

WHO Expert Committee on Biological Standardization

Report of the seventy-second and seventy-third meetings



World Health
Organization

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*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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Organization**

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This publication contains the collective views of an international group of experts and does not necessarily represent the decisions or the policies of WHO.

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WHO Expert Committee on Biological Standardization¹

Seventy-second meeting held via WebEx video conferencing on
19 to 23 October 2020

Seventy-third meeting held via Zoom video conferencing on
9 and 10 December 2020

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¹ Unless otherwise indicated, all participants attended both the seventy-second and seventy-third meetings in the capacities shown.

² The decisions of the Committee were taken in closed session with only members of the Committee present. Each Committee member had completed a Declaration of Interests form prior to the meeting. These were assessed by the WHO Secretariat and no declared interests were considered to be in conflict with full meeting participation.

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International Federation of Pharmaceutical Manufacturers & Associations

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Dr J. Bernat (seventy-third meeting only)

Dr M. Gencoglu (seventy-third meeting only)

International Society of Blood Transfusion

Professor E. Wood (seventy-second meeting only)

⁵ Unable to attend either meeting: European Medicines Agency.

⁶ Unable to attend either meeting: International Union of Immunological Societies.

International Society on Thrombosis and Haemostasis

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Coalition for Epidemic Preparedness Innovations

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Plasma Protein Therapeutics Association

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World Health Organization (WHO)

Access to Medicines and Health Products (MHP)

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Health Products Policy and Standards (MHP/HPS)

Dr C. Ondari, Director

Technical Standards and Specifications (MHP/HPS/TSS)

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Dr Y. Maryuningsih (*Lead for the blood products and in vitro diagnostics track*)

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Dr R. Isbrucker

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Dr D. Lei

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⁷ Unable to attend either meeting: Biotechnology Innovation Organization; Chinese Pharmacopoeia Commission; and Pharmaceutical and Medical Device Regulatory Science Society of Japan (formerly Society of Japanese Pharmacopoeia).

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Product and Delivery Research (UHL/IVB/PDR)

Dr B. Giersing (seventy-second meeting only)

INN and Classification of Medical Products (MHP/HPS/INN)

Dr R. Balocco (seventy-second meeting only)

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Dr C. Rodriguez Hernandez (seventy-second meeting only)

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Dr H. Langar (seventy-third meeting only)

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⁸ Unable to attend either meeting: WHO Regional Office for Africa and WHO Regional Office for South-East Asia.

Abbreviations

ACT	WHO Access to COVID-19 Tools (Accelerator)
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
Ag-RDT	antigen-detecting rapid diagnostic test
C-STFT	Committee for the Standardization of Thyroid Function Tests
CBER	Center for Biologics Evaluation and Research
CCP	COVID-19 convalescent plasma
CEPI	Coalition for Epidemic Preparedness Innovations
CHO	Chinese hamster ovary
COVID-19	coronavirus disease 2019
CRISPR	clustered regularly interspaced short palindromic repeats
cVDPV	circulating vaccine-derived poliovirus
CV	coefficient of variation
DNA	deoxyribonucleic acid
ECSP	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
EQA	external quality assessment
ErbB-2	epidermal growth factor receptor 2
EUL	emergency use listing
EV71	enterovirus 71
FDA	United States Food and Drug Administration
FLT3	FMS-like tyrosine kinase 3
FLT-ITD	FMS-like tyrosine kinase 3 internal tandem duplication
FMT	faecal microbiota transplantation
FNAIT	fetal/neonatal alloimmune thrombocytopenia



FSH	follicle-stimulating hormone
FVIII	blood coagulation factor VIII
FIX	blood coagulation factor IX
FXIII	blood coagulation factor XIII
GACVS	Global Advisory Committee on Vaccine Safety
GCV	geometric coefficient of variation
GRelP	good reliance practices
GRP	good regulatory practices
hCG	human chorionic gonadotrophin
HER2	human epidermal growth factor receptor 2
HFMD	hand, foot and mouth disease
HLA	human leukocyte antigen
HPA	human platelet antigen
hPSC	human pluripotent stem cell
HRP2	histidine-rich protein 2
HSV	herpes simplex virus
IDO1	indoleamine 2,3-dioxygenase 1
IFN α -2b	interferon alpha 2b
IFU	instructions for use
IGF-1	insulin-like growth factor 1
INN	international nonproprietary name(s)
IOP	inhibition of proliferation (assay)
IPV	inactivated poliomyelitis vaccine
IS	international standard(s)
ISBT	International Society of Blood Transfusion
ISCT	International Society for Cell & Gene Therapy
IU	International Unit(s)
IVD	in vitro diagnostic
LDH	lactate dehydrogenase

LH	luteinizing hormone
LMIC	low- and middle-income countries
mAb	monoclonal antibody
MAIPA	monoclonal antibody-specific immobilization of platelet antigen
MAPREC	mutant analysis by polymerase chain reaction and restriction enzyme cleavage
MERS-CoV	Middle East respiratory syndrome coronavirus
mRNA	messenger ribonucleic acid
MSC	mesenchymal stromal cell
NAT	nucleic acid amplification technique
NIBSC	National Institute for Biological Standards and Control
nOPV	novel oral poliomyelitis vaccine
NRA	national regulatory authority
OMCL	official medicines control laboratory
OPV	oral poliomyelitis vaccine
PCR	polymerase chain reaction
PDMP	plasma-derived medicinal product
qNMR	quantitative nuclear magnetic resonance
qPCR	quantitative polymerase chain reaction
RBP	reference biotherapeutic product
RDT	rapid diagnostic test
RNA	ribonucleic acid
RSV	respiratory syncytial virus
SAGE	Strategic Advisory Group of Experts (on Immunization)
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SBP	similar biotherapeutic product
SFTS	severe fever with thrombocytopenia syndrome
TCV	typhoid conjugate vaccine
TSH	thyroid stimulating hormone

VEGF165	vascular endothelial growth factor 165
WLA	WHO-listed authority
WNV	West Nile virus

1. Introduction

1.1 Introduction to the seventy-second meeting

The seventy-second meeting of the WHO Expert Committee on Biological Standardization was held from 19 to 23 October 2020 by WebEx video conferencing due to the restrictions imposed during the coronavirus disease 2019 (COVID-19) pandemic. 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 Committee members, meeting participants and observers.

Dr Simão began by noting that the Committee had met as recently as August 2020, primarily to address standardization activities related to COVID-19, and that much remains to be learnt about the pandemic. The WHO Solidarity Trial was continuing to facilitate the rapid and robust comparison of potential treatments. Recently published interim results indicated that the four small molecule treatments remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon had little or no benefit in terms of overall mortality or speed of recovery of hospitalized patients – to date only dexamethasone had proved to be effective against severe COVID-19.

Following the initial focus placed on the above therapeutic small molecules, the Committee would now have a key role to play as the emphasis shifted towards therapeutic and prophylactic biological medicines. There was clearly a need to address current gaps in the list of relevant WHO written standards and an urgent requirement for COVID-19 measurement standards for biological products. Reflecting on the important role played by the Committee during the Ebola emergency, Dr Simão expressed her gratitude to meeting participants for their expertise and commitment in helping to address the current public health emergency.

Dr Simão moved on to emphasize the equally crucial importance of WHO continuing to deliver on its non-COVID-19 work. Most WHO expert committees met in October and all had a number of non-COVID-19 issues to address. Dr Simão concluded by noting the correspondingly ambitious agenda of this meeting which covered a broad range of topics.

Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Simão for her opening remarks. Dr Knezevic reminded participants that WHO is responsible for providing leadership on global health matters, with the principal objective of attaining the highest possible level of health by all people. In this regard, setting norms and standards, and promoting their implementation, remains a core WHO function. In 2020, numerous COVID-19 activities had been initiated, including the standardization of related biologicals.

Dr Knezevic informed the Committee that the World Health Assembly, the supreme decision-making body of WHO, had held its Seventy-third

meeting virtually in May 2020. After noting the challenges of holding such virtual meetings, Dr Knezevic summarized the rules and procedures of the current meeting and provided details of the working arrangements. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would participate in the meeting from Monday 19 October to Thursday 22 October 2020. An open information-sharing session involving all participants, including non-state actors, would be held on Monday 19 October 2020. On Tuesday and Wednesday, the agenda would be divided into two parallel tracks covering vaccines and biotherapeutics in one track, and blood products and in vitro diagnostics in the other. All final decisions and recommendations regarding the adoption of written standards and the establishment of measurement standards would be made in a closed session held on Friday 23 October attended only by Committee members and WHO staff. Dr Knezevic expressed her thanks to WHO staff, members of WHO drafting and working groups, colleagues from WHO collaborating centres and custodian laboratories, and the many individual experts present for their invaluable support.

Following the conclusion of the open information-sharing session on Monday 19 October, the meeting officials were elected. 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 Diana Teo as Rapporteurs for the blood products and in vitro diagnostics track. Dr Klein was also elected as Vice-Chair of the plenary sessions. Dr Knezevic presented the declarations of interests completed by all members of the Committee and by WHO temporary advisers and participants. After evaluation, WHO had concluded that none of the interests declared constituted a significant conflict of interest and that the individuals concerned would be allowed to participate fully in the meeting.

The Committee then adopted the proposed agenda and timetable (WHO/BS/2020.2400).

1.2 Introduction to the seventy-third meeting

The seventy-third meeting of the WHO Expert Committee on Biological Standardization was held on 9 and 10 December 2020 by Zoom video conferencing due to the restrictions imposed during the COVID-19 pandemic. 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 expressed her gratitude to everyone attending this short but important meeting, particularly given the current level of demands at national

level, including the ongoing authorization of COVID-19 vaccines. The challenges presented by the pandemic were broad and complex, with the measures being worked on ranging from social behavioural aspects to the development of diagnostics, vaccines and therapeutics. Focusing on recent progress in COVID-19 vaccine development, Dr Simão expressed concern that the current tone of media reporting might induce a false sense of security and WHO messaging needed to be very clear. It currently appeared that there would likely be insufficient doses of vaccine in the coming year to immunize a large proportion of the global population. In the meantime, the continuing use of all available measures would remain crucial.

Dr Simão went on to note that although virtual meetings had proved to be highly useful in the current circumstances, a mix of face-to-face and virtual meetings would likely be adopted once the pandemic was over. Briefly reviewing the meeting agenda, Dr Simão emphasized that the work of the Committee was fundamental to the public health response to the current emergency, and cited the importance of the prospective WHO international reference standards due to be discussed at the present meeting in interpreting the results of clinical trials of candidate vaccines, therapeutic antibodies and convalescent plasma. Dr Simão concluded by highlighting a number of cross-cutting activities of other WHO advisory groups and expert committees.

Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Simão for her opening remarks. Dr Knezevic reiterated to 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. In this capacity, 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. Observing that the work of WHO on COVID-19 had to fit in with all its other strategic priorities in this area, Dr Knezevic briefly summarized the four strategic priorities of the WHO five-year action plan to improve the quality and safety of health products. She went on to summarize the main outcomes of the previous meetings of the Committee held in August and October 2020, which had included the adoption of five WHO written standards and the establishment of 19 new or replacement WHO measurement standards.

Dr Knezevic then provided details of the working arrangements for the present meeting. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would participate in the first three sessions of the meeting, the first of which would be an open information-sharing session involving all participants, including non-state actors. All final decisions and recommendations regarding the establishment of WHO measurement standards would be made in a closed session attended only by Committee members and WHO staff.

Following the conclusion of the open session, Dr Knezevic moved on to the election of meeting officials. In the absence of dissent, Dr Harvey Klein was elected to chair the second session and Professor Klaus Chichutek the third and fourth sessions. Dr Ian Feavers was elected as Rapporteur of the meeting.

The Committee then adopted the proposed agenda and timetable (WHO/BS/2020.2405).

2. General

2.1 Strategic directions in biological standardization: impact of COVID-19

2.1.1 WHO collaborations and partnerships related to COVID-19

Dr Carmen Rodriguez Hernandez updated the Committee on the regulatory work being carried out as part of the COVAX project, one of the pillars of the WHO Access to COVID-19 Tools (ACT) Accelerator that aims to ensure equitable access to vaccines, therapeutics and diagnostics. Emphasizing the importance of global regulatory alignment in the context of the COVID-19 pandemic, Dr Hernandez highlighted a number of challenges, including the rapid globalization of medicines and medical technologies, the inconsistency of regulatory requirements and processes, and the uneven distribution of regulatory capacity worldwide. Addressing such challenges would require global cooperation and a multilateral strategy to coordinate regulatory efforts. In this context, Dr Hernandez explained the role and key features of WHO prequalification and emergency use listing (EUL) processes in facilitating product-specific regulatory alignment.

The WHO vaccine prequalification team had made significant progress towards ensuring the timely and high-quality evaluation of COVID-19 vaccines worldwide. Specific activities included public consultation on the WHO Considerations for evaluation of COVID-19 vaccines document, and a call to manufacturers for expressions of interest in the prequalification and EUL of their candidate vaccines. In addition, a safety preparedness manual had been produced to provide guidance on pharmacovigilance, adverse event definitions, active surveillance, risk-management planning, periodic safety update reports and risk communications, while also addressing issues of regulatory reliance and work sharing. Reiterating the need for global cooperation, Dr Hernandez set out a WHO-led process for expediting in-country regulatory evaluation of dossiers, product approval and post-approval monitoring, and discussed the key issues to be considered by national regulatory authorities (NRAs) using this process. It was recognized that at present only limited information was available and based on a best-case scenario it would take 6–10 months from the start of Phase III studies to achieve WHO prequalification and EUL. On this basis, the first complete dossier submissions were anticipated in January 2021. After outlining a number of next steps in this area, Dr Hernandez concluded by emphasizing the importance of setting realistic expectations, including with regard to the time frame for achieving full reliance on WHO prequalification and EUL across low- and middle-income countries (LMIC).

Acknowledging the importance of these activities in ultimately ensuring equitable access to COVID-19 vaccines worldwide, the Committee sought

clarification of the process behind the development of the safety preparedness manual. It was informed that the manual had been the culmination of several workshops and working groups on vaccine safety, as well as an international webinar on vaccine pharmacovigilance. The final draft of the manual would be reviewed by the Global Advisory Committee on Vaccine Safety (GACVS). The Committee commented that review by the Strategic Advisory Group of Experts on Immunization (SAGE) would be important in the development of the proposed WHO-led process for expedited in-country regulatory approval.

2.1.2 Development of WHO guidance on regulatory considerations in the evaluation of monoclonal antibodies used for the prevention or treatment of COVID-19 and other infectious diseases

Dr Richard Isbrucker provided the Committee with an overview of the development status of monoclonal antibodies (mAbs) intended for use in the prevention or treatment of infectious diseases, including COVID-19. Because of their short development time, rapid impact and established safety profile the development of mAb products for use as COVID-19 therapeutics is considered to be a high priority, particularly as these are likely to become available sooner than vaccines. Dr Isbrucker noted that a range of mAb products had now been developed to prevent and treat other infectious diseases, with numerous other products (including further mAbs intended for use against COVID-19) currently in clinical evaluation.

Since 2013, a number of WHO guidance documents on mAbs had been published that focus primarily on the use of mAbs as biotherapeutics for noncommunicable diseases. Although manufacturing strategies for mAbs are similar regardless of their intended use, the target in the prevention or treatment of infectious disease is the pathogen rather than the host and the current guidance offers little advice on preclinical or clinical evaluation specific to pre-exposure prophylaxis or to post-infection treatment. It was envisaged that clarifying those issues unique to the development and evaluation of mAbs against infectious diseases – which would be of direct relevance for both NRA assessment and WHO prequalification – would improve access to such products.

It was therefore being proposed that a general WHO guidance document be developed on regulatory considerations in the evaluation of mAbs used for the prevention or treatment of infectious diseases (see section 3.1.1 below). The proposed approach would be to: (a) identify relevant sections of existing WHO guidance and clarify their application to this class of product; (b) provide greater clarity on the development of relevant similar biotherapeutic products (SBPs); (c) incorporate novel mAb manufacturing technologies not included in current guidance; and (d) address clinical trial design issues specific to anti-infective agents. The general guidance document would include disease-specific

supplements that would provide additional guidance on the assessment of mAbs against a particular pathogen – for example, on the availability of the relevant WHO international reference standards, the choice of infectious strains, use of animal models and clinical study design.

Noting that the related proposal to develop WHO guidance on mAbs used for the prophylaxis or treatment of respiratory syncytial virus (RSV) infection was also to be discussed later in the meeting (see section 3.1.2 below), the Committee agreed that the overall approach should be to develop general regulatory guidance for mAbs used for the prevention or treatment of infectious diseases, with consideration also given to the development of separate disease-specific supplements on mAbs used against RSV and COVID-19. The Committee highlighted that the guidance required on clinical trial design for prophylactic mAbs would differ from that needed for therapeutic mAbs and the proposed documents would need to reflect this.

2.1.3 **Update on the use of convalescent plasma as a potential therapy for COVID-19**

Dr Yuyun Maryuningsih highlighted that COVID-19 convalescent plasma (CCP) and hyperimmune immunoglobulin were currently being investigated as potential therapies for reducing mortality among COVID-19 patients. Dr Maryuningsih reminded the Committee that WHO interim guidance on maintaining a safe and adequate blood supply during the pandemic and on the collection of CCP had been developed in July 2020 in collaboration with international stakeholders (see section 2.1.4 below). This document strongly recommends restricting the use of CCP to randomized controlled trials. If such clinical studies are not possible then patient outcomes should be documented and blood samples from donors and recipients archived for future characterization. Provided that medical, legal and ethical safeguards are in place for both CCP donors and recipients, the guidance indicates that CCP can be made available on an experimental basis through local production. Two webinars had now been held, the first of which was intended to promote the interim guidance document and the second to provide specific guidance on the collection and use of CCP.

Following the emergency use authorization for CCP issued by the United States Food and Drug Administration (FDA) in September 2020, WHO had decided that it would not be necessary to update the interim guidance until the First WHO International Standard for anti SARS-CoV-2 immunoglobulin (see section 9.1.2 below) became available for the calibration of in vitro diagnostics (IVDs) used to measure CCP antibody content.

Other WHO collaborative activities had included the conducting of a survey at the WHO regional level to assess the availability and use of protocols for

CCP studies. The survey had revealed significant variation in the protocols used at institutional and national level, with differences noted in study design. It had been agreed that the International Society of Blood Transfusion (ISBT) would host a resource on their website consisting of information, policy statements and protocols for CCP studies. WHO had also worked with ISBT to initiate a meta-analysis of clinical CCP studies using the Cochrane Rapid Systematic Review methodology. This analysis had now been published and it had been concluded that too few randomized controlled trials had been conducted to be certain that CCP treatment was both safe and effective. In addition, WHO had been in close communication with the CoVIg Plasma Alliance, a plasma industry collaboration established to accelerate the development of a plasma-derived hyperimmune globulin therapy for COVID-19. During discussions, the Alliance had expressed interest in using the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (see section 9.1.2 below) to calibrate its internal antibody potency standards.

Reflecting on the lack of evidence for the safety and efficacy of CCP or hyperimmune serum, the Committee recognized the need for further randomized controlled trials and the importance of an anti-SARS-CoV-2 immunoglobulin standard for calibrating the potency of products in International Units (IU). A proposal to develop a WHO international standard for this purpose had been endorsed by the Committee at its previous meeting in August 2020 with establishment anticipated in December 2020 (see section 9.1.2 below).

