid methods contributing to the study, the candidate material was considered commutable with patient samples in six methods, partially commutable in three methods and not commutable with patient samples in seven methods. It was noted that candidate material was commutable with patient samples in two methods that are in worldwide use.

Although the Committee felt there was unquestionably a need for such a reference material, concern was expressed about the use of immunoassays rather than SI-traceable physicochemical reference methods to assign a mass value to a WHO-endorsed reference material. However, after considerable discussion, it was acknowledged that the industry would be highly unlikely to
accept a switch from SI units to IU. In the absence of a suitable physicochemical reference method, it was agreed that SI units would be assigned in this case. Recognizing that this situation was not unique to AMH reference materials, the Committee suggested that guidance on this issue be included in the revision of the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards.

The Committee considered the report of the study (WHO/BS/2019.2363) and, in light of the lack of commutability with patient samples in several methods, recommended that candidate material 16/190 be established as the First WHO International Reference Reagent for anti Müllerian hormone (human, recombinant) with a consensus geometric mean content of 489 ng/ampoule. The Committee further recommended that the assignment of SI units based on immunoassay data should be highlighted in the IFU.

8.1.4 Second WHO International Standard for insulin (human)

Insulin standards have been used for many years for the standardization of insulin immunoassays used in the assessment of β cell secretion and insulin resistance. The First WHO International Reference Preparation for human insulin, isolated and purified from human pancreas, was established in the 1960s and assigned an IU based on biological assays. Although now exhausted, the majority of insulin immunoassays are traceable to this standard. The current WHO international standards for human, bovine and porcine insulin were established in the mid 1980s with potencies defined in IU/mg. Today, insulin is commercially manufactured and an SI unitage can be assigned by physicochemical methods. The assignment of value to the current First WHO International Standard for insulin (human) had been based on a multi-method collaborative study by in vivo bioassay, and therefore needed to be updated to reflect the transition of insulin internationally to a well-characterized, mass-assigned molecule.

A candidate material (NIBSC code 11/212) was filled into ampoules in accordance with procedures recommended by WHO and an international collaborative study involving 19 laboratories in 11 countries was conducted to assign a value to the proposed replacement international standard. The study was conducted in three phases: (a) assignment of insulin content to the candidate material; (b) assessment of the suitability of the candidate material to serve as a WHO international standard for the calibration of diagnostic immunoassays; and (c) assessment of the suitability of the candidate material to serve as a WHO international standard for the calibration of secondary reference preparations used to assign potency to therapeutic preparations of insulin. Unitage was assigned based on mass balance and filling data.

The candidate material 11/212 is a well-characterized, mass-assigned, material which is immunoreactive and behaves in a similar manner to the
First WHO International Reference Preparation for human insulin. The results of immunoassay and commutability evaluations indicate that the candidate material would be a suitable replacement for this original reference preparation in the continued calibration of immunoassays for human insulin. Historically, the clinical reporting of insulin immunoassay results has been hampered by the use of different conversion factors. The proposed replacement international standard would, for the first time, enable the use of the internationally accepted activity conversion factor for pure insulin of 1 IU = 0.0347 mg. The results of an accelerated degradation study indicated that the candidate material would be sufficiently stable during storage at −20°C.

During discussion it was noted that, although rarely used, the existence of the “legacy” First WHO International Standard for insulin (human) complicated the nomenclature of the proposed replacement standard. Despite the potential of candidate material 11/212 to redefine the insulin standardization framework, the Committee felt that it should not be labelled as the first such standard. The Committee considered the report of the study (WHO/BS/2019.2366) and recommended that the candidate material 11/212 be established as the Second WHO International Standard for insulin (human), to be used in immunoassay, with an assigned content of 9.19 mg/ampoule ± 0.05 mg/ampoule (expanded uncertainty, k = 2). This value can be converted into IU using the internationally accepted specific activity of pure insulin (1 IU = 0.0347 mg).

8.1.5 First WHO international standards for HCT 15 cancer genome, MOLT-4 cancer genome and ATDB102 reference genome

Following its initial endorsement in 2017 of a proposal to develop a WHO international reference panel for cancer mutation detection, the Committee had been requested in 2018 to endorse a revised proposal in which three individual WHO international standards for cancer genomes would instead be developed. Discussion at the time had emphasized the need for a common approach to the development of such standards to increase the coverage of clinically relevant genomes. It was felt that the development of such materials would support next-generation sequencing (NGS) for multiple target detection and quantification in tumour DNA following the recent shift from single-marker to multiplex target analysis approaches. Despite the progress being made in this rapidly growing field, significant challenges remain in the successful clinical implementation of multiplex target assays, including the need for well-characterized multiple-target tumour/normal reference materials.

The Committee was informed that three candidate materials had been prepared comprising: (a) a single genomic preparation of PIK3CA originating from a colon adenocarcinoma (NIBSC code 18/118); (b) multiple genes (TP53, NRAS, PTEN, MAP2K1/MEK1) for acute T-lymphoblastic leukaemia (NIBSC code 18/130); and (c) wild-type representations of the above (NIBSC code
The candidate materials had been characterized and assessed for their suitability in an international collaborative study involving 35 laboratories in 22 countries using 38 different methods. Each laboratory was provided with triplicate coded samples of the three materials and asked to test at five different dilutions to allow for determination of the dilution response of each material. For ease of data analysis, the results were analysed as a single dataset, with NGS and droplet digital PCR data showing excellent concordance for each of the variants, despite the different methodologies used. Preliminary stability data at 7 months indicated no issue with stability for any of the candidate materials assessed at −150 °C, −20 °C and 56 °C. Samples will continue to be assessed on a regular basis (including in accelerated degradation studies) to ensure ongoing long-term stability, and assurance was given that the materials would be suitable for shipping at ambient temperatures.

During discussion, clarification was given that no single unit would be assigned to each material, as three different analyses had been performed on each, namely consensus variant percentage, consensus variant copy number per diploid human genome mass and consensus total copy number per diploid human genome mass. The corresponding values would be shown in the IFU accompanying each material, along with a table showing the expected values at each dilution. The Committee considered the report of the study (WHO/BS/2019.2368) and recommended that candidate materials 18/118, 18/130 and 18/164 be established as the First WHO International Standard for HCT 15 cancer genome, First WHO International Standard for MOLT-4 cancer genome and First WHO International Standard for ATDB102 reference genome, respectively.

8.2 Proposed new projects and updates – in vitro diagnostics

8.2.1 Proposed First WHO international standards for genomic epidermal growth factor receptor variants

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase which when activated leads to the stimulation of multiple downstream pathways, including those responsible for cell survival and proliferation. Variants of EGFR can lead to unregulated EGFR signalling and are associated with lung cancer, which is known to be the most common cancer with an estimated 2.09 million cases occurring globally in 2018. Therapies based on the use of tyrosine kinase inhibitors are available but patients receiving first-generation drugs such as erlotinib commonly develop therapeutic resistance with other therapies then being required. It is therefore crucially important to determine EGFR variant type in order to accurately diagnose patients, administer effective therapy and monitor treatment response. Diagnostic laboratories and assay manufacturers require standards for use in the calibration and determination of the sensitivity of
assays used for these purposes. Anticipated users include diagnostic laboratories and commercial organizations developing kits, assays or secondary standards.

It is intended, subject to obtaining the required licence, that the four most clinically relevant EGFR variants will be produced from CRISPR-engineered cell lines, together with wild-type EFGR derived from a human cell line to provide a sample diluent. It was further intended that these materials would be co-produced with the First WHO international standards for circulating tumour DNA, which the Committee had endorsed for development in 2018, and a parallel collaborative study conducted. This approach is intended to further facilitate the alignment of liquid biopsy based diagnostics with solid tumour based diagnostics as previously outlined in 2018. It was highlighted that the licence required for CRISPR engineering had not been sought at the time of the current proposal and that this would be a consideration in the proposed timeline. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

Having received assurance that patient samples would be included in the collaborative study regardless of whether a CRISPR licence was obtained for candidate material production, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop First WHO international standards for genomic epidermal growth factor receptor variants.

8.2.2 Proposed Second WHO International Standard for beta-2 microglobulin (lyophilized)

Beta-2 microglobulin (B2M) is a protein found on the surface of almost all cells in the body and is shed into the blood, particularly by B lymphocytes and tumour cells. Its concentration in the bloodstream rises due to conditions that increase cell production and/or destruction, or that activate the immune system. Elevated levels of B2M in the blood are frequently associated with cancers such as multiple myeloma and lymphoma, with inflammatory disorders and infections (for example, with HIV or cytomegalovirus), and with kidney disease. B2M is present in most body fluids and the current First WHO International Standard for beta-2 microglobulin (lyophilized) is used by clinical laboratories to measure B2M in blood, urine and occasionally cerebrospinal fluid.

The current standard was established by the Committee in 1985 and it is anticipated that stocks will run out by the end of 2020. There are currently about 70 ampoules of stock remaining plus 100 contingency ampoules, with requests now limited to 1–2 ampoules per customer per year. Prior to the imposing of this restriction in 2017, more than 120 ampoules per year were being issued following a steady upward trend in requests over the last 10 years. The envisaged source materials for evaluation as a replacement international standard will be samples of human plasma obtained as virus-inactivated fresh-frozen pooled
plasma and forwarded for Official Control Authority Batch Release purposes from a commercial source. In the absence of sufficient samples being provided, manufacturers will be contacted directly for a donation of material.

Collaborative study participants are likely to be manufacturers of B2M assay kits (usually ELISA kits) who will be asked to calibrate the candidate materials in terms of the current international standard by in vitro assay. Following the reporting back of data to NIBSC for statistical analysis, consensus will be reached on an assigned unitage. Patient samples containing a range of B2M concentrations will be included in the study to allow for commutability assessment. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

Noting the relatively high demand for this reference material and the stability of the current international standard, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a Second WHO International Standard for beta-2 microglobulin (lyophilized).

8.2.3 **Proposed First WHO International Standard for anti-Q fever serum (human)**

Query fever (Q fever) is a worldwide zoonotic infection caused by *Coxiella burnetii*. *C. burnetii* is considered to be one of the most infectious agents for humans, with a single bacillus capable of causing acute and chronic life-threatening disease. It is categorized as a class B bioterrorism agent by the United States Centers for Disease Control and Prevention. Q fever presents with influenza-like symptoms and is therefore often under reported. It has been estimated that there around 4000 acute cases occurred in the Netherlands between 2007 and 2010. The diagnosis of Q fever relies on the use of serology screening, with the immunofluorescence antibody assay being the preferred technique. Given the current large variability in the sensitivity and specificity of the assays used, the development of an international standard would facilitate assay harmonization and support immunogenicity studies of new vaccines.

Candidate materials will be derived from pooled sera obtained from patients in the Netherlands with historic Q fever infections. Patients with chronic Q fever will be excluded. Several international research groups will support the development of the reference material, with immunoglobulin G and immunoglobulin M testing expected to commence before the end of 2019. A small trial lyophilization will be conducted at NIBSC, followed by the full collaborative study in late 2020. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

During discussion, it was highlighted that following the Q fever outbreak in the Netherlands an in-house assay had been developed by Sanquin
and it was advised that this group should be approached for participation in the collaborative study. The Committee then endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for anti-Q fever serum (human).

8.2.4 Proposed First WHO International Standard for Rift Valley fever virus RNA for NAT-based assays

Rift Valley fever (RVF) is a viral zoonosis transmitted through contact with the blood and organs of infected animals, or through the bite of infected mosquitoes. Sporadic outbreaks have occurred in Africa, most recently in Mayotte Island and the Central African Republic. The largest outbreak to date occurred in Sudan in 2007. As well as its public health significance, the economic impact of RVF on farming-based communities can be severe due to livestock loss. Following its spread in African countries, the disease has the potential to emerge beyond its current geographical area. Currently, no human vaccine has been licensed and no treatment exists. Due to its outbreak potential WHO, CEPI and the United Kingdom Vaccine Network have listed RVF as a priority disease.

An international standard is therefore required for the standardization of the molecular assays (such as PCR, quantitative PCR and digital PCR) used to detect infection with RVF virus. Intended users of the standard include clinical and public health laboratories, assay kit manufacturers, vaccine manufacturers and research laboratories. It was proposed that RVF virus would be sourced before amplification at BSL3 and subsequent heat inactivation. Although the envisaged approach is straightforward, the existence of 15 different lineages causing disease will make selection of the most relevant material challenging. In addition, in the United Kingdom RVF virus is categorized as a schedule 5 agent under the Anti-terrorism, Crime and Security Act (2001) and shipping and security issues would need to be overcome. The Committee was informed, however, that starting materials had now been sourced and it was expected that the results of a proposed collaborative study would be submitted to the Committee for its consideration in 2021.

The Committee recommended that laboratories in the WHO African Region should be invited to participate in the collaborative study, and endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for Rift Valley fever virus RNA for NAT-based assays.

8.2.5 Proposed First WHO international reference reagents for microbiome analysis by NGS

NGS studies which survey the microbiome have demonstrated that significant changes in the microbiome are associated with a range of diseases such as infection, inflammatory bowel disease and colorectal cancer. Restoring the
microbiome from a state of dysbiosis (imbalance) to a healthy state has been proposed as a strategy for treating such diseases. Despite uncertainty regarding its mechanism of action, the most promising microbiome therapy to date is faecal microbiota transplantation (FMT) in which processed faecal material from a human donor is used to alter a patient’s gut microbiome. As of June 2019, there were 110 Phase II and 29 Phase III clinical trials investigating FMT as an intervention for a variety of diseases. In addition, four Phase IV clinical trials are currently under way on the use of FMT to treat antimicrobial resistant infection (n = 1) and ulcerative colitis (n = 3). A number of studies are also under way on the use of live biotherapeutic products based on manufactured bacterial strains, most notably Ser-109 (oral) and RBX2660 (enema solution) for the treatment of *Clostridium difficile*, both of which are in Phase III trials.

Studying changes in the microbiome is the principal way in which the outcome of microbiome therapy can be assessed and is largely based on NGS sequence analysis of DNA in samples extracted from a specific body site. Several studies have concluded that the effective standardization of NGS protocols will be a critical step for making progress in this field, and for effective translational research and product development. Despite this, no widely available NGS reference reagents are currently available for the study of the microbiome.

The Committee was informed that NIBSC had recently developed a method to accurately benchmark the performance of microbiome analytical pipelines using DNA from core species known to be present in each distinct human microbiome site. This method will potentially allow for accurate comparison of clinical trial and translational research studies. Two different approaches to process standardization were described, with one using 16S rRNA sequencing and the other using shotgun sequencing. In either case, it was proposed that standardization would be required at two stages in the process, namely the nucleic acid extraction process and the NGS bioinformatic analysis. It was recognized that this is a complex concept, and issues such as which strains to include in a reference reagent, what should be the measurand and how would the reference materials be used in conjunction with clinical outcome remained to be resolved.

The proposed NGS reference reagents consisted of DNA from 20 core species of a specific body site microbiome (the “mock community”). There were two reagents for each body site, one with even relative proportions of strains (mixed) and one with strains differing in relative proportions by two orders of magnitude (hi-lo) simulating the natural variety in the compositions of the same microbes across a cohort. The reagents would be compatible with a range of sequencing technologies, and six different sets would be needed to cover the analysis of six distinct microbiome sites in the human body (gut, lung, oral cavity, nasopharynx, skin and vagina).
Given the novelty of standardization in this area, this proposal stimulated considerable discussion. The Committee recognized that one critical challenge would be the assessment of cross-reactivity between species. Assurance was given however that the proposed reference reagents would include, where possible, more than one strain of the same species as well as multiple species from the same family. The Committee also queried how the DNA-extraction process would be standardized and agreed that, as it would be impossible to standardize the entire process at once, a multistep approach would be required, starting with the downstream processes. It was further explained that the longer term goals of the project would be to provide different types of starting material (for example, extracted DNA, matrix spiked materials or whole cell reagents) to mirror the different scenarios that may be experienced by the clinical laboratory. Clarification was provided that the strains had been sourced from a German strain bank and had therefore been well characterized with a publicly available bioinformatic pipeline. There was also some discussion regarding the potential use of the proposed reference reagents in microarrays. It was clarified that the proposed approach would not be appropriate as microarray assays require the species to be defined prior to detection. By contrast, NGS allows for the detection and measurement of “unknown” samples.

The Committee noted that this proposal exemplified the increasing demand for novel standards for biological therapies requiring innovative methods of testing, and endorsed the proposal (WHO/BS/2019.2378) to develop First WHO international reference reagents for microbiome analysis by NGS.

8.2.6 Proposed First WHO International Standard for Legionella urinary antigen

Infection caused by Legionella pneumophila serogroup 1 is currently diagnosed using ELISA-based kits and in-house tests that detect antigen in urine. These tests are prone to false positives, leading to unnecessary antibiotic use and expense in carrying out source investigations. Although many diagnostic laboratories use such kits for the detection of Legionella urinary antigen, there are currently no reference materials available for the calibration and validation of the assays used. As a result, the sensitivity and specificity of the various diagnostic tests currently used are unknown. The development of an international standard would allow for the comparison of different kits and methods, help reduce both intra- and inter-laboratory variation, and promote harmonization of testing.

The Committee was informed that Public Health England had donated a sample of human urine that was highly positive for Legionella antigen. It was intended that a candidate material would now be derived from the sample for characterization in a collaborative study involving diagnostic laboratories worldwide. Participating laboratories would be those that test for Legionella
urinary antigen on a regular basis and would be asked to evaluate the candidate material using their routine in-house methods in order to assign a unitage in IU. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

The Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for Legionella urinary antigen.

8.2.7 Proposed First WHO International Standard for Aspergillus fumigatus DNA for NAT-based assays

*Aspergillus fumigatus* is the most common etiological agent of aspergillosis. Invasive aspergillosis is a life-threatening infection that can affect many patient groups. Mortality rates of 35.6–60.5% for invasive aspergillosis at 90 days after diagnosis have been reported, with recent estimates indicating that there are around 250,000 cases worldwide each year. Early and accurate diagnosis is vital for good patient outcomes. PCR assays are the most commonly used assay type with six commercial kits on the market and a further four currently used under a “research only” label.

It was proposed that genomic DNA from the well-characterized AF293 strain of *Aspergillus fumigatus* be used as a candidate material for evaluation as an international standard. A collaborative study will be conducted involving 10–20 laboratories worldwide that routinely use NAT-based assays for the diagnosis of aspergillosis. The Committee was informed that the candidate material had already been donated by the Fungal PCR initiative. In order to maintain continuity of an arbitrary unit that has already been assigned and accepted in the community, a PCR calibrator previously developed by the Fungal PCR initiative would also be included in the study. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

The Committee noted that this would be the first fungal standard to be developed and endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for *Aspergillus fumigatus* DNA for NAT-based assays.

8.2.8 Proposed First WHO International Standard for cell-associated HIV nucleic acid and First WHO International Reference Panel for cell-associated HIV nucleic acid

In 2017, an estimated 36.9 million people were living with HIV and 1.8 million new infections were diagnosed globally, of which 180,000 were in children. Cell-associated HIV nucleic acids are widely used as clinical biomarkers to: (a) diagnose HIV infection in infants; (b) predict disease progression and response to treatment; (c) assess the size of the latent reservoir; and (d) monitor
patient status under pre-exposure prophylaxis. However, the current international standards for HIV do not support this area as they were developed for assays that detect free virus in the blood.

An initial study using DNA spiked into a cellular background revealed significant variability in cell-associated HIV DNA detection across a range of subtypes. In addition, the study also highlighted limitations in the cellular material chosen as it was apparent that the HIV genome was unstable in 8E5 cells, which is one of the reference reagents commonly used for molecular assay qualification. It was proposed that chronically infected cell lines containing HIV DNA and RNA would be spiked into plasma, lyophilized and evaluated in an international collaborative study for their suitability to serve as reference materials. A single international standard for HIV subtype C nucleic acid would then be developed along with a supporting international reference panel comprising a selection of subtypes and circulating recombinant forms. It was highlighted that the candidate reference materials would require comprehensive characterization of their nucleic acid content, and that the various analytes measured (for example, DNA, RNA or DNA+RNA) may require assignment of individual IU. In addition, while plasma would be the proposed diluent of choice, the evaluation of other matrices would also be necessary to address the potential problem of commutability. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

The Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for cell-associated HIV nucleic acid and a First WHO International Reference Panel for cell-associated HIV nucleic acid.

8.2.9 Update on the stability of the First WHO International Reference Panel for Ebola virus VP40 antigen

The Committee was updated on the stability of the First WHO International Reference Panel for Ebola virus VP40 antigen, which it had recommended for establishment in 2016. This material is intended to be used alongside other reference materials in the qualitative assessment of the performance of assays for the detection of Ebola virus VP40 antigen. At the time of its establishment, only limited data were available for determining its long-term stability and the Committee had recommended that the panel should be shipped at −20 °C until a more robust dataset was available for further consideration.

A study had now been conducted in which two freeze-dried preparations of bacterially expressed and purified full-length VP40 had been assessed for degradation across a range of temperatures from −20 °C to 56 °C. Accelerated degradation data from the point-of-care tests and potency assays indicated that the material continues to be sufficiently stable for continued use as the
First WHO International Reference Panel for Ebola virus VP40 antigen. Taken together, the point-of-care data showed that the two study samples retained their reactivities when held at temperatures as high as 37 °C for up to 3 months. ELISA results obtained from the same samples indicated potencies ranging from 0.86 to 0.92 relative to samples stored at −20 °C. When stored at −20 °C, the estimated loss in potency for either sample was less than 0.04% per year, and when stored at 4 °C, the loss was < 0.75% per year. The data also indicated that the reference material may be shipped at ambient temperatures. However, to avoid ambient temperatures approaching 37 °C, it was considered advisable to ship the material on ice packs when dispatching to tropical regions. It was highlighted in closing that this study had not been intended to address the separate issue of the biological relevance of Ebola virus antigens expressed in bacterial versus eukaryotic systems, and that further developmental assessment would be required before Ebola virus antigen preparations could serve as WHO reference materials for use in the WHO prequalification of point-of-care tests.

After due consideration of the data presented, the Committee recommended that the status of the First WHO International Reference Panel for Ebola virus VP40 antigen be upheld. It was further recommended that the material could be shipped at ambient temperature with the proviso that ice packs should be used when dispatching the materials to tropical regions.

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

9.1 WHO International Standards and Reference Reagents – vaccines and related substances

9.1.1 First WHO International Standard for EV71 inactivated vaccine, First WHO International Reference Reagent for EV71 genotype C4 inactivated vaccine and First WHO International Reference Reagent for EV71 genotype B4 inactivated vaccine

Enterovirus 71 (EV71) is the major causative agent of severe and fatal hand, foot and mouth disease (HFMD). Neurological complications induced by EV71 have become a serious public health problem in Asia-Pacific. For this reason, a number of vaccines for the effective prevention of HFMD caused by EV71 have been licensed with several others under development across this region. The First WHO International Standard for anti-EV71 serum (human) was established in 2015 to ensure that the methods used to measure serum neutralizing activity or antibody levels against EV71 were accurate, sensitive and reproducible.

The Committee was informed that an international collaborative study involving 14 laboratories from Europe, Asia and North America had been conducted to establish a First WHO International Standard for EV71 inactivated vaccine. Four candidate EV71 samples (NIBSC codes 18/116, 18/120, 18/122 and 18/156) were provided by three different manufacturers and analysed by participating laboratories using their in-house ELISA method and a common method (designed by NIBSC and NIFDC), alongside a low-titre vaccine sample. The Chinese National Standard for EV71 inactivated vaccine was also included in the study since it was the only reference available with an assigned potency value. Stability studies indicated that all four candidate materials were stable for up to 12 months at between −70 °C and 4 °C, and showed no loss of potency over 6 weeks at up to 45 °C.

There was largely good agreement between the laboratories in terms of EV71 antigen potency estimates whichever candidate material was used as a standard, and as a result all four candidate samples were considered to be suitable for use as an international standard. Levels of both intra-laboratory repeatability and inter-laboratory reproducibility were also generally very high. A second phase of the study was planned to evaluate these same candidate materials for use in in vivo potency assays.

One crucial issue not investigated in this study but requiring resolution in the near future was the relative contributions made by empty and full virus particles (both of which are present in EV71 vaccines) to the antigenic
and immunogenic properties of different EV71 vaccine products. Assays and reagents to specifically measure the quantity of empty and full virus particles present, along with analysis of their relative contribution to the antigenic and immunogenic properties of different EV71 vaccine products, will be fundamental to ensuring the appropriate standardization of EV71 vaccines used globally.

The Committee advised that real-time stability studies should continue for a number of years, though there was no need to continue the accelerated degradation studies. The Committee considered the report of the study (WHO/BS/2019.2362) and recommended that: (a) candidate material 18/116 be established as the First WHO International Standard for EV71 inactivated vaccine with an assigned unitage of 3625 IU/ampoule; (b) candidate material 18/120 be established as the First WHO International Reference Reagent for EV71 genotype C4 inactivated vaccine, with an assigned unitage of 300 IU/ampoule; and (c) candidate material 18/156 be established as the First WHO International Reference Reagent for EV71 genotype B4 inactivated vaccine, with an assigned unitage of 250 IU/ampoule.

### 9.1.2 Third WHO International Standard for tetanus toxoid for use in flocculation test

Tetanus is caused by a neurotoxin produced by the bacterium *Clostridium tetani*. Active immunization against tetanus is based on the use of tetanus toxoid, a chemically detoxified preparation of tetanus toxin, to induce protective antibody responses. The antigenic strength and purity of the bulk toxoid is evaluated by measurement of “limit of flocculation” (Lf) units. Tetanus vaccines are an essential component of the primary immunization schedule of children and have been part of the WHO Expanded Programme on Immunization (together with diphtheria and pertussis components) since its inception in 1974.

The Committee was informed that stocks of the Second WHO International Standard for tetanus toxoid for use in flocculation test, established in 2007, were now almost depleted. Currently, only around 200 ampoules remain with an annual average demand of around 450 ampoules. A project had therefore been initiated to calibrate and establish a replacement international standard. Candidate material (NIBSC code 16/302) had been provided to NIBSC for formulation and filling prior to freeze-drying. An international collaborative study involving 17 laboratories in 10 countries had then been undertaken. Calibration of the candidate material was performed using a Ramon spectroscopy flocculation method, standardized using the current WHO international standard, and a unitage of 971 Lf/ampoule assigned based on the results obtained. Intra-laboratory GCV ranged from 0% to 7% with an inter-laboratory GCV of 11.7%. Data from accelerated thermal degradation studies showed no temperature-dependent loss of activity after storage for 9 months,
indicating that the material would likely have good long-term stability. Further stability studies at later time points will be performed subject to establishment of the international standard.

The study also provided an opportunity to assess the use of alternative methods for measuring the tetanus antigen content of bulk purified toxoid. Thirteen participating laboratories were asked to determine the Lf value of candidate material 16/302 using an ELISA assay established at NIBSC (n = 9) or their own in-house methods (n = 4) for Lf determination. The results obtained from a total of 13 datasets indicated that each of the various methods used might provide suitable alternatives to the Ramon flocculation test, subject to validation. Subject to further investigation, the proposed replacement international standard might also be a suitable reference preparation for use with these methods.

The Committee considered the report of the study (WHO/BS/2019.2369) and, noting that its outcome had been straightforward, recommended that candidate material 16/302 be established as the Third WHO International Standard for tetanus toxoid for use in flocculation test, with an assigned potency of 970 Lf/ampoule. The Committee also recommended that further studies be undertaken to explore the use of this standard in ELISAs and potentially in other technologies such as Ramon spectroscopy.

9.1.3 Extension of use of the First WHO International Standard for antiserum to respiratory syncytial virus

The development of a vaccine against respiratory syncytial virus (RSV) remains a recognized global priority. Activity in this area has increased significantly in recent years, with at least 39 RSV candidate vaccines in development, 19 of which were now in human clinical trials. In 2017, the Committee had recommended the establishment of the First WHO International Standard for antiserum to respiratory syncytial virus. This international standard is recommended for use in the assessment of RSV subtype A neutralization titres in human serum. The Committee was informed that in order to investigate the possible extension of use of this standard to include neutralization titres against RSV subtype B, a further collaborative study had been conducted. In addition, given the previously noted effect of the use of guinea-pig complement in RSV assays, an evaluation had also been made of the impact of the use of such complement on assay titres and on the effectiveness of the international standard.

The international collaborative study had involved 11 laboratories in six countries, representing university laboratories, manufacturers/developers of RSV vaccines and public health laboratories. All laboratories used their own in-house virus neutralization assay and their own virus stocks. The study samples comprised the current international standard and its potential replacement
material, individual sera from naturally infected humans, a monoclonal antibody to RSV (palivizumab) and samples from the BEI Resources panel of human antiserum and immune globulin to RSV. Five of the laboratories returned data from neutralization assays conducted both with and without the inclusion of serum complement.

Study results indicated that inter-laboratory variability in neutralization titres was significantly reduced when values were expressed relative to both the current international standard and its potential replacement material. In addition, the use of complement did not affect the ability of the international standard to decrease such variability, with the use of the international standard also decreasing the variability between titres obtained from assays performed with or without complement, thus making these assays comparable.

The Committee considered the report of the study (WHO/BS/2019.2372) and recommended that the current First WHO International Standard for antiserum to respiratory syncytial virus (NIBSC code 16/284) and its potential replacement material (NIBSC code 16/322) be extended to include titres against RSV subtype B, with assigned unitages of 1000 IU/vial and 690 IU/vial of anti-RSV neutralizing antibodies respectively. The Committee agreed that the labelling of the current standard would not be changed but that the IFU would be modified to include the unitage for subtype B.

9.1.4 First WHO international standards for meningococcal serogroups W and Y polysaccharide

Invasive meningococcal disease causes mortality and morbidity worldwide, particularly in infants. With the incidence of meningococcal serogroup C (MenC) disease controlled in many countries through routine vaccination, an increasing focus has been placed on disease caused by other serogroups, including MenW and MenY. Prior to the establishment of international standards for MenC polysaccharides in 2011 and for MenA and MenX polysaccharides in 2015, standardization of the measurement of the polysaccharide content of plain polysaccharide or conjugate vaccines had been problematic due to the variety of assay methods and standards used by different manufacturers and control laboratories. These problems remain for the determination the MenW and MenY polysaccharide content of vaccines. It is intended that the proposed reference materials will be used to standardize the quantification of the MenW and MenY polysaccharide content of meningococcal polysaccharide (conjugate) vaccines and their intermediate components.