2.1.4 **Updating the WHO interim guidance document on maintaining a safe and adequate blood supply during the coronavirus disease 2019 (COVID-19) pandemic and on the collection of COVID-19 convalescent plasma**

Dr Maryuningsih informed the Committee of plans to update the current WHO interim guidance document on maintaining a safe and adequate blood supply during the COVID-19 pandemic and on the collection of CCP.⁹ The need to update this guidance had been driven by a number of evolving issues including: (a) the implications of vaccination against SARS-CoV-2 for donor deferral periods; (b) the prospective establishment of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (see section 9.1.2 below);

⁹ Guidance on maintaining a safe and adequate blood supply during the coronavirus disease 2019 (COVID-19) pandemic and on the collection of COVID-19 convalescent plasma. Interim guidance. World Health Organization: Geneva; 10 July 2020 ([https://www.who.int/publications/i/item/maintaining-a-safe-and-adequate-blood-supply-during-the-pandemic-outbreak-of-coronavirus-disease-\(covid-19\)](https://www.who.int/publications/i/item/maintaining-a-safe-and-adequate-blood-supply-during-the-pandemic-outbreak-of-coronavirus-disease-(covid-19)), accessed 31 December 2020).

(c) recent publications on convalescent plasma, including a Cochrane Rapid Systematic Review; and (d) the publication of new WHO considerations for implementing and adjusting public health and social measures in the context of COVID-19.

The views of the Committee were being sought on the updating of the interim guidance document, particularly with regard to the temporary deferral of donors following vaccination against SARS-CoV-2. This was now viewed as prudent for avoiding the theoretical risk of transmission of vaccine components to transfusion recipients. The prospective updated guidance would recommend that recipients of COVID-19 vaccines not based on the use of live virus should be deferred for 7 days. Support for this position had been expressed during consultations held with the drafting group established to develop the proposed WHO guidance document on regulatory considerations in the evaluation of messenger RNA vaccines (see section 3.4.5 below). In the case of recipients of live viral vaccines (for example, viral-vectored or live-attenuated virus vaccines), or in cases where it cannot be established which type of vaccine the potential donor received, donation should be deferred for 4 weeks, consistent with current practice. Potential donors suffering an adverse reaction to vaccination should be deferred until complete resolution of their symptoms. As some vaccines require more than one dose, the administration of each dose should be regarded as an independent event for the purposes of blood donor deferral. In settings where mass COVID-19 vaccination campaigns were anticipated, the updated guidance will recommend that the blood centre works closely with local health authorities to minimize the impact on blood donor availability.

Noting the lack of evidence either for or against the need for a 7-day deferral, the Committee discussed the impact such deferrals might have on the availability of donors in settings where mass COVID-19 vaccination was implemented. On a related note, the Committee felt that health care workers in blood transfusion centres should be considered as a priority group for such vaccination. The Committee also considered the likely positions of key stakeholders on the question of post-vaccination deferral. It was informed that the FDA would be unlikely to adopt the 7-day deferral period, while the European Centre for Disease Prevention and Control was awaiting the decision of the Committee so that its guidance would be consistent with that of WHO.

The Committee expressed its support for the updating of the WHO interim guidance document in general and specifically recommended inclusion of the proposed statement on the need for a 7-day post-vaccination deferral period as a prudent measure in protecting the health of blood donation recipients.

2.1.5 Issues around the implementation and use of WHO international standards

Dr Mark Page provided the Committee with an overview of a number of issues that can potentially undermine both the implementation and correct use of WHO international standards (IS) following their establishment. Despite being of recognized international importance and typically eagerly awaited by collaborators and stakeholders, WHO IS are not always used to the extent anticipated or in the intended manner. For a variety of reasons, the users of such standards are often unclear as to how best to calibrate their assays. This can result from the lack of a clear protocol, the choice of statistical software and a failure to appreciate the value of estimating relative potency. In addition, there is frequent confusion between the use of a standardized assay (that is, one which follows a standard operating procedure) and the use of an IS. Issues also exist in ensuring the appropriate use of IU in different types of assay (for example, neutralization versus binding assays) and in ensuring that IS are used correctly as calibrants rather than to validate or qualify assays. A mechanism is also needed to ensure that commercially manufactured kits are routinely calibrated in IU. In addition, the expectation of custodian laboratories is that IS stocks will be conserved for many years as laboratories will use the IS only infrequently to calibrate their own working standards for routine use in assays. However, in reality this is often not the case and a process may be needed to facilitate the production of such secondary standards by laboratories.

Dr Page then made particular reference to the prospective establishment and implementation of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin scheduled for consideration at the current meeting (see section 9.1.2 below). As many COVID-19 vaccines were now in development, it was anticipated that the level of demand for this IS would be high. National working standards were now being produced in China and the USA, while the centralized laboratory network administered by the Coalition for Epidemic Preparedness Innovations (CEPI) was using a working standard calibrated to the prospective IS. In addition, laboratories that had used a previously released working reagent (NIBSC code 20/130) in clinical and seroepidemiological studies such as the WHO Solidarity II studies would be able to recalculate their data into IU as this working reagent had been included in the collaborative study of the prospective IS and a conversion factor was available. Dr Page concluded by outlining a series of WHO and other events and initiatives during which the prospective IS would be publicized and implemented.

During discussion, a number of meeting participants commented on a general lack of appreciation of the important role of WHO IS and the need to remind the biopharmaceutical industry of the benefits of harmonizing biological activity measurements in IU for global public health. In this regard, the Committee acknowledged that the planned revision of the 2004 WHO

Recommendations for the preparation, characterization and establishment of international and other biological reference standards would provide an opportunity to present users of such standards with clear and up-to-date guidance.

With respect to the importance of secondary working standards in slowing the rate of depletion of IS the Committee discussed current WHO guidance in this area and the possibility of publishing a technical note on the purpose and correct use of IS. Additional approaches could include the holding of tailored webinars on different standardization issues in the vaccine, therapeutic and diagnostic fields, and the posting of online instructional “how to” videos on a dedicated social media channel. On the issue of the statistical software packages used in biological standardization projects, the Committee was informed that NIBSC routinely used CombiStats™. This open-access software package was available from EDQM and more than 800 licences had been issued worldwide to date. EDQM offered training in the use of this software package, including its use in relative potency assays. Subject to an end-user agreement, WHO also provides free statistical software developed by NIID in Japan and similar to CombiStats™. Discussion then took place on how best to publicize the availability of the upcoming COVID-19 reference standards and how to generate any specific guidance on their correct use that might be required.

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

2.2.1 Overview

Dr Knezevic reported on recent and planned WHO activities in the standardization and regulatory evaluation of vaccines and biotherapeutics. She reminded the Committee that two WHO written standards had been recommended for adoption at its previous meeting in August 2020 – the revised WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines; and an Amendment to the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines. At the present meeting, the following three written standards were to be considered by the Committee:

- Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines (see section 3.3.1 below);
- Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated) (see section 3.3.2 below); and
- Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics (see section 3.2.1 below).

There were also several written standards currently in development that were likely to be presented to the Committee within the next 2 years, including the revised WHO Guidelines on evaluation of similar biotherapeutic products (SBPs) (see section 3.1.4 below). In addition to the written standards proposed for adoption in 2020, 12 new WHO measurement standards and three replacement WHO measurement standards were being proposed for establishment by WHO. Such measurement standards continue to be crucial in the development, licensing and ongoing lot release of biological medicines.

Dr Knezevic then set out the approach that WHO is taking to address regulatory needs with regard to cellular and gene therapy products. Following an initial workshop in February 2020, plans to conduct a survey to assess the current global regulatory landscape and to develop a white paper on fundamental regulatory principles had been postponed due to the COVID-19 pandemic. Nevertheless, progress had been made in the development of WHO measurement standards in this area following the adoption of the first such materials in 2019. As a result, two proposals for the development of WHO international reference standards for mesenchymal stromal cells would be considered for endorsement by the Committee during the current meeting.

During the discussions of an ad hoc expert working group on COVID-19 animal models, established by the WHO R&D Blueprint team, the issue had been raised of the *in vitro* genetic stability of SARS-CoV-2 during propagation in different mammalian cell lines. Data on multiple isolates had now been analyzed, with initial observations suggesting that certain isolates might have been less genetically stable than others. Specifically, the serial propagation of SARS-CoV-2 in Vero E6 cells appears to be associated with the risk of deletions arising in critical virulence components of the virus. Such deletions appear to rapidly overrun the working stocks, which have subsequently been found to be less virulent in animal models. The outcomes of these discussions had now been published in the scientific press. Dr Knezevic concluded by presenting a list of further identified COVID-19 research gaps which had resulted in a call for proposals by WHO to identify suitable projects for WHO research grant support.

Recognizing that mRNA vaccines were among the more developmentally advanced COVID-19 candidate vaccines, and following discussions at its previous meeting, the Committee enquired about the progress made in developing WHO regulatory guidance on such vaccines and in drafting guidance specifically on COVID-19 mRNA vaccines. The Committee was informed that a preliminary draft document had been circulated to the drafting group but concerns remain that there is currently insufficient knowledge of COVID-19 to produce definitive disease-specific guidance. In response to a query on the possibility of developing WHO guidance on the plant-based production of COVID-19 mAbs and subunit

vaccines, Dr Knezevic referred to existing WHO guidance and noted that due to a lack of progress in this area there were no plans at present in this regard.

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

2.3.1 **Product-specific reference materials for blood coagulation factors VIII and IX**

Dr Mikhail Ovanosov provided the Committee with an overview of current issues in the standardization of assays used to assign potency values to blood coagulation factor VIII (FVIII) and factor IX (FIX) products. Around 30 such commercial FVIII and FIX concentrates have been assigned potencies in IU based on the use of the current WHO international standards. Coagulation factor activity in patient plasma is then estimated by clinical laboratories against the corresponding WHO plasma international standards. The standardization of the clinical assays used to estimate coagulation factor activity is crucial as any discrepancies in measurement will have implications for the safety and efficacy of such products. Although a number of genetically or chemically modified recombinant FVIII and FIX concentrates had been approved since 2013, assaying many of these products against the current WHO international reference standards or plasma calibrators results in substantial disagreement when using different reagents or kits. This would indicate the lack of commutability of such standards in the measurement of modified products in patient samples.

This challenging situation for clinical laboratories could be ameliorated by the development of publicly available product-specific reference materials such as WHO international reference reagents but this would also raise issues. The WHO biological standardization programme focuses on the provision of global primary standards and it is unclear how WHO would provide proprietary, product-specific reference materials. Moreover, the existence of multiple unique reference materials would potentially be confusing for stakeholders.

Dr Ovanosov updated the Committee on the key points discussed during a WHO hearing on the development of product-specific reference materials for FVIII and FIX products. The hearing had been organized to gather inputs from stakeholders as part of the sixth meeting of the WHO network of collaborating centres for blood products and IVDs in 2018. Broad agreement had been reached that product-specific reference materials were needed but would be considered as secondary standards. Opinions differed on whether it was the responsibility of WHO to provide such materials and WHO collaborating centres will communicate the outcomes of further discussion of the issue to the Committee for its advice. The Committee was informed that NIBSC had already started working with individual manufacturers to calibrate reference materials against the WHO international standards using the manufacturers' in-house assays.

While accepting the broad consensus among stakeholders that product-specific reference materials were required, the Committee noted that this would potentially create a completely new area of work. The Committee felt that although such reference materials were unquestionably important for patient care it would be premature for WHO to undertake their development and production. WHO should instead continue to consider its potential role with regard to such secondary standards while monitoring the progress of the prototype programme at NIBSC.

2.3.2 Development of guidance on the centralization of blood donation testing and processing

Dr Vee Armstrong outlined the development of a WHO guidance document that: (a) sets out the benefits and potential challenges of centralized blood donation testing and processing; and (b) provides a framework for assessing the suitability of centralized testing and processing of blood donations, and for planning the establishment of a centralized blood site. Dr Armstrong explained that the document is intended to support implementation of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023. Specifically, the centralization of blood testing and processing will contribute towards achieving the framework Strategic Objective 3 on ensuring functional and efficiently managed blood services, as well as Strategic Objective 4 on effective implementation of patient blood management.

The document provides a framework for blood establishments to assess their own suitability for centralization – regardless of whether they plan to establish a centralized facility or to send blood donations to centralized sites. It also provides an outline approach to planning and implementing such centralization, including technical information based largely on good manufacturing practices and good laboratory practices, and should also prove useful in managing emergency situations that affect blood supply and safety. In addition to the centralization of testing and processing of blood donations intended for use in transfusion the document also provides guidance on the optimization of resources and on the use of recovered plasma in the manufacture of plasma-derived medicinal products (PDMPs). Dr Armstrong noted that in many countries plasma extracted from whole blood donations was often discarded because of the lack of PDMP production facilities – a problem that could potentially be overcome by centralization. The guidance is intended primarily for use by management staff in blood establishments, ministries of health, NRAs and other policy-makers, with envisaged utility also for professional bodies and other nongovernmental organizations.

Acknowledging the importance of the issues addressed in the guidance document, the Committee enquired about the next steps following its publication

on the WHO website. It was informed that plans were in place to promote the document among regional advisers and countries through a series of webinars. The Committee also sought clarification on whether centralization would be at a country or regional level. It was informed that the document focuses primarily on centralization within countries and that regional centralization is difficult. However, it was anticipated that once countries centralize these activities within their borders and realize the significant benefits to be gained, consideration may be given to the possibility of expanding this to regional level.

2.3.3 Development of a WHO white paper on increasing access to PDMPs in LMIC

Dr Thierry Burnouf outlined the development of a WHO white paper intended to help address the ongoing inadequate supply of safe and effective PDMPs, particularly in LMIC. Dr Burnouf explained that the current situation was largely due to global shortages of some products, the high cost of imported PDMPs and the failure of local plasma to meet the requirements for contract or domestic fractionation. The shortage of safe and effective PDMPs in LMIC means that patients are often at risk of being treated with crude plasma or plasma fractions that have not been treated to remove bloodborne pathogens. In addition, efforts to increase whole blood collection to meet the need for red blood cells results in the generation of more plasma, which is then wasted.

Increasing the production of PDMPs from domestic plasma resources would improve the quality and safety of treatments given to patients in LMIC. The white paper therefore aims to provide high-level recommendations on reducing the wastage of domestic plasma that could otherwise be fractionated to make PDMPs. The paper targets policy-makers, NRAs, blood collection organizations, blood donors and their associations, clinicians and patients. The paper is aligned with World Health Assembly resolutions WHA58.13 and WHA63.12 on the provision of safe blood and blood components, and several PDMPs are on the WHO Model List of Essential Medicines. The development of this white paper will also support the implementation of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023.

Dr Burnouf concluded by outlining the process which had led to the development of the current document entitled *Increasing supplies of plasma-derived medicinal products in low- and middle-income countries through fractionation of domestic plasma* and provided a brief summary of its structure and content. Publication of the document was scheduled for the end of November 2020.

The Committee recognized the importance of the white paper which addresses issues, such as the wastage of plasma in LMIC, that have been of long-standing concern. It noted that the document also addresses the key issues of

plasma-sourcing capabilities and the commercial collection of plasma. The Committee emphasized the importance of involving experts, professionals and professional associations in the decision-making process as they understand the scientific issues as well as the situation on the ground. The Committee also noted that this white paper and the related WHO guidance document on the centralization of blood donation testing and processing discussed earlier (see section 2.3.2 above) were complementary, as one key anticipated benefit of centralization was the production of more plasma for fractionation.

The Committee also felt that the development of this white paper had highlighted the need to update several WHO guidelines in this area. For example, the WHO Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products do not include the latest advances in this area. Similarly, current WHO guidance on blood collection and good manufacturing practices would benefit from being updated. The Committee was informed that during the development of the workplan of the above WHO Action framework several such documents had been identified as requiring updating, including those on the clinical use of blood and on ensuring the preparedness of the blood supply system during emergencies. A review of all relevant documents would help to identify and prioritize all those now in need of updating.

2.3.4 Reference materials for *Plasmodium* spp. diagnostics

Dr Paul Bowyer reviewed the current landscape of malaria diagnostics with a particular focus on the requirement for secondary standards. Although microscopy remains the gold standard for the diagnosis of malaria in most regions, the number and quality of rapid diagnostic tests (RDTs) has rapidly increased in recent years. This has led to expanded access to malaria diagnosis in remote locations beyond the reach of microscopy services.

In 2018, an estimated 228 million cases of malaria occurred globally – the majority of which were caused by *Plasmodium falciparum*. However, *P. vivax*, *P. malariae*, *P. ovale wallikeri*, *P. ovale curtisi* and *P. knowlesi* are also known to frequently infect humans. Although speciation of the malaria parasite is incorporated into microscopic diagnosis in some developed countries, molecular methods for this purpose are largely unavailable in many parts of the world. WHO international reference standards to support malaria diagnosis have been established but only for *P. falciparum* and *P. vivax* as the two most prevalent species.

There are now more than 200 commercially available RDTs from 60 manufacturers. However, these are all based on the detection of a restricted set of antigen targets – namely, histidine-rich protein 2 (HRP2), lactate dehydrogenase (LDH) and aldolase. Dr Bowyer highlighted the success of the WHO-FIND malaria RDT evaluation programme in improving the quality of

malaria RDTs based on centralized evaluation at a single centre, primarily using patient-derived samples at defined parasitaemia. The mainstay of this system has been the use of a carefully calibrated reference collection of clinical isolates that has to be assembled each year at defined parasitaemia and calibrated to resemble previous collections. Although successful, this centralized approach is expensive with a number of inherent challenges and this had prompted investigation of the feasibility of developing a reference material framework to support a decentralized approach and to limit the need for the collection of clinical isolates. Dr Bowyer set out a new framework based on the use of cultured parasites and recombinant antigens to produce secondary standards calibrated in IU. This approach had been piloted collaboratively by NIBSC and FIND and Dr Bowyer concluded by outlining a number of preliminary findings and potential next steps.

Acknowledging the complexities in this area of diagnostics and its importance for public health, the Committee focused on whether the proposed approach was scientifically sound and whether it could support further development. The Committee supported the proposal in principle but was uncertain as to whether it should be involved in the development of secondary measurement standards. The concern was also expressed that some RDTs appeared to be unreliable at low levels of parasitaemia. Reassurance was given however that the WHO prequalification scheme ensured that this was not an issue for the most widely used RDTs.

2.4 Feedback from custodian laboratories

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

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

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

Dr Celia Witten updated the Committee on the recent activities of CBER as a custodian laboratory for biological reference materials. Activities in relation to SARS-CoV-2 had included participation in collaborative studies to evaluate prospective nucleic acid and antibody international standards. Agency experts had also participated in various WHO working groups including those working in the areas of viruses, reagents and immunoassays; animal models; and a core protocol for vaccines. Dr Witten went on to outline recent activities in the development of reference materials for the detection of adventitious viruses in biological products using high-throughput sequencing. Five such reference materials were to be proposed for establishment at the current meeting (see section 8.1.1 below).

Dr Witten also informed the Committee that CBER was working in collaboration with NIBSC to develop universal reagents based on human and mouse mAbs for use in the potency testing of inactivated poliomyelitis vaccines (IPV). A collaborative study to assess these reagents was anticipated in 2021. In addition, progress had been made in the development of a WHO international reference reagent for *Babesia microti* DNA – a project that had been endorsed by the Committee in 2018. Together with the Paul-Ehrlich-Institut and NIBSC, CBER had also co-organized the WHO hearing on the development of product-specific reference materials for FVIII and FIX products, the main outcomes of which had earlier been presented to the Committee (see section 2.3.1 above).

Dr Witten further noted the increased focus now being placed on the development of standards for cellular and gene therapy products, and reported that a WHO consultation held in February 2020 had agreed to the development of a white paper on the fundamental principles and issues relating to the regulatory oversight of such products. The project was at an early stage and progress had slowed due to the impact of the COVID-19 pandemic. Dr Witten concluded by highlighting the planned involvement of CBER in a number of upcoming WHO written and measurement standards projects, including the revision of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated), and the evaluation of new and replacement WHO measurement standards for blood coagulation factors VIII, X and XIII.

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

Dr Laurent Mallet outlined a number of EDQM standardization and COVID-19-related activities of relevance to the Committee. Since May 2006, EDQM has acted as the custodian laboratory for WHO international standards for antibiotics, with 23 such standards currently available – 10 of which had been replaced since 2006. No issues with this group of standards had been identified since the previous Committee meeting.

Dr Mallet 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. Dr Mallet specifically updated the Committee on the progress made in developing a sensitive and robust method for the detection of procoagulant activity in human normal immunoglobulins for intravenous administration. Experimental work had now been carried out by three subgroups examining Factor XIa chromogenic

assays, thrombin generation assays and non-activated partial thromboplastin time tests. Results from the studies into the first two assay types were expected by the end of the year, with the testing of a protocol for non-activated partial thromboplastin time tests planned for early 2021.

Dr Mallet also reviewed the progress made in the validation of an in vitro method as an alternative to the in vivo challenge assay for rabies vaccine. Following a small collaborative study coordinated by the European Partnership for Alternative Approaches to Animal Testing, an enzyme-linked immunosorbent assay (ELISA) method based on the use of two well-characterized mAbs had been selected. This method detects most of the virus strains used worldwide for human rabies vaccine production and can differentiate between sub-potent and compliant vaccines. In addition, prospective updates to the European monographs for tetanus vaccines will shortly remove the requirements for the in vivo test for irreversibility of tetanus toxoid and the test for specific toxicity in guinea-pigs. Furthermore, an ongoing collaborative study was now exploring the applicability of the binding and cleavage (BINACLE) assay as an alternative to the mandatory guinea-pig test for the absence of toxin in tetanus-toxoid-containing substance preparations or vaccines.

Dr Mallet concluded by briefly reviewing EDQM activities in relation to the development of vaccines and treatments for COVID-19. The European Pharmacopoeia was currently providing free temporary access to relevant texts and, in light of the number of developers with only limited regulatory experience, is offering training materials on their application. Assurance was given to the Committee that a text on viral-vectored vaccines would become available very soon. In addition, preparations were being made by the network of official medicines control laboratories (OMCLs) for the Official Control Authority Batch Release of COVID-19 vaccines and human blood derived medicinal products.

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

Dr Christian Schneider informed the Committee of a recent reorganization at the Medicines and Healthcare products Regulatory Agency with his appointment as Chief Scientific Officer of the agency and Dr Marc Bailey taking over as interim Director of NIBSC. Dr Schneider will continue to head the WHO collaborating centre.

Dr Schneider then provided an overview of the fast-track development process that had led to the expedited preparation of candidate SARS-CoV-2 RNA and anti-SARS-CoV-2 immunoglobulin reference materials in March and April 2020 respectively. It was anticipated that the reports of the collaborative evaluations of these candidate materials would be submitted for consideration by the Committee in December 2020 (see sections 9.1.1 and 9.1.2 below). Such unprecedented speed of development could be attributed to the prioritization

of resources and to having the platforms already in place to respond to such emergencies. Dr Schneider stressed that the fast-track development of COVID-19 reference materials would not have been possible without the support of key collaborators including CEPI and WHO, and noted that the holding of ad hoc meetings of the Committee had been vital in this regard.

Dr Schneider then updated the Committee on three further biological standardization issues. First, following the establishment of the First WHO International Reference Panel for lentiviral vector copy number in 2019, NIBSC was producing additional data to elucidate the currently unexplained discrepancies in data generated by quantitative polymerase chain reaction (qPCR) and digital PCR, and to assess whether value assignment based on lentiviral vector copies per cell is appropriate. It was envisaged that NIBSC would bring a revised proposal to the Committee in 2022. Second, NIBSC was carrying out an investigation into the stability of the Fourth WHO International Standard for tetanus vaccine following reports from a vaccine manufacturer of a shift in the effective dose 50 (ED₅₀) response. Such a shift (as opposed to a trend) would suggest an assay issue rather than a stability issue – a view supported by a review of internal data monitoring by three European Union OMCLs which indicated no stability issues. However, NIBSC will continue to work with the customer and with EDQM to resolve the problem. Finally, Dr Schneider reported that a Vi-poly-L-lysine assay developed by NIBSC had been found to be a suitable alternative to the commercial VaccZyme assay for the measurement of anti-Vi immunoglobulin in human serum and for the evaluation of typhoid vaccines based on the Vi antigen.

2.5 Cross-cutting activities of other WHO committees and groups

2.5.1 Feedback from the 55th meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations

Dr Sabine Kopp summarized the outcomes of the 55th meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP) which had been held virtually during the previous week. The ECSPP had been updated on a number of cross-cutting issues by other WHO groups and partner organizations, including the latest efforts to ensure that manufacturers and inspectors address the critical issue of antimicrobial resistance. The ECSPP had also reviewed several new and revised specifications and general texts for the quality control testing of medicines for inclusion in the International Pharmacopoeia. Following discussions, 10 guidance texts and 17 pharmacopoeial texts had been recommended for adoption. A number of proposals for new and updated quality assurance and regulatory guidance documents had also been reviewed. Further details on selected meeting outcomes would be presented separately to the Committee (see sections 2.5.2–2.5.4 below).

In response to an enquiry from the Committee regarding the impact of the COVID-19 pandemic on WHO activities in this area, Dr Kopp highlighted the close collaboration taking place with colleagues in the inspection team to manage the increased workload. Dr Kopp further noted that a number of pharmacopoeias had made relevant monographs available free of charge. The Committee also asked if there were any plans to revise the monographs for angiotensin receptor blockers to include mandatory nitrosamine testing. Dr Kopp provided assurance that although there were no monographs for these products at present the query had been duly noted and the Committee would be updated on developments.

2.5.2 **Good reliance practices in regulatory decision-making for medical products**

Dr Marie Valentin informed the Committee that a high-level document covering reliance activities in the field of regulatory oversight of medical products had been recommended for adoption at the 55th meeting of the ECSP. Dr Valentin outlined the importance of international cooperation in ensuring the safety, quality and performance of medicines. So-called good reliance practices (GRoP) allow for the optimal use of available resources, prevent duplication of efforts and help to focus regulatory activity where it is most needed. The importance of regulatory reliance had recently been highlighted by the demands of the COVID-19 pandemic. The WHO guidance document recommended for adoption addresses all regulatory functions as defined in the WHO Global Benchmarking Tool and spans the full life-cycle of a medical product. The key concepts and principles of GRoP had been set out along with a number of general considerations.

In response to questions from the Committee, Dr Valentin explained that it was envisaged that reliance could be achieved through either unilateral or mutual agreement, but that even a unilateral regulatory decision must be informed by regulatory documentation and verification of the sameness of the medical product. Dr Valentin also confirmed that the guidance document includes a section on regulatory competence that aims to support regulatory capacity-building and noted that regulatory reliance would be critical in the approval of COVID-19 vaccines. The Committee noted that reliance processes also benefit from open discussion of the differences between agencies – a matter that could potentially be addressed in subsequent revisions of the document.

2.5.3 **Good regulatory practices for regulatory oversight of medical products**

Dr Valentin further informed the Committee that a new WHO guidance document on good regulatory practices (GRP) had also been recommended for adoption at the 55th meeting of the ECSP. The purpose of this document

was to set out the high-level principles of GRP and to serve as a benchmark for countries in implementing GRP for medical products. The document will be relevant to all regulators regardless of resource level and capability and will help to establish effective oversight of medical products thereby promoting trust between regulators and other stakeholders.

The Committee acknowledged the importance of both the GRiEP and GRP documents and reflected on the challenges of promoting trust between agencies. Commenting on the observation that agencies in different jurisdictions often reached different regulatory outcomes, Dr Valentin felt that this was not necessarily a problem as risk–benefit considerations also varied from one region to another. With regard to the implementation of both GRiEP and GRP, particularly in LMIC, the Committee suggested that a network or forum might usefully be established to share ideas and overcome barriers.

2.5.4 Update on the WHO-listed authorities framework

Dr Hiiti Sillo informed the Committee that a policy document defining a WHO-listed authority (WLA) had been recommended for adoption at the 55th meeting of the ECSP and a framework had been developed for the transparent and evidence-based designation of regulators as WLAs. This will be followed by the development of operational guidance, including a performance evaluation framework and standard operating procedures.

Although the strengthening of regulatory systems remains the primary focus for WHO, the practice of regulatory reliance based on trust and confidence among agencies will be crucial in meeting the challenges posed by the current global regulatory environment. The WLA concept will encourage the continuous improvement of regulatory systems and the efficient use of resources, ultimately accelerating access to safe, effective and quality-assured medical products for all.

The Committee recognized the significant amount of work behind the development of the WLA framework. Although at present the framework was limited to medicines and vaccines, Dr Sillo noted that it would likely be expanded to include diagnostics and medical devices in the future. In response to further queries, Dr Sillo clarified that a minimum maturity level of 3 using the WHO Global Benchmarking Tool was required to become a WLA, though agencies would be able to apply by specialist function or product area. Further details on this and related aspects would be provided in the operational guidance in due course.

2.5.5 Progress report from the Global Advisory Committee on Vaccine Safety

Dr Christine Guillard updated the Committee on the progress made since the extraordinary GACVS meeting on COVID-19 vaccine safety in May 2020.

Meeting session topics had included COVID-19 vaccines in the pipeline and potential safety issues, the application of standardized templates for the risk–benefit assessment of vaccines, and vaccine risk–benefit communication and infodemic management during the COVID-19 response.

Dr Guillard outlined the development of a WHO COVID-19 vaccine pharmacovigilance preparedness guidance manual. Four working groups had been established and were meeting weekly to prepare a draft text for public consultation with a view to submitting the final version to GACVS in late 2020. Currently, the draft guidance comprises nine chapters on:

- COVID-19 vaccine platforms and their characteristics and safety profiles
- addressing adverse events
- regional and country preparedness for vaccine introduction
- stakeholders in pharmacovigilance and their roles and responsibilities
- establishing adverse event surveillance systems in countries
- responding to adverse events following vaccination
- establishing active surveillance systems during vaccine introduction
- data management
- COVID-19 vaccine safety communication.

Dr Guillard concluded by setting out the timeline for the further development, review and dissemination of the guidance manual. The final text would be reviewed at the next GACVS meeting in December 2020 and a global webinar held immediately after its broad dissemination. Training modules would also be developed to support implementation of the guidance.

2.5.6 Update on INN nomenclature for biologicals

Dr Raffaella Balocco updated the Committee on the use of international nonproprietary names (INN) for biologicals. The INN system had originally been developed to assign nonproprietary names primarily to small molecules but its continual adaption meant that it now encompasses more complex biological substances such as mAbs, nucleic acid based substances and cell-based therapeutics. Dr Balocco highlighted the steady increase over time in the overall number of INN requests received, including a disproportionate increase in the number received for biological medicines. Over the past decade, the annual number of INN applications made at each Consultation of International Nonproprietary Names had grown from around 80 to more than 200 in 2020. In recent years, requests for INN for cell therapies and cell-based gene therapies have contributed to this increase in biological INN requests. With

the development of new technologies such as CRISPR/Cas9 gene editing and personalized medicine the field was becoming ever more complex.

The INN programme was adapting to this increased complexity by adjusting the nomenclature scheme and revising the application process. The harmonization of INN definitions for cell-based substances was under way and a revised application form for INN for such substances had been approved at the 70th Consultation of International Nonproprietary Names in April 2020 and published on the INN programme website.

Acknowledging the importance of the work carried out by the INN programme, the Committee briefly discussed the challenge of developing a nomenclature for gene editing technologies.

2.5.7 Recent WHO activities in relation to snakebite envenoming

Dr David Williams briefed the Committee on recent work carried out in the area of snakebite envenoming. Despite the COVID-19 pandemic, progress had continued to be made, including the finalization of risk–benefit assessment technical reports for sub-Saharan African antivenom products. These reports indicate that the range of antivenom products is incomplete, with some pan-African polyvalent products lacking efficacy against particular venoms. In addition, some manufacturers of polyvalent products could not be confirmed to be compliant with current good manufacturing practices. WHO had also submitted a proposal to the Wellcome Trust for resources to support: (a) risk–benefit assessments of up to 13 antivenom products in Asia; and (b) the development of a pilot stockpile of WHO-recommended *Echis ocellatus* antivenom.

Dr Williams then set out some of the upcoming planned activities of the WHO antivenoms team. A new snakebite envenoming website – www.who.int/health-topics/snakebite – would be launched at the end of 2020 to: (a) bring together all related WHO resources into a single health-topic portal; (b) increase the accessibility and functionality of such resources for users; and (c) facilitate regular revision. The website will include a searchable snake distribution database, information on facilities in countries and the estimated length of time required to reach a treatment facility from a given location.

WHO also plans to revise its 2016 Guidelines for the production, control and regulation of snake antivenom immunoglobulins. The revised document is expected to be presented to the Committee for its consideration in 2021. In addition, a target product profile for sub-Saharan Africa will be developed to address the current lack of readily available guidance that defines both the preferred and minimally acceptable characteristics of products intended for both region-specific and species-specific deployment. Finally, the potential development of a WHO prequalification procedure for snake antivenom immunoglobulins was being investigated. This procedure will likely involve

determination of the minimum species coverage requirements for particular products and evidence-based establishment of minimum neutralizing potency against specific venom actions (for example, median effective doses against necrotic, haemorrhagic or procoagulant actions).

The Committee recognized the need to ensure the quality and consistency of antivenoms but questioned whether it was the right time to introduce WHO prequalification. Dr Williams clarified that, in practice, such prequalification was unlikely to be implemented within the next 3 years. The Committee also felt that the regional diversity of snake species and associated regional specificity of venoms would present a challenge for the prequalification of antivenom products intended for global distribution.

2.6 General matters of the Committee

2.6.1 Procedural aspects of WHO biological standardization activities

The Committee was provided with an overview of two draft documents prepared by the WHO Secretariat on the procedural aspects of WHO biological standardization activities. Once finalized, the first of these documents was intended for internal use by WHO and would set out in detail the function and procedures of the Committee, and the roles and responsibilities of its members. The second shorter document was intended to summarize the process through which WHO adopts and establishes its written and measurement standards, including the key issue of how various stakeholders can input into the prioritization and development of such standards. It was envisaged that ensuring the wide availability of the second document through its open dissemination on the public WHO website would serve to strengthen transparency among all stakeholders in this core WHO activity area.

Specific issues to be addressed included the need to increase awareness of the mechanisms for receiving stakeholder inputs and to improve reporting of the status of the written and measurement standards pipelines. In their capacity as members of WHO expert advisory panels, individual Committee members would be invited to review and comment upon both texts during their development.

3. International Recommendations, Guidelines and other matters related to the manufacture, quality control and evaluation of biological products

3.1 Biotherapeutics other than blood products

3.1.1 Development of WHO guidance on regulatory considerations in the evaluation of monoclonal antibodies used for the prevention or treatment of infectious diseases

A number of monoclonal antibody (mAb) products are now being used to prevent or treat infectious diseases, with many more currently in clinical development. Due to their short development time, rapid impact and established safety profile the potential use of mAbs as therapeutics for COVID-19 is considered a high priority. However, existing WHO guidance on mAbs largely focuses on therapeutic products for noncommunicable diseases. Despite commonalities in manufacturing strategies for recombinant mAbs regardless of intended use, current WHO guidance offers little advice on preclinical and clinical evaluation specific to mAbs used for infectious disease prevention or treatment.

At its previous meeting in October 2020 (see section 2.1.2 above) the Committee had expressed its support for a proposal to develop a WHO regulatory considerations document specifically on the evaluation of mAbs used against infectious diseases in order to both clarify the application of relevant existing WHO guidance and to highlight additional new manufacturing technologies or clinical requirements. An update on the progress made was duly provided to the Committee. The existing WHO guidance was now undergoing expert review to identify where additional guidance or clarity is required in relation to mAbs and antibody mimetic proteins used against infectious diseases. In order to broaden input into this review a consultation process had been initiated and drafting group members with appropriate regulatory experience were now being recruited. It was envisaged that a first draft document would be posted for public comment in early 2021.

The Committee was also updated on the outcomes of an initial review of existing WHO guidance relevant to mAbs used against infectious diseases. Although much of the current guidance relates to the production and regulation of mAb products there is little information provided on modern methods of mAb development and production, with direct implications for the evaluation of their quality and safety. In addition, the 1992 WHO Guidelines for assuring the quality of monoclonal antibodies used in humans were now outdated with a focus placed on the production of therapeutic antibodies from hybridomas – an approach unlikely to be used today. By contrast, the 2014 WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology remain up to date and contain information

relevant to the production of new mAbs for human use – though the primary focus is placed on products directed towards noncommunicable diseases rather than products intended for pre-exposure prophylaxis or treatment of infectious diseases.

When considered in combination with the more general WHO guidance on SBPs, good manufacturing practices for biologicals and the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products, the range of existing documents provides useful guidance on the regulation of recombinant DNA products – albeit with little tailored guidance on mAbs. Furthermore, specific guidance would seem to be required on: (a) mAbs used against RSV (see section 3.1.2 below); (b) mAbs used against SARS-CoV-2; (c) mAbs used to rapidly respond to emerging infections; and (d) mAbs as a general category of therapeutic in, for example, cancer treatment. A recent joint Wellcome and IAVI study on the accessibility of mAb-based products found that mAbs were predominantly licensed and available in high-income countries, with little availability in LMIC. In calling for greater global access to mAb products the study had identified the current lack of global regulatory harmonization as a significant obstacle to their wider availability. The Committee was further informed that during the WHO review it had become evident that new information would be required on the quality and manufacturing of mAbs regardless of their clinical application. Consequently, it was proposed that in addition to the regulatory considerations document on mAbs used against infectious diseases that WHO Guidelines on the quality and manufacturing of mAbs in general be developed.

Having been updated on the progress made and feedback received to date, the Committee reiterated its support for the continuation of this work. Following discussion of the important differences in clinical trial design for mAbs used for treatment compared to those used for prophylaxis, the Committee expressed its support for the proposed development of a WHO guidance document on regulatory considerations in ensuring the safety and efficacy of mAbs used against infectious diseases. This document should address common issues irrespective of the target pathogen and should encompass both prophylactic and therapeutic mAbs. It was also recommended that the format of the document should reflect the format used in recent WHO Guidelines, with sections covering nonclinical and clinical evaluation.

The Committee further acknowledged the need for significant updating of current WHO guidance in this area and expressed its support for the proposed development of separate WHO Guidelines on the manufacturing and quality assurance of mAbs regardless of clinical application. It was suggested that these guidelines should take into account the use of innovative production platforms, such as plant-based production methods, to promote research and development efforts under way in this area.

3.1.2 Development of WHO guidance on the quality, safety and efficacy of respiratory syncytial virus monoclonal antibodies

RSV is a major cause of lower respiratory infections worldwide, primarily affecting young children and the elderly. In recent years significant progress has been made in the research and development of prophylactic vaccines and mAb products against RSV. In 2016, the WHO Strategic Advisory Group of Experts on Immunization highlighted the public health importance of RSV disease and recommended strategies for its control. It further recommended that WHO should develop international guidance to support both regulators and the WHO prequalification process and to provide policy advice in this area. This led to the development of the WHO Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines which the Committee subsequently recommended for adoption in 2019. Furthermore, in light of the anticipated regulatory licensure and prequalification of mAb products against RSV corresponding WHO guidance was scheduled for development with support from the Bill & Melinda Gates Foundation.