In an international collaborative study involving 12 laboratories in 11 countries, two candidate materials (NIBSC codes 16/152 and 16/206 respectively) were assessed for their suitability to serve as quantitative standards for MenW and MenY polysaccharide using various physicochemical assays. To date, NIBSC had produced seven WHO international standards for bacterial
polysaccharides. The most recently produced of these, namely Men A, Men X and the Vi polysaccharide standards, had been assigned unitages based on quantitative nuclear magnetic resonance (qNMR) data. At the outset of the current collaborative study, the intention had been to assign the unitages of the proposed MenW and MenY standards on the same basis. However, following submission of the original report (WHO/BS/2018.2336) in 2018, the Committee had recommended that unitages be assigned based on the resorcinol assay and the revised report (WHO/BS/2019.2374) had therefore been updated accordingly.

Although real-time stability and accelerated thermal degradation studies were ongoing, the amount of polysaccharide per ampoule remained consistent under all conditions over a 24-month period. In accelerated thermal degradation studies, a decrease in molecular size of the polysaccharide had been observed after storage of the lyophilized material at 37 °C and 56 °C. The candidate material was however considered to be stable when stored at the lower temperatures of −70 °C, −20 °C and 20 °C.

The Committee reaffirmed the acceptability of assigning the unitages for MenW and MenY polysaccharides on the basis of resorcinol assay data. The Committee considered the updated report of the study (WHO/BS/2019.2374) and recommended that: (a) candidate material 16/152 be established as the First WHO International Standard for meningococcal serogroup W polysaccharide, with an assigned content of 1.015 ± 0.071 mg/ampoule (expanded uncertainty, k = 2.13); and (b) candidate material 16/206 be established as the First WHO International Standard for meningococcal serogroup Y polysaccharide, with an assigned content of 0.958 ± 0.076 mg/per ampoule (expanded uncertainty, k = 2.26).

9.2 Proposed new projects and updates – vaccines and related substances

9.2.1 Proposed First WHO International Reference Reagent for tetanus antitoxin for the flocculation test

Tetanus vaccines are among the most widely used and successful human vaccines, and are used in the primary immunization schedule of children and to reinforce immunity in adolescents and adults. The measurement of antigen content in Lf units using the flocculation test is a critical step in the production process of tetanus vaccines. The flocculation test is an immunological binding assay which measures the antigen content of toxoid in Lf units based on the formation of visible complexes between toxoid and antitoxin. As the tetanus toxoids used in the production of vaccines for human use must meet minimum requirements for antigenic purity then the standardization of the flocculation tests used is essential.
The current reference reagent for equine tetanus antitoxin for use in the standardization of flocculation tests was established in 1979 as a British Reference preparation and is widely used, with an average level of demand of 400 ampoules each year. As there are currently only around 1500 ampoules remaining, and as the current rate of use is likely to continue, there was now a requirement to initiate a project to replace this standard before stocks are depleted.

It is intended that equine tetanus antitoxin with an approximate potency of 1500 IU/mL will be purchased from a European manufacturer and its neutralizing potency determined by NIBSC. The suitability of the candidate material for use in the standardization of flocculation tests will then be demonstrated in a small collaborative study. The proposed reference material will be assigned a nominal potency value (in IU/mL) based on the NIBSC results obtained. It was anticipated that submission of the study outcomes for consideration by the Committee would take place in 2021.

The Committee noted that this appeared to be a straightforward replacement and endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Reference Reagent for tetanus antitoxin for the flocculation test.

9.2.2 Proposed First WHO International Standard for anti-Rift Valley fever virus immunoglobulin

As outlined above in section 8.2.4, the viral zoonosis Rift Valley fever (RVF) is listed as a priority disease for preparedness efforts by WHO, CEPI and the United Kingdom Vaccine Network due to its outbreak potential. Standardized and calibrated assays will be vital for the accurate evaluation of treatments, including antibody therapies and vaccines now in development, and for case management and surveillance. There is therefore a need for reference materials in this area.

It was proposed that a pool of plasma or serum from convalescent individuals would be solvent-detergent treated using a validated method to provide the research community with safe material for the standardization of serological assays, both enzyme immunoassays and neutralization assays. An international collaborative study involving 10–20 laboratories worldwide would then be conducted to assess the suitability of the candidate material. The anticipated users of the proposed international standard included clinical and public health laboratories, vaccine manufacturers, therapeutic antibody producers, assay kit manufacturers and research laboratories. It was highlighted that sourcing the material could be challenging, and that obtaining government approvals and setting up material transfer agreements with endemic countries may be time consuming. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.
The Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Standard for anti-Rift Valley fever virus immunoglobulin.

9.2.3 Proposed First WHO International Reference Reagent for recombinant pertussis toxoid

Pertussis toxin (PT) in its detoxified form is the main component of acellular pertussis vaccines. Currently, most such vaccines contain chemically detoxified PT. However, genetic detoxification resulting from mutations introduced into the A subunit of PT has been shown to produce toxoids that are non-toxic and may be more immunogenic than chemically detoxified PT. Recently, an acellular pertussis vaccine containing genetically detoxified recombinant PT (rPT) had been licensed for use in Thailand and is going through the registration process in other countries, with more such products in development.

Native/active PT is required as a positive working reference by manufacturers, control laboratories and research organizations for both in vivo and in vitro assays, and for research and development. Approximately 100 ampoules of the Second WHO International Standard for pertussis toxin are dispatched each year. A suitable rPT material could be used as a negative control in toxicity assays such as the Chinese hamster ovary cell clustering assay and for other aspects of vaccine antigen characterization, such as molecular weight, purity and the determination of antigen content by ELISA.

A manufacturer donation of rPT in liquid formulation will be filled and freeze-dried if found to be suitable following initial characterization. Approximately 10 laboratories will then be recruited to participate in an international collaborative study. It is anticipated that the rPT will have little or no activity in the assays used to assign values to native PT, and therefore it is unlikely that an rPT unitage will be assigned based on biological activity. The same concentration of rPT that exhibits “no PT toxicity” compared to that of native PT will be determined qualitatively. Lack of biological activity and product characterization will be determined using in vitro methods such as the Chinese hamster ovary cell clustering assay, carbohydrate binding of the molecule or SDS-PAGE. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2022.

The Committee was informed that although the anticipated distribution of the proposed reference material was quite low (around 20–30 ampoules per year) interest in its development had been expressed by several manufacturers in Europe and North America. Acknowledging that in the absence of biological activity the reference material would be based on mass units, the Committee endorsed the proposal (WHO/BS/2019.2378) to develop a First WHO International Reference Reagent for recombinant pertussis toxoid.
9.2.4 Proposed Second WHO international reference reagents for MAPREC analysis and NGS of poliovirus types 1, 2 and 3 (Sabin)

The MAPREC assay is the current in vitro test recommended by WHO for ensuring the safety of OPV3 and the consistency of production of OPV1 and OPV2. MAPREC is also required for process validation in the production of Sabin inactivated poliomyelitis vaccine (sIPV). This time-consuming molecular test is used to analyse single mutations in those positions of the virus genome which are either directly related to neurovirulence (in the case of poliovirus type 3) or associated with it (in the case of poliovirus types 1 and 2). In contrast, NGS allows mutations in all previously amplified positions of the virus genome to be easily determined. The increasing availability of high-throughput NGS technologies that allow for quantification of nucleotide polymorphisms in viral genomes is thus being explored as an alternative approach. A separate collaborative study to investigate the utility of NGS of virus stocks used in the manufacture of OPV had been conducted using the current WHO reference reagents for MAPREC analysis (see section 3.5.4 above). The results of this study have stimulated further interest in switching from MAPREC to NGS.

The study also highlighted the need to replace the current MAPREC reference reagents established in the 1990s. These are critical reagents required for both approaches and stocks are currently at risk of being exhausted within the next 3 years. The objective of this proposed project was to generate at least six new WHO reference reagents (two reference reagents for each of the three poliovirus types) for NGS, which will ultimately replace the MAPREC types 1, 2 and 3 reference reagents for use in both tests. Candidate materials will initially be assessed at NIBSC using MAPREC and NGS to verify their suitability and quality. Once these have been assessed and filled, a collaborative study will be conducted involving manufacturers and NCLs qualified to implement MAPREC and/or NGS using the candidate materials and a panel of materials of known value. It was anticipated that submission of the collaborative study outcomes for consideration by the Committee would take place in 2021.

The Committee recognized the challenges associated with the shipping of poliovirus reference materials once the disease had been eradicated, but acknowledged that this was also an issue for the current MAPREC reagents and noted that such reagents were only shipped to GAP-compliant laboratories. On the issue of how the proportion of mutations would be maintained and not revert during the growth of the virus, the Committee was assured that viruses would be mixed to achieve the correct percentage of mutations as confirmed by NGS. The Committee endorsed the proposal (WHO/BS/2019.2378) to develop replacement WHO international reference reagents for MAPREC analysis and NGS of poliovirus types 1, 2 and 3 (Sabin).
9.2.5 **Update on work towards the First WHO International Standard for antibody to the influenza virus haemagglutinin stem domain**

Monoclonal antibodies to the influenza virus haemagglutinin (HA) stem have been shown to be cross-reactive within and between influenza virus subtypes. A number of next-generation influenza vaccines currently in development therefore target the stem domain of the HA protein. Haemagglutinin stem-specific antibodies are measured using a variety of functional assays (such as virus neutralization or antibody-dependent cellular cytotoxicity assays) and binding assays such as ELISA. The resulting serological read-outs from clinical trials of HA stem-targeting vaccines may ultimately serve as correlates of protection. The harmonization of serological results would allow for the comparison of clinical trial outcomes and potentially aid in the defining of correlates of protection.

It was anticipated that the proposed WHO international standard would be used to standardize the measurement of antibodies binding the HA stem domain, thus facilitating vaccine development and the definition of correlates of protection for next-generation influenza vaccines. As outlined in the original proposal in 2017, the project was to be carried out in two phases. The first phase would be a pilot study to explore the potential of the candidate materials and the second phase would involve a larger collaborative study designed to assign unitage to the freeze-dried candidate materials. Ideally the latter would include samples from human clinical trials. Funding for the pilot phase had been obtained from the Bill & Melinda Gates Foundation and candidate material sourced. Ten human sera with high immunoglobulin G titres against the HA stem domain of Group 1 haemagglutinins have been identified and used to produce a trial pool. A pilot study involving 10 laboratories using various assay methods had been initiated with eight laboratories returning data so far. Interim data analysis indicates that the trial material can be used to normalize results and improve GCVs. The next steps are to complete the statistical analysis of the pilot study for all assay methods and plan the definitive collaborative study to determine whether use of a standard (pool of high-titre human sera) would result in better agreement of results between different laboratories and assays.

The Committee discussed the diversity of candidate universal influenza vaccines currently in development, which in addition to those based on HA-stem antigen also included nucleic acid based products. While acknowledging that the proposed antibody standard was specific to the Group 1 HA stem domain, it also discussed the use of different HA stem substrates for ELISA including the potential use of chimeric molecules. The Committee welcomed the progress made to date and recommended that the project should be progressed to completion.
Annex 1

WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological substances used in medicine

WHO Recommendations, Guidelines and other documents are intended to provide guidance to those responsible for the production of biological substances as well as to others who may have to decide upon appropriate methods of assay and control to ensure that 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 concerned with biological substances used in medicine are formulated by international groups of experts and are published in the WHO Technical Report Series\(^8\) as listed below. A historical list of Requirements and other sets of Recommendations is available on request from the World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

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

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

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

Technologies Standards and Norms
Department of Essential Medicines and Health Products
World Health Organization
20 avenue Appia
1211 Geneva 27
Switzerland

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\(^8\) Abbreviated in the following pages to “TRS”.
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⁹ Available online at: [https://www.who.int/biologicals/biotherapeutics/similar_biotherapeutic_products/en/](https://www.who.int/biologicals/biotherapeutics/similar_biotherapeutic_products/en/)
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Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines

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

bRSV bovine respiratory syncytial virus
BSA bovine serum albumin
CCID_{50} cell culture infectious dose 50%
EIA enzyme immunoassay
EOP end of production (cells)
ERA environmental risk assessment
ERD enhanced respiratory disease
FI-RSV formalin inactivated RSV
GMO genetically modified organism
GMP good manufacturing practice(s)
HPLC high-performance liquid chromatography
ICP immune correlate of protection
IgG immunoglobulin G
LRTI lower respiratory tract infection(s)
MCB master cell bank
MOI multiplicity of infection
MS master seed
MVA modified vaccinia Ankara
NAT nucleic acid amplification technique
NRA national regulatory authority
NP nasopharyngeal (swab or aspirate)
NS nasal swab
NW nasal wash (aspirate)
PCR polymerase chain reaction
PFU plaque-forming unit(s)
qPCR quantitative polymerase chain reaction
RDT rapid diagnostic test
RSV respiratory syncytial virus
RSV-F  respiratory syncytial virus fusion protein F
RSV-G  respiratory syncytial virus attachment protein G
RT-PCR reverse transcription polymerase chain reaction
Th1  type 1 T-helper (cell)
Th2  type 2 T-helper (cell)
T_{RM} resident memory T-cells
URTI upper respiratory tract infection(s)
WS working seed
WCB working cell bank
Introduction

Human respiratory syncytial virus (RSV) is a globally prevalent cause of lower respiratory tract infection (LRTI) in all age groups. In infants and young children the first infection may cause severe bronchiolitis that can sometimes be fatal. In older children and adults without comorbidities, repeated upper respiratory tract infections (URTIs) are common and range from subclinical infection to symptomatic upper respiratory tract disease.

In addition to the paediatric burden of disease, RSV is increasingly being recognized as an important pathogen in older adults, with infection leading to an increase in hospitalization rates among those aged 65 years and over, and to increased mortality rates among the frail elderly that approach the rates seen with influenza. The risk of severe disease in adults is increased by the presence of underlying chronic pulmonary disease, circulatory conditions and functional disability, and is associated with higher viral loads (1–6). RSV is also a nosocomial threat both to young infants and among immunocompromised and vulnerable individuals (7). High mortality rates have been observed in those infected with RSV following bone marrow or lung transplantation.

In the absence of safe and effective antiviral agents to treat RSV infection there is an unmet need for RSV vaccines. In recent years, increased understanding of the biology of RSV and associated technological advances have resulted in the entry of multiple candidate vaccines into clinical development, some of which may receive regulatory approval in the near future. The WHO Product Development for Vaccines Advisory Committee has highlighted the importance of ensuring that emerging RSV vaccines are suitable for licensure (8, 9) and meet policy decision-making needs to allow for their optimal use in low- and middle-income countries, in addition to high-income countries. A corresponding WHO roadmap has also been published (10).

There is therefore a recognized need for harmonized technical expectations to guide and facilitate the international development and assessment of candidate RSV vaccines. In response to this need, WHO convened a series of consultations with experts from academic institutes, industry, regulatory authorities and other stakeholders to review and discuss all aspects of RSV vaccine development (11, 12). Following this process of consultation, WHO brought together a group of experts to prepare draft WHO Guidelines on the quality, safety and efficacy of human RSV vaccines. In September 2018, WHO organized the first of a series of informal expert consultations attended by a wide range of stakeholders to further develop and refine the draft document. Inputs were also received from several rounds of public consultation following the posting of the draft document on the WHO Biologicals website during the course of 2018–2019. In May 2019, WHO organized a second informal consultation attended by experts and stakeholder representatives to review the
latest draft of the Guidelines and to propose further improvements prior to the submission of the Guidelines to the WHO Expert Committee on Biological Standardization.

The resulting current document has therefore been developed based on the experience gained to date in RSV vaccine development and on the contributions and outcomes of the international consultations described above. Unless otherwise specified, these WHO Guidelines are concerned only with human RSV strains and human RSV vaccines. The information provided may need to be updated as new data become available and as vaccines are licensed. The document therefore provides information and guidance on the production, quality control, nonclinical and clinical evaluation of candidate human RSV vaccines in the form of WHO Guidelines rather than WHO Recommendations as this format will allow for greater flexibility in response to future developments.

**Purpose and scope**

These WHO Guidelines provide guidance to national regulatory authorities (NRAs) and vaccine manufacturers on the manufacturing processes and nonclinical and clinical evaluation of human RSV vaccines required to assure their quality, safety and efficacy. The scope of the present document encompasses the leading technologies currently being used to develop prophylactic RSV vaccines at the clinical development stage (13). These include live-attenuated vaccines (including those based on genetically modified organisms (GMOs) such as chimeric virus vaccines), vaccines produced using recombinant viral and other vectored systems, and protein-based vaccines (including subunit and nanoparticle formulations with and without adjuvants). Some principles contained herein may also be applicable to vaccines manufactured using other platforms.

Despite possible overlaps, the quality, safety and clinical testing of RSV monoclonal antibody products involves a number of unique considerations and separate guidance will be needed with a specific focus on these products.

This document should be read in conjunction with other relevant WHO guidance, especially on the nonclinical (14, 15) and clinical (16) evaluation of vaccines, as well as relevant documents on the minimum requirements for an effective national pharmacovigilance system (17). Other WHO guidance should also be consulted as appropriate, including the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18).

In addition, despite covering a number of different technology platforms, the current document is limited in its scope and other WHO guidance documents should be consulted as relevant. This may include WHO guidance relevant to the manufacture of biologicals using pathogen-free embryonated eggs (19) if human RSV vaccines were to be produced in this way, or guidance on the
manufacture, quality control and release of bacille Calmette-Guérin (BCG) vaccines (20) in the case of BCG-vectored RSV vaccines.

It should be noted that there remain knowledge gaps in the scientific understanding of RSV vaccines which are being addressed by ongoing research and development. This document has been developed in the light of the available knowledge to date, and with regard to the currently most advanced candidate human RSV vaccines.

**Terminology**

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

**Adjuvant**: a substance or combination of substances used in conjunction with a vaccine antigen to enhance (for example, increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine.

**Adsorbed monovalent antigen bulk**: a batch of purified monovalent antigen bulk adsorbed on adjuvant. Different batches of adsorbed monovalent antigen bulks may be pooled before collection into a single vessel. If a novel adjuvant is used that does not involve adsorption of the antigen to the adjuvant, the term “adjuvanted monovalent bulk” may be used.

**Adventitious agents**: contaminating microorganisms of the cell culture or source materials, including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents and viruses that have been unintentionally introduced into the manufacturing process of a biological product.

**Candidate vaccine**: an investigational vaccine that is at the research and clinical development stage, and that has not yet been granted marketing authorization or licensure by a regulatory agency.

**Cell bank**: a collection of appropriate containers of cells whose contents are of uniform composition stored under defined conditions. Each container represents an aliquot of a single pool of cells.

**Cell bank system**: a system that consists of cell banks of defined population doubling or passage levels that generally include the **master cell bank** (MCB) derived from a cell seed and a **working cell bank** (WCB) derived from the MCB.

**Cell culture infectious dose 50% (CCID₅₀)**: the amount of virus sufficient to cause a cytopathic effect in 50% of inoculated replicate cell cultures, as determined in an end-point dilution assay in monolayer cell culture.

**Cell substrate**: cells used to manufacture a biological product. The cells may be primary cells or continuous cell lines and may be grown in monolayer or suspension culture conditions.
**Cell substrate qualification**: determination of the suitability of a cell substrate for manufacturing based on its characterization.

**Chimeric RSV vaccine**: a live-attenuated recombinant RSV vaccine expressing one or more RSV proteins in the context of the replication of viral or bacterial vectors. Examples of such vectors include, but are not limited to, Sendai virus, parainfluenza virus, bovine RSV, measles virus and BCG.

**Cytopathic effect**: a degenerative change in the appearance of cells, especially in tissue culture when exposed to viruses, toxic agents or non-viral infections.

**Drug product**: a pharmaceutical product type in a defined and sealed container-closure system that contains a **drug substance** typically formulated with excipients and prepared in the final dosage form and packaged for use. The collection of all vials of the drug product resulting from one working session constitutes the **final lot**.

**Drug substance**: the active pharmaceutical ingredient and associated molecules.

**End of production (EOP) cells**: cells cultured under conditions comparable to those used for production and derived from the MCB or WCB to a passage level or population doubling level comparable to or beyond the highest level reached for production.

**Expression construct**: a vector (plasmid or virus) capable of promoting the expression of the coding sequence(s) of recombinant protein(s) after introduction into host cells.

**Expression system**: the host cell with its expression construct and the cell culture process that is capable of expressing protein encoded by the expression construct. Expression systems may be bacterial-cell-based, baculovirus-insect-cell-based, mammalian-cell-based or yeast-cell-based.

**Final bulk**: a formulated vaccine preparation from which the final containers are filled. The final bulk may be prepared from one or more lots of purified **drug substance** formulated to contain all excipients and homogeneous with respect to composition.

**Final lot**: a collection of sealed final containers of the **drug product** that is homogeneous with respect to the risk of contamination during filling and freeze-drying. All final containers must, therefore, have been filled from a single vessel of final bulk in one working session, and if freeze-dried, processed under standardized conditions in a common chamber in one working session.

**Formalin-inactivated respiratory syncytial virus (FI-RSV) vaccine**: a formalin-inactivated whole-virion respiratory syncytial virus vaccine manufactured using the Bernett strain of RSV grown in African green monkey kidney cell cultures. Historically, the alum-adjuvanted product was causally related to vaccine-associated enhanced respiratory disease (ERD) noted in
vaccinated infants upon subsequent exposure to RSV during clinical trials conducted in the 1960s.

**Genetically modified organism (GMO):** an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

**Harvest:** the material collected from cell cultures and used to prepare the vaccine. The material may be culture supernatant, cells (which are often disrupted) or some combination of the two.

**Heterologous gene:** (a) in the context of an expression construct this term refers to the transgene from the disease-causing organism that is integrated into the backbone genomic sequence of the vector; (b) in the context of genes derived from RSV subtype A or B strains this term may be used to refer to the gene associated with the other subtype as in: “the gene for RSV-G\textsubscript{A} was expressed as well as the heterologous gene for subtype B viruses, RSV-G\textsubscript{B}”.

**Immune correlate of protection (ICP):** most commonly defined as a type and amount of immunological response that correlates with vaccine-induced protection against an infectious disease and that is considered predictive of clinical efficacy (16).

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

**Infant:** a child less than one year old.

**Live-attenuated RSV vaccine:** a vaccine derived either using conventional methods of attenuation (such as serial passage with or without chemical mutagenesis of RSV) or using recombinant methods to engineer an RSV strain recovered from plasmid complementary DNA (cDNA). Such a vaccine is capable of initiating an immune response following a mild infection lacking disease symptoms.

**Master cell bank (MCB):** a quantity of well-characterized cells of animal or other origin, derived from a cell seed at specific population doubling level or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions (such as the vapour or liquid phase of liquid nitrogen) in aliquots of uniform composition (18).

**Master seed (MS);** see also seed lot system below: a quantity of viral or bacterial material that has been derived from the same pre-master seed lot, has been processed as a single lot and has a uniform composition stored under defined conditions. Each container represents an aliquot of a single pool of viral or bacterial material of defined passage from which the working seed (WS) is derived.

**Monovalent bulk vaccine:** a quantity of vaccine derived either using a single harvest or using material pooled from one or more harvests and processed in a single production run.
**Monovalent vaccine**: a vaccine containing antigen or gene(s) encoding antigen derived from a single RSV strain or subtype.

**Multivalent vaccine**: a vaccine containing antigens or genes encoding antigens derived from more than one RSV strain or subtype.

**Nanoparticle vaccine**: a vaccine which contains or is manufactured using material(s) in the nanoscale range (1–100 nm) or engineered to have properties related to its structure or dimensions ranging in size from 1 to 1000 nm (21). Some nano-sized vaccines consist of self-assembled proteins or self-assembled virus-like particles. Others are prepared using technologies such as synthetic polymers, inorganic materials, liposomes or immunostimulating complexes, which are assembled with the respective antigen or antigens.

**Parental virus**: a virus that has been manipulated in some way to generate a viral seed with characteristics needed for vaccine production.

**Particle-based or subunit RSV vaccine**: an RSV vaccine that only contains certain antigens or subunits of RSV. Such vaccines may be produced by different expression systems.

**Plaque-forming unit (PFU)**: the smallest amount of virus sufficient to lyse host cells and cause a single visible focus of infection in a cell culture monolayer after proper staining of cells.

**Platform technology**: a standard method used for the manufacture of vaccines based on the use of heterologous gene inserts for different proteins either in an identical vector backbone or expressed from a recombinant cell line.

**Pooled harvest**: a homogeneous pool of two or more single production harvests (single harvest).

**Pre-master seed**: see also seed lot system below: a single pool of virus or viral/or bacterial-vectored particles of defined passage from which the master seed is derived.

**Purified bulk**: a batch of purified antigen of a single RSV subtype. Different batches of purified monovalent antigen bulks may be pooled into a single vessel.

**RSV-G and RSV-F**: the two major surface glycoproteins of RSV, namely the attachment (G) protein and the fusion (F) protein, with the latter present as a metastable pre-fusion F protein and a stable post-fusion F protein. These glycoproteins are the primary targets of neutralizing antibodies.

**RSV-naive**: denoting subjects who have not yet been exposed to RSV antigen by infection or vaccination.

**RSV-non naive/experienced**: denoting subjects who have experienced RSV infection in the past. Prior infection may be based on a previous clinical episode in which RSV was proven to be the etiological agent (for example, the subject had a positive culture, antigen detection or reverse transcription polymerase chain reaction (RT-PCR) test for RSV in the context of an RSV illness). Alternatively, or in addition, subjects may have immunological evidence
of prior RSV infection. In infants with persisting maternal antibody, evidence of prior infection may be based on finding RSV-specific immunoglobulin A (IgA) or immunoglobulin M (IgM) in serum or secretions, or evidence of immune memory (for example, a B-cell or T-cell memory response detected by ELISPOT). However, these serology tests may lack sensitivity and, if used alone, may underestimate the RSV-exposed population (22). In general, passively acquired maternal anti-RSV neutralizing or immunoglobulin G (IgG) binding antibodies are not known to persist in infants past their first birthday and most infants lose these maternal antibodies much earlier. Therefore, prior RSV infection in subjects at least 12 months of age may be identified by a single positive serology test for anti-RSV neutralizing or IgG binding antibodies.

**Seed lot system**: a system in which successive batches of vaccine are derived from the same **master seed** (MS) lot at a given passage level. For routine production, a **working seed** (WS) lot is prepared from the MS lot. The final product is derived from the WS lot and has not undergone more passages from the MS lot than the vaccine shown to be safe and effective in clinical studies. The seed lot system is usually based on the use of a **pre-master seed**, MS and WS.

**Single harvest**: a quantity or suspension derived from a batch of production cells inoculated with the same seed lot and processed together in a single production run.

**TSE-relevant animal species**: animals such as cattle, sheep, goats and other animals naturally susceptible to infection with transmissible spongiform encephalopathy (TSE) agents via the oral route.

**Vaccine-associated enhanced respiratory disease (ERD)**: severe lower respiratory tract disease following infection with wild-type RSV that occurs at a higher frequency in infants and children following immunization.

**Vaccine efficacy**: a measure of the protection induced by immunization in the vaccinated population sample. Vaccine efficacy is a measure of the reduction in disease attack rate (AR) between the control group that did not receive vaccination against the disease under study (ARU) and the vaccinated group (ARV). Vaccine efficacy is expressed as a percentage and is calculated from the relative risk (RR = ARV/ARU) of the disease comparing the vaccinated group to the unvaccinated control group as \([ (ARU-ARV)/ARU ] \times 100 \) or \([ 1-RR ] \times 100 \). This estimate may be referred to as absolute vaccine efficacy.

**Viral clearance**: an evaluation of the manufacturing process to determine and measure the effects of removal of virus particles and/or reduction of their infectivity through inactivation.

**Viral-vectored RSV vaccine**: a recombinant replication-deficient or conditionally replicating RSV vaccine that uses viral expression systems such as adenovirus or modified vaccinia Ankara (MVA) to express one or more antigens of RSV.
Working cell bank (WCB): a quantity of well-characterized cells of animal or other origin, derived from the MCB, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions (such as in the vapour or liquid phase of liquid nitrogen) in aliquots. One or more of the WCB containers is used for each production culture.

Working seed (WS); see also seed lot system above: for routine production, a WS lot is prepared from the MS lot under defined conditions and used to initiate production lot-by-lot. In the case of viral-vectored or live-attenuated vaccines, the final vaccine lot is derived from the virus WS lot and has not undergone more passages from the virus MS lot than the vaccine shown to be safe and effective in clinical studies. In the case of bacterial-vectored vaccines, a bacterial WS is derived from the bacterial MS.

General considerations

Respiratory syncytial virus (RSV)

RSV belongs to the genus Orthopneumovirus within the family Pneumoviridae and order Mononegavirales. Members of this genus include human RSV, bovine RSV (bRSV) and murine pneumonia virus. The RSV virion consists of a nucleocapsid packaged in a lipo-protein envelope derived from the host cell plasma membrane (23). RSV has a single-stranded, non-segmented negative-sense RNA genome consisting of between 15,191 and 15,288 nucleotides (23, 24).

The RSV envelope contains three viral transmembrane surface glycoproteins: the putative attachment glycoprotein G (RSV-G), the fusion glycoprotein F (RSV-F) and the small hydrophobic glycoprotein (RSV-SH). The non-glycosylated matrix M protein is present on the inner face of the envelope. RSV-F and RSV-G are the major targets of neutralizing antibodies and are the major protective antigens (23). The 574 amino acid RSV-F is a class I fusion protein that is cleaved into F2 and F1 fragments that form a trimer of heterodimers that mediates viral entry and syncytium formation. RSV-F on the virion surface exists in a metastable pre-fusion conformation that transitions to a stable post-fusion conformation spontaneously and during membrane fusion. There are at least five defined antigenic sites associated with neutralization on RSV-F. The 300 amino acid RSV-G is thought to form oligomers but whether dimeric or tetrameric forms are the dominant structures on the virus is not known; a monomeric secreted form of this protein is involved in immunomodulation and potentially acts as a decoy antigen that helps RSV evade host immunity (23). RSV-G can interact with CX3CR1 and other proteins but it is not required for virus entry and propagation in vitro and so its precise functional role in cell attachment is still the subject of debate. RSV-G is heavily glycosylated and has mucin-like domains on each end of the molecule surrounding a central conserved domain.
that is a target for neutralizing antibodies. The most extensive genetic diversity is found in the mucin domains of RSV-G (23, 25). The 65 amino acid RSV-SH is a pentameric ion channel and is analogous to the M2 protein in influenza viruses. Although it is not a target for neutralizing antibodies, anti-SH-specific antibodies can protect through antibody-Fc-mediated mechanisms (26).

There are two major antigenic subtypes of human RSV (RSV/A and RSV/B) determined largely by antigenic drift and duplications in RSV-G sequences, but accompanied by genome-wide sequence divergence, including within RSV-F (25, 27–29).

**Epidemiology**

Human RSV is a leading cause of respiratory disease globally. The virus causes infections at all ages. Young infants, including healthy full-term infants as well as those born prematurely, and those with chronic lung disease and congenital heart defects, have the highest incidence of severe disease, peaking at 1–3 months of age. By 2 years of age, almost all children will have been infected. Globally it is estimated that RSV causes > 30 million acute LRTI in young children annually, with over 3 million severe cases requiring hospitalization, making it the most common cause of hospitalization in children under 5 years of age. The global mortality attributed to RSV acute lower respiratory infection in young children is estimated to be as high as 150,000 per annum (30). In addition to the toll associated with acute RSV infection, the burden attributed to chronic disease (such as recurrent wheezing and asthma later in childhood) may be quite high. It is not precisely known if there is a direct causal relationship between early and severe RSV infection in infancy with asthma later in life or if symptomatic LRTI with RSV simply identifies those who are genetically predisposed to wheezing and/or asthma. Numerous factors may contribute to, and be involved in, the association between RSV bronchiolitis and wheezing illnesses later in childhood (31). In one multi-centre, randomized, placebo-controlled double-blind study it was demonstrated that monthly treatment of preterm infants with anti-RSV-F monoclonal antibody (palivizumab) during the RSV season decreased the number of parent-reported wheezing days and episodes during the first year of life even after treatment ended when compared to the number of days and episodes reported for the control group (32).

RSV infection does not elicit long-lasting sterilizing immunity and repeated URTI are common throughout life. Infections in adults can range from asymptomatic to life threatening, with severe infections more common in adults > 65 years of age and in those with underlying heart and lung problems (33). RSV transmission follows a marked seasonal pattern in temperate areas (with winter epidemics) but may occur during rainy seasons or all year round in the tropics (34–37).
The two major RSV subtypes (RSV/A and RSV/B) and multiple genotypes of each can either dominate or co-circulate during RSV epidemic seasons each year. The association between disease severity and a specific RSV subtype or genotype is variable with no consistent pattern having yet been discerned (38).

**Disease and diagnosis**

The incubation period for RSV is usually 3–6 days (ranging from 2 to 8 days). The virus typically enters the body through the eye or nose, or rarely through the mouth. The virus then spreads along the epithelium of the respiratory tract, primarily by cell-to-cell transfer. As the virus spreads to the lower respiratory tract it may produce bronchiolitis and/or pneumonia. Primary infections are often symptomatic and can range from mild URTI to a life-threatening LRTI. The course of the illness is variable, lasting from one to several weeks. Most infants show signs of improvement within 3–4 days after the onset of lower respiratory tract disease (39). RSV infection also occurs in adults where it is often a mild upper respiratory tract illness. However, in adults – particularly those > 65 years of age and those with comorbidities such as congestive heart failure or chronic obstructive pulmonary disease or immunocompromised individuals – severe illness may result (40–42). Fever and constitutional symptoms are less common than they are in influenza infection. Upper respiratory illness progresses over several days to lower respiratory symptoms of cough, new or increased sputum production, wheezing and shortness of breath. Abnormal breath sounds and/or radiographic pneumonia occur in 25–30% of cases. In patients with comorbidities, mortality ranges from 6.5% to 10% (43, 44).

RSV infection may be diagnosed by cell culture techniques or by the direct identification of viral antigen or virus genome through rapid diagnostic techniques. Diagnosis may be supported by serological testing – however, since this requires both acute and convalescent serum samples, serological diagnosis is not immediate.

**Immune response to natural RSV infection**

Innate and adaptive immune responses can contribute not only to the control and prevention of RSV infection but also to the pathogenesis of RSV disease. The repertoire of immune responses may vary substantially over the course of a lifetime. Providing a careful and complete description of the ontogeny and subsequent modulation of the human immune response against RSV in neonates, infants, children and adults remains an area of active investigation. Although a detailed account of all the many known parameters is beyond the scope of these Guidelines a number of in-depth reviews are available (45–49). In addition, a number of immune responses associated with protection or
potential pathology following RSV infection are discussed briefly below. These include: (a) virus neutralizing antibodies; (b) IgG and IgA antibodies in serum and on mucosal surfaces (including epitope-specific IgG responses); and (c) cell-mediated immunity involving RSV-specific CD8+ cytotoxic T-cell and CD4+ T-helper cell responses.

While there is no established immune correlate of protection (ICP), high concentrations of serum anti-RSV neutralizing antibodies are associated with a substantial decrease in the risk of severe lower respiratory tract disease following infection. This finding is based on the results of studies involving passively administered polyclonal or monoclonal antibodies, and on the clinical trials that led to the licensure of the monoclonal antibody palivizumab. The majority of neutralizing activity elicited in response to natural RSV infection in most individuals is directed against antigenic sites found exclusively on the pre-fusion conformation of RSV-F. There are antigenic sites on the post-fusion conformation of RSV-F that are shared with sites on the pre-fusion conformation and a smaller fraction of neutralizing activity is directed against those shared sites and against RSV-G (50, 51). This can vary between individuals and can be influenced by the way in which neutralization is measured. For example, using immortalized cells (Vero and HEp-2) to measure virus neutralization in vitro may underestimate the contribution made by anti-RSV-G antibodies in blocking virus attachment to cells mediated by RSV-G binding to its cognate receptor CX3CR1. In contrast, using primary human airway epithelial cells that express CX3CR1 but relatively low amounts of heparan sulfate on apical surfaces may provide a more sensitive and biologically relevant in vitro system for detecting anti-RSV-G-specific neutralizing antibodies. Some antibodies that bind specific epitopes present on the pre-fusion RSV-F trimer exhibit highly potent neutralizing activity relative to activity seen with antibodies directed against the shared epitopes retained on post-fusion RSV-F. Many of the antibodies directed against RSV-F are broadly neutralizing and cross-reactive with both RSV-FA and -FB proteins; however, some anti-RSV-F antibodies bind epitopes and neutralize RSV in a subtype-specific manner (52, 53).

Most post-infection human serum samples contain IgG antibodies to the central conserved region within the RSV-G protein – a region that mediates virus binding to the cellular receptor CX3CR1 (54–56). Antibodies that bind to this region of RSV-G react with both RSV subtypes and have been associated with protection against RSV infection in vivo and broad neutralizing activity in vitro. Antibody responses against RSV-G protein may also be subtype specific for RSV-GA or -GB protein, with specificity determined by substantial genetic variability within the mucin-like domains of this protein. RSV-G can bind glycosaminoglycans and C-type lectins and these interactions may facilitate virus infection and/or alter dendritic cell signaling (57).
Mucosal anti-RSV IgA antibodies have been demonstrated to correlate with protection against experimental challenge with wild-type RSV in adults (58, 59).

While antibodies may prevent RSV infection, cytotoxic CD8+ T-cells are involved in the clearance of virus-infected cells based on studies in animals and in immunocompromised individuals (60–62). Cytotoxic CD8+ T-cells may be elicited following natural RSV infection or following immunization if antigenic peptides are expressed in context with major histocompatibility complex class I proteins. CD8+ T-cells have been detected in bronchial alveolar lavage fluids and in the peripheral blood of infants and children following RSV infection (63, 64). Resident memory CD8+ T-cells ($T_{RM}$) with a CD3+ CD8+ CD103+ CD69+ phenotype have been recovered from lower airways using bronchoscopy in adults immediately following experimental RSV challenge, identified by re-stimulation with synthetic peptides representing sequences from RSV-N, -M and -NS2 proteins and confirmed using tetramer staining (60). In this study, $T_{RM}$ were also detected in the peripheral blood of adults 10 days after RSV challenge but at a lower frequency than those recovered by bronchoscopy. The presence of CD8+ $T_{RM}$ in adult lungs after RSV challenge was associated with reduced respiratory symptoms and lower viral loads (60).

CD4+ T-helper and T-regulatory ($T_{REG}$) cells modulate B and/or T-cell function. CD4+ T-helper cells in infants under 6 months of age are epigenetically programmed to have a dominant type 2 T-helper (Th2) cell cytokine response that may be antigen specific (65, 66). CD4+ Th2 cell responses are associated with cytokines that can lead to allergic inflammation. Such responses have been associated with severe disease in RSV-infected infants in some studies – suggesting that a dominant Th2 cell cytokine response following RSV exposure is not desirable in young infants (67). This is supported by the finding that genetic polymorphisms associated with clinically severe RSV disease are located in cytokine and cytokine receptor genes associated with Th2 cell responses (49). It has been suggested that cytokine responses during infancy may be skewed in favour of Th2 cell responses as a result of the down-regulation of type 1 T-helper (Th1) cell responses mediated by anti-inflammatory cytokines such as IL-10 (68). In the study involved, a specific subset of neonatal regulatory B (nBreg) cells produced anti-inflammatory IL-10 when infected with RSV via the B-cell receptor and CX3CR1. Neonates with severe RSV bronchiolitis had high numbers of RSV-infected nBreg cells that correlated directly with an increase in viral load and decrease in the frequency of memory Th1 cells (68).

**History of RSV vaccine development**

RSV vaccine development began in the 1960s with an unsuccessful formalin-inactivated RSV (FI-RSV) vaccine (69) that induced a severe – and in two cases lethal – lung inflammatory response during the first natural RSV infection after
Annex 2

vaccination of RSV-naive infants. This response to natural RSV infection has been referred to as vaccine-associated enhanced respiratory disease (ERD). The concerns over the FI-RSV vaccine hindered the development of alternative RSV vaccines for many years. Two major characteristics of vaccine-associated ERD have been defined and can be summarized as follows:

- Firstly, serological analyses of sera from the youngest infants with the most severe disease showed that these vaccinees had exhibited good induction of anti-RSV binding antibodies (as determined by complement fixation and ELISA) but weak induction of antibodies with neutralizing and fusion-inhibiting activities. Tissue sections from the lungs of the two vaccinees who died of RSV infection showed evidence of immune complex deposition and complement activation in small airways. These data suggest that weakly neutralizing antibodies induced by the FI-RSV vaccine left these infants vulnerable to infection and may have contributed to the risk of severe disease in vaccinees subsequently infected with RSV.

- Secondly, an allergic inflammation characterized by Th2-biased CD4+ T-helper cells producing IL-4, IL-5 and IL-13 associated with pulmonary eosinophilia, mucus production and neutrophilic alveolitis has been observed to various degrees in mice, cotton rats, calves and non-human primates immunized with FI-RSV or a similarly prepared antigen prior to challenge. The lung histopathology seen in the infants who died during the original FI-RSV vaccine trial showed similar neutrophilic alveolitis and pulmonary eosinophils in peribronchiolar infiltrates, suggesting that an overly exuberant allergic inflammatory response to the vaccine contributed to the complications seen thereafter.

However, a number of candidate vaccines have been proposed and evaluated over the last decade; some with promising results, and a number of observations have supported the feasibility of vaccination against RSV. Currently, there are no vaccines licensed for the prevention of RSV disease in any age group. Several candidates are at various stages of development with the most advanced of these in Phase III clinical efficacy trials. The vaccine construct and/or safety profile generated during nonclinical testing may help to determine acceptability for specific target populations. Understandably, prior experience with FI-RSV vaccine dictates the cautious approach that has been taken in vaccine development, especially regarding candidate vaccines designed to elicit active immunity in RSV-naive infants. It is widely recognized that safety data derived from clinical testing in RSV-experienced individuals (including adults, older children and toddlers) will not predict the risk of vaccine-associated ERD. It is also agreed that the
risk of vaccine-associated ERD among RSV-naive infants may vary according to the specific vaccine under consideration. For example, post-immunization surveillance of 175 very young infants given intranasal live-attenuated RSV vaccines did not identify a significant increase in risk of vaccine-associated ERD (83).

However, other candidate vaccines proposed for testing and use in RSV-naive infants should have a strong justification based on data derived from nonclinical testing that will discriminate the properties of the new candidate vaccine from those properties associated with FI-RSV vaccine. When evaluated in nonclinical tests, a candidate vaccine for RSV-naive infants should: (a) induce anti-RSV neutralizing antibodies; (b) avoid induction of non-neutralizing RSV antibodies and have a relatively low anti-RSV-F IgG ELISA binding-to-neutralizing antibody ratio; (c) avoid induction of allergic inflammation characterized by a Th2-biased CD4+ T-cell response (IL-4, IL-5, IL13 and/or mucus production); and (d) should not induce alveolitis after a valid, live RSV challenge. Evidence of the ability to elicit CD8+ T-cells in nonclinical testing may also be desirable to help distinguish the candidate vaccine from FI-RSV which does not elicit this response; while RSV-specific CD8+ T-cells may facilitate clearance of RSV-infected cells and promote Th1 responses, it is not known if this response is necessary for the prevention of vaccine-associated ERD. Pulmonary eosinophilia, while not thought to be causally related to vaccine-associated ERD, can be a marker of a dominant Th2 type cytokine response, and the presence of pulmonary eosinophils in animals after challenge should be heeded (84). In addition, immune complex deposition in the lungs of mice immunized with FI-RSV prior to a live RSV challenge was directly linked mechanistically to the pathology seen in lung tissues from the two fatal cases observed during the original FI-RSV vaccine trials (75). The exact predictive value of these animal models for determining the true risk of vaccine-associated ERD in humans will only be determined once these candidate vaccines proceed into clinical trials in RSV-naive infants. Nonclinical testing needs to be designed to control for potential confounding factors and results interpreted cautiously so as not to inadvertently dismiss vaccines with the potential to safely provide protection for very young and vulnerable infants.

For the reasons given above, and despite the fact that the current animal models do not accurately mimic all aspects of either human RSV disease or vaccine-associated ERD, it is expected that candidate vaccines with the immunopathological properties of FI-RSV will be evaluated in one or more animal models with appropriate positive and negative controls prior to testing in an RSV-naive infant population in order to demonstrate that the candidate vaccine meets the requirements (a)–(d) outlined above as applicable to each candidate vaccine. Several semi-permissive animal models that may be used for this safety assessment are discussed in Part B below. While no preference
is given to one animal model over the other, careful thought should be given to identify the model or models most compatible with the candidate vaccine under consideration. For example, it is known that tissue culture components in some vaccine preparations may provoke lung inflammatory responses characteristic of vaccine-associated ERD in rodent models following challenge (85–88). This problem can be avoided by using the neonatal calf model (89, 90). End-points for this safety analysis may include lung histopathology (to include an assessment of neutrophilic alveolitis and mucus production), pulmonary virus load (using infectious virus, genome copy number and/or reporter gene read-out) and measurement of vaccine-induced immune responses (to include neutralizing antibodies against RSV A and RSV B strains, cytokine secretion profile and phenotype of pulmonary T-cells post-challenge (including the presence or absence of CD8+ cytotoxic T-cells).

**International reference materials**

As the prospective vaccines differ in type, no international reference material for the various candidate vaccines is currently available.

However, a First WHO International Standard for antiserum to respiratory syncytial virus was established by the WHO Expert Committee on Biological Standardization, with an assigned unitage of 1000 IU/vial (91). This reference material is intended to be used in the standardization of virus neutralization methods for measuring antibody levels against RSV/A in human sera. It was subsequently shown that this reference material could also be used to measure antibody levels against RSV/B in human sera, with an assigned unitage of 1000 IU/vial (92). The use of the reference material will thus allow for the standardization of RSV neutralization assays independent of assay format and will facilitate comparability of immunogenicity among candidate RSV vaccines.

The WHO international standard is available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom. For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations (93) should be consulted.

**Expression of dose related to vaccine potency**

In the case of live-virus and chimeric viral or bacterial RSV vaccines, potency is typically expressed in terms of the number of infectious units of virus or culturable particles of bacteria contained in a human dose, using a specified tissue culture substrate or by inoculation on a solid medium, and based on the results of clinical trials.
In the case of subunit/particle-based RSV vaccines, potency is expressed using a suitable in vitro or in vivo method, which should be developed by the manufacturer. In the case of viral-vectored vaccines, potency is usually expressed using a combination of different methods.

International standards and reference reagents for the control of RSV vaccine antigen content and potency are not available. Therefore, product-specific reference preparations may be used. The dose related to vaccine potency should be calculated against a product-specific standard. Alternatively, until international reference preparations become available, assays based on plaque-forming units (PFU), the cell culture infectious dose 50% (CCID$_{50}$), colony forming units (CFU) or other relevant product-specific assays can be used to express the potency and dose of the vaccine. The dose should also serve as the basis for the establishment of parameters for stability and expiry date.

**Part A. Guidelines on the development, manufacture and control of RSV vaccines**

A.1  **Definitions**

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

Although there is no licensed RSV vaccine, the provision of a suggested international name will help in the harmonization of nomenclature after licensure. The international name should be “respiratory syncytial virus vaccine”. Depending on the construct of the antigen this should be further qualified (for example, live-attenuated, recombinant) including through the use of words such as “adjuvanted” and/or “adsorbed”, if relevant. The proper name should be the equivalent of the international name in the language of the country of origin, followed in parentheses by the virus subtype (where applicable) and name of the recombinant protein(s) when applicable.

A.1.2  **Descriptive definition**

A live-attenuated RSV vaccine which has been derived either through conventional attenuation of RSV or through recombinant biological methods should express antigens of RSV. The full proper name should identify the subtype of the parental virus from which it was derived and include gene-by-gene notations to identify deletions, insertions, mutations and changes in gene order relevant to the attenuation phenotype. The vaccine may be presented as a sterile aqueous suspension or solution, or as freeze-dried material. Likewise, a chimeric live-attenuated RSV vaccine (for example, recombinant bovine parainfluenza RSV chimera, recombinant Sendai-RSV chimera or recombinant BCG-RSV chimera) should contain the gene(s) for the RSV antigen. These
chimeric vaccines are produced by recombinant DNA technology. The vaccine may be presented as a sterile aqueous suspension or as freeze-dried material.

The description of a particle-based or subunit vaccine should identify the RSV antigen produced by recombinant DNA technology that is included in the vaccine. Particle-based RSV vaccines may form nanoparticles. A particle-based or subunit vaccine might be formulated with a suitable adjuvant. The vaccine may be presented as a sterile liquid suspension.

A replication-deficient viral-vectored RSV vaccine derived from a platform technology (such as adenovirus or MVA) is produced by recombinant DNA technology and the RSV antigen expressed by the vector should be identified. The vectored vaccine may be presented as a sterile liquid suspension or as freeze-dried material.

All of the above types of RSV vaccines are for prophylactic use.

A.2 General manufacturing guidelines

The general manufacturing recommendations contained in WHO good manufacturing practices for pharmaceutical products: main principles (94) and WHO good manufacturing practices for biological products (95) should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for each type of RSV vaccine. Manufacturing areas may be used on a campaign basis with adequate cleaning and changeover procedures between campaigns to ensure that cross-contamination does not occur.

Production steps involving manipulations of recombinant types which might involve live viruses should be conducted at a biosafety level consistent with the production of recombinant microorganisms, according to the principles of the WHO Laboratory biosafety manual (96). The basis for this is a microbiological risk assessment which results in the classification of activities into different biosafety levels. The respective classification level should be approved by the relevant authority of the country/region in which the manufacturing facility is located. The assessment should take into account both the backbone and the targeted RSV antigen involved.

Moreover, whenever in vivo tests are performed during vaccine development or manufacturing, it is desirable for ethical reasons to apply the 3Rs principles (Replacement, Reduction, Refinement) to minimize the use of animals where scientifically appropriate (97).

A.2.1 Considerations in the manufacturing of RSV vaccines

As there is currently no licensed RSV vaccine available, the following provisions should be considered.

During early clinical trials it is unlikely that data from sufficient batches will be available to validate/qualify product manufacture. However, as
development progresses data should be obtained from subsequent manufacture and should be used in support of an eventual application for the commercial supply of the product.

In addition to control during manufacture, each product should be adequately characterized at each stage of its development. The resulting attributes will facilitate understanding of the biology of the candidate vaccine and assessment of the impact of any changes in manufacturing that are introduced as development advances, or in a post-licensure setting. The immunogenicity of the product, when relevant and available, should also be included in the characterization programme (for example, as part of the nonclinical pharmacodynamic evaluation). When available, and in agreement with the NRA, platform technology data could be supportive and leveraged.

Prior to submitting a marketing authorization application, the manufacturing process should be adequately validated by demonstrating that at least three consecutively produced commercial-scale drug substance and drug product batches can be manufactured consistently. Drug product batches should be produced from individual drug substance batches. Adequate control of the manufacturing process may be demonstrated by showing that each lot meets predetermined in-process controls, critical process parameters and lot release specifications. Whenever important changes are made to the manufacturing process during vaccine development, a comparability exercise should be performed between batches manufactured according to the different manufacturing processes following the ICH Q5E guideline (98). This is extremely important if changes are introduced between the Phase III pivotal study batches and future commercial batches. Any materials added during the purification process should be documented, and their removal should be adequately validated, or residual amounts tested for, as appropriate. Validation should also demonstrate that the manufacturing facility and equipment have been qualified, cleaning of product contact surfaces is adequate, and critical process steps such as sterile filtrations and aseptic operations have been validated.

### A.3 Control of source materials

This section addresses the control of source materials for: (a) cell lines used as substrates (section A.3.1); (b) cell culture and virus propagation (section A.3.2); (c) live-attenuated/chimeric RSV vaccines (section A.3.3); (d) subunit/particle-based RSV vaccines (section A.3.4); and (e) viral-vectored RSV vaccines (section A.3.5).

#### A.3.1 Control of source materials for cell lines used as substrates

Candidate RSV vaccines have been produced in: (a) human cell lines (for example, human embryonic kidney cells – HEK 293, PERC6); (b) mammalian
cell lines (for example, Chinese hamster ovary cells (CHO-K1), African green monkey Vero cells); (c) primary chick embryo cells and embryonated chicken eggs; and (d) insect cell lines (for example, Sf9 derived from Spodoptera frugiperda and Hi-5 Rix4446 cells derived from Trichoplusia ni).

The use of a cell line should be based on a cell bank system (18). Sufficient information on the provenance of the cell bank should be recorded. A maximum number of passages or maximum population doubling level should be established, if applicable. This should be established for the MCB, WCB and the cells used for production. The cell bank or seed should be approved by the NRA.

The MCB is made in sufficient quantities and stored in a secure environment and is used as the source material for making the WCB. In normal practice the MCB is expanded by serial subculture up to a passage number (or population doubling level, as appropriate) selected by the manufacturer and approved by the NRA, at which point the cells are combined to give a single pool which is distributed into ampoules and preserved cryogenically to form the WCB.

Additional tests may include, but are not limited to, propagation of the MCB or WCB to or beyond the maximum in vitro age for production (end of production (EOP) cells), and examination for the presence of retroviruses, other adventitious agents and tumorigenicity when relevant (18). The MCB, WCB and EOP cells should be tested as described in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18).

If primary cells or eggs are used they should be produced using a controlled system (18). In the case of eggs, further guidance is available in section A.4.2.2 of the WHO Recommendations to assure the quality, safety and efficacy of influenza vaccines (human, live attenuated) for intranasal administration (19).

A.3.2  Control of source materials for cell culture and virus propagation

Only substances that have been approved by the NRA may be added. Whenever possible the use of materials of animal origin should be avoided.

If serum is used for the propagation of cells it should be tested to demonstrate the absence of bacteria, fungi and mycoplasmas – as specified in the 1995 amendment (99) to the WHO General requirements for the sterility of biological substances (100) – and freedom from adventitious viruses. Bovine serum should comply with the current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (101).

Detailed guidance on detecting bovine viruses in serum that is being considered for use in establishing an MCB and WCB are given in Appendix 1
of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18) and should be applied as appropriate. This same guidance may also apply to production cell cultures. As an additional monitor of quality, sera may be examined for endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation. Whatever process is used, the validation study should determine the consistency and effectiveness of the viral-inactivation process while maintaining serum performance. The use of non-inactivated serum should be strongly justified. The non-inactivated serum must meet the same criteria as the inactivated serum when tested for sterility and absence of mycoplasmal and viral contaminants.

The source(s) of animal components used in the culture medium (or used to produce culture medium components) should be approved by the NRA. Components derived from TSE-relevant animal species should comply with the current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (101).

Bovine or porcine trypsin used for preparing cell cultures (or used to prepare culture medium components) should be tested and found to be free of bacteria, fungi, mycoplasmas and adventitious viruses, as appropriate. The methods used to ensure this should be approved by the NRA. The source(s) of trypsin of bovine origin, if used, should be approved by the NRA and should comply with the current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (101).

In some countries irradiation is used to inactivate potential contaminant viruses in trypsin. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough so that the biological properties of the reagents are retained while being high enough to reduce virological risk. Consequently, irradiation cannot be considered to be a sterilizing process. The irradiation method should be validated by the manufacturer and approved by the NRA.

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free of the risk of contamination and should be subject to the usual considerations for any reagent of biological origin (18).

Human serum should not be used.

If human serum albumin derived from human plasma is used at any stage of product manufacture the NRA should be consulted regarding the relevant requirements, as these may differ from country to country. At a minimum, it should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (102). In addition, human albumin and materials of animal origin should comply with
the current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (101).

Penicillin and other beta-lactam antibiotics should not be used at any stage of manufacture because they are highly sensitizing substances in humans. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be approved by the NRA (95).

Non-toxic pH indicators may be added (for example, phenol red at a concentration of 0.002%).

A.3.3 Control of source materials for live-attenuated/chimeric RSV vaccines
A.3.3.1 Control of virus seed/chimeric seed
A.3.3.1.1 Vaccine virus strains/chimeric strains

Strains of live RSV – attenuated biologically, chemically or by recombinant DNA technology – should be thoroughly characterized. This should include information on the origin of the strain, cell culture passage history, method of attenuation (for example, by serial passages in animal species such as mice and chimpanzees), results of preclinical and clinical studies to prove attenuation, and whether the strains have been modified biologically, chemically or by molecular biological methods before generation of the master seed (MS). Furthermore, information on the complete genome sequence and on the passage level of the material used in clinical trials should be indicated. The respective strains should be approved by the NRA.

The strains of recombinant RSV used for the MS and working seed (WS) used to produce candidate vaccines should comply with the additional specifications given in section A.3.3.1.2 below.

For chimeric RSV vaccines (such as bovine parainfluenza RSV chimera or recombinant Sendai RSV chimera) the provisions laid down below in section A.3.5.1 apply.

A.3.3.1.2 Strains derived by molecular methods

In some countries, if a live-attenuated vaccine strain derived by recombinant DNA technology is used the candidate vaccine is considered to be a GMO and should comply with the regulations of the producing and recipient countries regarding GMOs.

The entire nucleotide sequence of any complementary DNA (cDNA) clone used to generate vaccine virus stocks should be determined prior to any nonclinical study or clinical trial. The cell substrate used for transfection to generate the virus should be appropriate for human vaccine production and should be approved by the NRA.
The production of RSV vaccine should be based on a virus seed lot system to minimize the number of tissue culture passages needed for vaccine production. This will involve the use of an MS and a WS. Seed lots should be prepared in the same type of cells using similar conditions for virus growth as those used for production of the final vaccine.

The virus WS should have a defined relationship to the virus MS with respect to passage level and method of preparation such that the virus WS retains the in vitro phenotypes and the genetic character of the virus MS. Once the passage level of the WS with respect to the MS is established it should not be changed without approval from the NRA.

The maximum passage level of the MS and WS should be approved by the NRA. The inoculum for infecting cells used in the production of vaccine should be from a virus WS with as few as possible intervening passages in order to ensure that the characteristics of the vaccine remain consistent with the lots used in clinical trials.

Virus seed lots should be stored in a dedicated temperature-monitored freezer that ensures stability upon storage. The duration of stability should be monitored by controlled testing at the selected storage temperature and conditions. It is recommended that a large virus WS lot be set aside as the basic material for use by the manufacturer for the preparation of each batch of vaccine.

Likewise, the production of chimeric BCG/RSV vaccine should be based on a seed lot system. For such vaccines the provisions laid down in section A.3 of the WHO Recommendations to assure the quality, safety and efficacy of BCG vaccines apply (20).

Control of cell cultures for virus seeds

In agreement with the NRA, tests on control cell cultures may be required and performed as described in section A.4.1 below.

Control of virus seed lots

The following tests should be performed on virus MS and WS lots.

Identity

Each virus MS and WS lot should be identified as RSV vaccine seed virus by immunological assay or by molecular methods approved by the NRA.

Genetic/phenotypic characterization

Each seed should be characterized by full-length nucleotide sequence determination and by other relevant laboratory and animal tests in order to provide information on the consistency of each virus seed. Molecular markers of
attenuation shall be identified and defined during the establishment of the viral seed (see Part B below). These tests are required to compare the new vaccine strain with the wild-type and/or parent virus. The sequence of the MS defines the consensus sequence of a vaccine strain.