In 2020, WHO commissioned a review of the global status of RSV mAb product development in order to identify the key scientific and regulatory issues to be addressed. In 1998, palivizumab had been approved by the FDA for the prevention of severe RSV disease in specified populations of infants and young children at high risk of infection. This product is currently available in high-income and some middle-income countries but not in low-income countries. Second-generation long-acting RSV mAb products were now in preclinical and clinical development. The extended half-life of these products will potentially protect infants over an entire RSV season following a single dose. Simplified and less expensive delivery requirements will also make them more likely to be suitable for use in LMIC and for all infants rather than just those at high risk. As discussed earlier in the meeting (see section 2.1.2 above) existing WHO guidance focuses on therapeutic mAbs for noncommunicable diseases and no WHO guidance is currently available specifically for mAbs used either therapeutically or prophylactically against infectious diseases. Increasingly, WHO was receiving requests for guidance on the quality, safety and efficacy of such products, which would also support the WHO prequalification process. In light of their potentially crucial role in rapidly responding to emerging infectious diseases, including those caused by the priority pathogens set out in the WHO R&D Blueprint as well as those for which no vaccines have been developed, the views of the Committee were now being sought.

Recalling its earlier discussion on the development of mAb products against SARS-CoV-2 (see section 2.1.2 above) the Committee reiterated its view that the overall approach should be to develop general regulatory guidance for mAbs used for the prevention or treatment of infectious diseases, with

consideration given to the development of separate disease-specific supplements on mAbs used against RSV and SARS-CoV-2.

3.1.3 **Outcomes of a WHO survey on the evolving regulatory landscape for similar biotherapeutic products**

It is anticipated that the increasing availability of SBPs worldwide will improve access to these medicines by increasing competition and bringing down prices. World Health Assembly resolution WHA67.21 on access to biotherapeutic products urges Member States and WHO to facilitate access to such products, while also ensuring their quality, safety and efficacy. Following the adoption of the WHO Guidelines on evaluation of similar biotherapeutic products (SBPs) in 2009, several workshops had been held to help countries incorporate the guiding principles set out in the Guidelines into their national regulations.

To assess the progress made during the past 10 years, and to identify challenges and country needs in the evolving regulatory landscape of SBPs, two WHO surveys had been conducted. The first of these, conducted in 2019, focused on the regulation, terminology, approval and development of SBPs. Based on analysis of the results it was concluded that considerable progress had been made since 2010. The WHO Guidelines had contributed significantly to setting the regulatory framework for SBPs within countries, increasing international regulatory convergence and improving consistency in the terminology used in the evaluation of SBPs. A range of SBPs had subsequently been approved in all participating countries.

The second survey, conducted in 2020 and involving 21 countries in all six WHO regions, identified a number of ongoing regulatory challenges in this area, along with potential solutions. Many of the reported obstacles to the development and regulation of SBPs relate to the reference biotherapeutic product (RBP). Such obstacles include limited access to information on the RBP, the high price of RBPs and insufficient quantities of RBPs in the country. Potential solutions include the exchanging of product information among NRAs, acceptance of RBPs licensed and sourced in other countries and the avoidance of unnecessary duplication of tests. In addition, the commonly reported lack of resources among NRAs might be addressed in the short term by sharing both workload and information, particularly in cases where another NRA had already assessed a particular product. Although a WHO project to prequalify selected SBPs would also benefit countries with insufficient regulatory capacity, this too was not viewed as an alternative to regulatory capacity-building in the longer term.

A further challenge identified during the survey was the quality of biotherapeutic products that were neither originator products nor SBPs but which had nevertheless been approved in several LMIC. Such products have

not been developed with reference to a licensed RBP as a comparator and may not have been compared clinically. The regulations governing these products are not consistent with the WHO Guidelines, which state that biotherapeutic products not shown to be similar to an RBP in a head-to-head study should not be described as “similar” or “biosimilar”. To address this, WHO recommends that NRAs reassess products approved under their pre-existing regulations to ensure they meet current regulatory expectations. Other challenges include inconsistencies in the designation of interchangeability and in the naming and labelling of SBPs, which are all crucial elements in ensuring effective pharmacovigilance.

The Committee was updated on the planned work of WHO in this area during 2021–2022 which would include the development of criteria to reduce unnecessary bridging studies and the revision of the 2009 WHO Guidelines on evaluation of similar biotherapeutic products (SBPs). As a first step in the revision of the Guidelines it was intended that an expert review would be conducted of the accrued experience, scientific considerations and evidence now available in this area. The Committee expressed its support for the planned activities outlined, including the proposed expert review (see section 3.1.4 below).

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

At its meeting in October 2020 (see section 3.1.3 above) the Committee had expressed its support for a proposed review of the current scientific evidence and experience gained in the regulatory evaluation of SBPs to inform the updating and revision of the 2009 WHO Guidelines on evaluation of similar biotherapeutic products (SBPs). At its meeting December 2020 the Committee was informed that this review was now in progress. The opportunity had been taken to review new developments and identify areas where the current guidance could be more permissive without compromising its basic principles, and where additional explanation could be provided regarding the possibility of reducing the amount of data needed for regulatory approval. The review had taken into account a number of national and regional guidelines and a number of sections in the current WHO Guidelines had been identified for potential updating and revision.

Analysis of the regional guidelines from Australia, Canada, Europe and the USA had indicated the feasibility of reducing the regulatory burden associated with the approval of SBPs. In particular, clinical data requirements could be reduced based on existing principles subject to new insights on the precise role of clinical safety and efficacy studies. However, any proposed reduction in clinical safety and efficacy requirements would inevitably require an increased

emphasis on quality and in vitro functional and human pharmacokinetic data. It was therefore proposed that the quality section of the current WHO Guidelines should be revised to provide more detail on the methods used to ensure a high level of similarity with the reference product. Key aspects to be covered would include the defining of acceptance ranges for product characterization, the comparison of reference products sourced from different jurisdictions and the risk assessment of quality attributes. In addition, the nonclinical section would be revised to reflect current practices and to provide more information on in vitro functional testing. Furthermore, the clinical section would be revised to more clearly describe the stepwise progression of clinical studies and to better reflect the experience gained in the last decade. It was intended that the revision of the WHO Guidelines would result in greater flexibility and reduced regulatory burden while continuing to ensure the quality, safety and efficacy of SBPs.

The Committee was informed that as the current WHO Guidelines had been adopted prior to the development of similar mAb products such products had not been included as examples, and this would also be addressed in the revised text. In addition, to ensure consistency across the range of related documents, WHO guidance specific to mAbs adopted after 2009, including the WHO Questions and Answers document on SBPs, would be revised in 2022. Having been updated on progress in this area, the Committee noted that the review of existing regional guidance had been comprehensive and reiterated its support for the continuation of the revision process outlined.

3.2 **In vitro diagnostics**

3.2.1 **Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics**

The assessment of applications for the approval and registration of biological products by NRAs is an essential step in ensuring their quality, safety and performance before they come to market. Since 2004, collaborative procedures based on reliance, work-sharing and joint review have been undertaken by WHO and NRAs to expedite national regulatory decisions on vaccines – with such procedures expanded in 2012 to encompass medicines. To date however such collaborative approaches have not been applied to the assessment and national registration of IVDs.

The proposed collaborative procedure submitted to the Committee for its consideration is intended to accelerate national regulatory processes relating to WHO-prequalified IVDs by allowing participating NRAs to take into consideration the outcomes of WHO prequalification dossier assessments, performance evaluations and manufacturing site inspection reports. The four key principles underlying the collaborative procedure are: (a) the voluntary

participation of NRAs and manufacturers; (b) the assessment by NRAs of the same product and same registration dossiers as those used for WHO prequalification; (c) the sharing of confidential information between WHO and participating NRAs to support NRA decision-making; and (d) monitoring by both the NRA and WHO to maintain harmonized product status. To assess the feasibility and impact of the collaborative procedure a pilot study had been conducted in six African countries. Two countries had been able to register the WHO-prequalified product within 90 days, demonstrating the utility of the collaborative procedure in accelerating national registration processes and thus facilitating the timely availability of IVDs.

Use of the procedure will potentially benefit all parties. NRAs will have access to WHO prequalification reports that are not in the public domain to support their decision-making process and save internal resources. The burden on manufacturers will also be reduced as the information to be submitted to NRAs will essentially be the same as that already submitted to WHO during prequalification, leading to faster and more predictable registration timelines. In addition, WHO will receive valuable feedback on its prequalification outcomes thus allowing for the improvement of its processes, while the timely availability of IVDs will result in quicker access to such products by health care workers and patients. The Committee was informed that the document had now been through two rounds of public consultation and had been amended to reflect the comments received.

The Committee expressed its broad support for what it agreed was a very useful document and recommended that it should be shared with the Strategic Advisory Group of Experts on In Vitro Diagnostics at the earliest opportunity. Although such a procedural resource did not typically come under its remit, the Committee welcomed its submission for review. After making a number of minor changes the Committee recommended that the document WHO/BS/2020.2399 be adopted and annexed to its report (Annex 4).

3.3 Vaccines and related substances

3.3.1 Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines

Typhoid fever continues to be endemic in many LMIC, particularly where access to safe water and basic sanitation is limited. Estimates of global disease burden range from 11 to 21 million cases and 145 000 to 161 000 deaths annually. Vaccines based on the Vi polysaccharide antigen have been available for several decades but have the same limitations as other polysaccharide vaccines in that they are poorly immunogenic in the young, confer short-lived immunity and cannot be boosted. Typhoid conjugate vaccines (TCVs) were developed to overcome these shortcomings and in 2013 the WHO Guidelines

on the quality, safety and efficacy of typhoid conjugate vaccines were adopted. Since that time, several TCVs have been licensed and more are in development. The revised document therefore provides recommendations for the evaluation of such vaccines rather than the previous guiding principles. Other important developments reflected in the updated text include the establishment of WHO Vi antigen and antibody measurement standards, publication of a WHO Strategic Advisory Group of Experts position paper on the use of TCVs, approval of funding for TCV introduction in Gavi-eligible countries and the WHO prequalification of Typbar-TCV in 2017.

The document presented to the Committee for its consideration reflects the impact of these and other developments on the production and quality control of TCVs, as well as on their nonclinical and clinical evaluation. Key changes made in the revised document include: (a) the addition of a section on the three associated WHO international standards established since 2013; (b) incorporation of recent advances in quality control methods; (c) modification of the section on animal challenge studies to reflect the development of human challenge studies; and (d) amendment of the clinical evaluation section to reflect the approval and WHO prequalification of TCVs in a number of countries. The Committee was informed that ongoing studies of immune responses and the search for an immune correlate of protection may necessitate the future updating of the clinical evaluation section as new data become available.

In addition, following the decision of the Committee in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing requirements for all vaccines in WHO written standards for biological products, the test is no longer included in the manufacturing recommendations provided in the current document. Furthermore, the document also makes reference to the recently updated WHO Guidelines on clinical evaluation of vaccines: regulatory expectations.

The Committee agreed that important developments in this area had warranted the full revision of the existing guidance and its change in status to WHO Recommendations. A brief discussion took place on whether the document should mention the biosafety level required for the production of the Vi polysaccharide. It was agreed that as this was covered by existing general WHO guidance on good manufacturing practices for pharmaceutical and biological products it did not need to be included in vaccine-specific guidance. Acknowledging the need to incorporate the 3Rs principles into all WHO written standards for biological products, the Committee supported the inclusion of the monocyte activation test as an in vitro alternative to the rabbit pyrogenicity test. Furthermore, the Committee suggested text to make it clearer that the need for pyrogenicity testing should be based on risk assessment. After making a number of additional minor changes to the text the Committee recommended that the document WHO/BS/2020.2387 be adopted and annexed to its report (Annex 2).

3.3.2 Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated)

Enterovirus 71 (EV71) is associated with hand, foot and mouth disease (HFMD) throughout the world and has caused epidemics in Asia, Europe and North America. Manifestations of the disease range from asymptomatic infection to mild HFMD to neurological disease with severe central nervous system complications and cardiopulmonary failure. In severe cases mortality rates can be high, especially in children aged 5 years and younger. From 2008 the number of cases of HFMD increased in China where it became a major public health problem. Several vaccines against EV71 were currently under development and three inactivated EV71 vaccines have been licensed in China. Since 2016 around 72.6 million doses of EV71 vaccine had been administered to children with no serious adverse reactions observed. Compared with the combined number of deaths from HFMD during the period 2010–2015, the number of deaths in 2018 and 2019 fell by 93% and 96% respectively. A number of other EV71 vaccines were now being developed in China and other countries.

Following discussions with regulators and other stakeholders a drafting group had been established to produce the first WHO written standard for inactivated EV71 vaccines. The resulting WHO Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated) take into account existing WHO guidance on the evaluation of similar vaccines (such as IPV and hepatitis A vaccines) and refer to recently established WHO international standards for anti-EV71 serum and EV71 inactivated vaccine. The document provides recommendations to regulators, vaccine developers and manufacturers on the research, evaluation, manufacture and quality control of EV71 vaccines based on chemically inactivated whole EV71 produced in cell culture but does not cover recombinant and other forms of subunit vaccines, vectored vaccines, virus-like particles or bivalent EV71-CA16 vaccines. The document takes into account current WHO guidance on the nonclinical and clinical evaluation of vaccines, good manufacturing practices for biological products, characterization of cell banks, nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines, and lot release. Specific issues addressed include: (a) the extent to which a vaccine based on one viral genogroup will protect against other genogroups; (b) the lack of a convenient and reliable animal model; and (c) the potential for potency assay complications resulting from the existence of both full and empty particle forms in products.

Noting that the EV71 vaccines used to date appear to be efficacious and safe with post-marketing surveillance revealing only mild reactions, the Committee enquired about the effectiveness of such vaccines in clinical use. It was informed that effectiveness data would be published soon indicating a 93% reduction in disease incidence. This impact was attributed in part to the level of

herd protection afforded by the vaccine. The Committee accepted that there had been a dramatic decline in the number of cases but felt that the situation might be complicated by the emergence of other viruses that cause HFMD and that this would likely depend on how the vaccine was used.

The Committee then reviewed the text of the proposed Recommendations in detail, noting the comments received during the public consultation process – most of which had related to Part A. Referring to the Cutter incident in the 1950s, which had been caused by the incomplete inactivation of a poliomyelitis vaccine, the question of exactly when during the manufacturing process the EV71 virus should be inactivated was discussed at length. The Committee felt that the timing of filtration steps in relation to inactivation would be crucial in ensuring complete inactivation and was satisfied that two filtration steps would be adequate. This would be consistent with the filtration and inactivation of polioviruses used in the production of IPV. The relevant text of the document was modified accordingly. After making a number of further changes to the text, the Committee recommended that the document WHO/BS/2020.2388 be adopted and annexed to its report (Annex 3).

3.3.3 **Revision of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated)**

The current WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) were adopted in 2012. Since then there have been several developments in relation to the production and quality control of oral poliomyelitis vaccines (OPVs). In particular, genetically stable novel OPV (nOPV) production strains have been developed to prevent the emergence of circulating vaccine-derived polioviruses (cVDPVs). Several nOPVs against type 2 polioviruses were currently in clinical trials and were being considered under the WHO prequalification and EUL procedure. In addition, trials of nOPVs against type 1 and type 3 polioviruses were expected to commence shortly, while the feasibility of a trivalent nOPV was being evaluated in animal studies. In the context of the Global Polio Eradication Initiative, the introduction of nOPVs will be vital in preventing the emergence of cVDPVs.

In addition to the introduction of novel vaccine production strains, advances have also been made in the quality control methods for OPVs. Confirming the superior safety of nOPV relies on demonstrating the consistency of the vaccine strain at the molecular level. As incidental mutations can emerge, careful monitoring is currently required using both *in vivo* and *in vitro* methods. One recent collaborative study has now highlighted the potential utility of high-throughput sequencing technologies in monitoring the consistency of vaccine production. The application of such technologies may ultimately lead to the elimination of the *in vivo* mutant analysis by polymerase chain reaction and

restriction enzyme cleavage (MAPREC) test subject to further clarification and the development of agreed acceptability criteria for vaccine manufacturers and national control laboratories. Other developments include the recognition that the reproductive capacity at elevated temperature (rct40) test, which is also used to ensure the molecular consistency of OPV bulk, is insufficiently sensitive, with its requirement for wild-type control strains also complicating GAPIII compliance.¹⁰

Following the eradication of poliomyelitis, live poliovirus will continue to be required for many years for neutralization and other quality-control tests used to evaluate OPV and immunoglobulin products. In order to eliminate the risk of infection of laboratory workers and maintain GAPIII compliance without the need for expensive containment facilities, safer S19 poliovirus strains have now been designed which are hyper-attenuated and extremely stable genetically. Such strains will increasingly be used for the quality control of OPVs and related products.

The proposed revision of the WHO Recommendations would involve expanding the scope of the document to include more details on the use of nOPV and other safer production strains while comprehensively updating the sections on the manufacturing, quality control, and nonclinical and clinical evaluation of OPVs in line with the developments outlined above. The revision would be undertaken in consultation with OPV/nOPV developers, manufacturers, regulators and other stakeholders.

Following further discussion of some of the potential advantages of the developments outlined in this area, the Committee expressed its support for the revision of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated).

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

The current WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines were adopted in 2010. This document recommends that virus master and working seed lots should be tested for viscerotropism, immunogenicity and neurotropism in monkeys. Following reported discrepancies in the clinical scoring of monkeys during the assessment of working seed lots, one manufacturer had requested that the neurotropism assessment be aligned with that used during neurovirulence testing of OPV seed

¹⁰ 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. Geneva: World Health Organization; 2015 (https://apps.who.int/iris/bitstream/handle/10665/208872/WHO_POLIO_15.05_eng.pdf?sequence=1&isAllowed=y, accessed 29 December 2020).

lots. In this approach, clinical signs are recorded but do not form part of the assessment or pass/fail criteria. At its seventy-first meeting in August 2020, the Committee had agreed that a drafting group should be established to consult with as many manufacturers and other stakeholders as possible on the proposed amendment of the 2010 WHO Recommendations.

The Committee was updated on the progress made to date. A survey of five yellow fever vaccine manufacturers and four NRAs had led to a number of further issues being raised that would also need to be addressed during amendment of the WHO Recommendations. Currently the manufacture of all yellow fever vaccines is based on old technology involving the use of live attenuated viruses derived from the 17D lineage developed in the 1930s. Three substrains are used that are genetically and phenotypically distinct but are indistinguishable in terms of induced immune response. All such vaccines are currently manufactured in embryonated eggs and have a long history of safety and efficacy. However, some manufacturers are now developing cell culture production methods while others are investigating the use of different production platforms altogether. As the current WHO Recommendations are based exclusively on the use of live 17D virus produced in embryonated chicken eggs, new guidance would be needed to support the innovative approaches now being developed.

The requested alignment of the neurotropism assessment of yellow fever vaccines would be among the issues to be addressed in the amended WHO Recommendations. The currently specified approach is associated with several technical challenges including: (a) a paucity of data on the performance of the test; (b) the difficulties inherent in conducting a collaborative study involving non-human primates; (c) the lack of an international reference standard and consequent use of different reference materials; (d) reported discrepancies between clinical and histopathological assessments; (e) inconsistencies between staff in the scoring of clinical and histopathological observations; and (f) the sourcing of animals from different locations.

It was anticipated that work on the amended WHO Recommendations would commence in early 2021 and, following public consultation, a proposed text would be presented to the Committee for its review in October 2021. The Committee discussed the possibility of discrepant lots of yellow fever vaccine being placed on the market but concluded that as the neurovirulence test was performed on the seed lot, any discrepant lots would not be used in production. Moreover, evidence indicated that product lots were of consistent quality, suggesting that the discrepancies observed probably arose from the test itself. The Committee agreed that there was a need to address the issues identified in the current WHO Recommendations and supported the proposed timeline for their amendment.