Mutations introduced during the derivation of each vaccine strain should be maintained in the consensus nucleotide sequence, unless spontaneous mutations induced during tissue culture passage were shown to be without effect in nonclinical and small-scale clinical trials. Some variations in the nucleotide sequence of the virus population during passaging are to be expected but the determination of what is acceptable should be based on experience in production and clinical use.

The genetic stability of the vaccine seed to a passage level comparable to final bulk and preferably beyond the anticipated maximum passage level should be demonstrated. Phenotypic characterization should focus on the markers for attenuation/modification and expression of the RSV antigens. For example, if attenuation is associated with temperature sensitivity, cold adaptation, plaque-size or host-range restriction, the phenotype of the candidate vaccine virus associated with attenuation should be shown to be conserved at passage levels required for manufacture and ideally beyond.

For any new MS and WS it is recommended that the first three consecutive bulk vaccine lots should be analyzed for consistency of manufacturing and for identity of the active substance based on relevant quality parameters.

A.3.3.3.3 Sterility tests for bacteria, fungi, mycoplasmas and mycobacteria

Each virus MS and WS lot should be shown to be free from bacterial, fungal, mycoplasmal (or spiroplasmal if insect cells are used) and mycobacterial contamination using appropriate tests as specified in the WHO General requirements for the sterility of biological substances (99, 100). Nucleic acid amplification techniques (NATs), either alone or in combination with cell culture and with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods after suitable validation and agreement with the NRA (15).

A.3.3.3.4 Tests for adventitious agents

Each virus MS and WS lot should be tested in cell culture for adventitious agents relevant to the passage history of the seed virus. Where antisera are used to neutralize RSV or the vector virus the antigen used to generate the antiserum should be produced in cell culture from species different from that used for production of the vaccine and should be free from adventitious agents. Suitable indicator cells should be selected to enable the detection of viruses. The choice of indicator cells should be guided by the species and legacy of the production
cell substrate, taking into consideration the types of viruses to which the cell substrate could potentially have been exposed. Infection with such viruses should then be tested for using a suitable assay method. For test details, refer to section B.11 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18).

Each virus MS lot should also be tested in animals if the risk assessment indicates that this test provides a risk mitigation taking into account the overall testing package (103). The animals used might include guinea-pigs, adult mice and suckling mice. For test details, refer to section B.11 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18). For ethical reasons it is desirable to apply the 3Rs principles (Replacement, Reduction, Refinement) to minimize the use of animals where scientifically appropriate (97).

New molecular methods with broad detection capabilities are available for adventitious agent detection. These methods include: (a) degenerate NAT for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and (c) high-throughput sequencing. These methods may be used to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and agreement from the NRA.

A.3.3.3.5 Tests in experimental animals

As outlined in Part B below, studies should, when relevant, be performed in animals to determine that the MS virus displays attenuating features which are maintained throughout subsequent vaccine process steps. For certain candidate vaccines it may be required to test at least once during nonclinical development for these features in a relevant animal model. For an MS virus to be identified as attenuated the criteria for determining attenuation should be clearly defined.

The NRA may decide that such testing does not need to be repeated each time a new WS lot is derived.

A.3.3.6 Virus titration for infectivity

The infectivity of each virus MS and WS lot should be established using an assay acceptable to the NRA. Manufacturers should determine the appropriate titre necessary to produce vaccine consistently. Depending on the results obtained in preclinical studies, plaque assays, immunofocus assays or CCID₅₀ with read-outs such as quantitative PCR (qPCR) may be used. All assays should be validated.
A.3.3.4 Control of bacterial seeds
For the control of bacterial seeds, the provisions laid down in the WHO Recommendations to assure the quality, safety and efficacy of BCG vaccines (20) apply.

A.3.4 Control of source material for subunit/particle-based RSV vaccines
A.3.4.1 Cells for antigen production
A.3.4.1.1 Recombinant yeast and bacteria cells
The characteristics of the parental cells and the recombinant strain (parental cell transformed with the recombinant expression construct) should be fully described and information should be recorded on the testing carried out for adventitious agents and on the genetic homogeneity of the MCB and WCB. A full description of the biological characteristics of the host cell and expression vectors should be given. This should include genetic markers of the host cell, the construction, genetics and structure of the expression vector, and the origin and identification of the gene being cloned. Some techniques (for example, sequencing) allow for the entire construct to be examined, while others (for example, restriction-enzyme mapping) allow for assessment of segments of respective plasmids (104, 105). The molecular and physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail (105).

The nucleotide sequence of the gene insert and adjacent segments of the vector, along with restriction-enzyme mapping data for the vector containing the gene insert, should be provided, as required, to the NRA.

Cells must be maintained in a frozen state that allows for recovery of viable cells without alteration of genotype. The cells should be recovered from the frozen state, if necessary in selective media, such that the genotype and phenotype consistent with the recombinant (modified) host and vector are maintained and clearly identifiable. Cell banks must be identified and characterized by appropriate tests.

Data – for example on plasmid restriction-enzyme mapping, nutritional requirements or antibiotic resistance (if applicable) – that demonstrate the genetic stability of the expression system during passage of the recombinant WCB up to or beyond the passage level used for production should be provided to, and approved by, the NRA. Any instability of the expression system occurring in the seed culture during expansion or after a production-scale run should be documented. Stability should also be monitored to confirm cell viability after retrieval from storage, and to confirm maintenance of the expression system. These studies may be performed as part of the routine use of the expression system in production or may include samples specifically taken for such a purpose.
A.3.4.1.1 Tests on recombinant yeast and bacterial MCB and WCB

Such MCBs and WCBs should be tested for the absence of bacterial and fungal contamination by appropriate tests, as specified in the WHO General requirements for the sterility of biological substances (99, 100), or by an alternative method approved by the NRA, to demonstrate that only the bacteria or yeast production strain is present, and that the MCB and WCB are not contaminated with other bacteria or with fungi.

A.3.4.1.2 Recombinant mammalian cells

If recombinant mammalian cells are used, the cell substrates and cell banks should conform with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18) and the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (105) and should be approved by the NRA.

A.3.4.1.3 Insect cells

WCBs of insect cells may be used for recombinant baculovirus seed lot production and antigen expression. If insect cells are used for expression of the RSV vaccine antigen with a baculovirus-based expression vector, the cell substrates and cell banks should conform with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18), as appropriate to insect cells, and should be approved by the NRA.

A.3.4.1.3.1 Tests on insect MCB and WCB

Testing of insect MCBs and WCBs should be performed in accordance with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18). It is important to show that the cell banks are free from bacteria, fungi, mycoplasmas, mycobacterium species and adventitious agents relevant to the species that may be present in raw materials used in their derivation. For insect cells a special emphasis is placed on potential insect-borne human pathogens (for example, arboviruses). Moreover, the risk from insect cell lines inherently contaminated with viral agents should be assessed (106, 107).

Insect viruses have not been well characterized compared with other potential adventitious agents, and less information about them is therefore available, especially on their infectivity, replicative life-cycles and pathogenicity, if any. It should be kept in mind that infection of insect cells with some insect viruses may occur without showing cytopathic effect. Testing may involve specific NAT-based assays such as PCR and other nonspecific tests such as
co-cultivation. The specificity and sensitivity of assays should be determined by the manufacturer and approved by the NRA.

Full characterization may be performed on either the MCB or the WCB, with more limited testing on the other, depending on the strategy chosen for testing (18). Scientific advice on the testing strategy should be sought from the NRA.

A.3.4.2 Recombinant baculovirus MS and WS lots

The recombinant baculovirus expression vector used in the production of RSV vaccine contains the coding sequence of the respective RSV antigen and should be identified by historical records. The historical records will include information on the origin and identity of the gene being cloned, details on the method of construction, and the nucleotide sequence of the selected baculovirus expression vector in the context of the final construction.

The production of vaccine should be based on a seed lot system. Recombinant baculovirus seed lots should be stored in a dedicated temperature-monitored refrigerator or freezer at a temperature shown formally by the manufacturer to ensure stability for the duration of the planned period of storage.

Only recombinant baculovirus seed lots that are approved by the NRA should be used. The recombinant baculovirus MS lot should be made in sufficient quantities to meet anticipated needs for long-term production and should be stored in a secure environment. The MS lot is used as the source material for making the manufacturer’s recombinant baculovirus WS lot. Either the MS or the WS should be fully characterized and tested extensively for adventitious agents, while the other may be subjected to more limited testing. The testing strategy and seed lots should be approved by the NRA.

It is recommended that a large lot of recombinant baculovirus WS should be set aside as the basic material for use by the manufacturer in the preparation of each batch of the vaccine. The recombinant baculovirus WS lot should be prepared based on a defined number of passages from the recombinant baculovirus MS lot using a method and a passage level from the MS lot approved by the NRA. Once the acceptable passage level of the WS is established it may not be changed for future lots of WS without approval from the NRA.

A.3.4.2.1 Tests on recombinant baculovirus MS and WS lots

The expression construct should be analyzed using NAT-based assays in conjunction with other tests performed on the purified recombinant protein for assuring the quality and consistency of the expressed RSV antigen. The genetic stability and stability of expression of the expression construct should be demonstrated from the baculovirus MS up to at least the highest passage level used in production, but preferably beyond this level (104, 105).
A.3.4.2.1.1 **Identity**

Each baculovirus MS and WS lot should be identified for the inserted RSV gene using an appropriate molecular method approved by the NRA.

A.3.4.2.1.2 **Sterility tests for bacteria, fungi, mycoplasmas and mycobacteria**

The provisions laid down in section A.3.3.3.3 above apply.

A.3.4.2.1.3 **Tests for adventitious agents**

Each recombinant baculovirus seed should be tested in cell cultures for adventitious agents appropriate to the origin and passage history of the seed baculovirus. For tests on recombinant baculovirus-permissive indicator cells, the neutralization of baculovirus is necessary. Antisera used for this purpose should be free from antibodies that may neutralize adventitious agents and should preferably be generated by the immunization of specific-pathogen-free animals with an antigen from a source (other than the production cell line) which has itself been tested for freedom from adventitious agents. The inoculated indicator cells should be examined microscopically for cytopathic changes. At the end of the examination period the cells should also be tested for haemadsorbing viruses (see section A.4.1.1 below).

It should be noted that the infection of indicator cells with insect viruses may not reveal any cytopathic effect. Additional tests such as PCR, electron microscopy and co-cultivation may therefore be performed. It is important to show that recombinant baculovirus seeds are free of adventitious agents relevant to the insect species used in their derivation with a special emphasis placed on potential insect-borne human pathogens (for example, arboviruses). The specificity and sensitivity of the assays used should be determined by the manufacturer and approved by the NRA.

In general, recombinant baculovirus seeds should be assessed for the presence of adventitious agents that may have been introduced during their production, including those that may have been present in the source materials used at each production stage of the MS and WS lots. For details on these tests see the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18). Whenever in vivo tests are performed it is desirable for ethical reasons to apply the 3Rs principles (Replacement, Reduction, Refinement) to minimize the use of animals where scientifically appropriate (97).

New molecular methods with broad detection capabilities are being developed for adventitious agent detection. Such methods may be used at the discretion of the NRA to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and agreement from the NRA (see section A.3.3.3.4 above).
A.3.4.2.1.4 **Test of control cells used for production of seeds**
Tests on control cell cultures should be undertaken as described in section A.4.1 below.

A.3.4.2.1.5 **Virus titration for infectivity**
Each recombinant baculovirus seed should be assayed for its infectivity in the insect cell line chosen for antigen manufacture using a sensitive assay. The detailed procedures used to carry out the tests and interpret their results should be approved by the NRA.

A.3.5 **Control of source materials for viral-vectored RSV vaccines**

A.3.5.1 **Virus vector MS and WS**
The use of any viral vector should be based on a seed lot system, analogous to the cell banking system used for production cells.

The rationale behind the development of the viral-vectored vaccine should be described. The origin of all genetic components of the vaccine and their function should be specified; overall, this should allow for a clear understanding of the functionality of the vaccine and how it is attenuated or made replication-incompetent by genetic engineering. All intended and unintended genetic modifications (such as site-specific mutations, insertions, deletions and/or rearrangements to any component) should be detailed in comparison with their natural counterparts. For a vaccine construct that incorporates genetic elements to control the expression of a transgene (for example, in a tissue-specific manner) evidence should be provided on product characterization and control to demonstrate such specificity. RNA editing should be discussed if relevant.

All steps from the derivation of the material that ultimately resulted in the candidate vaccine to the virus MS level should be described. A diagrammatic description of the components used during vaccine development should be provided and annotated. The method of construction of the viral-vectored vaccine should be described and the final construct should be genetically characterized according to the principles discussed in this section.

The cloning strategy should ensure that if any antibiotic-resistance genes are used during the development of the initial genetic construct these are absent from the viral vaccine seed.

The complete nucleotide sequence of the gene insert and of the vector should be provided and may be supplemented by restriction-enzyme mapping of the vector containing the gene insert. The genetic stability of the vector with the recombinant construct during amplification associated with manufacture should be demonstrated. The stability of a recombinant vector should be assessed by comparing the sequence of the vector at the level of a virus pre-
master seed or MS to its sequence at, or preferably beyond, the anticipated maximum passage level. Any modifications to the sequence of the heterologous insert should be demonstrated to have no impact on the resulting antigenic characteristics of the vaccine.

A.3.5.1.1 Tests on virus MS and WS

A.3.5.1.1.1 Identity

Both the vector and the RSV-specific genetic components of the virus MS and WS should be identified by immunological assay or by molecular methods acceptable to the NRA.

A.3.5.1.1.2 Genetic and phenotypic characterization

The virus MS should be characterized as fully as possible. If this characterization is limited (for example, because of limited quantities of material) the virus WS should be fully characterized in addition to the limited characterization of the MS. It should be noted that it would not be feasible to manufacture the vaccine directly from the virus MS in these circumstances.

Virus MS characterization will include a description of the genetic and phenotypic properties of the vaccine vector. This should include a comparison with the parental viral vector/virus and is particularly important where vector modification might affect the attenuation or replication competency, pathogenicity and tissue tropism or species specificity of the vaccine vector compared with the parental vector.

Genetic characterization will involve a complete nucleotide sequence analysis of the vaccine vector which might be supplemented by restriction enzyme mapping, southern blotting, PCR analysis or DNA fingerprinting. Promoter elements involved in expression of the RSV-derived gene(s) (including relevant junction regions) should be described and delineated.

The genetic stability of the vaccine seed to a passage level comparable to final vaccine bulk, and preferably beyond the anticipated maximum passage level, should be demonstrated.

Phenotypic characterization should focus on the markers for attenuation/modification and expression of the heterologous antigen, and should be performed in vitro under conditions that allow for the detection of revertants (including the emergence of replication-competent vectors from replication-incompetent vectors during passage) and of changes to the stability of the heterologous gene insert during replication of the recombinant vector. However, other studies – including antigenic analysis, infectious titre, ratio of genome copies to infectious units (for replicating vaccines) and in vitro yield – should also form part of the characterization. For replicating vectors, in vivo growth characteristics in a suitable animal model may also be informative and should
be performed if justified. For some vectors (for example, adenoviral vectors) the particle number should be measured in addition to the infectivity titre.

A subset of the above studies should be applied to the virus WS lot and justification for the chosen subset should be provided.

A.3.5.1.3 Sterility tests for bacteria, fungi, mycoplasmas and mycobacteria
The provisions laid down in section A.3.3.3.3 above apply.

A.3.5.1.4 Tests for adventitious agents
Information should be given on the testing for adventitious agents, as outlined in section A.3.3.3.4 above. The methods used and results obtained should be acceptable to the NRA.

A.3.5.1.5 Virus titration for infectivity
The infectivity of each virus MS and WS lot should be established as outlined in section A.3.3.3.6 above.

A.3.5.2 Cell substrates
The cell substrate used for the manufacture of a viral-vectored RSV vaccine should be based on controlled primary cells or a cell banking system using continuous cell lines as outlined in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18). See section A.3.1 above for further information.

A.4 Control of production for live-attenuated/chimeric RSV vaccines
A.4.1 Control of cell cultures
In cases where a mammalian or other animal cell line used for propagation of the vaccine has been thoroughly characterized and has been used for the production of other vaccines, the NRA might decide that no control cells are necessary.

In cases where mammalian or other animal cells are used for propagation of the vaccine and the NRA requires the use of control cells, the following procedures should be followed. From the cells used to prepare cultures for the production of vaccine a fraction equivalent to at least 5% of the total cell suspension, or 500 mL of cell suspension or 100 million cells should be used to prepare uninfected control cell cultures.

These control cultures should be observed microscopically for cytopathic and morphological changes attributable to the presence of adventitious agents for at least 14 days (at the temperature used for the production cell culture).
after the day of inoculation of the production cultures or until the time of final virus harvest, whichever is the longer. At the end of the observation period, supernatant fluids collected from the control culture should be pooled and tested for adventitious agents as described below. Samples that are not tested immediately should be stored at $-60^\circ\text{C}$ or lower until such tests can be conducted.

If adventitious agent testing of control cultures yields a positive result, the harvest of virus from the parallel vaccine-virus-infected cultures should not be used for vaccine production. For the test to be valid, no more than 20% of the control culture flasks should have been discarded, for any reason, by the end of the test period.

A.4.1.1 Test for haemadsorbing viruses
At the end of the observation period a fraction of control cells comprising not less than 25% of the total should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the guinea-pig red blood cells have been stored prior to use in the haemadsorption assay, the duration of storage should not have exceeded 7 days and the storage temperature should have been in the range $2–8^\circ\text{C}$.

In some countries the NRA requires that additional tests for haemadsorbing viruses should be performed using red blood cells from other species, including those from humans (blood group O), monkeys and chickens (or other avian species). For all tests, readings should be taken after incubation for 30 minutes at $2–8^\circ\text{C}$, and again after further incubation for 30 minutes at $20–25^\circ\text{C}$. The test using monkey red blood cells should be read once more after additional incubation for 30 minutes at $34–37^\circ\text{C}$.

For the test to be valid, no more than 20% of the control culture flasks should have been discarded, for any reason, by the end of the test period.

A.4.1.2 Test for adventitious agents in control cell culture fluids
Supernatant culture fluids from each of the control cell culture vessels should be tested for adventitious agents. A 10 mL sample of the pool should be tested in the same cell substrate (but not the same cell batch) as that used for vaccine production, and additional 10 mL samples tested in relevant cell systems.

Each sample should be inoculated into cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1:4. The area of the cell sheet should be at least 3 cm$^2$ per mL of pooled fluid. A least one bottle of each type of cell culture should not be inoculated in order to serve as a control.

The inoculated cultures should be incubated at a temperature of $35–37^\circ\text{C}$ and should be examined at intervals for cytopathic effects over a period of at least 14 days.
Some NRAs require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least an additional 7 days. Furthermore, some NRAs require that these cells should be tested for the presence of haemadsorbing viruses.

The tests are satisfactory if no cytopathic changes attributable to adventitious agents are detected in the test sample. For the test to be valid, no more than 20% of the culture flasks should have been discarded, for any reason, by the end of the test period.

A.4.1.3 Identity of cells
Depending on the type of cells used at the production level, the cells – especially those propagated from the WCB – should be identified by means of tests approved by the NRA.

Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), immunological tests (for example, major histocompatibility complex assays), cytogenetic tests (for example, for chromosomal markers) and tests for genetic markers (for example, DNA fingerprinting or short tandem repeats).

A.4.2 Production and harvest of monovalent bulk vaccine
A.4.2.1 Cells used for virus inoculation
On the day of inoculation with the seed virus, each production cell culture flask (or bottle) or control cell culture flask should be examined for cytopathic effects potentially caused by infectious agents. If the examination shows evidence of an adventitious agent, all cell cultures should be discarded.

If animal serum is used in the growth medium, the medium should be removed from the cell culture either before or after inoculation with the virus WS. The cell cultures should be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin and other beta-lactam antibiotics should not be used at any stage of manufacture because they are highly sensitizing substances in humans. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be approved by the NRA (95).

A.4.2.2 Virus inoculation
Cell cultures are inoculated with virus WS at a defined optimal multiplicity of infection (MOI). After viral adsorption, cell cultures are fed with maintenance medium and are incubated at a temperature within a defined range and for a defined period.
The MOI, temperature range and duration of incubation will depend on the vaccine strain and the production method, and specifications should be validated by each manufacturer.

**A.4.2.3 Monovalent bulk vaccine**

Monovalent bulk vaccine is harvested within a defined period post-inoculation. A monovalent bulk vaccine may be the result of one or more single harvests or multiple parallel harvests. Samples of monovalent harvest pools should be taken for testing and should be stored at a temperature of −60 °C or below. The manufacturer should submit data to support the conditions chosen for these manufacturing procedures.

The monovalent harvest pool may be clarified or filtered to remove cell debris and stored at a temperature that ensures stability before being used to prepare the final bulk for filling. The sponsor should provide data to support the stability of the bulk throughout the duration of the chosen storage conditions, as well as to support the choice of storage temperature.

Harvests derived from continuous cell lines should be subjected to further purification to minimize the amount of cellular DNA, and treatment with DNase to reduce the size of retained host cell DNA is also recommended.

**A.4.2.4 Tests on monovalent bulk vaccine**

**A.4.2.4.1 Identity**

A test for identity should be performed if this has not been done on the single harvest or pooled harvest.

**A.4.2.4.2 Sterility tests for bacteria, fungi, mycoplasmas and mycobacteria**

A sample of each monovalent bulk or virus culture supernatant should be tested for bacterial, fungal, mycoplasmal and mycobacterial sterility as specified in the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.

NAT-based assays, alone or in combination with cell culture and with an appropriate detection method, might be used as an alternative to one or both of the pharmacopeial mycoplasma detection methods after suitable validation and with the agreement of the NRA (18).

The method used for testing for mycobacteria should be approved by the NRA. NAT-based assays might be used as an alternative to the microbiological culture method for mycobacteria after validation by the manufacturer and with the agreement of the NRA.
A.4.2.4.3 **Tests for adventitious agents**

If the single harvests are not pooled on the same day they are harvested then a test for adventitious agents should be performed on each single harvest.

A.4.2.4.4 **Virus titration for infectivity**

In the case of pooling of viral harvests, the virus content of each single harvest should be tested with an infectivity assay. Minimum acceptable titres should be established for the use of a single harvest in the preparation of a virus pool or final bulk, and to confirm the consistency of production. A reference preparation should be included to validate the titration assay.

A.4.2.4.5 **Residual bovine serum albumin content**

If bovine serum is used during production, then residual bovine serum albumin (BSA) content should be measured and a maximum permitted concentration should be set and approved by the NRA.

In some countries tests are carried out to estimate the amount of residual animal serum in the purified bulk or in the final vaccine. Other serum proteins may also be measured.

A.4.2.4.6 **Test for consistency of virus characteristics**

Recombinant RSV candidate vaccine lots should be tested and compared to the MS, WS or other suitable comparator to ensure that the vaccine virus has not undergone critical changes during its multiplication in the production culture system.

Relevant assays should be identified in nonclinical studies and may include, for example, virus yield in cell culture, growth in primary human bronchial epithelial cells or plaque morphology. Other identifying characteristics may also be applicable.

Assays for assessing the attenuation of recombinant RSV should also be conducted and the results compared to the control results.

The test for consistency may be omitted as a routine test once the consistency of the production process has been demonstrated on a significant number of batches and in agreement with the NRA. Where there is a significant change in the manufacturing process, the test should be reintroduced.

A.4.3 **Final bulk**

A.4.3.1 **Preparation of final bulk**

Only monovalent bulk vaccine meeting the recommendations for sterility, freedom from adventitious agents and virus content should be pooled. The operations necessary for preparing the final bulk should be conducted in a manner that avoids contamination of the product.
In preparing the final bulk, any excipients (such as diluent or stabilizer) that are added to the product should have been shown, to the satisfaction of the NRA, not to impair the safety and efficacy of the vaccine in the concentration used.

A.4.3.2 Tests on final bulk
A.4.3.2.1 Test for residual materials
The manufacturer should demonstrate by testing each final bulk or by validating the manufacturing process that any residual materials used in the manufacturing process – such as animal serum, antibiotics, residual cellular DNA and DNase – are consistently reduced to a level acceptable to the NRA.

The host cell protein profile should be examined as part of the characterization studies (105).

For viruses grown in continuous-cell-line cells, the final bulk material should be tested for the amount of residual cellular DNA, and the total amount of cell DNA per dose of vaccine should not be more than the upper limit agreed by the NRA. Where technically feasible, the size distribution of the DNA should be examined as a characterization test, taking into account the amount of DNA detectable using state-of-the-art methods approved by the NRA.

A.4.3.2.2 Bacterial and fungal sterility
Except where it is subject to in-line sterile filtration as part of the filling process, the final bulk suspension should be tested for bacterial and fungal sterility according to the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.

A.4.3.2.3 Storage
Prior to filling the final bulk suspension should be stored under conditions shown by the manufacturer to allow the final bulk to retain the desired viral potency.

A.4.4 Control of production of chimeric RSV vaccines
For chimeric RSV vaccines grown on cell culture, the provisions laid down in sections A.4.1–A.4.3 above apply.

For chimeric RSV vaccines grown in eggs, most of the provisions laid down in the WHO Recommendations to assure the quality, safety and efficacy of influenza vaccines (human, live attenuated) for intranasal administration (19) apply.

For chimeric BCG/RSV vaccines, most of the provisions laid down in sections A.4 of the WHO Recommendations to assure the quality safety and efficacy of BCG vaccines (20) apply.
A.5 Control of production for subunit/particle-based RSV vaccines

A.5.1 Production up to single antigen harvest

A.5.1.1 Production of antigen if recombinant yeast or bacteria are used

Microbial purity in each fermentation vessel should be monitored at the end of the production run by methods approved by the NRA.

Any agent added to the fermenter or bioreactor with the intention to feed cells or to increase cell density should be approved by the NRA. Penicillin and other beta-lactam antibiotics should not be used at any stage of manufacture because they are highly sensitizing substances in humans. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be approved by the NRA.

Genetic integrity and stability of the expression vector during the process of vaccine manufacture shall be confirmed by appropriate methods in order to ensure consistency of vector-based protein expression.

A.5.1.2 Production of antigen if mammalian or insect cells are used

Some mammalian cell lines have been generated which constitutively express the desired antigen.

In other technologies, cell cultures are expanded to an appropriate scale and are inoculated with the respective expression vector (for example, recombinant baculovirus) at a defined MOI. After adsorption the cell cultures are fed with maintenance medium and incubated within a defined temperature range and for a defined period of time.

The range of MOI, temperature, pH and incubation period will depend on the cell substrate and the specific characteristics of the expression vector. A defined range should be established by the manufacturer and approved by the NRA.

A single harvest is obtained within a defined time period post-inoculation. Several antigen harvests may be pooled. If multiple antigen harvests are pooled, each single antigen harvest should be sampled for testing, stabilized and stored under suitable conditions until pooling. Penicillin and other beta-lactam antibiotics should not be used at any stage of manufacture because they are highly sensitizing substances in humans. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be approved by the NRA.
Samples of single harvest pools should be taken for testing and stored at a temperature of −60 °C or below.

A.5.1.3 Tests of control cell cultures (if applicable)
When control cells are included in the manufacturing process, the provisions laid down in section A.4.1 above apply. However, it should be noted that the control cell cultures should be incubated under conditions that are essentially similar to those used for the production cultures, with the agreement of the NRA. For insect cells, the incubation time of at least 14 days might not apply because of the specifics of cells cultivated in suspension but it should not be less than the time of collection of the single antigen harvest.

A.5.1.3.1 Tests for haemadsorbing viruses
The provision laid down in section A.4.1.1 above applies. However, for cells cultivated in suspension the test for presence of haemadsorbing viruses is not technically feasible. A test for presence of haemagglutinating agents using guinea-pig red blood cells is therefore required with spent control cell culture fluid.

A.5.1.3.2 Tests for other adventitious agents
The provisions laid down in section A.4.1 above apply.

A.5.1.3.3 Identity of cells
The provisions laid down in section A.4.1.3 above apply.

A.5.2 Purified antigen bulk
The purification process can be applied to a single antigen harvest, part of a single antigen harvest or a pool of single antigen harvests, and should be approved by the NRA. The maximum number of harvests that may be pooled should be defined by the manufacturer and approved by the NRA. Adequate purification may require several purification steps based on different biophysical and/or biochemical principles and may involve disassembly and reassembly of nanoparticles. The entire process used for the purification of the antigen should be appropriately validated and should be approved by the NRA. Any reagents added during the purification processes (such as DNase) should be documented.

The purified monovalent antigen bulk should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity. Intermediate hold times should be validated by the manufacturer and approved by the NRA.
A.5.2.1 Tests on the purified antigen bulk

All quality control release tests for the purified antigen bulk should be validated and should be shown to be suitable for the intended purpose. Assay validation or qualification should be appropriate for the stage of the development life-cycle. Additional tests on intermediates during the purification process may be used to monitor consistency and safety.

A.5.2.1.1 Identity

A test for identity should be performed using a suitable method.

A.5.2.1.2 Purity

The degree of purity of the antigen bulk and levels of residual host cell proteins should be assessed by suitable methods. In the case of yeast-derived products these tests may be omitted for routine lot release upon demonstration that the purification process consistently eliminates the residual components from the monovalent bulks to the satisfaction of the NRA.

A.5.2.1.3 Protein content

Each purified antigen bulk should be tested for total protein content using a suitable method. Alternatively, the total protein content may be calculated from measurement of an earlier process intermediate.

A.5.2.1.4 Antigen content

The antigen content may be measured on the purified monovalent antigen bulk or the adsorbed monovalent antigen bulk by an appropriate method.

The ratio of antigen content to protein content may be calculated and monitored for each purified antigen bulk.

International standards and reference reagents for the control of RSV vaccine antigen are not available. Therefore, product-specific reference preparations may be used.

A.5.2.1.5 Bacterial and fungal sterility

The purified antigen bulk should be tested for bacterial and fungal sterility, as specified in the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.

Alternatively, if the antigen is directly adsorbed onto an adjuvant and no samples can be drawn, the test can be performed on the related adsorbed antigen bulk, if properly justified.
A.5.2.1.6 *Percentage of intact RSV antigens*

If the integrity of certain RSV proteins (for example, the F protein) is a critical quality parameter, this should be carefully monitored. The percentage of intact RSV protein trimer should be assessed in comparison to a reference standard. Using a suitable panel of monoclonal antibodies, the percentages of F protein that exist in the pre-fusion state and post-fusion state could be individually assessed. Such assays could be used to assure consistency of manufacture.

A.5.2.1.7 *Nanoparticle size and structure*

In the case of particle-based vaccines such as F protein nanoparticle vaccines, the size and structure of the nanoparticles are to be established and monitored. This test may be omitted for routine lot release once consistency of production has been established, in agreement with the NRA.

Suitable methods for assessing nanoparticle size and structure include dynamic light scattering, size-exclusion chromatography–high-performance liquid chromatography (SEC–HPLC), transmission electron microscopy and disc centrifugation size analysis. Disc centrifugation size analysis allows for the determination of the hydrodynamic radius of particles which sediment in a sucrose gradient when referenced against spherical particles of known sizes.

A.5.2.1.8 *Tests for reagents used during production or other phases of manufacture*

A test should be carried out to detect the presence of any potentially hazardous reagents used during manufacture using methods approved by the NRA. This test may be omitted for routine lot release upon demonstration that process consistency eliminates the reagent from the purified monovalent antigen bulks, subject to the agreement of the NRA.

A.5.2.1.9 *Tests for residual DNA derived from the expression system*

The amount of residual host cell DNA derived from the expression system should be determined in the purified antigen bulk by suitable sensitive methods. The level of host cell DNA should not exceed the maximum level agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18).

These tests may be omitted for routine lot release upon demonstration that the process consistently inactivates the biological activity of residual DNA or reduces the amount and size of the contaminating residual DNA present in the purified antigen bulk, as agreed upon with the NRA.
A.5.2.1.10 Test for residual bovine serum albumin content

If bovine serum is used during production then the residual BSA content should be measured and a maximum permitted concentration should be set and approved by the NRA.

A.5.2.1.11 Viral clearance study

When a cell substrate is used for the production of RSV antigens then the production process should be validated in terms of its capacity to remove and/or inactivate adventitious viruses – as described in the Q5A guidelines (108). This validation is performed during manufacturing development or as part of process validation and is not intended as an assessment for lot release.

If a replicating viral vector such as baculovirus is used then the production process should be validated for its capacity to eliminate (by removal and/or inactivation) residual recombinant virus. The provisions listed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18) should be taken into consideration.

A.5.2.2 Purified adjuvanted bulk

The purified antigens may be adsorbed onto an adjuvant such as an aluminum salt, in which case the adjuvant and the concentration used should be approved by the NRA. If an alternative adjuvant or additional adjuvant is used, this should also be approved by the NRA.

If a novel adjuvant is used that does not involve adsorption of the antigens to the adjuvant then the term “adjuvanted antigen bulk” may be used.

A.5.2.2.1 Storage

Until the adsorbed antigen bulk is formulated into the final bulk the suspension should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity, if applicable. Hold times should be approved by the NRA.

A.5.2.2.2 Tests of adsorbed antigen bulk

If applicable, all tests and specifications for adsorbed antigen bulk should, unless otherwise justified, be approved by the NRA.

A.5.2.2.2.1 Bacterial and fungal sterility

Each adsorbed antigen bulk should be tested for bacterial and fungal sterility, if applicable, as specified in the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.
A.5.2.2.2 Bacterial endotoxins
For a vaccine intended to be administered parenterally the adsorbed antigen bulk should be tested for bacterial endotoxins using a method approved by the NRA. The total amount of residual bacterial endotoxins should not exceed that found in vaccine lots shown to be safe in clinical trials or the amount found in other lots used to support licensing. The test may be omitted once production consistency has been demonstrated after agreement from the NRA. If it is inappropriate to test the adsorbed antigen bulk, the test should be performed on the purified antigen bulk prior to adsorption and should be approved by the NRA.

A.5.2.2.3 Identity
The adsorbed antigen bulk should be identified as the correct RSV antigen by a suitable method (for example, an immunological assay), if applicable.

A.5.2.2.4 Adjuvant concentration
Adsorbed antigen bulk should be assayed for adjuvant content until production consistency is demonstrated, if applicable.

A.5.2.2.5 Degree of adsorption
The degree of adsorption (completeness of adsorption) of the adsorbed antigen bulk should be assessed, if applicable. This test may be omitted upon demonstration of process consistency and should be approved by the NRA.

A.5.2.2.6 pH
If applicable, the pH value of the adsorbed antigen bulk may be monitored until production consistency is demonstrated, and should be approved by the NRA.

A.5.2.2.7 Antigen content
The antigen content of the adsorbed antigen bulk should be measured using appropriate methods, if applicable. If this test is conducted on purified antigen bulk, it may be omitted from the testing of the adsorbed antigen bulk.

International standards and reference reagents for the control of RSV-F antigen content and conformation are not available. Therefore, product-specific reference preparations may be used.

A.6 Control of production for viral-vectored RSV vaccines
The manufacture of vaccine vectors starts with the amplification of the vaccine vector seed stock in a suitable cell line. The number of passages between the virus WS lot and final viral-vectored vaccine product should be kept to a
minimum and should not exceed the number of passages used for production of the vaccine shown in clinical studies to be satisfactory, unless otherwise justified by the manufacturer and authorized by the NRA. A maximum number of passages should be defined for which the identity of the vaccine has been demonstrated.

After harvesting of the culture product, the purification procedure can be applied to a single harvest or to a pool of single monovalent harvests. The maximum number of single harvests that may be pooled should be defined on the basis of validation studies.

If applicable to the vector platform, a control cell culture should be maintained simultaneously and in parallel to the production cell culture. Cells should be derived from the same expansion series but no virus vector should be added to the control cells. The growth medium and supplements used in culturing should be identical to those used for the production cell culture. All other manipulations should be as similar as possible.

A.6.1 Tests on control cell cultures (if applicable)

When control cells are included in the manufacturing process due to limitations on the testing of primary cells or viral harvests, or is required by the NRA, the procedures described in section A.4.1 above should be followed.

A.6.1.1 Tests for haemadsorbing viruses

The provision laid down in section A.4.1.1 above applies.

A.6.1.2 Tests for other adventitious agents

The provisions laid down in section A.4.1.2 above apply.

A.6.2 Single virus harvest

The method used to harvest the vaccine vector should be described and the virus titre ascertained. A reference preparation should be included to validate the titration assay. Minimum acceptable virus titres should be established for both single virus harvests and for pooled single harvests.

The integrity of the integrated heterologous gene should be confirmed. An expression assay method should be described and should be performed on production harvest material or downstream (for example, on purified final bulk). For example, a Western blot analysis (or other method to confirm that the integrated gene is present and expressed) should be included in the testing of every batch.
A.6.2.1 Control tests on single virus harvest

Unless otherwise justified, an identity test should be performed on each crude or purified single harvest, whichever is the most appropriate. This should include the identity of the expressed heterologous antigen and of the vector virus.

Tests for adventitious agents should be performed on each single harvest according to the relevant parts of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18). Additional testing for adventitious viruses may be performed using validated NAT-based assays or other methods such as next generation sequencing.

New molecular methods with broad detection capabilities are being developed for adventitious agent detection and may also be used once validated to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and agreement from the NRA (see section A.3.3.3.4 above).

Single virus harvests should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, as specified in the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.

Due to the very high titres of single harvests of viral-vectored vaccines, alternatives to the classical testing for adventitious agents may be applied with the approval of the NRA.

Provided that cell banks and viral seed stocks have been comprehensively tested to demonstrate freedom from adventitious agents, the possibility of delaying in vitro testing for adventitious agents (viral pathogens and mycoplasmas) at the cell harvest or bulk substance stages, or replacing it with validated PCR tests or other NAT-based methods such as next generation sequencing, could be discussed and agreed upon with the NRA. The method of production should be taken into account when deciding upon the specified viruses being sought.

Additional considerations for this approach are that no animal-derived raw materials are used during manufacture, and that the manufacturing facility operates under a good manufacturing practices (GMP) certificate (where applicable), with assurances that prevention of cross-contamination is well controlled in the facility. Samples should be retained for testing at a later date if required.

A.6.3 Pooled monovalent virus harvest

Single virus harvests may be pooled to form virus pools from which the final bulk vaccine will be prepared. The strategy for pooling single virus harvests should be described. Minimum acceptable titres should be established for the
use of a single virus harvest in the preparation of a virus pool or final bulk. All processing of the virus pool should be described in detail.

A.6.3.1 Control tests on pooled virus harvests

Virus pools should be tested to demonstrate freedom from bacteria, fungi, mycobacteria (if applicable) and mycoplasmas, as specified in the WHO General requirements for the sterility of biological substances (99, 100). Alternatively, if single virus harvests have been tested to demonstrate freedom from bacteria, fungi, mycobacteria (if applicable) and mycoplasmas then these tests may be omitted on the pooled virus harvests.

A.6.4 Monovalent bulk vaccine

The monovalent bulk vaccine can be prepared from one or several virus pools with the same antigen, or it may be derived from a single virus harvest. Substances such as diluents or stabilizers or any other excipients added during preparation of the monovalent bulk or the final bulk vaccine should have been shown not to impair the potency and safety of the vaccine in the concentrations employed.

Penicillin and other beta-lactam antibiotics should not be used at any stage of manufacture because they are highly sensitizing substances in humans. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be approved by the NRA (95).

A.6.4.1 Control tests on monovalent bulk

The monovalent bulk vaccine should be tested and consideration given to using the tests listed below, as appropriate for each individual product. Alternatively, if the monovalent bulk will be held for a short period of time then some of the tests listed below could, if appropriate, be performed on the final bulk or final lot instead. If sufficiently justified, some tests may be performed on an earlier intermediate instead of on the monovalent bulk. All quality-control release tests for monovalent bulk should be validated and shown to be suitable for the intended purpose. Assay validation or qualification should be appropriate for the stage of the development life-cycle. Additional tests on intermediates during the purification process may be used to monitor for consistency and safety.

A.6.4.1.1 Purity

The degree of purity of each monovalent bulk vaccine should be assessed using suitable methods. The purity of the bulk should be ascertained for fragments, aggregates or empty particles of the product, as well as for contamination by residual cellular proteins. Residual cellular DNA levels should also be assessed.
The content and size of host cell DNA should not exceed the maximum level agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (18).

Process additives should also be controlled. In particular, if any antibiotics are added during vaccine production, the residual antibiotic content should be determined and should be within limits approved by the NRA.

These tests may be omitted for routine lot release upon demonstration that the process consistently clears the residuals from the monovalent bulk vaccine, subject to the agreement of the NRA.

A.6.4.1.2 Potency

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

A.6.4.1.2.1 Particle number

For relevant vectors (for example, adenovirus vectors) the total number of virus particles per mL (quantitated by a technique such as qPCR or HPLC) should be provided for each batch of monovalent bulk.

A.6.4.1.2.2 Infectivity

The infectious virus titre as a measure of active product should be tested for each batch of monovalent bulk. Direct methods such as a plaque-forming assay or indirect methods such as qPCR (if suitably correlated with a direct measure of infectivity) could be considered. If the particle number can be determined, the particle/infectivity ratio should also be specified.

A.6.4.1.2.3 Expression of the heterologous antigen in vitro

If not otherwise justified, the ability of the viral particles to express the heterologous gene should be demonstrated using a suitable method, for example based on the use of an antigen-specific antibody (and/or conformation-specific antibody if detecting RSV pre-fusion F antigen) after growth of the vector in a suitable cell line.

A.6.4.1.3 Identity

Tests used for assessing relevant properties of the viral vector – such as antigen expression, restriction-enzyme mapping, PCR with a specific probe or sequencing – will generally be suitable for assessing the identity of the product.
A.6.4.1.4  *Bacterial and fungal bioburden or sterility*

Each monovalent bulk should be tested for bacterial and fungal bioburden or sterility. Bioburden testing should be justified in terms of product safety. Sterility testing should be carried out as specified in the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.

A.6.4.1.5  *Bacterial endotoxins*

For vaccine intended to be administered parenterally, each monovalent bulk should be tested for bacterial endotoxins using a method approved by the NRA. At the concentration of the final formulation of the vaccine, the total amount of residual endotoxins should not exceed that found in vaccine lots shown to be safe in clinical trials or the amount found in other lots used to support licensing. The test may be omitted once production consistency has been demonstrated after agreement from the NRA.

A.6.4.1.6  *Reversion to replication competency or loss of attenuation*

The viral-vectored RSV vaccines under development are either replication-incompetent in human cells or adequately attenuated to prevent disease symptoms related to the viral vector backbone. Although manufacturers generally provide theoretical justifications for why reversion to competency or virulence is unlikely to occur, low levels of viral particles may emerge that have gained the complementing gene from the production cell line by an unknown or poorly characterized mechanism. It is not known whether such viral particles represent a safety concern. Consequently, it should be shown that the vaccine virus is still replication-incompetent or fully attenuated (whichever is relevant) in initial batches of the monovalent bulk.

    After demonstrating this, it may be possible to omit such tests in future batches provided a sufficient justification is made (which should include discussion of why reversion to competency or loss of attenuation would be unlikely in future batches).

A.6.5  *Final bulk vaccine*

Appropriate quantities of monovalent bulk vaccines should be pooled, mixed and formulated (if required) to form a homogeneous solution to manufacture the final bulk vaccine. The final bulk can be made up of one or more batches of a single monovalent vaccine to give the final vaccine product.

    If an antimicrobial preservative is used, it should not impair the safety or potency of the vaccine; the intended concentration of the preservative should be justified and its effectiveness should be validated (109).
A.6.5.1 **Control tests on final bulk vaccine**

The following tests should be performed on the final bulk vaccine unless it can be demonstrated that they are not necessary, for example where filling operations are performed immediately after manufacture of the final bulk, and on the same site:

A.6.5.1.1 **Identity**

See section A.6.4.1.3 above.

A.6.5.1.2 **Preservative**

Where applicable, the amount of antimicrobial preservative should be determined using a suitable method.

A.6.5.1.3 **Bacterial and fungal sterility**

Each final bulk should be tested for bacterial and fungal sterility. Sterility testing should be carried out as specified in the WHO General requirements for the sterility of biological substances ([99](#), [100](#)) or by an alternative method approved by the NRA.

A.7 **Filling and containers**

The relevant manufacturing recommendations contained in WHO good manufacturing practices for pharmaceutical products: main principles ([94](#)) and WHO good manufacturing practices for biological products ([95](#)) should apply to the RSV vaccine filled in the final form.

Care should be taken to ensure that the materials from which the container and, if applicable, the closure are made do not adversely affect the quality of the vaccine under the recommended storage conditions. 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. Assessment of extractables and/or leachables might also be required for container systems used for long-term storage of bulks and formulated bulks.

If multi-dose vaccine vials are used then the vaccine may contain preservative; the use of which should be compliant with the WHO Policy Statement: multi-dose vial policy ([109](#)), as is the case for reconstituted vaccines such as BCG and measles vaccines. In addition, the multi-dose container should prevent microbial contamination of the contents after opening. The extractable volume of multi-dose vials should be validated.

The manufacturer should provide the NRA with adequate data to prove that the product is stable under appropriate storage and shipping conditions (see section A.13 below).
A.8  **Control tests on final lot**

Where applicable or appropriate, the following tests should be performed on the final lot unless otherwise justified and agreed with the NRA. All tests and specifications should be approved by the NRA. The specifications should be defined on the basis of the results of tests on lots that have been shown to have acceptable performance in clinical studies.

A.8.1  **Inspection of final containers**

Every final container in each final lot should be inspected visually and/or in an automated manner, and those showing abnormalities (for example, improper sealing, clumping or the presence of particles) should be discarded and recorded for each relevant abnormality. A maximum limit should be established for the percentage of containers that can be rejected before triggering investigation of the cause, potentially resulting in batch failure.

A.8.2  **Identity**

An identity test should be performed on at least one final labelled container from each filling lot – in the case of freeze-dried vaccines, after reconstitution according to the manufacturer’s instructions for preparing the vaccine for human administration. However, it is not necessary to perform the genetic identity test on the final lot.

For multivalent vaccines each antigen component should be identified.

A.8.3  **Appearance**

The appearance of the liquid or freeze-dried vaccine should be described with respect to form and color (for example, viscosity of suspension). In the case of freeze-dried vaccines a visual inspection should be performed on the freeze-dried vaccine, the diluent and the reconstituted vaccine.

A.8.4  **pH**

The pH of the final lot should be tested and an appropriate limit should be set to guarantee virus stability. In the case of freeze-dried vaccines the pH should be measured after reconstitution of the vaccine with the diluent.

A.8.5  **Osmolality**

The osmolality of the final lot may be tested, if appropriate. The osmolality test may be omitted if performed on the final bulk. Alternative tests (for example, freezing point) may be used as surrogate measures for ionic strength/osmolality.
A.8.6 **Bacterial and fungal sterility**
Each final lot should be tested for bacterial and fungal sterility, as specified in the WHO General requirements for the sterility of biological substances (99, 100) or by an alternative method approved by the NRA.

A.8.7 **Bacterial and fungal contamination**
For chimeric BCG/RSV vaccines, samples from each final lot should be tested for bacterial and fungal contamination by appropriate tests as specified in Part A section 5.2 of the WHO General requirements for the sterility of biological substances (100) or by an alternative method approved by the NRA.

A.8.8 **Preservative**
Each final lot should be tested for the concentration of preservative, if added.

A.8.9 **Residual moisture**
The residual moisture in a representative sample of each freeze-dried lot should be determined by a method approved by the NRA. The upper limit for moisture content should be approved by the NRA based on the results of stability testing. Moisture levels of 3% or less are generally considered to be acceptable.

A.8.10 **Pyrogenic substances**
Each final lot should be tested for pyrogenic substances, if appropriate. Tests for bacterial endotoxin (for example, the limulus amoebocyte lysate (LAL) test) should be performed. However, if there is interference in the test – for example, because of the addition of an immunostimulant such as 3-O-desacyl-4’-monophosphoryl lipid A – a test for pyrogens should be performed. The classical rabbit pyrogen test should now be replaced by a validated monocyte-activation test approved by the NRA.

A.8.11 **Adjuvant content**
Each final lot should be assayed for adjuvant content, if added. Where aluminum compounds are used, the amount of aluminum should not exceed 1.25 mg per human dose.

A.8.12 **Protein content**
The protein content should be determined, if appropriate. Alternatively, this may be calculated from an earlier process intermediate.
A.8.13  **Degree of adsorption**
The degree of adsorption to the adjuvant (completeness of adsorption) of each antigen present in the final bulk should be assessed, if applicable (for example, if the adjuvant is aluminum salts) and the lower limit should be approved by the NRA.

This test may be omitted for routine lot release upon demonstration of process consistency, subject to the approval of the NRA.

A.8.14  **Potency**
An appropriate in vitro or in vivo quantitative test for potency should be performed using samples representative of each final vaccine lot. In the case of freeze-dried vaccines, potency should be determined after the freeze-dried product has been reconstituted with the approved diluent.

The potency test used and method of data analysis should be approved by the NRA. Vaccine potency should be compared with that of a reference preparation, and the limits of potency should be agreed with the NRA. The reference preparations used should be approved by the NRA.

Until international standards for the potency of RSV vaccines become available, manufacturers should establish a product-specific reference preparation that is traceable to a lot of vaccine, or to bulks used in the production of such a lot, shown to be efficacious in clinical trials. The performance of this reference preparation should be monitored by trend analysis using relevant test parameters and the reference preparation should be replaced when necessary. An acceptable procedure for replacing reference preparations should be in place (110).

For multivalent vaccines it may be necessary to perform potency tests on the monovalent bulks if analytical methods cannot distinguish between the different monovalent vaccines in the final lot.

A.8.15  **Purity**
Testing for purity should be performed on the monovalent bulk or final bulk vaccine. However, limited purity testing of the final lot may be required even if purity is tested on the final bulk vaccine if, after taking the manufacturing process and nature of the vaccine into consideration, it is considered possible that the purity may have changed. This should be considered on a case-by-case basis.

A.8.16  **Bacterial concentration**
If appropriate, in the case of chimeric BCG/RSV vaccines, the total bacterial content of the reconstituted vaccine should be estimated for each lot by a validated method approved by the NRA and should have a value range approved by the NRA. The estimate of total bacterial content may be made either directly, by
determining the dry weight of the organism, or indirectly by an opacity method that has been calibrated in relation to the dry weight of the organism.

A.8.17  Extractable volume
It should be demonstrated that the nominal volume on the label can consistently be extracted from the containers.

A.8.18  Aggregates/particle size
If the RSV vaccine consists of nanoparticles which might be susceptible to aggregation then each final lot should be examined for particle size/aggregate content at lot release and across the shelf-life. This test may be omitted for routine lot release upon demonstration of process consistency, subject to the approval of the NRA.

A.8.19  Viability
If appropriate, in the case of chimeric BCG/RSV vaccines, the number of culturable particles of each final lot should be determined by an appropriate method approved by the NRA – see section A.6.7 of the WHO Recommendations to assure the quality, safety and efficacy of BCG vaccines (20).

A.8.20  Thermal stability
If appropriate, a thermal stability test should be performed. The purpose of the thermal stability test is to demonstrate the consistency of production. Additional guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (111).

For live-attenuated and/or viral-vectored vaccines, at least three containers of each final vaccine lot should be incubated at the appropriate temperature for the appropriate time (for example, 37 °C for 7 days). The geometric mean titre (GMT) of infectious virus in the containers should not have decreased during the period of exposure by more than a specified amount (for example, 1 log₁₀) that has been justified by the production data and approved by the NRA. Titration of non-exposed and exposed containers should be carried out in parallel. A reagent for intra-assay validity control should be included in each assay.

For chimeric BCG/RSV vaccines, each final lot should be tested for thermal stability by a validated method approved by the NRA. After production consistency has been demonstrated, this test may be omitted on subsequent final lots subject to NRA approval. If performed, the test should involve the determination of the number of culturable particles before and after the samples have been held at appropriate temperatures and for appropriate periods. For example, the thermal stability test may be carried out by taking samples of the
vaccine and incubating them at 37 °C for 28 days (20). The percentage decrease in the number of culturable particles is then compared with that of samples of the same vaccine lot stored at the recommended temperature. An upper limit on the acceptable percentage decrease in culturable particles compared to the untreated vaccine should be approved by the NRA.

A.8.21 Residual antibiotics
If any antibiotics were added during production then residual antibiotic content should be determined and should be within limits approved by the NRA.

A.8.22 Diluent
The recommendations given in WHO good manufacturing practices for pharmaceutical products: main principles (94) should apply to the manufacturing and control of diluents used to reconstitute freeze-dried RSV vaccines. An expiry date should be established for the diluent on the basis of stability data. For lot release of the diluent, tests should be carried out for identity, appearance, pH, extractable volume, sterility, endotoxin and the content of key components.

A.8.23 Safety test
If appropriate, for chimeric BCG/RSV vaccines, tests to confirm the absence of virulent mycobacteria and a test for excessive dermal activity should be performed – see section A.6.4 of the WHO Recommendations to assure the quality, safety and efficacy of BCG vaccines (20).

A.9 Records
The recommendations given in WHO good manufacturing practices for pharmaceutical products: main principles (94) should apply, as appropriate to the level of development of the candidate vaccine.

A.10 Retained samples
A sufficient number of samples should be retained for future studies and needs. Vaccine lots that are to be used for clinical trials may serve as a reference material in the future, and a sufficient number of vials should be reserved and stored appropriately for that purpose.

A.11 Labelling
The labelling recommendations provided in WHO good manufacturing practices for biological products (95) should be followed as appropriate. The label of the carton enclosing one or more final containers, or the leaflet accompanying the container, should include:
- the name of the vaccine;
- in the case of live-attenuated vaccines, a statement on the nature of the preparation, specifying the strain of RSV or recombinant RSV that the vaccine has been prepared from;
- in the case of live-attenuated/chimeric vaccines, the minimum number of infective units per human dose, the nature of any cellular systems used for the production of the vaccine, and whether the vaccine strain was derived by molecular methods;
- in the case of subunit, particle-based and viral-vectored vaccines, a statement that specifies the nature of the cells and/or any expression system used for the production of the vaccine;
- in the case of subunit and particle-based vaccines, a statement that specifies the nature and content of adjuvant contained in one human dose;
- in the case of subunit, particle-based and viral-vectored vaccines, the volume of one recommended human dose, and the amount of active substance(s) contained in one recommended human dose;
- the immunization schedule, and the recommended route(s) of administration;
- the number of doses if the product is issued in a multi-dose container;
- a statement to the effect that product contact with disinfectants should be avoided;
- a statement concerning the photosensitivity of the vaccine based on photostability data;
- if applicable, a statement indicating the volume and nature of diluent to be added to reconstitute the vaccine, specifying that the diluent to be used is that supplied by the manufacturer – and a statement to the effect that after the vaccine has been reconstituted it should be used without delay or, if not used immediately, stored under conditions of time and temperature formally shown not to affect stability, and protected from light for a maximum period defined by stability studies;
- the name and concentration of any preservative added;
- a statement on the nature and quantity, or upper limit, of any antibiotics present in the vaccine;
- the temperature recommended during storage and transport;
- the expiry/retest date;
any special dosing schedules;
contraindications, warnings and precautions, and information on concomitant vaccine use and on potential adverse events.

A.12 **Distribution and transport**
The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (94) and WHO good manufacturing practices for biological products (95) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (112).

A.13 **Stability testing, storage and expiry date**
The recommendations given in WHO good manufacturing practices for biological products (95) and WHO Guidelines on stability evaluation of vaccines (111) appropriate for the respective RSV vaccine should apply. Furthermore, the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions might apply (113). 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.

A.13.1 **Stability testing**
Adequate stability studies form an essential part of vaccine development. Guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (111). Stability testing should be performed at different stages of production – namely on stored intermediates (including single harvests, monovalent bulk vaccine and final bulk) and on the final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. It is advisable to assign a shelf-life to all in-process materials during vaccine production, particularly to stored intermediates such as single harvests, purified bulk and final bulk.

Accelerated thermal stability tests may be undertaken on each final lot to give additional information on the overall characteristics of the vaccine and may also be useful in assessing comparability when the manufacturer plans to make changes to manufacturing.

For vaccine licensure, the stability of the vaccine and its final container at the recommended storage temperatures should be demonstrated to the satisfaction of the NRA on at least three lots of the final product (or, in the case of adsorbed vaccine, on the adsorbed antigen bulks). During clinical trials fewer data are likely to be available. However, the stability of the vaccine...
under the proposed storage conditions should be demonstrated for at least the expected duration of the product in the clinical trial and information should be supplemented and updated when more data become available (114).

Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (111). Data should be provided to the NRA according to local regulatory requirements.

The final stability-testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection and sharing of stability data, and criteria for rejecting vaccines(s).

In-use stability should also be specified and justified with adequate data under real-time conditions.

The formulation of vaccine and adjuvant (if used) should be stable throughout its shelf-life. Moreover, the stability of the antigen-adjuvant adsorption (if specified) should be demonstrated for the duration of the shelf-life. Acceptable limits for stability should be agreed with the NRA.

**A.13.2 Storage conditions**

Before being distributed by the manufacturing establishment or before being issued from a storage site, the vaccine should be stored for no longer than a fixed length of time and at a temperature shown by the manufacturer to be compatible with a minimal loss of potency. The maximum duration of storage should be fixed with the approval of the NRA based on the results of stability studies, and should be such as to ensure that all quality specifications for the final product, including the minimum potency specified on the container or package, are maintained until the end of shelf-life. During clinical trials, this period should ideally be at least equal to the expected duration of vaccine administration.

**A.13.3 Expiry date**

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

Additional stability studies are needed during development to support a determination of the shelf-life of the vaccine after thawing prior to administration, both before and after the addition of diluent, if relevant. The shelf-life for the vaccine and the diluent (if used) may differ.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals are inoculated.
A.13.3.1 Expiry of reconstituted vaccine

In the case of single-dose containers of freeze-dried vaccines which require reconstitution, the reconstituted vaccine should be used immediately. Multi-dose containers should be kept in the dark at 2–8 °C and the expiry time for use of an opened container should comply with the WHO Policy Statement: multidose vial policy (109). If a preservative is used, data supporting the stability and efficacy of antimicrobial preservation should be generated and approved by the NRA.

Part B. Nonclinical evaluation of RSV vaccines

B.1 General remarks

Nonclinical evaluation of RSV vaccines includes all in vivo and in vitro testing prior to and during clinical development. Consideration should be given to the number and types of preclinical pharmacological studies to be conducted, with the expectation of streamlining and limiting such studies to those that provide results directly relevant to the proposed clinical programme. Sponsors may consult NRAs to identify the most relevant studies for their regulatory submission.

Before proceeding to human testing, there should be adequate information suggestive of the safety and potential efficacy of the vaccine, including product characterization, immunogenicity studies, and toxicity and safety testing in animals. The continuation of some nonclinical testing would be expected in order to maintain adherence to current GMP and to support further clinical development (14, 115).

The following sections describe the type of nonclinical information required to support the initiation of a specific clinical study, or that should be submitted in a marketing authorization application. Guidance on the designing, conducting and analysis of nonclinical studies is available in the WHO guidelines on nonclinical evaluation of vaccines (14) which should be consulted.

B.2 Process development and product characterization

The general principles described in the WHO guidelines on nonclinical evaluation of vaccines (14) regarding vaccine production, testing and stability are broadly applicable to RSV vaccines. The production process should be adequately controlled at critical steps to ensure consistency of manufacture. Vaccine antigens and the end product should be well defined and thoroughly characterized to confirm that vaccine lots used in nonclinical studies are qualified.

Vaccine lots used in nonclinical studies may be at research grade or manufactured under GMP. Ideally, the lots tested are clinical lots. If this is not
feasible, they should at least be comparable to clinical lots with respect to the concurrent clinical lot specification.