3.3.5 Development of WHO guidance on regulatory considerations in the evaluation of messenger RNA vaccines

Novel RNA-based vaccine platform technologies offer the potential to very rapidly develop vaccines against priority pathogens during public health emergencies. Recently, this potential has been realized during the COVID-19 pandemic with messenger RNA (mRNA) vaccines entering clinical trials and in some countries receiving emergency use authorization. At its meeting in August 2020, the Committee had been provided with an overview of the current status of mRNA vaccine development and had expressed its support for the development of a WHO guidance document on regulatory considerations in the evaluation of such vaccines. At its December 2020 meeting the Committee was updated on the progress made in the development of the guidance document and was presented with a detailed outline of the current draft text and key associated issues.

Following the establishment of a WHO drafting group a pre-draft version of the proposed document had been circulated among a working group of experts drawn from academia, industry, regulatory agencies and other stakeholders. Specific issues highlighted for attention in the document had included the potential for immune activation resulting from the vaccine formulation itself, assessment of vaccine potency, and the crucial importance of nonclinical safety and proof-of-concept assessments. In addition, several concerns had been raised in relation to the proposed inclusion of an appendix specifically on COVID-19 mRNA vaccines, particularly given the rapidly evolving situation. Such an appendix was likely to contain speculative information, would likely be out of date by the time the document was adopted and would not be written from a regulatory perspective. Based on the feedback received from the working group, a first draft of the document had now been developed which would be further revised during a series of informal consultations and public reviews prior to its submission for consideration by the Committee in October 2021.

The Committee was informed that the development of guidance on this novel class of vaccine presented a number of challenges. While the general manufacturing methods for mRNA encapsulated in lipid nanoparticles are understood, details of the associated quality control methods are not publicly available. In addition, compared with other vaccine platform technologies, there is currently only very limited experience of appropriate nonclinical and clinical assessment methods. In relation to these and other issues highlighted the advice of the Committee was now being sought.

The Committee agreed that the scope of the document should be restricted to mRNA vaccines for the prevention of infectious diseases, including those based on self-amplifying technology, but that RNA-expressed mAbs

should not be included. Recognizing the rapidly changing situation with respect to COVID-19 vaccine development and evaluation, it also agreed that a COVID-19-specific appendix would not be appropriate or necessary as long as sufficient information was included within the general text to support the development of such vaccines. Conversely, given that the guidance was being developed in the context of the current COVID-19 pandemic, any specific issues identified and experience gained during the development of COVID-19 mRNA vaccines should be reflected in the main text and should be supported, where possible, by published scientific evidence.

The Committee further advised that guidance on a number of specific quality issues should be included, such as guidance on assessing the integrity of the mRNA, characterizing the mRNA in terms of the level of capping and polyadenylation, and ensuring the consistency of lipid encapsulation. Accepting that assessing the potency of mRNA vaccines would largely be based on the consistency of the product demonstrated by physicochemical methods, the Committee requested that the document also provide sufficient advice on the use of cell-based bioassays for potency assessment. The Committee concluded by expressing its broad support for the proposed approach to the nonclinical section, acknowledging the need for practical advice on assessing the safety and immunogenicity of candidate mRNA vaccines without introducing improbable theoretical concerns.

4. International reference materials – biotherapeutics other than blood products

All reference materials established at the meeting are listed in Annex 5.

4.1 WHO international reference standards for biotherapeutics other than blood products

4.1.1 Third WHO International Standard for interferon alpha 2b

Interferon alpha 2b (IFN α -2b) is a pleiotropic cytokine involved in the regulation of numerous antiviral, anti-proliferative, anti-tumour and immunomodulatory cellular activities via the JAK/STAT signalling cascade. Various therapeutic products are currently 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, and malignant melanoma. Although the clinical use of IFN α -2b has declined over time, interest in new therapeutic applications in the treatment of certain ophthalmic disorders and more recently of COVID-19 have been reported. The current Second WHO International Standard for interferon alpha 2b had been established in 1999 and stocks were now running low. In 2019, the Committee therefore endorsed a proposal to develop a replacement standard. Due to anticipated difficulties in sourcing new material, it had been proposed that a freeze-dried preparation of IFN α -2b lyophilized in 1995 and included in the previous collaborative study could serve as a replacement standard subject to demonstration of suitable stability and assignment of potency relative to the current international standard. The proposed candidate material (NIBSC code 95/656) was an *E. coli* expressed clinical-grade protein, similar to that used for the current international standard.

The approach taken was to calculate the potency of candidate material 95/656 relative to the current international standard using the antiviral assay data generated by laboratories that had evaluated both preparations during the previous international collaborative study. The resulting potency estimate was then verified by in-house studies using a reporter gene assay rather than traditional antiviral assays. The potency of 95/656 relative to the current international standard was estimated at 24 149 IU based on data from the antiviral assay, with a similar estimate obtained using the reporter gene assay. The potency of an additional preparation, that had also been included in the original collaborative study, was estimated in the same way and was found to be in good agreement, thus providing further assurance of the reliability of potency estimates derived in this way. The stability of the candidate material 95/656 was assessed in accelerated thermal degradation studies also using the reporter

gene assay. The candidate material was found to be highly stable when stored at the recommended temperature of -20°C .

The Committee enquired about comparability of the reporter gene assay with the long-established antiviral assay. Assurance was given that the reporter gene assay generated comparable results and was inherently less variable, producing consistently lower coefficients of variation (CVs) than the antiviral assay. The Committee considered the report of the study (WHO/BS/2020.2389) and recommended that the candidate material 95/656 be established as the Third WHO International Standard for interferon alpha 2b with an assigned unitage of 24 000 IU/ampoule.

4.1.2 First WHO International Standard for bevacizumab

Vascular endothelial growth factor 165 (VEGF165) is an important molecule required for the highly complex and coordinated process of angiogenesis. The upregulation of VEGF165 expression drives pathological angiogenesis as well as vascular leakage in cancers and eye neovascular disorders. Consequently, VEGF165 is recognized as a key target for anti-angiogenic therapy and four different VEGF165 antagonists, including bevacizumab, have been approved for human use. Bevacizumab, the first VEGF165 antagonist to be developed, is a full-length recombinant humanized mAb produced in Chinese hamster ovary (CHO) cells. The expiration of its patent in the USA, and imminent expiration in Europe, has resulted in bevacizumab becoming a target for developers of SBPs. Two such SBPs have now been licensed and a number of further products are currently in late-stage clinical development worldwide. Versions of anti-VEGF165 mAb products, including non-originator products, are already available in Argentina, India and the Russian Federation. It was envisaged that the establishment of a WHO international standard would facilitate global harmonization of the *in vitro* potency assessment of bevacizumab products and support monitoring of their bioactivity throughout their life-cycle. Based on its categorization as a VEGF165 antagonist, the Committee had endorsed a proposal to develop a WHO international standard in 2016.

An international collaborative study involving 25 laboratories in 11 countries was conducted to evaluate the suitability of a manufacturer-donated candidate material (NIBSC code 18/210) to serve as a WHO international standard for bevacizumab bioactivity. The study evaluated both VEGF165 neutralizing and VEGF165 binding activities in a range of cell-based bioassays and direct binding assays. Study results indicated that all of the study preparations exhibited both VEGF165 neutralizing and binding activity. The inter-laboratory variability of potency estimates was significantly reduced when the candidate material was used in comparison with in-house reference standards. Limited data based on storage of the pilot fill of the candidate material for 26 months

indicated that it was likely to be stable; however, stability monitoring of the material will continue.

The Committee commented that this standard was likely to be in high demand and after considering the report of the study (WHO/BS/2020.2391) recommended that the candidate material 18/210 be established as the First WHO International Standard for bevacizumab with an assigned unitage of 1000 IU/ampoule for VEGF165 neutralizing activity and 1000 IU/ampoule for VEGF165 binding activity. It was noted that this international standard was intended for use in controlling bioassay performance, supporting the calibration of secondary or local standards and facilitating the harmonization and consistency of potency assessment. 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 bevacizumab products or to serve as a reference product for biosimilarity determination.

4.1.3 First WHO International Standard for trastuzumab

Trastuzumab is a therapeutic mAb administered either as a monotherapy or combined with other chemotherapies for the treatment of breast and gastric cancers. It selectively inhibits the signalling of human epidermal growth factor receptor 2 (ErbB-2, also known as HER2) and is therefore most effective against cancers associated with high levels of HER2 expression. Trastuzumab also seems to exert anti-tumoral effects through several mechanisms not yet fully elucidated, including the downregulation of HER2 expression, promotion of its degradation, and inhibition of growth and cell proliferation, as well as through mAb Fc effector functions, such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and FcγRIIIa binding activity.

Trastuzumab is on the WHO Model List of Essential Medicines. Since its patent expired, five SBPs have been approved with more than 30 others currently under development worldwide. In emerging markets, a number of non-originator versions of the mAb have also been approved using local regulatory pathways that are not always consistent with the published WHO guidance on SBPs which calls for a rigorous comparability exercise. The *in vitro* bioactivity of trastuzumab is routinely measured by the manufacturer during production and quality control using proprietary reference materials and bioactivity units. Trastuzumab products are, nevertheless, dosed and labelled in mass units with no reference to its biological activity. Suitably calibrated bioassays are essential for both the regulatory approval of post-manufacturing process changes and assessment of novel SBPs. Despite this, there are currently no higher order reference preparations for calibrating either local potency standards or trastuzumab bioassays.

It is intended that the WHO international standard will define bioactivity in IU and facilitate the harmonization and traceability of bioactivity data, in turn supporting the development of products that are consistent in quality and efficacy, both pre- and post-marketing, across the world. A preparation of trastuzumab expressed in CHO cells was assessed for its suitability to serve as a WHO international standard in an international collaborative study involving 25 laboratories worldwide. Of these, 18 assessed the inhibition of proliferation activity, 13 the ADCC activity and 7 the HER2 binding activity of the candidate material (NIBSC code 19/108) and other study materials. For the measurement of ADCP and Fc γ RIIIa binding activity, only two laboratories returned data for each assay.

The overall results indicated that the candidate material 19/108 would be suitable to serve as a potency standard for trastuzumab. Use of the candidate material harmonized the inter-laboratory reporting of trastuzumab bioactivities using in-house potency assays for inhibition of proliferation (IOP), ADCC and HER2 binding activities. This contrasted with the poor inter-laboratory agreement of potency estimates observed when estimates were expressed relative to the in-house reference standards. Accelerated and real-time stability studies conducted over 14 months indicated no loss of activity in the candidate material when used in IOP, ADCC and HER2 binding assays.

The Committee considered the report of the study (WHO/BS/2020.2401) and recommended that the candidate material 19/108 be established as the First WHO International Standard for trastuzumab with an assigned unitage of 1000 IU/ampoule for IOP, ADCC and HER2 binding activities. It also recommended that the standard be assigned a unitage of 1000 IU/ampoule for ADCP and Fc γ RIIIa binding activities as, despite the limited data generated on these activities, this would facilitate the continuity of unitage when a replacement standard was eventually required. The Committee noted that this international standard was intended for use in controlling bioassay performance, supporting the calibration of secondary or local standards and facilitating the harmonization and consistency of potency assessment. 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 trastuzumab products or to serve as a reference product for biosimilarity determination.

4.1.4 **Sixth WHO International Standard for chorionic gonadotrophin (human)**

Human chorionic gonadotrophin (hCG) is produced by the developing embryo during pregnancy. Its early role is to support the corpus luteum thereby maintaining the levels of progesterone required for pregnancy. Urinary derived

hCG is an important biotherapeutic used to promote the final maturation of ovarian follicles and ovulation in the treatment of infertility, as well as in assisted reproductive technology. The measurement of hCG is used for pregnancy testing and for the diagnosis of ectopic pregnancy, in prenatal screening for Down's syndrome and as a marker for a range of other clinical conditions. The Committee was informed that stocks of the current Fifth WHO International Standard for chorionic gonadotrophin (human), which is widely used for the calibration of bioassays and immunoassays used to evaluate therapeutic hCG preparations, were almost exhausted.

A candidate material of purified intact hCG (NIBSC code 18/244) had been filled into ampoules and evaluated by both bioassay and immunoassay in an international collaborative study involving 16 laboratories in nine countries. Six laboratories took part in the bioassay arm of the study, which evaluated the candidate material against the current WHO international standard. Inter-laboratory agreement was good with an overall geometric mean of 158.5 IU/ampoule obtained for the candidate material (95% confidence interval = 153.5–163.8 IU/ampoule; inter-laboratory geometric coefficient of variation (GCV) = 5.6%). For the immunoassay arm of the study, 11 laboratories returned data on the potency of the candidate material; again relative to the existing international standard. Estimates were again in good agreement with an overall geometric mean of 185.8 IU/ampoule (95% confidence interval = 183.3–188.4 IU/ampoule; inter-laboratory GCV = 3.1%). The candidate material was also assessed relative to the WHO Reference Reagent for intact hCG (NIBSC code 99/688) to give an assigned content in molar units. The geometric mean of the immunoassay estimates obtained was 0.413 nmol/ampoule (95% confidence interval = 0.399–0.428 nmol/ampoule) with good inter-laboratory agreement once again observed (GCV = 7.4%).

No significant loss of activity was observed in samples of the candidate material stored at elevated temperatures for 7 months; indicating high stability. An assessment of commutability, using a difference-in-bias approach, indicated that the candidate material and the current standard behaved similarly in the immunoassays, with both materials commutable with patient samples in 11 out of 17 assay methods. In one laboratory the candidate material was commutable but the current standard was not, and in the remaining five laboratories neither material was commutable with human serum samples.

The Committee considered the report of the study (WHO/BS/2020.2395) and recommended that the candidate material 18/244 be established as the Sixth WHO International Standard for chorionic gonadotrophin (human) with assigned unitages of 159 IU/ampoule for bioassay and 186 IU/ampoule (corresponding to 0.41 nmol/ampoule) for immunoassay.

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

4.2.1 **Proposed First WHO International Standard for vascular endothelial growth factor 165 (human)**

The current WHO Reference Reagent for vascular endothelial growth factor 165 (VEGF165) is used primarily to calibrate and harmonize potency assessments of VEGF165 preparations. Such preparations serve either as therapeutic products in cellular and gene therapies or as critical reagents in the potency evaluation of VEGF165 antagonists, including those approved for the treatment of cancers and neovascular intraocular diseases. The above WHO international reference standard is therefore used by manufacturers and regulatory laboratories to control the performance of potency assays for both VEGF165 antagonist products and VEGF165 therapeutic preparations. The Committee was informed that as stocks of the current reference reagent are rapidly becoming depleted and limits were being imposed on its distribution, a replacement was now required. A bulk preparation of recombinant human VEGF165 expressed in Sf21 insect cells had now been filled and lyophilized and post-fill testing was in progress. It was proposed that an international collaborative study be conducted to assess the suitability of this preparation as a replacement for the existing reference reagent. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee noted that as WHO reference reagents are usually replaced with a WHO international standard, the current reference reagent would prospectively be replaced with a first international standard rather than a second reference reagent. The Committee felt that this was an otherwise straightforward replacement and endorsed the proposal (WHO/BS/2020.2396) to develop a First WHO International Standard for vascular endothelial growth factor 165 (human).

4.2.2 **Proposed Fourth WHO International Standard for thyroid-stimulating hormone (human, pituitary)**

The measurement of serum thyroid-stimulating hormone (TSH) by immunoassay is an important component in the diagnosis of thyroid disorders and in the subsequent monitoring of therapy. The Committee was reminded that the present WHO international standard, consisting of a native pituitary TSH preparation, is known to have commutability issues in current immunoassays, resulting in a lack of harmonization of laboratory measurements of TSH. A Committee for the Standardization of Thyroid Function Tests (C-STFT) had been established by the International Federation of Clinical Chemistry and Laboratory Medicine to address this issue based on the comparison of multiple assay methods using

a panel of clinical serum samples, with unitages defined by reference to the current WHO international standard. The current standard also continues to be used for the internal calibration of immunoassays for TSH and for quality control purposes.

Following continued demand for the current standard, which was established in 2003, there is now a need to replace the depleted stocks. In 2003, two preparations of pituitary TSH from the same batch of material had been prepared and evaluated as a potential replacement of the previous second WHO international standard. One of these preparations had become the current third international standard and it was now proposed that the second preparation be re-evaluated as its potential replacement in an international collaborative study. The anticipated main users of the replacement standard, and likely study participants, would be manufacturers of TSH immunoassays, and clinical and quality control laboratories. The replacement of the WHO international standard would also coincide with the development of a new serum panel by C-STFT. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

Noting the importance of replacing the current international standard and the clarity of the proposal, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a Fourth WHO International Standard for thyroid-stimulating hormone (human, pituitary).

4.2.3 Proposed Sixth WHO International Standard for follicle-stimulating hormone and luteinizing hormone (human, urinary)

The therapeutic product menotrophin is a mixture of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) extracted from the urine of postmenopausal women. Menotrophin is used for the treatment of infertility and for ovarian hyperstimulation in assisted reproductive technologies. Urinary FSH/LH activity is defined in IU and the current international standard is therefore essential for the correct potency labelling of therapeutic preparations. Stocks of the current standard established in 2012 are now nearing exhaustion and at the current rate of use will need to be replaced before mid-2022. It was intended that highly purified urinary FSH/LH donated by manufacturers would be evaluated in an international collaborative study as a potential replacement standard using two bioassays: the rat ovarian weight gain assay for FSH and the immature rat seminal vesicle weight gain assay for LH. A consensus unitage relative to the current international standard will be determined from the resulting data. Study participants would likely be menotrophin manufacturers or contract research laboratories that perform FSH/LH bioassays on behalf of manufacturers. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee commented on the importance of this widely used standard and after due consideration endorsed the proposal (WHO/BS/2020/2396) to develop a Sixth WHO International Standard for follicle-stimulating hormone and luteinizing hormone (human, urinary).

5. International reference materials – blood products and related substances

All reference materials established at the meeting are listed in Annex 5.

5.1 WHO international reference standards for blood products and related substances

5.1.1 WHO International Reference Reagent for anti-human platelet antigen-15b immunoglobulin G (human)

Alloantibodies against human platelet antigens (HPAs) cause fetal/neonatal alloimmune thrombocytopenia (FNAIT), a disease in which fetal/neonatal platelets are destroyed by immunoglobulin G alloantibodies from the incompatible, HPA-sensitized mother. Alloantibodies are also involved in platelet refractoriness (the repeated failure to achieve the desired level of blood platelets following transfusion) and in post-transfusion purpura (the delayed reaction to a transfusion caused by recipient alloantibodies to incompatible transfused platelet antigens). Identification of HPA antibody specificity is essential in the diagnosis and treatment of patients. In severe cases of thrombocytopenia requiring platelet transfusion, it is crucial that the transfused platelets are negative for the target alloantibody specificity. HPA antibody detection has thus become commonplace in blood transfusion centres and larger hospitals.

HPA-15 is immunogenic and alloantibodies against HPA-15b can be detected in patients receiving multiple transfusions and in mothers with FNAIT. The monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay is considered to be the “gold standard” for anti-HPA immunoglobulin G detection due to its high accuracy in identifying antibody specificity. However, despite some success in the harmonization of MAIPA assays, the detection of anti-HPA-15 antibodies can be unreliable and is associated with variable sensitivity due to the low concentration and lability of the CD109 glycoprotein on which HPA-15b is located. It is envisaged that the establishment of a minimum potency reference reagent for anti-HPA-15b would support validation of the sensitivity of the MAIPA assay used for its detection. The need for such an international reference material had been highlighted in 2017 following laboratory proficiency testing organized by NIBSC. Furthermore, in 2018, at the 19th International Platelet Immunology Workshop organized by the International Society of Blood Transfusion it had been concluded that a more standardized approach to the CD109 MAIPA assay was required.