For recombinant DNA-derived antigens one intrinsic aspect is to demonstrate the stability of their conformation(s) using suitable methods. These methods include negative staining and electron microscopy and/or a direct antibody-binding assay, ideally using a standardized panel of monoclonal antibodies with well-defined epitope specificities. Any instability of the expressed proteins occurring during storage or after a production-scale run should be documented. Serological investigation based on antibody-competition assays using post-immunization sera may also provide informative data regarding the presence and stability of antigenic sites exposed in a given conformational state.

For live-attenuated vaccines, the suitability of an attenuated vaccine strain needs continuous careful review to ascertain attenuation and phenotypic stability. A complete genetic sequence should be obtained to document the attenuating mutations within the virus genome that may correlate with its attenuated phenotype. Since each virus passage may introduce new mutations, studies should determine if the genetic basis of attenuation is stable over the entire manufacturing process and during replication in vaccinees. These studies should also define the phenotype of the vaccine strain as far as is practical. The critical phenotypic markers, including replication efficiency in animal models and/or primary human bronchial epithelial cells or other relevant cultured cells, temperature sensitivity and/or cold adaptation in vitro, are useful for detecting reversion events.

Candidate vaccines based on live viral-vectored vaccines are associated with similar safety issues, including degree of attenuation in vivo and replication in vitro, genetic stability of the virus and the potential risk of reversion to virulence, and should be characterized accordingly. Neurovirulence testing is not normally needed for vectored RSV vaccines unless vaccine constructs with gene deletions or modifications of the vector are suspected to have the potential for neurovirulence.

Guidance on the general principles of the nonclinical assessment of vaccine adjuvants can be found in the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (15).

B.3 Nonclinical immunogenicity and protective activity

There is no animal model that precisely mimics RSV disease in humans. Despite this, it is acceptable to demonstrate vaccine immunogenicity in animal models as the rationale to support advancing a candidate vaccine to the clinical setting. Assessment of immunogenicity in animals should consider the construct designed or the type of vaccine. For certain vaccines (including protein-
based ones) it is generally recommended that the serum antibodies with RSV-neutralizing activity be assessed in immunogenicity studies because antibodies directed against RSV-F or RSV-G neutralize the virus in vitro and have been associated with a protective effect in animal models and/or in humans. Consideration should be given to the choice of RSV subtype (A or B) as well as to the cell type to be used for assessing neutralizing antibody responses when a vaccine construct is specifically designed to target RSV-G alone.

Candidate vaccines may be designed to elicit cellular immunity. The cellular response is typically assessed by evaluating the effect of the vaccine on the number or functional specificity of CD8+ cytotoxic T-lymphocytes and/or type 1 CD4+ T-helper (Th1) cells. For other products, induction of an effective mucosal immune response may be an intended mechanism of protection – for example, for a vaccine administered by the intranasal route. This is the case for some live-attenuated or replication-competent vectored vaccines. Therefore, a product-specific approach to the evaluation of candidate vaccines should be taken.

For vaccines that include an adjuvant, information to support the adjuvant selection and its inclusion in the vaccine formulation should be provided, based for example on demonstrated adjuvant activity and the beneficial effect assessed in terms of the magnitude and/or the type, broadness and duration of the functional immune response induced (15). The passive transfer of antibodies, generated in response to vaccination, to RSV-naive animals that are subsequently challenged with RSV can provide evidence for antibody-mediated protection and may be explored. In such cases, early discussion with the NRA is recommended.

For a multivalent candidate RSV vaccine the immune responses to each of the vaccine antigens targeted should be assessed.

Careful characterization of vaccine-induced immune responses in animal models is recommended, whenever feasible, during the assessment of vaccine-associated ERD anticipated for certain vaccines (see General considerations above).

Protective activity in challenged animals may be evaluated during the assessment of vaccine-associated ERD risk (see section B.5.2 below). However, experience has shown that such data, especially those derived from rodents, are not necessarily predictive of immune protection in humans.

B.4 Pharmacokinetic studies

Studies to determine serum concentrations of antigens are not needed. Specific studies such as local deposition studies at the site of injection, distribution studies or viral shedding studies may be necessary, especially in the case of novel adjuvants, new formulations or alternative route of administration (for example, intranasal route).
For live viral-vectored vaccines for which no prior experiments have been done, biodistribution should be studied in a full set of tissues and organs, including the brain. Such a study is unnecessary if supportive data generated for the same vector but using different gene insert(s) are available and in cases where the construction of the vector is not suspected to result in altered tissue tropism. Testing in one species is considered sufficient if scientifically justified. Crossing of the blood–brain barrier might be an indication of potential neurovirulence and should trigger additional safety testing (115).

B.5 Nonclinical toxicity and safety testing

B.5.1 Preclinical toxicology

Toxicology studies for RSV vaccines should be undertaken based on the guidance on the general principles of toxicity assessment provided in the WHO guidelines on nonclinical evaluation of vaccines (14). Toxicological testing should aim to identify any untoward effect associated with a vaccine dose, or as a consequence of replication and tissue tropism of vaccine virus in the case of a replicating vaccine, by careful analysis of all major organs as well as tissues near to and distal from the site of administration. Toxicology studies should support the safety of the starting dose, dosing schedule, route of administration and proposed rate of dose escalation.

When a new adjuvant for which no experience exists in relation to human use is included in the formulation of a vaccine it is advisable that the adjuvant alone be characterized in accordance with the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (15).

If a candidate vaccine is intended to be used for the immunization of pregnant women or women of childbearing age, a single developmental and reproductive toxicity study in one relevant species should be performed. The timing of submission of such data varies by geographical region or country. Some NRAs require the exclusion of women of childbearing potential from large-scale clinical trials prior to the completion of developmental and reproductive toxicity study. In other cases, NRAs can allow the recruitment of women of childbearing potential into early clinical trials if highly effective birth control methods are used by trial participants.

Any change introduced into the manufacturing or formulation of a vaccine during product development, when judged to be significant, may require partial or full re-evaluation in preclinical toxicity testing (14, 116, 117).

B.5.2 Preclinical safety

Studies with live-attenuated and live viral-vectored vaccines entail the identification of markers of attenuation that can assist during the clinical evaluation phases. The primary purpose of such studies is to demonstrate that
the vaccine is less virulent in the animal host than comparable wild-type viruses, and that the vaccine does not exhibit any unexpected harmful tissue tropism and damage (see sections B.2 and B.4 above).

The need for potential vaccine-associated ERD risk assessment in animal models is determined by the type of RSV vaccine and/or the target population(s) and should be considered on a case-by-case basis. For example, for candidate vaccines with immunological characteristics similar to that of FI-RSV and developed for the active immunization of RSV-naive infants, a preliminary assessment of the vaccine-associated ERD risk is crucial (see General considerations above). Such investigations may not be needed for live-attenuated RSV vaccines based on existing experience and if agreeable to the relevant NRAs. For live chimeric RSV vaccines experience is more limited and for many new candidate vaccines does not exist at the time of writing these Guidelines, and a more cautious approach should therefore be taken. This testing is not required for RSV vaccines indicated for use in RSV-experienced/non-naive populations.

When the conducting of such a study is justified, the inclusion of adequate controls will have to be considered – for example, a group given intranasal RSV infection needs to be included as a negative control, and when appropriate another group should receive FI-RSV at a dose level shown to cause ERD as a positive control. Furthermore, RSV disease enhancement has been noted to occur in the absence of RSV antigen, thus raising doubts regarding the validity of data resulting from the use of these models. Therefore, it may be important to address inflammatory responses that may be due to host cell proteins or components of the cell culture medium used to produce the vaccine and/or the RSV challenge virus (85). Another important consideration is the choice of the vaccine dose. It may be necessary to examine serum antibody responses and lung histopathology after RSV challenge over a range of vaccine doses. In some cases, the dose may need to be optimized so that it is capable of inducing a measurable immune response to the vaccine while also permitting some degree of viral replication in the lungs of vaccinated animals after challenge. Since surrogate read-outs of vaccine-associated disease exacerbation vary by animal model, the weight given to each of the factors discussed above for consideration should be tailored according to the animal model used – for example, the confounding effect of cell culture serum has mainly been reported in rodent models. Although the measurement of viral titres in the lungs of affected animals does not predict enhanced pulmonary pathology, this parameter is broadly suited to assessment of the protective effect of the candidate vaccine. To enhance regulatory acceptance, it is recommended that the relevant NRA be engaged in discussion of the design of preclinical testing for vaccine-associated ERD risk at an early stage of product development.
Irrespective of the animal model used for RSV challenge prior to vaccine-associated ERD risk assessment, lung sections should be scored by a pathologist/person blinded to the group assignment; the method used to summarize and compare lung histopathology scores should be adequately described.

A brief review of some representative animal models is provided below. It is important to note that the mechanism of action for human vaccine-associated ERD is not fully understood, and that current small-animal models primarily reproduce some immunopathological features of human ERD. Accordingly, the interpretation of these data should be undertaken with extreme caution.

B.5.2.1 Mouse model
Mice are relatively resistant to human RSV infection and require high titres of challenge inocula for significant lung pathology (for example, above $10^6$ PFU) (118). The small airway epithelium of mice is not as extensively infected as it is in humans and most virus replication occurs in type 1 pneumocytes. Notwithstanding these limitations, the mouse model is attractive because of the relatively low cost and the availability of extensive molecular tools. Certain strains, such as BALB/c mice, have been extensively used to explore the mechanisms underlying FI-RSV-associated ERD, such as patterns of CD4+ Th2 activities after vaccination and RSV challenge, immune complex deposition, pulmonary eosinophilia and induction or absence of RSV-specific CD8+ cytotoxic T-lymphocytes (89, 118–120). Other informative parameters displayed by the challenged mice may include body weight loss, illness and changes in respiratory physiology.

In addition, there are a number of models based on the use of genetically modified mice that may provide unique insights into pathogenesis.

B.5.2.2 Cotton rat model
Cotton rats are more susceptible to human RSV infection than mice and have been widely used to characterize vaccine-associated ERD (121). In this model, virus replication in the lower airway is primarily limited to bronchiolar epithelium, closely resembling human infection. Several key histological features of disease exacerbation have been reproduced in cotton rats, including neutrophilic alveolitis and peribronchiolitis primarily caused by lymphocyte infiltration. In addition, interstitial pneumonitis appears to be another marker specific to the enhanced pulmonary pathology.

B.5.2.3 Non-human primate model
African green monkeys are one of the non-human primate species that has been used to model FI-RSV-associated ERD. Enhanced pulmonary pathology that closely resembles ERD in humans has been demonstrated in this model, as
manifested by severe infiltration of lymphocytes, macrophage, eosinophils and polymorphonuclear cells into parenchyma and the peribronchiolar areas of the lung. However, data on this model are limited and clinical disease presentation in vaccinated monkeys is of limited comparability to humans (122).

Similarly, cynomolgus macaques display lung eosinophilia and production of type 2 cytokines after FI-RSV immunization and RSV challenge. Although fatal outcomes may occur in FI-RSV-immunized macaques, the histological presentation observed in fatal human cases is not duplicated as there are no inflammatory lesions in the lungs at necropsy (123).

Despite sharing a high degree of similarity with the human immune system, non-human primates do not reproduce all of the immunological features seen in humans, as significant RSV-neutralizing antibody responses can be induced in FI-RSV-immunized monkeys. In addition, their limited availability, high cost and ethical considerations further present practical limitations on the use of non-human primates. It is also worth noting that human RSV is semi-permissive in non-human primates and the inoculum needs to be very large, with challenge viruses matched to their hosts and able to appropriately inhibit type 1 interferon and accomplish all other immune-evasion strategies. Typically, several mL of high-titre virus stock need to be given in each nostril and sometimes intratracheally – which does not reflect the type of transmission that occurs in humans.

B.5.2.4 Calf model

Calves are a natural host for bRSV and efficiently replicate the virus in the upper and lower respiratory tract. Infection with bRSV in calves causes a spectrum of clinical disease resembling the disease observed in RSV-infected human infants, such as fever, nasal discharge, cough, and tachypnea with chest retractions, wheezing, hypercapnia and hypoxemia (124). Severe lower respiratory tract disease occurs mostly in calves less than 6 months of age. Studies to model FI-RSV disease exacerbation in calves have demonstrated a similar clinical and histopathological presentation to that observed in the original human trials, including detection of poorly neutralizing antibodies. The features unique to enhanced pulmonary pathology include proliferative alveolitis, alveolar syncytium and septal fibrosis. However, these outcomes could not be consistently reproduced in all studies reported (89).

Since the fusion protein ectodomains of bRSV and human RSV share significant homology and since other viral proteins are highly conserved across strains, the calf model challenged with bRSV may have value in demonstrating the protective efficacy of a vaccine based on human RSV-F and for assessing the risk of vaccine-associated ERD. However, an absence of protection in this model may not be predictive of the human situation.
Disadvantages of this model include the need to use a different (that is, non-human) RSV strain, the need for very large inocula and the need for expertise in working with large animals. Due to the large size of the lung and the potentially unequal distribution of signs of disease within it, there are also concerns that sampling errors could interfere with an accurate evaluation of pathology.

B.6 Environmental risk assessment

Inactivated or protein-based RSV vaccines are unlikely to result in significant risk to the environment and thus are exempted from specific environmental risk assessment (ERA) studies. However, live vaccines attenuated by genetic modification or live viral vectors pose a potential risk of spread to a third party – that is, unvaccinated humans and/or animals. For such vaccines, an ERA may be required as part of the preclinical evaluation. Data on the phenotype of live-attenuated vaccine virus or live viral vectors (including their degree of attenuation and replication, their genetic stability, the potential for reversion to a virulent virus and the possibility of shedding following vaccine administration) will contribute at least in part to the ERA.

In addition, the issue of the vector’s potential for recombination with other infectious agents that might coincidentally occur in vaccinees should be addressed as appropriate. For live-attenuated RSV vaccines, one study has suggested that the rate of RSV recombination can be very low and may not pose a concern for vaccine safety (125).

Part C. Clinical evaluation of RSV vaccines

C.1 Introduction

Clinical studies for RSV vaccines should be conducted in accordance with the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (114) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (16). This section focuses only on issues that are most relevant or specific to the clinical evaluation of RSV vaccines, regardless of the vaccine construct. Guidance is provided on assays for the measurement of immune responses to vaccination and for laboratory confirmation of clinical cases of RSV disease in efficacy trials. The discussion of clinical programmes is generally applicable across age and population groups but specific attention is given to trials that evaluate the safety, immunogenicity and efficacy of vaccines intended for:

- active immunization of infants and toddlers (aged 28 days to 23 months), including those who were born prematurely;
Annex 2

- active immunization of pregnant women, with the primary aim of protecting the infant in the first months of life;
- active immunization of older adults (for example, aged ≥ 50), including subjects with comorbidities.

Sponsors may wish to investigate the use of RSV vaccines in other populations. These may include neonates (0–27 days), children from 2 years of age and adults and/or subjects with comorbidities or immunodeficiencies predisposing to the development of RSV disease. Safety and immunogenicity data should be obtained in each target population in accordance with sections C.2 and C.4 below. Section 6 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (16) considers the possible need for efficacy trials and the extrapolation of results of vaccine efficacy trials between populations.

At the time of preparing the current guidance, no vaccines against RSV had been approved. Subjects who have already received any RSV vaccine should not be enrolled into clinical trials intended to be confined to RSV-naive subjects. Furthermore, depending on the trial objectives, it may be necessary to exclude subjects who have received any RSV vaccine from trials intended to be confined to RSV-experienced subjects.

C.2 Immunogenicity trials

C.2.1 Assays

General guidance on the use and validation of assays for measuring immune responses is provided in the WHO Guidelines on clinical evaluation of vaccines; regulatory expectations (16). This section provides specific guidance on assays of relevance to the investigation of immune responses to RSV vaccines, some of which may be selected for use in individual clinical development programmes according to the vaccine construct.

C.2.1.1 Humoral immunity

C.2.1.1.1 Neutralizing antibodies

Serum RSV neutralization assays occur in a multitude of formats (91, 126). Sponsors should provide detailed information on the identity of the cell substrate, virus challenge strain and whether neutralization is modulated by complement, stating the type and concentration if used in the assay (127, 128). Neutralization assays may use laboratory-adapted strains representative of RSV/A (such as A2, Long, or Tracy) and RSV/B subtypes (such as 18537, 9320 or B1) and/or contemporary RSV isolates like RSV/A/Ontario/2010 (ON1) and RSV/B/BA viruses of the Buenos Aires lineage (73, 129–132) or other contemporary strains as they become available. The use of both RSV/A and RSV/B viruses will help to
verify the ability of a vaccine to elicit antibodies capable of broadly neutralizing RSV strains irrespective of subtype.

The read-out for the assay (for example, cytopathic effect, plaque counts, fluorescence, luminescence or gene copy number) should be described. Adequate controls should be used to define a valid test and to justify the pooling of data across assay runs. When calculating neutralization end-points, the final serum dilution should take into account the addition of the challenge virus. The method used to calculate end-point titres should be provided. Generally, it is recommended that the end-point should be derived from the linear portion of the titration curve.

The results should be reported in International Units (IU) along with information on the performance of the international standard. The First WHO International Standard for antiserum to respiratory syncytial virus (91–93) has been established to facilitate comparison of RSV neutralizing antibody responses across different neutralization assay formats, thereby permitting a closer comparison of the responses elicited by various candidate vaccines. To date, the international standard has been validated for the harmonization of RSV neutralization assays using post-infection adult and paediatric sera, as well as adult vaccinee sera representing three candidate RSV-F vaccines with similar antigen conformations assayed against RSV/A and RSV/B strains. The international standard harmonizes output when RSV/A and RSV/B strains are used, with or without the inclusion of complement in the assays.

C.2.1.1.2 RSV-binding antibodies

Enzyme immunoassays (EIAs) that measure anti-RSV IgG antibodies are commercially available. Sponsors may also develop in-house tests suited to the individual vaccine. If commercial assays are used it is recommended that kits are derived from the same manufacturing lot or otherwise qualified by appropriate bridging studies in order to minimize variability in results.

- Use of EIA to measure anti-RSV-F IgG – it is recommended that RSV-F antigens used to capture anti-F binding antibodies be of high quality, with a well-characterized conformation and proven stability. During assay development, the appropriate antibody reagents should be used to confirm the predominant conformation of RSV-F antigen present by assessing the ability to bind antibodies specific for epitopes on the pre-fusion and/or post-fusion conformations of RSV-F, with appropriate bridging studies performed to confirm the suitability of each new lot of RSV-F antigen prior to use. Some pre-fusion RSV-F epitopes are specific to RSV/A or RSV/B pre-F protein. Therefore, in some cases it may be necessary to test for IgG antibodies that bind pre-F antigens in a subtype-specific manner (53, 74, 133, 134).
Purified recombinant proteins or synthetic peptides may be used in EIA to detect antibody responses against antigens such as RSV-G/A and RSV-G/B proteins. Antibody responses against RSV proteins not included in the vaccine may support surveillance for RSV exposures/infections during follow-up (135).

Antibody responses to a specific protein or epitope may be detected using competitive binding studies based on EIA formats or biosensor technology wherein antibody binding to the antigen of interest is evaluated in the presence of a competitor (136).

C.2.1.2 Cell-mediated immunity

C.2.1.2.1 CD8+ T-cell responses

Ideally, CD8+ T-cells are collected at 7–14 days after a vaccine dose in adults for determination of sensitization by in vitro stimulation with RSV antigens (60, 137). A similar sampling window may apply to infants and children based on the finding that CD8+ T-cell responses in peripheral blood peaked between 11 and 15 days after onset of symptoms in RSV-infected infants (64). The optimal sampling time for detecting CD8+ T-cells may vary by vaccine platform and a broader window may be considered if supported by data for the vaccine under study.

C.2.1.2.2 CD4+ T-cell responses

CD4+ T-cells in infants less than 6 months of age are epigenetically programmed to have a dominant Th2 cytokine response that may be antigen specific (65, 66). In some cases, it may be appropriate to evaluate CD4+ T-cell responses in RSV-naive infants in early phase clinical testing to determine the ratio of Th2 cytokines (such as IL-4, IL-5 and IL-13) to Th1 cytokines (such as IL-2 and IFN-γ) following in vitro re-stimulation (using overlapping peptides representative of vaccine antigens, inactivated virus or purified protein antigen). CD4+ T-cell memory response in RSV-naive infants given a priming dose of vaccine may be detected as early as 10–14 days after this first dose but these memory responses should persist and may also be detected using samples collected at later time points.

C.2.2 Trial population and design

Regardless of the target population(s) for a candidate RSV vaccine, the first trials are expected to be conducted in healthy adults to provide data on safety and immunogenicity in RSV-experienced male and non-pregnant female subjects. For live viral-vectored vaccines these initial studies should provide an indication of whether pre-existing or vaccine-associated immune responses to the vector
have an impact on responses to the RSV antigen and whether such issues need to be explored in sequential trials in target populations.

C.2.2.1 Infants and toddlers

Unless justified based on accumulated evidence specific or relevant to the candidate vaccine, a safety and immunogenicity trial should be conducted in RSV-experienced subjects before considering a trial in RSV-naive subjects. A definition of RSV-naive and RSV-experienced subjects should be included in the protocol. For example, RSV-naive subjects could be defined as having no documented history of RSV disease and no immunological evidence of prior exposure to RSV. It may also be possible to apply an age cut-off based on relevant epidemiological data to minimize the risk that RSV-naive subjects are falsely determined to be RSV-experienced.

The potential for maternal antibody to interfere with the immune response to active immunization of infants should be assessed from the relationship between pre- and post-vaccination immune parameters. If maternal antibody has a negative effect on the infant immune response, consideration could be given to administering an additional dose (for example, 6 months after completion of the primary series) and comparing the response with that to a single dose administered to unvaccinated subjects of the same age to assess whether vaccinated infants were primed.

C.2.2.2 Pregnant women

Data obtained in non-pregnant women of childbearing potential should be used to select the initial dose regimen(s) to be tested in pregnant women. Dose regimens for pregnant women may aim to maximize the difference in RSV neutralizing antibody titres in cord blood between infants born to vaccinated and unvaccinated mothers whilst maintaining an acceptable safety profile. Analysis of cord blood antibody levels in infants by time elapsed between maternal vaccination and delivery may assist in determining the optimal timing of maternal vaccination.

Documenting the RSV neutralizing antibody decay curve in vaccinated women following delivery (for example, for 3–6 months) may give an early indication of the need to revaccinate women during each pregnancy. Consideration should be given to investigating the safety and immunogenicity of revaccination post-delivery or during a subsequent pregnancy whenever the opportunity arises in the post-approval period.

Documenting the RSV neutralizing antibody decay curve in infants until titres are below the limit of quantitation of the assay may give an early indication of the maximum duration of protection that might be expected.
C.2.2.3 Older adults

It is important that data are obtained from all age subgroups (for example, < 65, 65–74, 75–84 and ≥ 85 years) within the target population in safety and immunogenicity trials to explore the possibility that age-subgroup-specific regimens may be needed. Unless otherwise justified, it is recommended that trials should document the safety and immunogenicity of additional doses administered at intervals – for example, after 1–2 years since the primary dose(s) – to randomized subsets of subjects. This information can be used to support a revaccination strategy if this is later concluded to be appropriate from the results of efficacy trials.

C.3 Efficacy trials

In the absence of RSV vaccines licensed and widely recommended for use in the target population of a candidate RSV vaccine, vaccine efficacy trials should compare rates of RSV disease between groups randomized to the candidate vaccine or to no vaccination against RSV. At least one trial should be conducted in each target population subgroup proposed for the candidate vaccine (for example, in infants ± toddlers, in pregnant women and/or in older adults) depending on the perceived suitability of the candidate vaccine for these population subgroups.

The WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (16) provide guidance on the need for, and design of, efficacy trials when there is a licensed vaccine available that is widely recommended for use in the target population for a candidate vaccine. The Guidelines also discuss situations in which efficacy may be inferred from immunogenicity data.

Before commencing efficacy trials in target populations, sponsors may consider the possible value of conducting a human challenge study.

In accordance with Statistical principles for clinical trials, ICH Topic E9 (138), consideration should be given to the stratification of subjects at randomization by important known or suspected prognostic factors measured at baseline. The factors on which randomization has been stratified should be accounted for in the analysis.

Efficacy trials in infants and toddlers and in older adults may aim to recruit subjects just before the expected RSV season (where seasonality occurs) to accumulate the required number of cases for the primary analysis as quickly as possible.

It is recommended that efficacy trials that evaluate passive protection of infants born to mothers vaccinated during pregnancy should recruit women who are expected to deliver shortly before, or in the early weeks of, the RSV season. In this way, their infants can be followed for efficacy through one RSV season during which maternal antibody levels will decay. See also section C.4.2 below.
It is recommended that post-vaccination blood samples are obtained at least from a randomized subset of subjects, and preferably from all subjects, at a fixed point in time to allow for an exploration of vaccine efficacy according to immunological parameters and possible identification of an ICP.

Depending on the population(s) in which efficacy is to be evaluated, sponsors should consider the range of clinical and laboratory parameters that could be captured in addition to those to be included in the primary case definition (see section C.3.2 below). Data on additional parameters could be used to support secondary or exploratory analyses of efficacy in subgroups defined by the presence or absence of specific clinical and/or laboratory findings, including cases caused by RSV/A compared to RSV/B. In this regard, the efficacy trials will not be powered to determine efficacy against RSV/A or RSV/B and the numbers of cases due to each subtype is expected to vary depending on the regions in which trials are conducted and the seasons. Therefore, data demonstrating the ability of the vaccine to elicit broadly comparable immune responses to subtypes A and B will be important in supporting an expectation of efficacy regardless of RSV type.

C.3.1 Trial populations
C.3.1.1 Infants and toddlers
Selection criteria should include the minimum gestational age at birth and the minimum and maximum ages at the time of enrolment.

It is not expected to be feasible to determine baseline RSV serostatus prior to enrolment into efficacy trials. To allow for a retrospective analysis of vaccine efficacy according to RSV-naive and RSV-experienced status, it is recommended that baseline blood samples are obtained at least from a randomized subset of subjects and preferably from all subjects.

C.3.1.2 Pregnant women
The minimum and maximum gestational stage and the method used for estimating this should be specified in the protocol and applied across all trial sites.

Consideration should be given to information expected to be available on placental insufficiency at trial sites. If such data are expected to be widely available, protocols should state whether pregnant women with any evidence of placental insufficiency are eligible for enrolment. For example, if there are cord blood data to suggest that vaccination increases the anti-RSV neutralizing antibody transferred to the fetus despite placental insufficiency, it may be appropriate to include these women.
C.3.1.3 **Older adults**

To support use of a candidate RSV vaccine without an upper age limit, trials should aim to ensure that the trial population covers a wide age range. For example, it may be reasonable to aim for at least 25% of the total trial population to be aged > 75 years. It is recommended that exclusion criteria are kept to a minimum to ensure that subjects have a range of comorbidities.

C.3.2 **Efficacy trial end-points**

C.3.2.1 **Primary case definition**

The primary case definition should require both clinical and laboratory criteria to be met.

C.3.2.1.1 **Clinical criteria**

The primary case definition could be any RSV disease or could be confined to RSV LRTI, which could also be defined by severity.

The list of clinical signs and symptoms and the number to be met must be tailored to the age range of the trial population (for example, a definition applicable to infants would not be appropriate for older adults). Information on clinical presentations from epidemiological studies of RSV and/or from completed clinical trials may be helpful when selecting the minimum signs and symptoms to be met. For example, in an efficacy trial in infants and young children, information could be captured on respiratory rates, oxygen saturation, lower chest wall indrawing, new-onset apnoea, acute ventilatory failure and inability to feed. For objective measurements, such as respiratory rate and oxygen saturation, the actual values should be recorded at intervals during the illness.

Sponsors are advised to take account of proposals for classifying RSV disease severity in different age groups that come from well-recognized public health or professional bodies. For example, WHO has published suggested clinical criteria for defining severe RSV disease in infants and toddlers (11). Published clinical scores suitable for application to RSV disease could also be considered.

C.3.2.1.2 **Laboratory criteria**

Laboratory confirmation of a case may be based on a protocol-defined commercially available rapid diagnostic test (RDT) for RSV. These tests may be based on the amplification of RSV nucleic acid sequences – for example, RT-PCR (11, 12). It is recommended that the same RDT (for example, a NAT-based assay from a single manufacturer that can detect low levels of virus) is used at all sites if multiple testing sites are permitted for early phase studies. In pivotal clinical trials it is recommended that testing is conducted in a central laboratory using a single validated RDT (see also section C.3.3 below).
The sponsor should justify the RDT(s) chosen based on their performance characteristics (sensitivity and specificity). RDTs should be able to discriminate between RSV/A and RSV/B strains. The test method should be able to detect a low copy number (for example, < 10^3 gene copies per mL or < 50 gene copies per reaction) of the target RSV sequence. In clinical trials involving live-attenuated RSV vaccines, a NAT-based assay should have the ability to differentiate between vaccine and wild-type RSV strains. For example, some live-attenuated RSV strains used in candidate vaccines are gene-deletion mutants so that amplification of a target sequence from within the deleted gene can be used to distinguish vaccine strains from wild-type viruses.

During clinical trials, arrangements should be in place to collect samples from suspected cases as early as possible after onset of clinical features suggesting a possible RSV infection. Licensed test kits specify the type of sample to be collected and most frequently recommend the use of nasal or nasopharyngeal swab and/or nasal wash samples. Other samples such as nasal secretions (mucus), sputum, tracheal aspirates, bronchial alveolar lavage samples and postmortem lung tissues may be used for virus detection if the test method is modified and validated for this use. In most cases, collection of nasal swabs (NS), nasopharyngeal (NP) swabs or nasal wash (NW) aspirates will be acceptable to trial subjects. Whilst NS may be more sensitive in detecting RSV shedding (139), NW aspirates may be better at detecting virus when quantities are low (140–143). The protocol for sample collection should provide the details of the collection method, including issues such as type of swab (which may be very important for some assays) and swabbing site/action so that the protocol is applied consistently across all study sites and all trials in any one clinical development programme. Training of site personnel in sample collection may be required.

Negative controls (for example, collection medium blanks) should be processed and tested with clinical samples to ensure that no cross-contamination occurs. A human cellular DNA target sequence (such as GAPDH) may be used as an internal control to monitor the quality of the collected samples. Alternatively, upon thawing and prior to further processing, NS or NW samples may be spiked with a barcode-tagged RNA sequence to serve as a unique sample identifier and internal control to monitor efficiency of RNA extraction.

C.3.2.2 Secondary case definitions

Alternative case definitions should be defined as necessary for the purposes of the secondary analyses.

C.3.3 Case ascertainment

It is generally recommended that active surveillance is used to identify cases meeting the primary and other case definitions (16). The method of case ascertainment should be tailored to the geographical distribution of trial sites.
and should include instructions to subjects and caregivers on trigger signs and symptoms of possible RSV disease and on presentation to site staff and/or participating health care facilities.

Nevertheless, some trial subjects who develop RSV disease may present at health care facilities not participating in the trial, where confirmation of the diagnosis of RSV may occur using different laboratory tests to those listed in the trial protocols. It is recommended that sponsors plan prospectively for these occurrences. Every effort should be made to capture these cases and to obtain and record clinical and laboratory data from the non-participating health care facilities. The protocol and statistical analysis plan (SAP) should clarify how these cases may be included in the primary or any predefined secondary analyses (for example, it may be appropriate that only those cases for which certain data elements are available should be included in certain analyses).

C.3.4 Analysis of efficacy

If the primary analysis is based on a primary end-point defined as all RSV disease (that is, regardless of severity – see section C.3.2.1 above) then secondary analyses should be conducted based on RSV LRTI, severe RSV disease and/or other case definitions.

In infants and toddlers and in older adults, the primary analysis may be confined to RSV cases with onset after a stated minimum number of days after completion of the assigned dose(s). In such cases, it is important that a secondary analysis compares numbers of cases that occur at any time from randomization. Additionally, a secondary analysis should address the time between the last assigned dose (scheduled or completed) and the onset of disease.

In trials that evaluate protection afforded by maternal antibody, the primary analysis may be confined to infants born a minimum number of weeks after their mothers were vaccinated. If this is the case, a secondary analysis of efficacy should be conducted in all infants regardless of the time that elapsed between maternal vaccination and delivery.

Some additional considerations for population subgroups are provided below.

C.3.4.1 Infants and toddlers

In efficacy trials that enrol RSV-naive subjects it is essential that detailed information on case severity is captured so that the clinical presentations of cases that occur in vaccinated and unvaccinated cohorts can be compared (whether in the primary or secondary analyses) to assess the risk of vaccine-associated ERD (see section C.4.1 below).

There is interest in evaluating whether RSV vaccination impacts on the rate of asthma and symptomatic wheezing in children. This could be investigated
in the post-licensure period. This would require a clear definition of symptomatic wheezing (for example, including pulmonary function criteria in children old enough to undergo testing), along with long-term structured follow-up to maintain high retention of the original clinical trial population, to determine whether there is any detectable benefit, and if so its duration.

C.3.4.2 Pregnant women

Some infants born to mothers who were vaccinated during pregnancy may be eligible for routine use of an anti-RSV monoclonal antibody in line with local guidance, in which case it may be appropriate to exclude them from the primary analysis of efficacy if they have received such a monoclonal. If excluded from the primary analysis, cases of RSV disease in these subjects should be captured and included in a secondary analysis of efficacy in the all-randomized population.

C.3.4.3 Older adults

It is recommended that, unless an ICP has been established, subjects should continue to be followed for RSV disease to assess the potential need for revaccination and the intervals at which this may be required to maintain protection. One approach may be to sub-randomize subjects initially allocated to the vaccine group to receive or not receive revaccination and to follow these cohorts further for RSV cases. Data to support advice on revaccination may not be available until after first licensure and may be modified as additional data emerge.

C.4 Safety aspects

C.4.1 Infants and toddlers

Safety data (for example, on local and systemic reactogenicity) obtained from trials in RSV-experienced subjects of various ages may be poorly predictive of the safety profile in RSV-naive subjects. Therefore, a cautious approach is recommended when commencing trials that enrol RSV-naive subjects.

Historical data indicate that the potential risk of vaccine-associated ERD is highest in (and perhaps confined to) RSV-naive infants. Therefore, it is particularly important that there is a large representation in the safety database of infants known or expected (for example, from epidemiology data) to be RSV-naive.

Any vaccine-associated ERD would be expected to occur with the first natural RSV infection after completion of vaccination. Unless otherwise justified based on evidence relevant to the candidate vaccine construct, it is recommended that the duration of follow-up for RSV disease in all trials that include RSV-naive subjects should be sufficient to maximize the likelihood that
subjects will have been exposed to wild-type RSV. The duration of follow-up for RSV disease to address this issue in any one trial should be decided upon based on knowledge of the rate of natural exposure with increasing age in the region(s) in which the trial is conducted. The assessment of risk in any one trial that includes RSV-naive subjects – even if this is a preliminary evaluation conducted as part of a relatively small immunogenicity trial – should be completed before initiating the next trial(s) in which RSV-naive subjects will be exposed to the candidate vaccine.

Additional safety considerations for trials with live-attenuated RSV vaccines in RSV-naive infants include the need to assess the duration and magnitude of virus shedding. Depending on the results, consideration should be given to conducting a study to assess the risk of transmission to RSV-naive close contacts. Until the risk has been assessed and/or based on results, precautions should be put in place to minimize the risk of transmission of the vaccine virus from vaccinees during the period of virus shedding to contacts who are under 1 year of age and/or are immunocompromised.

C.4.2 Pregnant women

The threshold for determining tolerability of a vaccine during pregnancy is usually lower than that applicable to non-pregnant adults. The risk of local and systemic reactions to vaccination (including fever) should be assessed in non-pregnant women before proceeding to the vaccination of pregnant women. The rates of premature delivery, complications of pregnancy or labour and the condition of infants at birth should be compared between the vaccinated and unvaccinated groups.

Routine safety assessments of infants should be conducted for 6–12 months after birth.

As discussed in section C.3 above, trials involving maternal vaccination should follow-up infants to assess protection against RSV disease through one season (or the equivalent period in non-seasonal regions). The data collected on the severity of cases of RSV disease should be reviewed to assess whether there is any signal for vaccine-associated ERD in infants born to vaccinated mothers compared to those born to unvaccinated mothers.

C.4.3 Older adults

The tolerability of a vaccine may differ between subgroups of older persons by age subgroup and degree of frailty. Therefore, it is important that safety data are obtained from all age subgroups that are to be included in the target population for routine use. If post-licensure data indicate that revaccination at intervals may be required then the safety profile of repeated dosing should be documented (see section C.2.2.3 above).
Part D. Guidelines for NRAs

D.1 General
The general recommendations for control laboratories given in the WHO Guidelines for national authorities on quality assurance for biological products (144) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (145) should apply after the vaccine product has been granted a marketing authorization. These recommendations specify that no new biological substance should be released until consistency of batch manufacturing and quality has been established and demonstrated. The recommendations do not apply to material for clinical trials.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of the RSV vaccines, should be discussed with and approved by the NRA.

The NRA may obtain the product-specific working reference from the manufacturer to be used for lot release until the international or national standard preparation is established.

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

D.2 Release and certification
A vaccine lot should be released to the market only if it fulfils all national requirements and/or satisfies Part A of these WHO Guidelines (145). A summary protocol for the manufacturing and control of RSV vaccines, based on the model summary protocol provided in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A Lot Release Certificate signed by the appropriate NRA official should then be provided if requested by a manufacturing establishment, and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these WHO Guidelines. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccine. A model NRA Lot Release Certificate is provided below in Appendix 2.
Authors and acknowledgements

The first draft of these WHO Guidelines was prepared by Dr J. Beeler, United States Food and Drug Administration, the USA; Dr E. Kretzschmar, Paul-Ehrlich-Institut, Germany; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr Y. Sun, Paul-Ehrlich-Institut, Germany and Dr T.Q. Zhou, World Health Organization, Switzerland. This draft document was then reviewed at a WHO informal consultation on the development of WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines, held in Geneva, Switzerland, 18–19 September 2018 and attended by: Dr J. Beeler, United States Food and Drug Administration, the USA; Dr O.G. Engelhardt, National Institute for Biological Standards and Control, the United Kingdom; Dr B.S. Graham, National Institutes of Health, the USA; Dr D. Higgins, PATH, the USA; Dr B.L. Innis, PATH, the USA; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr E. Kretzschmar, Paul-Ehrlich-Institut, Germany; Dr C. Li, National Institutes for Food and Drug Control, China; Dr M. Nunes, University of Witwatersrand, South Africa and Chris Hani Baragwanath Hospital, South Africa; Dr P.A. Piedra, Baylor College of Medicine, the USA; Dr R. Pless, Health Canada, Canada; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr T. Ruckwardt, National Institutes of Health, the USA; Mr B.K. Samantaray, Central Drugs Standard Control Organization, India; Dr K. Shirato, National Institute of Infectious Diseases, Japan; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr N. Wairagkar, Bill & Melinda Gates Foundation, the USA; and Dr M. Alali, Dr D. Feikin, Dr S. Hirve, Dr I. Knezevic, Dr O.C. Lapujade, Dr E. Sparrow and Dr T.Q. Zhou, World Health Organization, Switzerland; representatives of the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA): Dr I. Dieussaert, GSK Vaccines, Belgium; Dr P. Dormitzer, Pfizer Vaccines, the USA; Dr D. Guris, Merck & Co. Inc., the USA; Dr S.S.S. Sesay, Sanofi Pasteur, France; Dr F. Takeshita, Daiichi Sankyo Co., Ltd, Japan; and Dr M. Widjojoatmodjo, Janssen Vaccines & Prevention B.V., Netherlands; representatives of the Developing Countries Vaccine Manufacturers Network (DCVMN): Ms C. Ma, Lanzhou Institute of Biological Products Co., Ltd, China and Dr K. Wu, BravoVax, China; and representatives of other industries: Dr A.B. Fix and Dr L. Fries, Novavax, the USA; and Dr C. Heery, Bavarian Nordic, Inc., the USA. Following the WHO consultation, comments were also received during September and October 2018 from: Dr M. Alali, World Health Organization, Switzerland; Dr I. Dieussaert, GSK Vaccines, Belgium; Dr D. Feikin, World Health Organization, Switzerland; Dr L. Fries, Novavax, the USA; Dr T. Fumihiko, Daiichisankyo, Japan; Dr B.S. Graham and Dr T. Ruckwardt, National Institutes of Health, the USA; Dr D. Guris, Merck & Co., Inc., USA; Dr D. Higgins and
Dr B. Innis, PATH, the USA; Dr W.V. Molle, Sciensano, Belgium; Dr S.S.S. Sesay, Sanofi Pasteur, France; Dr E. Sparrow, World Health Organization, Switzerland; and Dr M. Widjojoatmodjo, Janssen Vaccines & Prevention B.V., Netherlands.

Taking into consideration the comments received, a second draft was prepared by Dr J. Beeler, United States Food and Drug Administration, the USA, Dr E. Kretzschmar, Paul-Ehrlich-Institut, Germany, Dr M. Powell, Health Products Regulatory Authority, Ireland, Dr Y. Sun, Paul-Ehrlich-Institut, Germany and Dr T.Q. Zhou, World Health Organization, Switzerland. The second draft was posted on the WHO Biologicals website for a first round of public consultation from 22 January to 28 February 2019 and comments were received from: Dr M.C. Annequin, Agence nationale de sécurité du médicament et des produits de santé, France; Dr P. Dormitzer, Pfizer Vaccines, the USA; Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Dr D. Feikin, World Health Organization, Switzerland; Dr C. Heery, Bavarian Nordic, Inc., the USA; Dr D. Higgins, PATH, the USA; Dr N. Hosken, PATH, the USA; Dr J.L. Hurwitz, St. Jude Children’s Research Hospital, the USA; Dr D. Kapse, Serum Institute of India Pvt. Ltd, India; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr C. Li, National Institutes for Food and Drug Control, China; Dr D.L. Skea, Sanofi Pasteur, France (on behalf of IFPMA); Dr K. Smits, Federal Agency for Medicines and Health Products, Belgium; Dr W. Van Molle, Sciensano, Belgium; and Dr L.R. Yeolekar, Serum Institute of India Pvt. Ltd, India.

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Based on the outcomes of the above informal consultation, a fourth draft was prepared by Dr J. Beeler, United States Food and Drug Administration, the USA, Dr E. Kretzschmar, Paul-Ehrlich-Institut, Germany, Dr M. Powell, Health Products Regulatory Authority, Ireland, Dr Y. Sun, Paul-Ehrlich-Institut, Germany and Dr T.Q. Zhou, World Health Organization, Switzerland. The resulting document WHO/BS/2019.2355 was then posted on the WHO Biologicals website for a second round of public consultation from June to September 2019 and written comments received from: Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Dr D. Higgins, PATH, the USA; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr J. Southern, Advisor to the South African Health Products Regulatory Authority; Dr W. Van Molle, Sciensano, Belgium; Dr L. Yeolekar, Serum Institute of India Pvt. Ltd, India; and Dr W. Weston (on behalf of IFPMA provided the consolidated comments of Daiichi Sankyo, Japan; GSK Vaccines, Belgium; Janssen Vaccines & Prevention B.V., Netherlands; Pfizer Vaccines, the USA; and Sanofi Pasteur, France). Special input was also sought on scientific issues from: Dr B.S. Graham, National Institutes of Health, the USA; and Dr R.A. Karron, Johns Hopkins University, the USA.

In response to the comments received during the second round of public consultation, a number of proposed amendments were presented by Dr J. Beeler, United States Food and Drug Administration, the USA; Dr E. Kretzschmar, Paul-Ehrlich-Institut, Germany; Dr M. Powell, Health Products Regulatory Authority, Ireland; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; and Dr T.Q. Zhou, World Health Organization, Switzerland to the WHO Expert Committee on Biological Standardization which made further changes to document WHO/BS/2019.2355.

References


Appendix 1

Model summary protocol for the manufacturing and control of RSV vaccines

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

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

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

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

- International name:
- Trade name/commercial name:
- Product licence (marketing authorization) number:
- Country:
- Name and address of manufacturer:
- Name and address of product licence-holder, if different:
- Vector(s) (if applicable):
- Virus strain(s) (if applicable):
- Batch number(s):


- Type of container:
- Number of filled containers in this final lot:
- Number of doses per container:
- Preservative and nominal concentration (if applicable):
- Summary of the composition (summary of qualitative and quantitative composition of the vaccine, including any adjuvant and other excipients):
- Target group:
- Shelf-life approved (months):
- Expiry date:
- Storage conditions:

2. Control of source material
2.1 Virus and viral/bacterial vector seeds (where applicable)
2.1.1 Seed banking system

- Name and identification of virus or viral/bacterial vector:
- Origin of all genetic components (if applicable):
- Construction of virus or viral/bacterial vector:
- Nucleotide sequence of the transgene and flanking regions:
- Antigenic analysis, infectivity titre, yield (in vitro/in vivo):
- Comparison of genetic and phenotypic properties with parental vector:
- Seed bank genealogy with dates of preparation, passage number and date of coming into operation:
- Tests performed for detection of adventitious agents at all stages of development:
- Tests for bacteria, fungi, mycoplasma, mycobacteria (for virus and viral vector seeds):
- Virus titration for infectivity (for live-attenuated RSV vaccines):
- Freedom from TSE agents:
- Details of animal or human components of any reagents used in the manufacture of seed banks, including culture medium:
- Genetic stability at the level of a virus/bacterial pre-master seed or viral/bacterial master seed to its sequence at, or preferably beyond, the anticipated maximum passage level:
• Confirmation of approval for use by manufacturer, and the basis for that approval:

2.1.2 Tests on working seed lot production (for chimeric BCG/RSV-vaccines)

• Antimicrobial sensitivity:
• Delayed hypersensitivity (if applicable):
• Identity:
• Bacterial and fungal contamination:
• Absence of virulent mycobacteria:
• Excessive dermal reactivity (if applicable):

2.2 Cell cultures (where applicable)

2.2.1 Cell banking system

• Name and identification of cell substrate:
• Origin and history of cell substrate:
• Details of any manipulations (including genetic manipulations) performed on the parental cell line in the preparation of the production cell line:
• Cell bank genealogy with dates of preparation, passage number and date of coming into operation:
• Confirmation of approval for use by manufacturer, and the basis for that approval:
• Tests performed for detection of adventitious agents at all stages of development:
• Test for absence of bacterial and fungal contamination (if of yeast and bacterial origin):
• Sterility test (bacteria, fungi, mycoplasmas, virus):
• Details of animal or human components of any reagents used in manufacture of cell banks, including culture medium:
• Freedom from TSE agents:
• Genetic stability (if genetically manipulated):

2.2.2 Primary cells (if generated)

• Source of animals and veterinary control (for example, specify if animals or eggs are sourced from closed, pathogen-free colonies):
3. Control of vaccine production

3.1 Control of production cell cultures/control cells (where applicable)

3.1.1 Information on preparation

- Lot number of master cell bank:
- Lot number of working cell bank:
- Date of thawing ampoule of working cell bank:
- Passage number of production cells:
- Date of preparation of control cell cultures:
- Result of microscopic examination:

3.1.2 Tests on cell cultures or control cells

- Identity:
- Haemadsorbing viruses:
- Adventitious agents:
- Sterility (bacteria, fungi, mycoplasmas):

3.2 Control of purified antigen bulk (where applicable)

- Identity:
- Purity:
- Protein content:
- Antigen content:
- Sterility (bacteria and fungi):
- Percentage of intact RSV antigens:
- Nanoparticle size and structure:
- Reagents during production of other phases of manufacture:
■ Residual DNA derived from the expression system (if applicable):
■ Residual bovine serum antigen content:
■ Viral clearance (during manufacturing development):

3.3 **Control of adsorbed antigen bulk (where applicable)**

■ Lot number of adsorbed antigen bulk:
■ Date of adsorption:
■ Volume, storage temperature, storage time and approved storage period:
■ Sterility (bacteria and fungi)
■ Bacterial endotoxin:
■ Identity:
■ Adjuvant:
■ Degree of adsorption:
■ pH:
■ Antigen content:

3.4 **Control of virus and viral/bacterial vector harvests or pooled harvests (where applicable)**

3.4.1 **Information on manufacture**

■ Batch number(s):
■ Date of inoculation:
■ Date of harvesting:
■ Lot number of virus/bacterial master seed lot:
■ Lot number of virus/bacterial working seed lot:
■ Passage level from virus/bacterial working seed lot:
■ Methods, date of purification if relevant:
■ Volume(s), storage temperature, storage time and approved storage period:

3.4.2 **Tests**

■ Identity:
■ Sterility (bacteria, fungi, mycoplasmas and mycobacteria) (if applicable):  
■ Adventitious virus tests:
■ Bacteria/fungi/mycoplasmas (for recombinant BCG/RSV vaccines):
- Virus titration for infectivity (if applicable):
- Residual bovine serum albumin (if applicable):
- Tests for consistency of virus characteristics (if applicable):
- Determination of attenuation (if appropriate):

3.5 Control of monovalent vaccine bulk (where applicable)

3.5.1 Information on manufacture

- Batch number(s):
- Date of formulation:
- Total volume of monovalent bulk prepared:
- Virus/bacteria harvest used for formulation:
- Lot number/volume added:
- Virus/bacteria concentration:
- Name and concentration of added substances (for example, diluent, stabilizer if relevant):
- Volume(s), storage temperature, storage time and approved storage period:

3.5.2 Tests

- Identity:
- Purity (if applicable):
- Residual host cell protein (if not done on final bulk or final product):
- Residual host cell DNA (if non-primary cell lines; if not done on final bulk or final product):
- Potency:
- Particle number (if relevant, for example for adenovirus):
- Infectious virus titre and particle-to-infectivity ratio (if relevant, for example for adenovirus):
- Expression of heterologous antigen in vitro:
- Replication competence (if relevant, for example for adenovirus):
- pH:
- Preservative content (if applicable):
- Endotoxin:
- Sterility or bioburden:
3.6 Control of final vaccine bulk (where applicable)

3.6.1 Information on manufacture

- Batch number(s):
- Date of formulation:
- Total volume of final bulk formulated:
- Monovalent bulk vaccine used for formulation:
- Volume(s), storage temperature, storage time and approved storage period:
- Lot number/volume added:
- Virus/bacteria concentration:
- Name and concentration of added substances (for example, diluent, stabilizer if relevant):

3.6.2 Tests on virus or viral vector bulk

- Identity (if applicable):
- Sterility or bioburden (if applicable):
- Concentration of antimicrobial agent, if relevant:
- Total protein:
- Residual DNA (for cell-culture vaccine):
- Ovalbumin (for egg-based vaccine):

3.6.3 Tests on bacterial vector bulk

- Bacterial and fungal contamination:
- Absence of virulent mycobacteria (if not performed on final lot):
- Bacterial concentration:
- Number of culturable particles:

4. Filling and containers

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

5. Control tests on final vaccine lot

- Inspection of final containers:
- Identity:
- Appearance:
- pH (if applicable):
- Osmolality (if applicable):
- Sterility (if applicable):
- Bacterial and fungal contamination (for chimeric BCG/RSV vaccines):
- Preservative (if applicable):
- Residual moisture content (for freeze-dried product):
- Pyrogenic substances (if applicable):
- Adjuvant content (if applicable):
- Protein content (if applicable):
- Degree of adsorption (if applicable)
- Potency:
- Particle number (if relevant, for example for adenovirus):
- Infectious virus titre and particle-to-infectivity ratio (if relevant, for example for adenovirus):
- Expression of heterologous antigen in vitro:
- Purity (if applicable):
- Bacterial concentration (for chimeric BCG/RSV vaccines):
- Tests for viability (for chimeric BCG/RSV vaccines):
- Extractable volume (if applicable):
- Aggregates/particle size (if applicable)
Viability (for chimeric BCG/RSV vaccines)
Thermal stability test (if applicable):
Residual antibiotics (if relevant):
Diluent (if applicable):
Safety (for chimeric BCG/RSV vaccines):

6. Certification by the manufacturer

Name of Head of Production (typed) __________________________

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

I certify that lot no. __________________________ of RSV vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A\(^1\) of the WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines.\(^2\)

Name (typed) __________________________
Signature __________________________
Date __________________________

7. Certification by the NRA

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

\(^1\) With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

Appendix 2

Model NRA Lot Release Certificate for RSV vaccines

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

Certificate no. _______________________

The following lot(s) of RSV vaccine produced by ________________________\(^1\) in ________________________,\(^2\) whose lot numbers appear on the labels of the final containers, meet all national requirements\(^3\) and Part A\(^4\) of the WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines\(^5\) and comply with WHO good manufacturing practices for pharmaceutical products: main principles,\(^6\) WHO good manufacturing practices for biological products,\(^7\) and Guidelines for independent lot release of vaccines by regulatory authorities.\(^8\)

The release decision is based on ________________________\(^9\)

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container used;

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1. Name of manufacturer.
2. Country of origin.
3. If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.
4. With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
9. Evaluation of the product-specific summary protocol, independent laboratory testing and/or specific procedures laid down in a defined document, and so on as appropriate.
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other appropriate authority)

Name (typed) _______________________________________________
Signature _______________________________________________
Date _______________________________________________
Annex 3

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

Amendment to Annex 3 of WHO Technical Report Series, No. 993

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Introduction

At its sixty-fifth meeting in 2014, the WHO Expert Committee on Biological Standardization adopted the current WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1). These WHO Recommendations provide guidance on the manufacture and control, nonclinical and clinical evaluation, and lot release of inactivated poliomyelitis vaccines (IPV). The Recommendations include guidance on the use of several assays requiring the handling of live poliovirus and were produced at a time when only limited data and experience with Sabin-based IPV (sIPV) were available, and when no specific biocontainment requirements for IPV manufacturing had been adopted. Since then, the third revision of the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) (2) was adopted by the World Health Assembly in 2015 to provide guidance on the implementation of biosafety and biosecurity measures at facilities handling poliovirus in the post-eradication era. In addition, WHO Guidelines for the safe production and quality control of poliomyelitis vaccines (3) were adopted by the Committee in 2018. These Guidelines further align the biocontainment requirements for the production of both sIPV and IPV derived from wild-type strains (wIPV) with good manufacturing practices (GMP) and GAPIII requirements. Currently, a step-wise implementation of GAPIII is taking place according to the WHO GAPIII Containment Certification Scheme (4). As few laboratories have the high-containment facilities required by GAPIII, global capacity for performing assays using live poliovirus, particularly type 2, is limited.

Given the urgent need for sIPV in order to increase global IPV supply, as well as recent progress in sIPV development, the Committee at its meeting in 2018 also recommended that the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1) be amended accordingly. The amendments provided in the current document comprise:

- modified definitions of “virus sub-master seed lot” and “virus working seed lot” to cover all poliovirus strains;
- updated information on the WHO international standards now available to support quality control testing;
- modified requirements for confirming the genetic stability of attenuated vaccine seeds and monovalent virus pools to provide flexibility for vaccine developers;

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11 In the current document the use of the abbreviation “IPV” refers to IPV derived from any strain. “wIPV” refers to IPV derived from wild-type strains only and “sIPV” refers to IPV derived from Sabin strains only.
- inclusion of additional cell substrates that can be used for the effective-inactivation test based on published studies (5);
- deletion of the general safety (innocuity) test in line with the decision made by the Committee in 2018 to discontinue the inclusion of this test in all WHO Recommendations, Guidelines and other guidance documents for biological products published in the WHO Technical Report Series (6);
- updated recommendations for the evaluation of sIPV immunogenicity in nonclinical and clinical studies to provide much needed flexibility, and thus facilitate the development and licensure of new vaccines.

No attempt was made at this time to review the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1) in their entirety and only the above issues have been addressed.

Amendments

Replace the following two paragraphs which appear under “General considerations” in the current WHO Recommendations (pages 95–96) with the replacement text shown below:

Original text to be replaced

Given these uncertainties, assurance is required in relation to the characteristics of the live-attenuated Sabin virus before inactivation in order to justify the implementation of containment measures that may be different from those required for wIPV production (18). Production conditions should be validated by the full range of tests including in vivo and in vitro testing of the master seed and working seed and successive monovalent bulks (with the number to be approved by the NRA) to ensure that the attenuated phenotype of the Sabin strains in monovalent pools is maintained. Subsequently, a limited range of tests, such as mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency. The number of pools of each type tested each year should be justified and agreed by the NRA. Furthermore, it is important that, at intervals to be agreed with the NRA, pools should be tested with the full range of tests to ensure that production conditions remain satisfactory.

In addition to the Sabin strains that are used in the manufacture of OPV, alternative attenuation methods utilizing recombinant DNA technology are being investigated (25–29). Strains derived by such a methodology may have properties specifically designed to be suitable for the safe production of vaccine (for example,
the inability to replicate in the human gut). Such strains should be considered as they become available and may require specific characterization. Biocontainment requirements for such strains will need to be determined on a case-by-case basis. Only virus strains that are approved by the NRA should be used.

Replacement text

Once the circulation of wild-type polioviruses has been stopped, vaccine-manufacturing establishments will remain a major source of risk for the potential release of virulent polioviruses back into populations. Therefore, enhanced containment measures must be implemented as described in the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) (2) and in the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines (3). According to GAPIII, the required containment conditions for wild and Sabin strains are effectively identical. However, it is still important to prevent the incorporation of mutations during the vaccine production process to ensure that production consistency is achieved. It is known that upon cultivation under inappropriate conditions (for example, at higher temperature or lower multiplicity of infection) Sabin strains can undergo molecular changes resulting in reversion to virulence. Therefore, in order to minimize the likelihood of such changes, the Sabin strains must be propagated under defined and well-controlled conditions – for example, the conditions used to manufacture OPV (7).

Additional tests should also be performed on seed viruses and validation lots to confirm production consistency. This can be achieved by comparing the consensus nucleotide sequences of monovalent pools (or viral harvests) with those of the starting seed viruses and confirming that no mutations occurred during virus growth that changed the consensus sequence. Any viral preparation includes molecules that contain mutations that are present in only a small fraction of the entire population. Consensus sequences represent nucleotides that are found in the majority of molecules. Confirming that the consensus sequences of monovalent pools or viral harvests are the same as that of the starting seed virus is sufficient to demonstrate the genetic stability of the virus during production. The seed viruses used by some manufacturers may be different from the original WHO Sabin seeds12 and may have consensus sequences that differ by several nucleotides. Alternatively, instead of nucleotide sequencing analysis, the maintenance of attenuation could be confirmed by performing in vivo tests (monkey or transgenic mouse neurovirulence tests) as described in the WHO

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12 NCBI nucleotide sequence accession numbers for WHO Sabin seed materials are AY184219, AY184220 and AY184221, for type 1, 2 and 3 polioviruses respectively.
Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7). Subsequently, these additional tests may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency. The number of pools of each serotype tested each year should be justified and agreed to by the NRA.

In addition to Sabin strains, alternative attenuation methods utilizing recombinant DNA technology are being investigated (8-12). Strains derived by such a methodology may have properties specifically designed to be suitable for the safe production of vaccine (for example, the inability to replicate in the human gut and/or significantly reduced likelihood of reverting to virulence). Such strains should be considered as they become available and may require extensive characterization. Biocontainment requirements for such strains and the necessary in-process tests will be determined individually, for each new candidate strain, based on scientific evidence (3). Recommendations on the appropriate level of containment for such strains will be made by the WHO Containment Advisory Group (CAG), after reviewing detailed information on their biological characteristics (3, 4). For example, CAG has previously determined (13) that:

... sufficient data has been provided to conclude the series of S19-poliovirus strains (S19 with capsid region, P1 of wild-type and Sabin vaccine strain polioviruses of all serotypes) and the parallel series of viruses with the substitution of an asparagine by a serine at amino acid 18 in the non-structural protein 2A to allow better growth in Vero cells could be considered for use, outside of the containment requirements of Annex 2 or Annex 3 of GAPIII, as applicable for IPV production, rat neutralization IPV potency assays, human serum neutralization test for poliovirus antibody determination and potency testing for immunoglobulin (human) lot control and release.