An international collaborative study had therefore been conducted involving 25 laboratories in 16 countries to evaluate a freeze-dried, recalcified plasma preparation (NIBSC code 18/220) for its suitability as a minimum potency WHO international reference reagent for HPA-15b detection. Participants tested

doubling dilutions of the material in glycoprotein-specific assays used for the detection of anti-HPA-15 antibodies and recorded the highest dilution at which the antibody could be detected. Study results indicated that a 1 in 8 dilution should be assigned to candidate material 18/220 as a minimum potency, with a positive result at this dilution validating the sensitivity of the test method.

Stability studies indicated that the candidate material would be stable for long-term storage at $-20\text{ }^{\circ}\text{C}$ and would be sufficiently stable to allow for the shipment of ampoules at ambient temperature. As the material contained an anti-human leukocyte antigen (anti-HLA) component, the instructions for use (IFU) would make it clear that it was only to be used in methods that are glycoprotein CD109 specific or where it can be ensured that the anti-HLA antibodies would not cause a false-positive reaction (for example, following the pre-treatment of platelets to remove HLA epitopes).

Following discussion of the performance of a number of the study laboratories and of the dilution chosen to establish minimum potency, the Committee considered the report of the study (WHO/BS/2020.2390) and recommended that the candidate material 18/220 be established as the WHO International Reference Reagent for anti-human platelet antigen-15b immunoglobulin G (human) with a 1 in 8 dilution corresponding to the minimum potency for assay validation purposes.

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

5.2.1 Proposed Second WHO International Standard for blood coagulation factor XIII plasma

Blood coagulation factor XIII (FXIII) is a transglutaminase that circulates in plasma as a heterotetramer consisting of two A and two B subunits. The active A subunit functions by cross-linking fibrin thus stabilizing its structure. Congenital and acquired FXIII deficiencies are severe and potentially life-threatening bleeding disorders. The accurate measurement of FXIII is crucial for both diagnosis and characterization of the deficiency. In addition, the treatment of patients with plasma-derived or recombinant FXIII is vitally dependent upon use of the correct dosing. The current WHO international standard is used to standardize the measurement of FXIII potency (in terms of both activity and antigen content) in: (a) the plasma of patients for the purpose of diagnosis; and (b) in plasma-derived and recombinant therapeutic concentrates, and fibrin sealants. The Committee was informed that stocks of this international standard were now running low and, with approximately 200 ampoules dispatched each year, were anticipated to be exhausted by 2023.

It was proposed that an international collaborative study be conducted to evaluate a candidate material for its suitability as a replacement international

standard. The study, to be conducted in association with the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, would involve 10–15 laboratories worldwide. The source material will be plasma of a similar composition to the current international standard and will be obtained from NHS Blood and Transplant. The material would be calibrated relative to the current international standard using specific substrate-based assays for FXIII activity, A2B2 antigen and total FXIII-B subunit antigen. Calibration would also be carried out relative to locally sourced normal plasma pools to confirm the continuity of the IU. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee felt that the proposed collaborative study was straightforward and endorsed the proposal (WHO/BS/2020.2396) to develop a Second WHO International Standard for blood coagulation factor XIII plasma.

5.2.2 Proposed Ninth WHO International Standard for blood coagulation factor VIII concentrate

Blood coagulation factor VIII (FVIII) is an essential blood-clotting factor in the coagulation cascade and FVIII deficiency results in the severe bleeding disorder haemophilia A. Effective treatment is via substitution therapy using FVIII concentrated from donated plasma or recombinant products. The current WHO international standard is used in the measurement of FVIII activity and is vital for the diagnosis and treatment of haemophilia A. The Committee was informed that stocks of the current standard were running low and, with approximately 1200 ampoules distributed each year, were likely to be exhausted by 2023.

It was proposed that an international collaborative study be conducted to evaluate a number of candidate materials for their suitability to serve as a replacement standard. A large number of FVIII products have now been licensed based on a diverse range of formulations, including plasma-derived products, fourth generation recombinant products, and modified and extended half-life products. Potential replacement materials would be sourced from the manufacturers of therapeutic FVIII products. Calibration will be carried out using clotting and chromogenic assays in a multicentre study involving approximately 40 laboratories. Due to the wide variety of licensed FVIII products, a larger number of candidate materials than usual would need to be assessed and it was anticipated that several of these would only prove suitable as product-specific standards. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee discussed the prospect of replacing the current plasma-based international standard with a recombinant, modified or extended half-life material. It agreed that this was unlikely on this occasion but that the inclusion of such candidate materials would provide useful information on

their comparative performance against the plasma-based material, and may also inform the development of product-specific materials. Having confirmed the number and international distribution of the laboratories to be involved in the collaborative study, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a Ninth WHO International Standard for blood coagulation factor VIII concentrate.

6. International reference materials – cellular and gene therapies

All reference materials established at the meeting are listed in Annex 5.

6.1 Proposed new projects and updates – cellular and gene therapies

6.1.1 Update on the development of a First WHO International Reference Reagent for mesenchymal stromal cell identity for flow cytometry

Following its endorsement in 2018 of the proposed development of a First WHO International Reference Reagent for mesenchymal stromal cell identity for flow cytometry, the Committee was provided with an update of progress in this area. Mesenchymal stromal cells (MSCs) are multipotent “adult” stem cells found in skeletal and adipose tissue. Assessment of the identity of such cells is a key requirement for products entering clinical trials. In 2006, the International Society for Cell & Gene Therapy (ISCT) defined the minimum criteria recommended for use in determining MSC identity. However, a subsequent survey of producers highlighted considerable variations in the testing parameters and release criteria used for MSC marker detection by flow cytometry. Such variations are likely to have adverse affects on product consistency and quality, and thus on clinical outcomes, highlighting the need for appropriate international reference materials. Although more than 1000 clinical trials were now under way into the use of MSCs in the treatment of a wide variety of diseases, no such reference materials are currently available.

The Committee was informed of a recent successful collaborative study of a potential flow cytometry reference reagent. Fixed freeze-dried MSCs derived from human pluripotent stem cells (hPSCs) were tested for MSC markers using the in-house flow cytometry methods of each collaborating laboratory. A high degree of consistency between laboratories was observed in terms of the expression of both the positive and negative markers required for compliance with ISCT identity requirements. However, the current scale of production of an hPSC-derived MSC reference reagent would be limited and the repeated generation of MSCs from stocks of progenitors would potentially lead to drift and instability of the material. It was instead being proposed that the prospective MSC reference reagent be produced using MRC-5 cells, which have been shown to express the required profiles of MSC identity markers. However, it is currently not known if MRC-5 cells express MSC markers at the required levels or if they exhibit the immunomodulatory and anti-inflammatory properties of MSCs detected by functional assays. As MSCs derived from MRC-5 cells may therefore not be appropriate for potency assessment based on the use of such functional

assays, it was being separately proposed that MSCs derived from hPSCs be generated for investigation as a WHO international standard for this purpose (see section 6.1.2 below). The candidate reference reagent for MSC identity would be assessed in an international collaborative study involving 15–20 laboratories conducting flow cytometry using their own reagents, equipment and protocols.

Having been assured that the use of the prospective reference reagent would be restricted to flow cytometry, the Committee reiterated its support for the proposed development of a First WHO International Reference Reagent for mesenchymal stromal cell identity for flow cytometry.

6.1.2 **Proposed First WHO International Standard for mesenchymal stromal cells**

MSCs can be derived from a wide range of tissue types including bone marrow, adipose, umbilical cord and other birth-related tissue. In addition to the need to assess MSC identity (see section 6.1.1 above) numerous clinical trials exploring the use of MSCs for the treatment of a wide variety of different diseases have also highlighted the need for standardized MSC potency assays. Despite current consensus that MSCs function through conserved immunomodulatory and anti-inflammatory mechanisms, the effects of MSC origin on these functions are not clear. In 2013 the ISCT highlighted the need for a standardized potency assay based on the induction of the indoleamine 2,3-dioxygenase 1 (IDO1) gene following priming with interferon-gamma and tumour necrosis factor-alpha. The ISCT subsequently proposed the use a “matrix approach” to MSC standardization involving flow cytometry, RNA-based evaluation of gene expression and functional assays.

Following the recent development of MSCs derived from hPSCs, and in light of ethical issues and the inherent variability of MSCs derived from donor material, it was proposed that an hPSC-derived MSC standard be generated for use as a WHO international reference standard in assays for IDO1 response, lymphocyte differentiation and RNA-based assays of MSC marker expression. The candidate material would be assessed in an international collaborative study involving 15–20 laboratories and would be validated against primary human MSCs. The prospective standard was expected to be used by developers and manufacturers of clinical products during in-process testing for the purposes of quality assurance, and should help to ensure the consistent safety and efficacy of MSC-based therapies.

Noting the importance of functional potency assays in establishing the efficacy of MSC-based advanced therapy medicinal products, and the number of such products currently in clinical development, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a First WHO International Standard for mesenchymal stromal cells.

7. International reference materials – in vitro diagnostics

All reference materials established at the meeting are listed in Annex 5.

7.1 WHO International reference standards for in vitro diagnostics

7.1.1 Second WHO International Standard for insulin-like growth factor 1 (recombinant, human)

Insulin-like growth factor 1 (IGF-1) is produced primarily in the liver in response to growth hormone and elicits anabolic effects on a variety of cell and tissue types. The measurement of circulating IGF-1 is used in the diagnosis of growth disorders and various immunoassay methods are commercially available – the majority of which are calibrated to the WHO First International Standard for insulin-like growth factor 1 (recombinant, human). The Committee was informed that stocks of the current international standard were close to exhaustion and would need to be replaced to ensure the continued calibration of IGF-1 assays.

A candidate material (NIBSC code 19/166) of therapeutic grade recombinant human IGF-1 had been prepared for evaluation in a two-phase international collaborative study involving 16 laboratories in 11 countries. In Phase I, the candidate material was assigned a value in SI units against a primary calibrant using high-performance liquid chromatography assays. Phase II had then been conducted to provide confirmatory data on the immunoreactivity of the candidate material and to evaluate its suitability to serve as a WHO international standard for the calibration of commercially available IGF-1 immunoassays. Phase II had also been designed to assess the commutability of the candidate material with patient serum and plasma samples. In Phase I, 23 valid assays performed in eight laboratories produced an overall geometric mean IGF-1 content for the candidate material 19/166 of 33.0 µg/ampoule (expanded uncertainty = 30.5–35.6 µg/ampoule; $k = 2.36$). Phase II data indicated that the candidate material was immunoreactive and behaved similarly to the current international standard in the immunoassays used in the study. Phase II data also demonstrated the commutability of the candidate material with patient samples in nine of the 12 immunoassays performed. No significant loss of activity was observed in samples stored at elevated temperatures over a period of 9 months, indicating good long-term stability when stored at -20°C .

The Committee considered the report of the study (WHO/BS/2020.2393) and, having been assured of the representative international distribution of the participant laboratories, recommended that the candidate material 19/166 be established as the Second WHO International Standard for insulin-like growth

factor 1 (recombinant, human) with an assigned content of 33.0 µg/ampoule (expanded uncertainty = 30.5–35.6 µg/ampoule; $k = 2.36$).

7.1.2 **First WHO International Standard for herpes simplex virus type 1 DNA for NAT-based assays; and First WHO International Standard for herpes simplex virus type 2 DNA for NAT-based assays**

Herpes simplex virus (HSV) types 1 and 2 are large enveloped linear double-stranded DNA viruses belonging to the Alphaherpesvirinae (a subfamily of Herpesviridae) and are prevalent worldwide. HSV causes facial lesions in the form of cold sores and genital ulcers, and is an important pathogen of the central nervous system, manifesting either as meningitis or encephalitis. It is recommended that clinical diagnosis should be confirmed by type-specific laboratory testing as both prognosis and counselling depend upon the type of HSV infection. For the diagnosis of patients seeking treatment for genital ulcers or other mucocutaneous lesions, or central nervous system and systemic infections, nucleic acid amplification technique (NAT)-based assays are preferred due to their high sensitivity. The early accurate diagnosis and treatment of viral central nervous system infections decreases morbidity and mortality rates, especially among immunocompromised patients. Many diagnostic assays allow for the simultaneous detection of both HSV-1 and HSV-2, with most diagnostic laboratory assays reporting results in a qualitative format; though with significant variation in their sensitivity to each type. There is therefore a need for standardization to allow for the accurate determination of assay sensitivity for both HSV-1 and HSV-2.

An international collaborative study involving 25 laboratories had been conducted to evaluate the suitability of two candidate materials (NIBSC codes 16/368 and 17/122) to serve as WHO international standards for the calibration of secondary reference materials used in the standardization of NAT-based assays, and to assess their commutability. The candidate materials comprised lyophilized preparations of whole virus laboratory cultured strains of HSV-1 (16/368) or HSV-2 (17/122). The potency of each candidate material was determined using a diverse range of NAT-based assay methods consisting of both qualitative assays ($n = 12$) and quantitative assays ($n = 20$). All qualitative assays were commercial, with nine of the 20 quantitative assays developed in house. A wide range of amplification and extraction platforms were used.

Study results indicated that inter-laboratory agreement on combined mean potency estimates for high-titre samples using both qualitative and quantitative assays was improved when potencies were expressed relative to the candidate materials. However, it was also noted that the standard deviation of potencies for lower-titre clinical samples largely increased when calculated relative to both candidate materials. Further evaluation of the candidate

materials alongside a larger number of clinical samples (and using a wider range of assays) in an external quality assessment (EQA) scheme indicated a moderate improvement in harmonization across participating laboratories and methods when the clinical sample results were expressed relative to each candidate standard. Both candidates were assessed for stability – either for up to 24 months (16/368) or 21 months (17/122) – with no loss in titre observed.

The Committee highlighted the relative performance of the candidate materials in the collaborative and EQA studies and was assured that good harmonization of results had been observed in both studies. The Committee considered the report of the study (WHO/BS/2020.2392) and, after receiving confirmation that the source materials consisted of laboratory-cultured strains, recommended that: (a) the candidate material 16/368 be established as the First WHO International Standard for herpes simplex virus type 1 DNA for NAT-based assays with an assigned unitage of 7.19 log₁₀ IU/vial; and (b) the candidate material 17/122 be established as the First WHO International Standard for herpes simplex virus type 2 DNA for NAT-based assays with an assigned unitage of 7.31 log₁₀ IU/vial.

7.1.3 **First WHO International Standard for West Nile virus lineage 1 RNA for NAT-based assays; and WHO International Reference Reagent for West Nile virus lineage 2 RNA for NAT-based assays**

West Nile virus (WNV) is a flavivirus whose global distribution is maintained through a transmission cycle involving mosquito vectors and birds. The virus was first isolated in the West Nile district of Uganda in 1937 and is commonly found in Africa, Europe, the Middle East, North America and West Asia. Over the last 20 years a substantial increase in the incidence of disease has been observed in North America and Europe. Most infections remain asymptomatic but approximately 20% cause West Nile fever, with < 1% of such cases resulting in neurological disease and/or death, primarily among the elderly and immunocompromised. Human-to-human transmission may also occur through transfusion or transplantation from an infected donor. Blood donors from endemic regions are commonly screened using NAT-based assays, which can detect viral load 2–18 days post infection. Commercial diagnostic assays used for this purpose are qualitative and can detect both WNV lineages 1 and 2 simultaneously – however, the lower limits of detection may vary for each lineage. In the absence of international standards in this area, some laboratories have produced their own in-house controls but the need for standardization has been long recognized. In 2012, the Committee had endorsed a proposal to develop WHO international reference standards for each of the two lineages.

An international collaborative study had now been conducted involving 13 laboratories in seven countries. The candidate materials consisted

of laboratory-grown whole WNV lineage 1 and lineage 2 strains lyophilized in negative human plasma (NIBSC codes 18/206 and 18/208 respectively). Combined study results obtained using both qualitative and quantitative assays indicated overall mean potencies of $7.20 \log_{10}$ IU/vial and $6.93 \log_{10}$ IU/vial for the WNV lineage 1 candidate material (18/206) and WNV lineage 2 candidate material (18/208) respectively. Laboratory reported mean potency estimates showed good agreement across the majority of assays, indicating the possible standardization benefits of the existing in-house reference materials. Even better agreement was achieved by expressing the relative potency of samples relative to the proposed WHO international reference standard for their corresponding lineage, demonstrating the requirement for two separate lineage-specific WHO international reference standards.

Accelerated degradation studies for both candidate materials were ongoing. An analysis of the initial data obtained indicated that candidate material 18/206 fitted the Arrhenius model but in the case of candidate material 18/208 the pattern of response observed was insufficient to predict the loss of potency and further data were being generated. Legal constraints on the permitted work involving Schedule 5 pathogens had made it impossible to assess the commutability of the candidate materials.

After reviewing the issues raised during public consultation the Committee discussed the question of whether the availability of two WHO international standards each with their own unitage would lead to confusion as current regulatory requirements stipulate that assay sensitivity should be expressed in IU as a single value. Consequently, after considering the report of the study (WHO/BS/2020.2397), the Committee recommended that: (a) the candidate material 18/206 be established as the First WHO International Standard for West Nile virus lineage 1 RNA for NAT-based assays with an assigned unitage of $7.20 \log_{10}$ IU/vial; and (b) candidate material 18/208 be established as the WHO International Reference Reagent for West Nile virus lineage 2 RNA for NAT-based assays with no assigned unitage.

7.2 Proposed new projects and updates – in vitro diagnostics

7.2.1 Proposed First WHO International Standard for anti- β 2GPI immunoglobulin G autoantibodies

Anti- β 2GPI immunoglobulin G autoantibodies in patient serum are a biomarker of antiphospholipid syndrome and their detection is essential for the diagnosis of this disease which affects 40–50 people per 100 000 population worldwide. Antiphospholipid syndrome causes arterial/deep vein thrombosis, thrombosis in the brain and miscarriage. Currently, around 17 assays are commercially available assays with various in-house methods also used. All such methods exhibit variability in assay cut-off values for qualitative outcomes and in the

quantitative values observed in EQA schemes. The calibration of kits based on traceability to an international standard would improve the comparability of results, monitoring of disease progression and the management of therapeutic strategies.

The Committee was informed that the European Joint Research Commission had prepared 2500 vials of lyophilized defibrinated plasma containing autoantibodies from two patients with antiphospholipid syndrome. In addition to making this material available as a working reagent, it was being proposed that part of the batch be used to develop a WHO international standard with an assigned unitage in IU/vial. The need to ensure that the material was compliant with WHO requirements for the quality, stability and homogeneity of international standards was recognized. It was also intended that commutability would be assessed using other patient samples. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2021.

The Committee questioned whether other materials would be available for assessment as material from only two patients was not a representative pool. Clarification was given that, although further materials may be made available, fully investigating the impact of the heterogeneity of the autoantibody pool would require assessment of hundreds of samples and was considered to be impractical. After further consideration, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a First WHO International Standard for anti- β 2GPI immunoglobulin G autoantibodies.

7.2.2 Proposed First WHO International Standard for epidermal growth factor receptor genomic variant C797S

Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor that plays a key role in cell survival and proliferation. Mutations in the EGFR gene may result in dysfunctional signalling leading to uncontrolled cell proliferation and the development of cancer. EGFR variants are particularly associated with lung cancer and are especially prevalent in non-small cell lung cancer. Third-generation EGFR tyrosine kinase inhibitors such as osimertinib are effective against resistant tumours with T790M mutations; however, resistance to third-generation inhibitors results in disease progression, with the EGFR C797S variant being one of several resistant variants identified to date. The proposed EGFR C797S reference standard would facilitate accurate and sensitive genotyping thereby supporting diagnosis, appropriate therapy selection and the monitoring of treatment response. It was envisaged that the proposed WHO international standard would be added to the four WHO international standards for EGFR receptor variants endorsed by the Committee in 2019. The proposed WHO international standard would be used for the calibration of secondary

standards used in molecular methodologies such as qPCR, digital PCR and high-throughput sequencing. It was also expected to be used to calibrate commercially available assays or kits, including a number of FDA-approved companion diagnostics.