As indicated above, there are S19 versions containing Sabin capsid sequences and S19 versions containing wild-type capsid sequences (S19-Sabin and S19-wild respectively) for the three poliovirus serotypes. The immunological properties of S19-Sabin and S19-wild are identical to the respective Sabin and wild strains. Therefore they can be used instead of Sabin and wild strains in the tests recommended in this current document (3, 13).

Replace the definition of “Virus sub-master seed lot” in the “Terminology” section with the following text:

Virus sub-master seed lot: a quantity of virus suspension produced by a single passage from the virus master seed and made at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate time frame,
and that has been processed at the same time to ensure its uniform composition. The virus sub-master seed lot should be characterized to the extent necessary to support the development of the **virus working seed lot**. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.1).

**Replace the definition of “Virus working seed lot” in the “Terminology” section with the following text:**

**Virus working seed lot**: a quantity of virus of uniform composition derived from the virus master seed lot or virus sub-master seed lot made at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate time frame and used at a passage level approved by the NRA for the manufacturing of vaccine.

**Replace section A.1.3 “International reference materials” with the following text:**

### A.1.3 International reference materials

A WHO International Standard is available for use in in vitro assays to measure the D-antigen content of wIPV. However, several studies have revealed differences in the reactivity of antibody reagents used in various ELISA methods used by manufacturers and control laboratories to measure the potency of wIPV and sIPV products. This has resulted in high inter-laboratory variability in the potency results obtained for IPV products when using a heterologous reference – that is, a wild-type reference for sIPV or a Sabin reference for wIPV. For this reason, a new WHO International Standard specific to sIPV products was established in 2018 and a new sIPV antigen unit – the Sabin D-Antigen Unit (SDU) – was defined (14). This new unit is independent of the D-antigen unit (DU) used to express the potency of wIPV. Both wIPV and sIPV International Standards are intended for use in calibrating secondary reference preparations of IPV, which are then used in potency tests to calculate the D-antigen content of IPV products. The D-antigen content of wIPV and sIPV should be expressed in DU or SDU respectively. However, manufacturers of existing sIPV products, including those already licensed and those in late-stage development, that use potency values expressed in DU measured against their internal standards can continue to use these values with the approval of the NRA. It is recommended that these manufacturers also determine the potency of their sIPV products in SDU using the sIPV International Standard, and establish the correlation between “SDU” and “DU”. This can serve as a useful quality characteristic to ensure product comparability.
At present there is no standardized ELISA assay available as a resource for laboratories, and efforts are under way to develop standardized reagents and protocols. International standards and reference reagents for the control of in vivo potency assays in rats are also currently being evaluated.

An International Reference Preparation (IRP) of poliomyelitis vaccine (inactivated) was established by the WHO Expert Committee on Biological Standardization in 1962 (15). This preparation was a trivalent blend prepared in 1959 in primary monkey kidney cells from type-1 (Mahoney), type-2 (MEF) and type-3 (Saukett) strains of poliovirus.

After preparation of the IRP, significant advances in the production and control of IPV occurred and vaccines of increased potency and purity were developed. An enhanced potency IPV (PU78-02) from the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) was widely used as a reference preparation for control purposes. When stocks of this reagent were almost exhausted, a new reference material (91/574) was established by the WHO Expert Committee on Biological Standardization in 1994 as the second WHO International Reference Reagent for in vivo and in vitro assays of IPV (16). Potencies of 430, 95 and 285 DU/mL were assigned, respectively, to poliovirus types 1, 2 and 3 of this preparation. A separate aliquot of the preparation, established by the European Pharmacopoeia Commission as the Biological Reference Preparation (BRP) batch 1, has an identical assigned titre (17). Material from a concentrated trivalent bulk from a commercially available IPV vaccine was established as the BRP batch 2 in 2003, with assigned potencies of 320, 67 and 282 DU/mL for types 1, 2 and 3 respectively (18). Following inconsistency in the performance of some vials of 91/574, the use of this reference was discontinued in 2010. In 2013, the Third WHO International Standard for inactivated poliomyelitis vaccine (12/104) was established by the WHO Expert Committee on Biological Standardization using BRP batch 2 as the reference in the study. Potencies of 277, 65 and 248 DU/mL were assigned to poliovirus types 1, 2 and 3 respectively (19).

A collaborative study conducted in 2015–2016 found the International Standard for conventional IPV (12/104) unsuitable for measuring the antigen potency of sIPV. A relatively high proportion of invalid assays and large differences between laboratory potency results were observed when using 12/104 as a reference to measure the potency of sIPV products. Assay validity and inter-laboratory variability improved when an sIPV sample was used as a reference to determine the potency of sIPV study samples. The decision was made to establish a new WHO International Standard specific to sIPV products. The First WHO International Standard for Sabin inactivated poliomyelitis vaccine (17/160) was established by the WHO Expert Committee on Biological Standardization in 2018.
A unitage of 100 SDU/mL was assigned to each of the three poliovirus serotypes (14). The value 100 is an arbitrary unit.

There are still gaps in the scientific knowledge required for the further standardization of IPV products. Some differences have been noted in the antigenic profile of different IPV products, highlighting the importance of product-specific assessment of future IPV products, particularly sIPV products, against current international standards (20–24).

A WHO International Standard for anti-poliovirus sera, types 1, 2 and 3 is available for the standardization of neutralizing antibody tests for poliovirus (25).

The First WHO International Standards for anti-poliovirus sera of types 1, 2 and 3 were established by the WHO Expert Committee on Biological Standardization in 1963 from serotype-specific polyclonal antisera produced by the hyper-immunization of rhesus monkeys with live virus suspensions. Each of the standards was specific to one serotype only. They were established through a collaborative study (26) and assigned a unitage of 10 IU/ampoule for each of the three serotypes.

In around 1989, stocks of these international standards ran very low (especially for type 3) and a replacement was selected following a collaborative study (27). This Second WHO International Standard for anti-poliovirus sera types 1, 2 and 3 (66/202) was established by the WHO Expert Committee on Biological Standardization in 1991 (28). In contrast to the previous standards, the replacement standard was a single serum that exhibited activity against each of the three poliovirus serotypes. The following unitages were assigned: 25 IU of anti-poliovirus serum (type 1) human; 50 IU of anti-poliovirus serum (type 2) human; and 5 IU of anti-poliovirus serum (type 3) human. Following the exhaustion of stocks of 66/202, the Third WHO International Standard for anti-poliovirus sera, types 1, 2 and 3 (82/585) was established by the WHO Expert Committee on Biological Standardization in 2006 with assigned unitages of 11, 32 and 3 IU/vial of neutralizing antibody to types 1, 2 and 3 polioviruses respectively (25).

All of the above WHO international standards are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom. For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations (29) should be consulted.
Replace section A.3.1.3.3 “Additional tests on seeds from Sabin strains and other attenuated strains derived by recombinant DNA technology” with the following text:

A.3.1.3.3 Additional tests on seeds from Sabin strains and other attenuated strains derived by recombinant DNA technology

If live-attenuated Sabin strains are used for vaccine production, established master seeds validated for OPV production should be used as starting materials. A list of seeds used for OPV production is provided in Appendix 1 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7). In order to maintain production consistency, the conditions used for virus seed propagation – including the temperature and duration of incubation, the multiplicity of infection, and so on – should be well defined and controlled. The conditions used to manufacture OPV described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) are suitable for sIPV production. In addition, tests should be performed to ensure that viruses grown in cell culture do not undergo major genetic changes. Complete consensus nucleotide sequences of Sabin virus master seed and working seed used in the manufacture of IPV should be determined and demonstrated to be identical, as detailed in General considerations above. Alternatively, instead of nucleotide sequence analysis, other in vitro tests (MAPREC or deep sequencing) or in vivo tests (monkey or transgenic mouse neurovirulence tests) could be used to confirm genetic stability, as described in sections A.3.2.4 and A.3.2.5 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) and below in section A.4.4.2.7 of this amendment document. The specifications for these tests must be established in agreement with the NRA.

Suitable in vitro tests should be performed on the master seed produced from attenuated strains derived by recombinant DNA technology. Such tests may include full genome characterization by determining consensus nucleotide sequences, or deep sequencing techniques and demonstration of genetic or phenotypic stability on passage under production conditions. Tests should be validated for this purpose using appropriate standards and materials, and should be approved by the NRA. The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology using in vivo neurovirulence tests should be considered and scientifically justified, in agreement with the NRA.

Any new virus working seed derived from an established master seed, including Sabin strains and other attenuated strains derived by recombinant DNA technology, along with at least three consecutive purified monovalent pools, should be analysed to monitor virus molecular characteristics – for example, through nucleotide sequence analysis (see section A.4.4.2.7.1 of this amendment document).
Replace section A.4.4.2.7 “Additional tests for purified monovalent pools produced from Sabin vaccine seeds or from other attenuated seeds derived by recombinant DNA technology” with the following text:

A.4.4.2.7 Additional tests for purified monovalent pools produced from Sabin vaccine seeds or from other attenuated seeds derived by recombinant DNA technology

Production conditions – particularly Sabin virus growth conditions (for example, the temperature and duration of incubation, the multiplicity of infection, and so on) – should be well defined and controlled in order to maintain the genetic stability of the virus. The conditions used to manufacture OPV described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) are suitable for this purpose. The production conditions should be validated by testing a sufficient number of consecutive purified monovalent virus pools using suitable methods (with the number of pools used and tests selected to be approved by the NRA) to confirm the maintenance of consistent properties. Such tests may be applied to a proportion of the monovalent virus pools produced each year in order to ensure production consistency. The number of pools of each serotype tested each year should be justified and should be agreed with the NRA.

Suitable tests for Sabin strains include nucleotide sequencing to demonstrate that there is no difference between the complete consensus nucleotide sequences of the seed virus and purified monovalent pools, as detailed in General considerations above. Alternatively, instead of nucleotide sequence analysis, the tests described in section A.3.2.4 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) could be used to confirm the stability of the attenuated properties of the virus and to monitor the consistency of virus molecular characteristics. Suitable tests include the in vitro and in vivo tests described in section A.4.4.2.7.1 of this amendment document and in section A.4.4.2.7.2 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1).

The use of the rct40 test to confirm attenuated phenotype is discouraged as it is insufficiently sensitive.

Suitable tests should be performed on purified monovalent pools produced from attenuated strains derived by recombinant DNA technology. Tests may include full genome characterization by nucleotide sequencing or deep sequencing techniques. Such tests should be validated for this purpose by the use of appropriate standards and materials, and should be approved by the NRA.
Replace the following opening paragraph of section A.4.4.2.7.1 “Tests to monitor virus molecular characteristics (consistency)” with the replacement text shown below:

Original text to be replaced

In vitro tests such as MAPREC, which are used to determine the molecular consistency of production of monovalent pools, should meet the specifications for the test used (45).

Replacement text

Characterization of the molecular properties of Sabin virus and strains derived by recombinant DNA technology grown for IPV production may provide an additional tool for monitoring consistency of manufacture. If IPV manufacturers choose to implement this approach, the consistency of the molecular characteristics of Sabin virus and strains derived by recombinant DNA technology can be monitored by determining the proportion of mutations present at specific nucleotide positions (mutational profiles) using deep sequencing or MAPREC as described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7). The specifications for interpreting these tests should be established and approved by the NRA.

Replace the following opening paragraph of section A.4.5.2 “Test for effective inactivation” with the replacement text shown below:

Original text to be replaced

Two samples should be taken of a volume equivalent to at least 1500 human doses of each inactivated purified monovalent pool. One sample should be taken at the end of the inactivation period and the other not later than three quarters of the way through this period. After removal or neutralization of the inactivating agent, the samples should be tested for the absence of infective poliovirus by inoculation into tissue cultures. Kidney cells from some monkey species, such as those of the genera Macaca, Cercopithecus and Papio sp., appear to be more sensitive than others. If other tissue culture systems, including continuous cell lines (for example, L20B), are used, they should have been shown to possess at least the same sensitivity to poliovirus as those specified above by inoculating with partially formalin-inactivated virus (as opposed to infectious, untreated virus) as formalin treatment changes the biological properties of poliovirus (see below). When primary monkey kidney cells are used for this test, the two samples should be inoculated into culture vessels of tissue cultures derived from different batches of cells.

Replacement text
Replacement text

Two samples should be taken of a volume equivalent to at least 1500 human doses of each inactivated purified monovalent pool. One sample should be taken at the end of the inactivation period and the other not later than three quarters of the way through this period. After removal or neutralization of the inactivating agent, the samples should be tested for the absence of infective poliovirus by inoculation into tissue cultures. Kidney cells from some monkey species, such as those of the genera *Macaca*, *Cercopithecus* and *Papio* spp. – as well as mouse L20B cells expressing human poliovirus receptor, and HEp-2 (Cincinnati) cells – all appear to possess adequate sensitivity. If other tissue culture systems are used, they should have been shown to possess at least the same sensitivity to poliovirus as those specified above by inoculating with partially formalin-inactivated virus (as opposed to infectious, untreated virus) as formalin treatment changes the biological properties of poliovirus (see below). When primary monkey kidney cells are used for this test, the two samples should be inoculated into culture vessels of tissue cultures derived from different batches of cells.

Delete section A.6.4 “General safety test (innocuity)” – see section 3.1.3 in WHO Technical Report Series No. 1016 (6)

Replace section B.4 “Evaluation of immunogenicity in animal models” with the following text:

B.4 Evaluation of immunogenicity in animal models

Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in suitable animal models (for example, rats). Proof-of-concept nonclinical studies should include the comparison of immunogenicity between a candidate IPV and a licensed IPV based on serotype-specific serum neutralizing antibody titres against suitable poliovirus strains. These studies may also assist in the selection of D-antigen content to be tested in the dose-finding studies in humans. However, it is important to note that immunogenicity data in animals do not reliably predict the antigen content that might be appropriate for inclusion as a single human dose in the final vaccine formulation. An assay using transgenic mice may be performed to compare the immune response and protection against virulent challenge induced by a candidate IPV to that induced by a licensed IPV (30, 31). In vivo tests are also important tools to be used as characterization tests to demonstrate comparable manufacturing processes when major changes are introduced.

In view of antigenic differences between different poliovirus strains of the same serotype and limited clinical experience with IPV derived from
Sabin strains or other attenuated strains, it is important to assess the neutralizing antibody titres induced by a candidate IPV derived from Sabin or other attenuated strains against heterologous poliovirus strains that differ from the production strains in antigenicity. For practical reasons, wild-type poliovirus strains used in the manufacture of wIPV (or their S19-wild equivalents) are considered suitable heterologous challenge strains for a candidate sIPV. Any other heterologous strain, demonstrated to be antigenically different from production strains, may be used if justified and approved by NRA. When comparing serum neutralizing antibody titres between a candidate sIPV and a licensed IPV for the purpose of selecting D-antigen content for clinical studies, it is important to bear in mind that the titre of sIPV-induced neutralizing antibody measured against homologous production strains or their S19 equivalents will most likely be higher than that measured against heterologous strains (for example, strains used for wIPV production or their S19 equivalents). It is vital that the D-antigen content selected for further clinical studies is sufficient to induce protective immunity against heterologous strains. Adequate antigen content is required for better cross-protection against heterologous strains.

When an adjuvant is included in the formulation, manufacturers should provide a rationale and immunogenicity data to support the use of an adjuvant in the vaccine.

Replace section C.2.1 “Assessment of the immune response” with the following text:

C.2.1 Assessment of the immune response

The presence of neutralizing antibodies against polioviruses is considered a reliable correlate of protection against poliomyelitis. However, immunity induced by one serotype does not provide protection against the other two serotypes. A serum neutralizing antibody titre of ≥ 8 is considered to be a marker of clinical protection against poliomyelitis (34). The demonstration of an immune response to IPV vaccination should be based upon the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for polio antigen is defined as:

- for subjects seronegative at the pre-vaccination time point, post-vaccination antibody titres of ≥ 8;
- for subjects seropositive at the pre-vaccination time point, a ≥ 4-fold rise in post-vaccination antibody titres. In the event that the pre-vaccination titre is due to maternal antibodies, a 4-fold rise above the expected titre of maternal antibodies based
on the pre-vaccination titre declining with a half-life of 28 days indicates seroconversion, or post-vaccination antibody titres of ≥ 8, whichever is higher.

The assay used to assess serum neutralizing antibodies in the clinical samples should follow the key parameters described in the WHO Manual for the virological investigation of poliomyelitis (35), with the exception of the challenge poliovirus strains. The challenge poliovirus strains should be carefully selected, as this may affect the results (and the interpretation of the results) of clinical studies as further discussed below in section C.2.4 of this amendment document. IPV developers are encouraged to use appropriate genetically modified poliovirus strains that can be manipulated outside of containment facilities (for example, S19 strains) as challenge viruses. The level of neutralizing antibody present in a serum sample is expressed as a titre, which is the reciprocal of the highest serum dilution that inhibits the viral cytopathic effect in 50% of cell cultures. A reference serum, calibrated against or traceable to the appropriate International Standard (see section A.1.3 of this amendment document) should be used to control assay performance (36, 37).

Replace section C.2.2 “Comparative immunogenicity studies” with the following text:

C.2.2 Comparative immunogenicity studies

A candidate IPV should be directly compared with at least one licensed IPV in prospective controlled studies. The choice of comparator vaccine is crucial for interpreting the results of a non-inferiority study, and should take into account the available evidence supporting its efficacy. Currently licensed wIPV products are better suited as comparator vaccines as they are similar to IPV products shown to be efficacious against paralytic poliomyelitis both in clinical studies and during more than 60 years of use in controlling poliomyelitis (38, 39). This recommendation is in line with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (40).

Non-inferiority studies to evaluate immunogenicity after completion of the primary vaccination series in the target population (for example, naive infants) are required for regulatory approval of a candidate IPV. Persistence of the serum neutralizing antibodies after completion of the primary series should also be investigated to determine whether and when a booster dose might be required. However, data on long-term antibody persistence might not be available prior to regulatory approval. The waning of antibodies over time is inevitable and should not be interpreted as indicating the need for a booster dose per se.
Replace section C.2.4 “End-points and analyses” with the following text:

C.2.4 End-points and analyses

The primary study analysis should be based on the rate of seroconversion (see section C.2.1 of this amendment document) measured at approximately 4 weeks following completion of the primary infant immunization. The primary study objectives should be based on the demonstration of the non-inferiority of the seroconversion rates achieved with the candidate IPV versus the comparator vaccine.

It is well recognized that the antigenic properties of different poliovirus strains of the same serotype are different, and that the titre of IPV-induced neutralizing antibody measured against homologous strains is generally higher than that measured against heterologous strains (32). Therefore, the level of neutralizing antibody present in clinical samples should in principle be assessed against heterologous poliovirus strains that differ in antigenicity when compared to the production strains to ensure that the conclusions of the clinical studies are applicable to different poliovirus strains. However, if the serum-neutralizing antibodies induced by a candidate sIPV are measured against the Sabin strains for the entire study population then, at a minimum, a subset of the clinical samples should be assessed against heterologous strains to ensure that the adequate seroconversion rate is reached. For practical reasons, wild-type poliovirus strains used in the manufacture of wIPV (or their S19-wild equivalents) are considered to be suitable heterologous challenge strains for assessing a candidate sIPV. In order to minimize bias, the subset should be randomly assigned from the total randomized test vaccine (candidate) and control vaccine (comparator) groups according to study protocol.

The clinical study protocol, including the sample size of the subset, should be discussed and agreed upon with the NRA prior to commencement of the study.

The requirement for assessing neutralizing antibody titres against heterologous poliovirus strains may be waived for technology-transfer products if such data have already been generated at a different site, and the comparability of the products used at these sites has also been demonstrated. However, such a waiver should be discussed with, and approved by, the NRA.

Other strategies may also be acceptable, provided that the data and analysis demonstrate adequate seroconversion rates, induced by a candidate IPV derived from attenuated strains, against heterologous poliovirus strains.

Comparison of geometric mean titres (GMTs) and reverse cumulative distributions of individual titres against all poliovirus strains tested at 4 weeks
post-primary should also be performed. While it may be that the GMT(s) for one or more poliovirus serotypes induced by the candidate IPV derived from attenuated strains is lower than that induced by the comparator vaccine, it is not clear if a lower GMT at 4 weeks post-primary affects long-term antibody persistence. Consequently, any significant differences in observed GMT should be carefully considered by the NRA and a decision should be supported by additional studies of antibody persistence (see section C.2.2 of this amendment document) and/or by a commitment to post-marketing studies – see section C.5 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1).

The minimum D-antigen content required for the candidate vaccine at the end of its shelf-life should be based on the D-antigen content of clinical lots shown to induce acceptable immune responses in clinical studies (for example, lots used in the dose-finding study).

Further guidance on demonstrating non-inferiority is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (40).

**Authors and acknowledgements**

The first draft of this document was prepared by Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, Technologies, Standards and Norms, World Health Organization, Switzerland. In addition, Dr R. Sutter, Dr M. Eisenhawer and Dr C. Sein, Department of Polio Operations and Research (WHO/POL), World Health Organization, Switzerland and Dr G. Enwere, Dr M. Janssen and Dr O. Lapujade, Vaccine Prequalification Team, World Health Organization, Switzerland contributed to the discussions and provided inputs into the first draft.

The draft document was then posted on the WHO Biologicals website during April and May 2019 for a first round of public consultation and comments were received from: Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Ms Q. Guan, Beijing Institute of Biological Products, China; Dr H-S Kim, LG Chem Ltd, Republic of Korea; Dr J. Li and Dr M. Sun, Institute of Medical Biology at the Chinese Academy of Medical Sciences, China; Dr K. Mahmood, PATH, the USA; Dr K. Maithal, Cadila Healthcare Ltd (Zydus Cadila), India; Dr W. Meng, Sinovac Biotech Ltd, China; Dr P. Minor, London, the United Kingdom; Dr J. Modlin, Dr D. Vaughn, Dr A. Chakrabarti and Dr A. Bandyopahiyay, Bill & Melinda Gates Foundation, the USA; Dr M. Pallansch, Centers for Disease Control and Prevention, the USA; Dr M. Song, National Institute of Food and Drug Safety Evaluation, Republic of Korea; Dr R. Sutter, Dr M. Eisenhawer and Dr C. Sein (WHO/POL); Dr T. Wu and Dr M. Baca-Estrada, Health Canada, Canada; Dr W. Wulandari, National
Agency of Drug and Food Control, Indonesia; Dr H. Zheng, Beijing Minhai Biotechnology Co., Ltd, China; and Dr C. Cahill, (on behalf of the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) provided the consolidated comments of subject matter expert reviewers from Janssen Pharmaceuticals, Sanofi Pasteur, GSK and Takeda Pharmaceutical Company Ltd).

Taking into consideration the comments received, a second draft document was prepared by Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, Technologies, Standards and Norms, World Health Organization, Switzerland.

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In response to the comments received during the second round of public consultation, a number of proposed amendments were presented by Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin and Dr M. Majumdar, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, Technologies, Standards and Norms, World Health Organization, Switzerland to the WHO Expert Committee on Biological Standardization which made further changes to document WHO/BS/2019.2354.

References


Annex 4

Biological substances: WHO International Standards, Reference Reagents and Reference Panels

The provision of global measurement standards is a core normative WHO activity. WHO reference materials are widely used by manufacturers, regulatory authorities and academic researchers in the development and evaluation of biological products. The timely development of new reference materials 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 reference preparations 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.13 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 Standards, Reference Reagents and Reference Panels for biological substances is available at: http://www.who.int/biologicals.

At its meeting held on 21–25 October 2019, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list. Each of the preparations shown in this table should be used in accordance with their instructions for use (IFU).

### Additions

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<tr>
<td><strong>Antibiotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B*</td>
<td>953 IU/mg</td>
<td>Third WHO International Standard</td>
</tr>
<tr>
<td><strong>Biotherapeutics other than blood products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Darbepoetin</td>
<td>100 000 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td><strong>Adalimumab</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro biological activity: 500 IU/ampoule of TNF-α neutralizing activity</td>
<td></td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>500 IU/ampoule of ADCC activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 IU/ampoule of CDC activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 IU/ampoule of binding activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapeutic drug monitoring: 50 µg/ampoule</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood products and related substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prekallikrein activator</td>
<td>30 IU/ampoule</td>
<td>Third WHO International Standard</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>1013 IU/ampoule</td>
<td>Fourth WHO International Standard</td>
</tr>
<tr>
<td>Anti-tetanus immunoglobulin (human)</td>
<td>45 IU/ampoule</td>
<td>Second WHO International Standard</td>
</tr>
</tbody>
</table>

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14 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. Materials identified by an * in the above list are held and distributed by the European Directorate for the Quality of Medicines & Healthcare, Strasbourg 67081, France. Materials identified by an ** in the above list are held and distributed by the Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, the USA. Materials identified by an *** in the above list are held and distributed by the Paul-Ehrlich-Institut, 63225 Langen, Germany.
<table>
<thead>
<tr>
<th>Preparation</th>
<th>Unitage</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assignment of additional analyte for blood coagulation factor XIII-B subunit antigen (total) to the current First WHO International Standard for blood coagulation factor XIII</td>
<td>0.98 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Establishment of RBC13–30 blood group genotyping alleles (lyophilized) as a companion set to the WHO international reference reagent collection for blood group genotyping**</td>
<td>[no assigned units]</td>
<td>First WHO International Reference Panel</td>
</tr>
<tr>
<td>Red blood cell transfusion relevant bacterial reference strains***</td>
<td>[no assigned units]</td>
<td>First WHO Repository of red blood cell transfusion relevant bacterial reference strains</td>
</tr>
</tbody>
</table>

### Cellular and gene therapies

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Unitage</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentiviral vector copy number</td>
<td>0 LV copies/cell</td>
<td>First WHO International Reference Panel</td>
</tr>
<tr>
<td></td>
<td>1.42 LV copies/cell (low)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.76 LV copies/cell (high)</td>
<td></td>
</tr>
<tr>
<td>Lentiviral vector integration site analysis</td>
<td>[no assigned units]</td>
<td>First WHO International Reference Reagent</td>
</tr>
</tbody>
</table>

### In vitro diagnostics

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Unitage</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C virus RNA for NAT-based assays</td>
<td>$2.57 \times 10^5$ IU/vial (equivalent to $5.41 \log_{10}$ IU/vial)</td>
<td>Sixth WHO International Standard</td>
</tr>
<tr>
<td>Human papillomavirus type 6 DNA for NAT-based assays</td>
<td>$1 \times 10^7$ IU/ampoule (equivalent to $7.0 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Human papillomavirus type 11 DNA for NAT-based assays</td>
<td>$1 \times 10^7$ IU/ampoule (equivalent to $7.0 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Preparation</td>
<td>Unitage</td>
<td>Status</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Human papillomavirus type 31 DNA for NAT-based assays</td>
<td>$1.6 \times 10^7$ IU/ampoule (equivalent to $7.2 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Human papillomavirus type 33 DNA for NAT-based assays</td>
<td>$1.6 \times 10^7$ IU/ampoule (equivalent to $7.2 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Human papillomavirus type 45 DNA for NAT-based assays</td>
<td>$1 \times 10^7$ IU/ampoule (equivalent to $7.0 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Human papillomavirus type 52 DNA for NAT-based assays</td>
<td>$7.9 \times 10^6$ IU/ampoule (equivalent to $6.9 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Human papillomavirus type 58 DNA for NAT-based assays</td>
<td>$7.9 \times 10^6$ IU/ampoule (equivalent to $6.9 \log_{10}$ IU/ampoule)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Anti-Müllerian hormone (human, recombinant)</td>
<td>489 ng/ampoule</td>
<td>First WHO International Reference Reagent</td>
</tr>
<tr>
<td>Insulin (human)</td>
<td>$9.19 \text{ mg/ampoule} \pm 0.05 \text{ mg/ampoule}$ (expanded uncertainty, $k = 2$)</td>
<td>Second WHO International Standard</td>
</tr>
<tr>
<td>HCT 15 cancer genome</td>
<td>[no assigned units]</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>MOLT-4 cancer genome</td>
<td>[no assigned units]</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>ATDB102 reference genome</td>
<td>[no assigned units]</td>
<td>First WHO International Standard</td>
</tr>
</tbody>
</table>

**Vaccines and related substances**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Unitage</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV71 inactivated vaccine</td>
<td>3625 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>EV71 genotype C4 inactivated vaccine</td>
<td>300 U/ampoule</td>
<td>First WHO International Reference Reagent</td>
</tr>
<tr>
<td>EV71 genotype B4 inactivated vaccine</td>
<td>250 U/ampoule</td>
<td>First WHO International Reference Reagent</td>
</tr>
<tr>
<td>Tetanus toxoid for use in flocculation test</td>
<td>970 Lf/ampoule</td>
<td>Third WHO International Standard</td>
</tr>
</tbody>
</table>
### Preparation

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Unitage</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assignment of additional analyte for antiserum to respiratory syncytial virus subtype B to the current First WHO International Standard for antiserum to respiratory syncytial virus</td>
<td>1000 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Meningococcal serogroup W polysaccharide</td>
<td>1.015 ± 0.071 mg/ampoule (expanded uncertainty, k = 2.13)</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Meningococcal serogroup Y polysaccharide</td>
<td>0.958 ± 0.076 mg/ampoule (expanded uncertainty, k = 2.26)</td>
<td>First WHO International Standard</td>
</tr>
</tbody>
</table>
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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

To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO's books contribute to achieving the Organization's principal objective – the attainment by all people of the highest possible level of health.

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

For further information, please contact: WHO Press, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (tel. +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int; order on line: www.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 substances, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of general issues brought to the attention of the Committee. The next part of the report, of particular relevance to manufacturers and national regulatory authorities, outlines the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines; and an Amendment document to the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) were adopted on the recommendation of the Committee.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: antibiotics; biotherapeutics other than blood products; blood products and related substances; cellular and gene therapies; in vitro diagnostics; and vaccines and related substances.

A series of annexes are then presented which include an updated list of all WHO Recommendations, Guidelines and other documents on biological substances used in medicine (Annex 1). The above two WHO documents adopted for publication on the advice of the Committee are then presented as part of this report (Annexes 2 and 3). Finally, all additions and discontinuations made during the 2019 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 4. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.