The proposed source material was CRISPR-engineered HT1080 cells containing the C797S variant. An international collaborative study would be conducted to evaluate the material using various diagnostic genotyping methods. The resulting quantitative data will be used to establish consensus values for percentage EGFR variant and copy number in the material. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

After considering the potential impact of the need to obtain a CRISPR licence on the proposed project schedule, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a First WHO International Standard for epidermal growth factor receptor genomic variant C797S to supplement the four WHO international standards for EGFR receptor variants previously endorsed by the Committee for development.

7.2.3 **Proposed First WHO International Standard for FMS-like tyrosine kinase 3 genomic variant FLT3-ITD**

FMS-like tyrosine kinase 3 (FLT3) is a receptor kinase expressed on the surface of many haematopoietic progenitor cells. Mutations of the FLT3 gene occur in approximately 30% of acute myeloid leukaemia cases, with the FLT3 internal tandem duplication (FLT3-ITD) representing the most common type of FLT3 mutation. As FLT3-ITD mutations are a prognostic indicator of adverse disease outcome there is a need for accurate and sensitive FLT3 genotyping to allow for correct patient diagnosis, matching to optimal therapy, and monitoring of treatment response and prognosis. Such genotyping would be supported by the availability of a WHO international standard based on a well-characterized lyophilized genomic DNA extracted from a single cell line clone harbouring the variant gene. It was proposed that an international collaborative study be conducted to evaluate a candidate material using various diagnostic genotyping methods including PCR and high-throughput sequencing for use as a non-assay-specific WHO international standard for the calibration of secondary standards for specific molecular methodologies. The anticipated users of the international standard were diagnostic laboratories and commercial organizations developing kits, assays or secondary standards. The candidate material would be a CRISPR-modified genomic DNA bearing the FLT3-ITD variant and diluted in wild-type genomic DNA – an approach already established for other cancer standards. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

After due consideration, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a First WHO International Standard for FMS-like tyrosine kinase 3 genomic variant FLT3-ITD.

7.2.4 Proposed First WHO International Standard for *Huaiyangshan banyangvirus* RNA for NAT-based assays

Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne infection caused by *Huaiyangshan banyangvirus*, a phlebovirus of the order Bunyvirales. The virus was first isolated in 2009 in China, with cases subsequently reported in Japan, the Republic of Korea, Viet Nam and Taiwan, China. The WHO R&D Blueprint has identified SFTS as a priority emerging disease. The case fatality rate of SFTS is estimated at 6–30% and there is currently no licensed vaccine or therapeutic with only supportive patient care currently available. Accurate diagnosis during early infection is essential for initiating such care which can reduce the high fatality rate. At present, two commercially available in vitro diagnostic kits based on real-time qPCR are used to diagnose infection along with numerous in-house assays. The harmonization and further development of such diagnostic assays would be supported by the development of a common WHO international standard.

It is intended that a candidate material consisting of *Huaiyangshan banyangvirus* RNA packaged in lentiviral particles will be evaluated in an international collaborative study to evaluate its suitability for use as a WHO international standard. The vector construct would contain sequences representative of the whole genome. Such chimeric constructs are designed to be safe, non-infectious and non-replicative using the approach previously applied to the development of a WHO international reference reagents for Ebola virus RNA for NAT-based assays. The study would be designed to characterize the reactivity and specificity of the candidate material and to assess its potency in typically used assays. The study would be conducted jointly by NIBSC and the Korea Research Institute of Standards and Science. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in either 2022 or 2023.

The Committee discussed the possibility of *Huaiyangshan banyangvirus* causing asymptomatic infection and wondered if transmission might therefore occur via blood transfusion. Although clarification was given that no known instances of bloodborne transmission had been recorded, the Committee noted that blood services did not actively test for this pathogen. The Committee also raised concerns regarding the limited geographical distribution and number of intended participant laboratories in the prospective study. The Committee recommended that laboratories in Thailand should be contacted and requested that further information on the study participants be presented at a future

meeting. Notwithstanding these issues, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop a First WHO International Standard for *Huayangshan banyangvirus* RNA for NAT-based assays.

8. International reference materials – standards for use in high-throughput sequencing technologies

All reference materials established at the meeting are listed in Annex 5.

8.1 WHO International reference standards for use in high-throughput sequencing technologies

8.1.1 WHO international reference reagents for adventitious virus detection in biological products by high-throughput sequencing

In 2010 the discovery of porcine circovirus in a licensed rotavirus vaccine using high-throughput sequencing alerted regulators and manufacturers to the potential utility of such technologies in detecting a broad range of adventitious viruses in biological products. Today, the use of such technologies is increasingly being encouraged as an alternative to conventional virus detection assays in WHO, European Pharmacopeia and FDA guidance. The technology has a number of significant inherent advantages, including its potential to reduce the use of animals in product testing and its quick turnaround time. There is, however, international recognition of the need to standardize the application of high-throughput sequencing technologies in the development and quality control of biological medicines.

An international collaborative study involving eight laboratories in six countries had been conducted to assess the suitability of five reference virus stocks for use as WHO international reference standards for adventitious virus detection by high-throughput sequencing. The individual viral stocks had been prepared by the American Type Culture Collection and included porcine circovirus type 1, mammalian orthoreovirus type 1, feline leukaemia virus, RSV and Epstein-Barr virus (CBER codes SC-VR-6000P to SC-VR-6004P respectively). The five viruses were selected to provide a broad representation of different virus families, each with distinct physicochemical properties relevant to their preparation and detection during the entire workflow of high-throughput sequencing. During the study, a high-titre adenovirus 5 stock (corresponding to 10^9 genome copies/mL) was spiked with each prospective reference material (at 10^4 genome copies/mL of each virus) to assess the breadth of virus detection in a matrix mimicking a viral vaccine seed. Each laboratory used the same starting virus stocks to create their own sample material and then used their own protocols for sample processing, cDNA synthesis, library preparation, sequencing, and bioinformatic analysis. Study results showed that at the spiking level of 10^4 genome copies per mL all five viruses were detected by the participating laboratories, clearly indicating the suitability of the approach for evaluating inter- and intra-laboratory consistency with regard to the sensitivity and specificity of detection. The results of real-time stability studies indicated

that the liquid viral stocks would be suitable for long-term storage at -80°C with no changes observed in either genome copy number (after 24–36 months) or infectious titre (after 24 months).

The Committee enquired about the possibility of endogenous retroviruses (or residual viral nucleic acid) from the cell line affecting the results. Assurance was given that endogenous viruses from the host cell line can be eliminated through bioinformatic analysis. It was also clarified that the materials would consist of five separate fills rather than a single mixture of the five viruses. Furthermore, although stocks of the materials were relatively low funding was available to develop replacement materials in the near future. The Committee then emphasized the need for consistency in the terminology used for international reference standards in this area. This was particularly important in light of the recent endorsement by the Committee of a proposal from NIBSC to develop similar reference materials that would presumably be submitted for establishment in the near future. The Committee considered the report of the study (WHO/BS/2020.2394) and recommended that the candidate materials coded SC-VR-6000P to SC-VR-6004P be established as WHO international reference reagents for adventitious virus detection in biological products by high-throughput sequencing with the following assigned unitages:

- porcine circovirus type 1 (SC-VR-6000P) = 2.7×10^{11} genome copies/mL
- mammalian orthoreovirus type 1 (SC-VR-6001P) = 1.4×10^{10} genome copies/mL
- feline leukaemia virus (SC-VR-6002P) = 5.3×10^{10} genome copies/mL
- human respiratory syncytial virus (SC-VR-6003P) = 1.0×10^9 genome copies/mL
- Epstein-Barr virus (SC-VR-6004P) = 3.7×10^8 genome copies/mL

The materials were being recommended for use as a panel of five viruses to demonstrate the breadth and sensitivity of the high-throughput sequencing technologies used but could also be used individually for the detection of specific viruses or virus families.

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

8.2.1 Proposed WHO international reference reagents for DNA extraction for microbiome analysis

High-throughput sequencing studies have demonstrated that significant changes in the microbiome are associated with a number of diseases. For example, changes in the human gut microbiome have been associated with

inflammatory bowel disease and colorectal cancer. Restoring the microbiome to a healthy state has been proposed as a therapeutic strategy, with faecal microbiota transplantation (FMT) widely regarded as one such promising approach. Research into such therapies is largely based on high-throughput sequencing analysis of DNA extracted from samples taken from a specific body site microbiome to estimate its relative microbial composition. A number of studies have concluded that the current lack of effective standardization of high-throughput sequencing protocols and analysis represents the single largest barrier to translational research and product development. Presently, 126 Phase II clinical trials and 29 Phase III clinical trials involving the human microbiome and FMT are under way worldwide. The lack of accredited reference materials for microbiome analyses has led to major differences in the results obtained by conceptually similar studies – a concern for both product developers and regulatory authorities.

In 2019, the Committee had endorsed a proposal to develop DNA-based reference reagents for use in standardizing the sequencing and bioinformatic steps of microbiome analyses. Subsequently, a whole-cell prospective reference material was developed by NIBSC for use as a control reagent during the DNA extraction step. This material consists of acetone-fixed bacterial cells representing the same strains as those used in the DNA-based reference reagents. It was proposed that the candidate material be evaluated in an international collaborative study involving at least 15 laboratories worldwide using the same DNA extraction kit and optionally a method of their own choice. The extracted DNA would be characterized using three physicochemical measures (DNA yield, purity and integrity) and four measures of bias to specific taxa (sensitivity, false-positive abundance, diversity and similarity). The aim of the study would be to establish go/no-go criteria based on this seven-measure reporting system. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee acknowledged the importance of developing international reference materials for molecular methods and innovative therapeutic approaches such as those now emerging in the microbiome field. After reviewing preliminary data obtained using different microbiome DNA extraction kits, the Committee expressed its surprise at the substantial differences between kits in the predicted microbiome composition of the candidate reference material. Noting the importance of this work for ongoing and future clinical studies, the Committee endorsed the proposal (WHO/BS/2020.2396) to develop WHO international reference reagents for DNA extraction for microbiome analysis.

9. International reference materials – standards for use in public health emergencies

All reference materials established at the meeting are listed in Annex 5.

9.1 WHO international reference standards for use in public health emergencies

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

Primary clinical diagnostic testing to confirm COVID-19 is based on the detection of SARS-CoV-2 RNA using NAT-based assays. Following the rapid increase in cases of COVID-19 worldwide, an unprecedented effort has been made by independent laboratories and diagnostic test manufacturers to meet the demand for testing capability. As of November 2020, more than 350 NAT-based assays had been released onto the market. As part of its response to the COVID-19 pandemic WHO highlighted the need for an international standard for SARS-CoV-2 RNA. The availability of such an international standard with an assigned potency in IU would significantly facilitate assay calibration, allowing for greater comparability and harmonization of global SARS-CoV-2 RNA detection. Such harmonization of data reporting would also support the evaluation and defining of key assay parameters such as sensitivity and limits of detection.

An accelerated international collaborative study had therefore been conducted to assess the suitability of two candidate SARS-CoV-2 preparations to serve as a WHO international standard for the harmonization of COVID-19 NAT-based assays. One of the lyophilized candidate materials was a synthetic preparation consisting of chimeric lentiviral particles containing whole-genome SARS-CoV-2 RNA (NIBSC code 20/138) and the other an inactivated SARS-CoV-2 preparation (NIBSC code 20/146). Seventeen laboratories in nine countries returned 32 sets of data representing 21 methods, including in-house and commercial assays based on digital PCR, real-time PCR and transcription mediated amplification technology. A comparable reduction in inter-laboratory variation was achieved when results were expressed relative to each of the candidate materials. Although the synthetic RNA preparation provided greater overall harmonization, it did not perform well in a test based on the E-gene and was not considered to be suitable as a standard. Despite showing great promise in the study, wider evaluation was needed before such materials could be recommended as international standards.

The unprecedented speed at which the candidate materials were developed, and the collaborative study completed, inevitably imposed limitations. Restrictions on the ability to source clinical samples and distribute

a BSL3 pathogen meant it was not feasible to fully evaluate commutability in the expedited timeframe available. Similarly, the shortened timeframe meant that only limited data could be generated on the stability of the candidate materials to date. Accelerated degradation study data evaluated at 3 months indicated that the candidate materials were sufficiently stable for storage at -20°C and shipment at ambient temperature in temperate climates, with ice packs or dry ice possibly required elsewhere.

The Committee sought clarification regarding the method used to assess the degradation of RNA during the stability studies and was informed that this had been done using a PCR method. Recognizing the challenges posed by sequence variability among different SARS-CoV-2 strains and the diversity of test formats, the Committee was further assured that material based on the whole viral genome should be sufficient to standardize a wide range of NAT-based assays.

The Committee commended the comprehensive nature of the study and the speed with which the prospective standards had been produced. However, the Committee also expressed its concern that the existence of two different standards would be problematic and would potentially cause confusion among users. After considering the report of the study (WHO/BS/2020.2402) the Committee therefore recommended that the candidate material 20/146 be established as the First WHO International Standard for SARS-CoV-2 RNA for NAT-based assays with an assigned unitage of $7.40 \log_{10}$ IU/ampoule. The Committee further suggested that the candidate material 20/138 could be distributed by NIBSC as a secondary working standard calibrated against the newly established international standard.

9.1.2 **First WHO International Standard for anti-SARS-CoV-2 immunoglobulin; and First WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin**

Accurate serological assays will be vital for understanding the real impact of the COVID-19 pandemic as asymptomatic or mildly symptomatic cases often go undetected. In addition, the rapid development of COVID-19 vaccines and therapeutics depends upon reliable serological assays for their evaluation. The establishment of WHO international reference standards for SARS-CoV-2 antibodies would facilitate the standardization of such assays thus strengthening the comparability and harmonization of datasets obtained by different laboratories worldwide. In turn, this would help to establish the protective antibody levels needed to develop efficacious vaccines and therapeutics, while also improving the comparability of data collected during epidemiological and immunological surveillance studies.

An international collaborative study involving 44 laboratories in 15 countries had therefore been conducted to evaluate a candidate material

(NIBSC code 20/136) for its suitability to serve as a WHO international standard for anti-SARS-CoV-2 immunoglobulin. The candidate material, comprising pooled plasma obtained from 11 COVID-19 convalescent patients, was evaluated across 125 different assays, including ELISAs, neutralization assays, flow cytometry assays, lateral flow immunoassays, inhibition assays and one double antigen binding assay. The candidate material was assessed blind as part of a panel of samples, which also included plasma and serum taken from convalescent patients, to assess both its commutability and performance in harmonizing assay results.

In addition, the study evaluated four pools of convalescent plasma representing a range of antibody titres (low to high) along with one negative control plasma pool obtained from healthy individuals prior to 2019. Taken together, these five materials (NIBSC codes 20/150, 20/148, 20/144, 20/140 and 20/142) constituted the proposed WHO international reference panel intended to facilitate assessment of the factors contributing to assay variability. A further single plasma sample that had been distributed since April 2020 as an NIBSC working reagent was also evaluated alongside the candidate materials to allow for the retrospective assignment of IU to data that had already been generated using this reagent.

Study results indicated that inter-laboratory variation for almost all the positive samples was reduced when neutralizing antibody titres were expressed relative to the candidate material 20/136. The material only failed to improve inter-laboratory variability for the two samples with the lowest detectable neutralizing antibody titres, probably because of the proximity of these titres to the limit of detection of such assays. Expressing ELISA titres relative to the candidate material resulted in even greater reduction in inter-laboratory variability, likely because of the wide range of units used to report antibody titres obtained from different ELISAs. The proposed WHO international reference panel also proved fit for purpose in all assays, with only a small number of participants failing to detect the lowest titre samples. Across all methods used in the study, the antibody titres of the proposed panel members were ranked correctly with very few exceptions.

The stability of the candidate material 20/136 was being assessed in ongoing accelerated thermal degradation and real-time studies. Analysis of real-time data generated to date indicated minimal loss of potency up to 2 weeks. Long-term stability was estimated using the Arrhenius model, which predicted a loss in potency of 0.288% per year when stored at -20°C . These results suggest that the candidate material is sufficiently stable for use as a WHO international standard. Stability studies were not conducted for each member of the proposed WHO international reference panel due to the short timeframe of the study and increased workload caused by the COVID-19 pandemic. These were however

formulated in the same way as the proposed WHO international standard and are assumed to have a similar stability profile.

Acknowledging the amount of work involved in this study and the speed with which it had been conducted, the Committee highlighted the importance of these candidate standards, including in the establishing of immune correlates of protection against COVID-19. The Committee indicated its satisfaction with the proposed assignment of units for neutralizing antibodies to the candidate material 20/136 but expressed concern that the assignment of the same unitage for antibody binding assays based on different antigens would allow for the inappropriate use of the standard to compare relative antibody titres against different antigens. However, recognizing its potential utility in the harmonization of such assays, the Committee requested that further statistical analysis be conducted to confirm the suitability of the material for this purpose, with a view to recommending the assignment of a unitage for antibody binding activity at its next meeting. Given the urgent need for such a standard during the ongoing COVID-19 pandemic, the Committee recommended that the material be made available immediately via the custodian laboratory as an NIBSC working reagent for the harmonization of antibody binding assays. Data to support its use in antibody binding assays should be provided in the IFU and webinar-based and other technical assistance provided to users.

The Committee further considered the report of the study (WHO/BS/2020.2403) and recommended that the candidate material 20/136 be established as the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin with an assigned unitage of 250 IU/ampoule for neutralizing antibody activity against SARS-CoV-2 antigens. The Committee further recommended that the collection of candidate materials of varying titre (NIBSC codes 20/150, 20/148, 20/144, 20/140 and 20/142) be established as the First WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin (with no assigned unitage) to facilitate the assessment of serological assays used to measure SARS-CoV-2 antibodies.

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

9.2.1 Proposed First WHO International Standard for SARS-CoV-2 antigen

Rapid and accurate detection of infection with SARS-CoV-2 is essential for supporting the clinical management of patients and for guiding and monitoring infection control procedures. Although NAT-based assays are the recommended “gold standard” approach for virus detection they are generally dependent upon the availability of expensive equipment and trained operators, thus making them less accessible in low-resource settings. Antigen-detecting rapid diagnostic tests (Ag-RDTs) are relatively inexpensive and can quickly

identify infected individuals. The sensitivity of Ag-RDTs is however lower than that of NAT-based assays as the target analyte is not amplified. As with NAT-based assays, the clinical sensitivity of Ag-RDTs is highly dependent upon the characteristics of the individual product, the patient population, and the quality of sampling and sample processing.

WHO interim guidance has now been published on the use of antigen detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays, and numerous Ag-RDTs are currently in development in this rapidly evolving field. The Committee was informed that a common reference reagent was now required to: (a) facilitate the development, assessment and comparability of Ag-RDTs, including through accurate determination of their limit of detection; and (b) serve as the basis for the calibration of secondary standards for use in the quality control of Ag-RDTs; and (c) support other regulatory and quality assurance activities such as EQA initiatives and post-market surveillance. It was proposed that a pilot study be conducted to explore the potential suitability of several source materials as candidate standards prior to their full evaluation in an international collaborative study. Pilot study materials would include inactivated whole virus and recombinant N and S proteins. The full collaborative study would then involve at least 15 laboratories worldwide, including national control laboratories, manufacturers, and clinical and academic laboratories. Issues raised by this proposal included the potential antigenic variability of the N and S protein antigens and the commutability of the candidate materials with clinical material.

The Committee was asked for its opinion on two options for making the proposed reference material available to users as soon as possible. In the first option, the custodian laboratory would assign a unitage based on the results of the pilot study to an NIBSC “calibrator” for immediate deployment to users, while at the same time preparing the candidate material for evaluation in the international collaborative study. In the second option, a feasibility study would first be conducted by a subset of the collaborative study participants to develop an interim “working standard” for deployment that would subsequently be submitted to the Committee for consideration of its suitability for establishment as a WHO international standard, subject to the satisfactory outcome of the full collaborative study.

Noting the extensive variability reported for Ag-RDTs and the large number of novel products now in development, the Committee underlined the urgent need for this standard. It expressed its support for the proposed feasibility study to produce the interim working standard but did not agree with the assignment of a corresponding interim unitage. Having reviewed the details of the prospective international collaborative study common to both options, the Committee endorsed the proposal (WHO/BS/2020.2404) to develop a First WHO International Standard for SARS-CoV-2 antigen.

10. International reference materials – vaccines and related substances

All reference materials established at the meeting are listed in Annex 5.

10.1 WHO International reference standards for vaccines and related substances

10.1.1 First WHO International Standard for anti-MERS-CoV immunoglobulin G (human)

Middle East respiratory syndrome coronavirus (MERS-CoV) is associated with sporadic zoonotic infections as well as human-to-human transmission primarily in health care settings. Symptoms of infection include fever, cough, shortness of breath, and in most cases pneumonia. As of 31 March 2020, 2562 cases had been reported resulting in 881 deaths, corresponding to a case fatality rate of approximately 35%. The virus was first described in Saudi Arabia in 2012 and is one of the top ten priority pathogens with outbreak potential identified by the WHO R&D Blueprint. The establishment of a WHO international standard for anti-MERS-CoV immunoglobulin would facilitate the standardization of MERS serological methods, thereby supporting the reliable evaluation of vaccines and therapeutics now in development.

Following endorsement by the Committee in 2018 of a proposal to develop international reference standards in this area, a candidate material (NIBSC code 19/178) was prepared consisting of a lyophilized pool of sera from two MERS-recovered patients. This candidate material had now been evaluated in an international collaborative study involving 11 laboratories in seven countries using a total of 22 methods, including the most frequently used ELISA and virus neutralization assays. The material was evaluated as part of a blinded panel of samples that had also included four serum samples from individual MERS-recovered patients, a candidate working reagent, a pool of sera from two MERS-recovered patients and two anti-MERS immunoglobulin G preparations derived from trans-chromosomal cattle previously immunized with either MERS-CoV spike protein or irradiated virus.

When the potencies of study samples were reported relative to the candidate material 19/178, inter-assay and inter-laboratory variation for both binding and neutralization assays were reduced. In addition, the geometric mean value for each sample for both ELISA and neutralization assays when expressed in relative units were very close, supporting the use of the same arbitrarily chosen unitage for both assay types. The stability of the candidate material was assessed using a pseudotype-based neutralization assay. Data obtained from both real-time and accelerated stability studies indicated that

the candidate material is sufficiently stable to serve as a WHO international standard and could be shipped at ambient temperature.

Noting that this type of study would also be crucial in supporting the development and evaluation of COVID-19 vaccines, the Committee pointed out that the various assays would be detecting different antigens, with some targeting the nucleoprotein and others the spike protein. It was agreed that although this should not delay the establishment of the international reference standard, further and more-detailed work would be required post establishment. The Committee considered the report of the study (WHO/BS/2020.2398) and recommended that the candidate material 19/178 be established as the First WHO International Standard for anti-MERS-CoV immunoglobulin G with an assigned unitage of 250 IU/ampoule.

10.2 Proposed new projects and updates – vaccines and related substances

10.2.1 Proposed Second WHO International Standard for meningococcal serogroup C polysaccharide

Meningitis and septicaemia caused by *Neisseria meningitidis* can be prevented by immunization with polysaccharide conjugate vaccines that target the bacterial capsule. The first such vaccines were approved in 1999 and are now typically administered as part of tetravalent formulations that provide protection against serogroup A, C, W and Y organisms. The current First WHO International Standard for meningococcal serogroup C polysaccharide, recommended by the Committee for establishment 2011, is used by manufacturers and control laboratories in the quantification of the polysaccharide content of vaccine products. The Committee was informed that stocks of this international standard will be exhausted in the next 2–3 years and therefore needs to be replaced. It was proposed that an international collaborative study be conducted to assign a value in SI units of polysaccharide per ampoule to a manufacturer-donated candidate material and to assess the suitability of the material to serve as a replacement WHO international standard.

Several such bacterial polysaccharide standards have now been established over a number of years and one key issue is the way in which SI units have been assigned to this group of standards. The earlier standards had been assigned unitages based on the resorcinol assay, which is widely used by manufacturers and control laboratories. However, in terms of metrology this is not considered to be a suitable primary method and should therefore no longer be used for this purpose. More recent standards have been assigned SI units based on quantitative nuclear magnetic resonance (qNMR) spectroscopy as the primary method. However, the use of qNMR spectroscopy in such studies has been associated with a higher degree of uncertainty of measurement among

participating laboratories compared to the resorcinol assay. As part of the proposed collaborative study, laboratories performing qNMR spectroscopy will therefore be provided with a comprehensive protocol for performing the analysis along with a certified reference material to reduce inter-laboratory variability. As part of the study design, resorcinol assays will also be used to ensure continuity with the current WHO international standard. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee noted that the proposal appeared to be straightforward and indicated its support for the steps to be taken to improve the consistency of results obtained with the primary method. The Committee endorsed the proposal (WHO/BS/2020.2396) to prepare a Second WHO International Standard for meningococcal serogroup C polysaccharide.

Annex 1

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

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

Recommendations and guidance on biological products are formulated by international groups of experts and published in the WHO Technical Report Series¹¹ 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 and other documents may be obtained free of charge as offprints by writing to:

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

¹¹ Abbreviated in the following pages to “TRS”.

Recommendations, Guidelines and other documents	Reference
Animal cells, use of, as in vitro substrates for the production of biologicals	Revised 2010, TRS 978 (2013)
BCG vaccines (dried)	Revised 2011, TRS 979 (2013)
Biological products: good manufacturing practices	Revised 2015, TRS 999 (2016)
Biological standardization and control: a scientific review commissioned by the UK National Biological Standards Board (1997)	Unpublished document WHO/BLG/97.1
Biological substances: International Standards and Reference Reagents	Revised 2004, TRS 932 (2006)
Biotherapeutic products, changes to approved biotherapeutic products: procedures and data requirements	Adopted 2017, TRS 1011 (2018)
Biotherapeutic products, similar	Adopted 2009, TRS 977 (2013)
Biotherapeutic products, similar: WHO Questions and Answers ¹²	Adopted 2018; online document
Biotherapeutic protein products prepared by recombinant DNA technology	Revised 2013, TRS 987 (2014); Addendum 2015, TRS 999 (2016)
Blood, blood components and plasma derivatives: collection, processing and quality control	Revised 1992, TRS 840 (1994)
Blood and blood components: management as essential medicines	Adopted 2016, TRS 1004 (2017)
Blood components and plasma: estimation of residual risk of HIV, HBV or HCV infections	Adopted 2016, TRS 1004 (2017)
Blood establishments: good manufacturing practices	Adopted 2010, TRS 961 (2011)
Blood plasma (human) for fractionation	Adopted 2005, TRS 941 (2007)
Blood plasma products (human): viral inactivation and removal procedures	Adopted 2001, TRS 924 (2004)
Blood regulatory systems, assessment criteria for national	Adopted 2011, TRS 979 (2013)

¹² Available online at: https://www.who.int/biologicals/expert_committee/OA_for_SBP_ECBS_2018.pdf?ua=1

Recommendations, Guidelines and other documents	Reference
Cholera vaccines (inactivated, oral)	Adopted 2001, TRS 924 (2004)
Dengue tetravalent vaccines (live, attenuated)	Revised 2011, TRS 979 (2013)
Diphtheria, tetanus, pertussis (whole cell), and combined (DTwP) vaccines	Revised 2012, TRS 980 (2014)
Diphtheria vaccines (adsorbed)	Revised 2012, TRS 980 (2014)
DNA vaccines, plasmid	Revised 2020, TRS 1028 (2021)
Ebola vaccines	Adopted 2017, TRS 1011 (2018)
Enterovirus 71 vaccines (inactivated)	Adopted 2020, TRS 1030 (2021)
<i>Haemophilus influenzae</i> type b conjugate vaccines	Revised 1998, TRS 897 (2000)
Haemorrhagic fever with renal syndrome (HFRS) vaccines (inactivated)	Adopted 1993, TRS 848 (1994)
Hepatitis A vaccines (inactivated)	Adopted 1994, TRS 858 (1995)
Hepatitis B vaccines prepared from plasma	Revised 1994, TRS 858 (1996)
Hepatitis B vaccines (recombinant)	Revised 2010, TRS 978 (2013)
Hepatitis E vaccines (recombinant)	Adopted 2018, TRS 1016 (2019)
Human immunodeficiency virus rapid diagnostic tests for professional use and/or self-testing Technical Specifications Series for WHO Prequalification – Diagnostic Assessment	Adopted 2017, TRS 1011 (2018)
Human interferons prepared from lymphoblastoid cells	Adopted 1988, TRS 786 (1989)
Influenza vaccines (inactivated)	Revised 2003, TRS 927 (2005)
Influenza vaccines (inactivated): labelling information for use in pregnant women	Addendum 2016, TRS 1004 (2017) to Annex 3, TRS 927 (2005)
Influenza vaccines (live)	Revised 2009, TRS 977 (2013)
Influenza vaccines, human, pandemic: regulatory preparedness	Adopted 2007, TRS 963 (2011)
Influenza vaccines, human, pandemic: regulatory preparedness in non-vaccine-producing countries	Adopted 2016, TRS 1004 (2017)

Recommendations, Guidelines and other documents	Reference
Influenza vaccines, human, pandemic: safe development and production	Adopted 2018, TRS 1016 (2019)
In vitro diagnostics (WHO-prequalified), collaborative procedure between WHO and NRAs for assessment and accelerated national registration	Adopted 2020, TRS 1030 (2021)
In vitro diagnostic medical devices, establishing stability of, Technical Guidance Series for WHO Prequalification – Diagnostic Assessment	Adopted 2017, TRS 1011 (2018)
Japanese encephalitis vaccines (inactivated) for human use	Revised 2007, TRS 963 (2011)
Japanese encephalitis vaccines (live, attenuated) for human use	Revised 2012, TRS 980 (2014)
Louse-borne human typhus vaccines (live)	Adopted 1982, TRS 687 (1983)
Malaria vaccines (recombinant)	Adopted 2012, TRS 980 (2014)
Measles, mumps and rubella vaccines and combined vaccines (live)	Adopted 1992, TRS 840 (1994); Note 1993 TRS 848 (1994)
Meningococcal polysaccharide vaccines	Adopted 1975, TRS 594 (1976); Addendum 1980, TRS 658 (1981); Amendment 1999, TRS 904 (2002)
Meningococcal A conjugate vaccines	Adopted 2006, TRS 962 (2011)
Meningococcal C conjugate vaccines	Adopted 2001, TRS 924 (2004); Addendum (revised) 2007, TRS 963 (2011)
Monoclonal antibodies	Adopted 1991, TRS 822 (1992)
Monoclonal antibodies as similar biotherapeutic products	Adopted 2016, TRS 1004 (2017)
Papillomavirus vaccines (human, recombinant, virus-like particle)	Revised 2015, TRS 999 (2016)
Pertussis vaccines (acellular)	Revised 2011, TRS 979 (2013)
Pertussis vaccines (whole-cell)	Revised 2005, TRS 941 (2007)
Pharmaceutical products, storage and transport of time- and temperature-sensitive	Adopted 2010, TRS 961 (2011)

Recommendations, Guidelines and other documents	Reference
Pneumococcal conjugate vaccines	Revised 2009, TRS 977 (2013)
Poliomyelitis vaccines (inactivated)	Revised 2014, TRS 993 (2015); Amendment 2019, TRS 1024 (2020)
Poliomyelitis vaccines (oral)	Revised 2012, TRS 980 (2014)
Poliomyelitis vaccines: safe production and quality control	Revised 2018, TRS 1016 (2019) Amendment 2020, TRS 1028 (2021)
Quality assurance for biological products, guidelines for national authorities	Adopted 1991, TRS 822 (1992)
Rabies vaccines for human use (inactivated) produced in cell substrates and embryonated eggs	Revised 2005, TRS 941 (2007)
Reference materials, secondary: for NAT-based and antigen assays: calibration against WHO International Standards	Adopted 2016, TRS 1004 (2017)
Regulation and licensing of biological products in countries with newly developing regulatory authorities	Adopted 1994, TRS 858 (1995)
Regulatory risk evaluation on finding an adventitious agent in a marketed vaccine: scientific principles	Adopted 2014, TRS 993 (2015)
Respiratory syncytial virus vaccines	Adopted 2019, TRS 1024 (2020)
Rotavirus vaccines (live, attenuated, oral)	Adopted 2005, TRS 941 (2007)
Smallpox vaccines	Revised 2003, TRS 926 (2004)
Snake antivenom immunoglobulins	Revised 2016, TRS 1004 (2017)
Sterility of biological substances	Revised 1973, TRS 530 (1973); Amendment 1995, TRS 872 (1998)
Synthetic peptide vaccines	Adopted 1997, TRS 889 (1999)
Tetanus vaccines (adsorbed)	Revised 2012, TRS 980 (2014)
Thiomersal for vaccines: regulatory expectations for elimination, reduction or replacement	Adopted 2003, TRS 926 (2004)
Thromboplastins and plasma used to control oral anticoagulant therapy	Revised 2011, TRS 979 (2013)

Recommendations, Guidelines and other documents	Reference
Tick-borne encephalitis vaccines (inactivated)	Adopted 1997, TRS 889 (1999)
Transmissible spongiform encephalopathies in relation to biological and pharmaceutical products ¹³	Revised 2005, WHO (2006)
Tuberculins	Revised 1985, TRS 745 (1987)
Typhoid vaccines, conjugated	Revised 2020, TRS 1030 (2021)
Typhoid vaccines (live, attenuated, Ty21a, oral)	Adopted 1983, TRS 700 (1984)
Typhoid vaccines, Vi polysaccharide	Adopted 1992, TRS 840 (1994)
Vaccines, changes to approved vaccines: procedures and data requirements	Adopted 2014, TRS 993 (2015)
Vaccines, clinical evaluation: regulatory expectations	Revised 2016, TRS 1004 (2017)
Vaccines, regulatory considerations: use of human challenge trials	Adopted 2016, TRS 1004 (2017)
Vaccines, lot release	Adopted 2010, TRS 978 (2013)
Vaccines, nonclinical evaluation	Adopted 2003, TRS 927 (2005)
Vaccines, nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines	Adopted 2013, TRS 987 (2014)
Vaccines, prequalification procedure	Adopted 2010, TRS 978 (2013)
Vaccines, stability evaluation	Adopted 2006, TRS 962 (2011)
Vaccines, stability evaluation for use under extended controlled temperature conditions	Adopted 2015, TRS 999 (2016)
Varicella vaccines (live)	Revised 1993, TRS 848 (1994)
Yellow fever vaccines (live, attenuated)	Revised 2010, TRS 978 (2013)
Yellow fever vaccines, laboratories approved by WHO for the production of	Revised 1995, TRS 872 (1998)
Yellow fever virus, production and testing of WHO primary seed lot 213-77 and reference batch 168-736	Adopted 1985, TRS 745 (1987)

¹³ Available online at: <http://www.who.int/biologicals/publications/en/whotse2003.pdf>

Annex 2

Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines

Replacement of Annex 3 of WHO Technical Report Series, No. 987

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Recommendations published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations 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 recommendations set out below.

Abbreviations

ADH	Adipic acid dihydrazide
<i>C. freundii</i> s.l.	<i>Citrobacter freundii</i> sensu lato
CI	confidence interval
CRM ₁₉₇	cross-reactive material 197
CTAB	hexadecyltrimethylammonium bromide
DT	diphtheria toxoid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (also abbreviated to EDAC)
ELISA	enzyme-linked immunosorbent assay
HPAEC-CD	high-performance anion exchange chromatography with conductivity detection
HPAEC-PAD	high-performance anion exchange chromatography with pulsed amperometric detection
HPLC	high-performance liquid chromatography
HPSEC	high-performance size-exclusion chromatography
IgA	immunoglobulin A
IgG	immunoglobulin G
IU	International Unit
K _D	(distribution constant)
LAL	<i>Limulus</i> amoebocyte lysate (test)
LPS	lipopolysaccharide
MAT	monocyte activation test
MW	molecular weight
NCL	national control laboratory
NMR	nuclear magnetic resonance
NRA	national regulatory authority
qNMR	quantitative nuclear magnetic resonance
SAGE	WHO Strategic Advisory Group of Experts
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

S. Typhi	<i>Salmonella enterica</i> serovar Typhi
TCV	typhoid conjugate vaccine
TT	tetanus toxoid
Vi-rEPA	Vi polysaccharide conjugated to recombinant exoprotein A of <i>Pseudomonas aeruginosa</i>

Introduction

The WHO Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines were developed following a series of international consultations in 2012 and 2013, and were adopted by the WHO Expert Committee on Biological Standardization at its Sixty-fourth meeting in October 2013 (1). Since that time, there have been several major developments with respect to typhoid conjugate vaccines (TCVs), including:

- The establishment of WHO international standards for Vi antigens and Vi antibodies (human).
- The licensing of TCVs in some countries.
- The publication of a WHO Strategic Advisory Group of Experts (SAGE) position paper in 2018 recommending the use of TCVs from 6 months to 45 years of age, and that the introduction of TCVs into routine immunization programmes be prioritized in countries with the highest burden of typhoid disease or with a high burden of antimicrobial-resistant *Salmonella* Typhi.
- Approval of funding support by Gavi, the Vaccine Alliance, for the introduction of TCVs in Gavi-eligible countries starting in 2019.
- WHO Prequalification in 2017 of the Typbar-TCV¹⁴ produced by a manufacturer in India.

The impact of these developments on the production and quality control of TCVs and on their nonclinical and clinical evaluation is reflected in the present revision. As TCVs have been licensed since the development of the original WHO Guidelines in 2013, the current document provides recommendations for the evaluation of such vaccines rather than guiding principles. As a consequence of the increasing demand for TCVs, together with the above-mentioned Gavi decision on funding, new vaccine developers and manufacturers are entering the field and should benefit from updated WHO recommendations. Further clinical evaluation of TCVs, the detailed investigation of immune responses to these vaccines and the search for a true immunological correlate or surrogate of protection are ongoing and Part C of this document may therefore require further updating as new data become available.

Other significant changes reflected in the current document include the updating in 2017 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (2) which provide methodological considerations for the clinical evaluation of vaccine immunogenicity, efficacy and safety. Manufacturers

¹⁴ See: WHO Prequalified Vaccines at: https://extranet.who.int/gavi/PQ_Web/

and regulators should also take note of the decision of the Committee in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing requirements for all vaccines in WHO Recommendations, Guidelines and other guidance documents for biological products (3). This test is therefore not included in the manufacturing recommendations provided in Part A of the current document.

Terminology

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

Activated carrier protein: a carrier protein that has been chemically or physically modified and prepared for conjugation to the polysaccharide.

Activated polysaccharide: purified polysaccharide that has been modified by a chemical reaction or a physical process in preparation for conjugation to the activated carrier protein.

Carrier protein: the protein to which the Vi polysaccharide is covalently linked for the purpose of eliciting a T-cell-dependent immune response to the Vi polysaccharide.

Final bulk: the homogeneous preparation from one or more lots of **purified bulk conjugate** in a single container from which the final containers are filled, either directly or through one or more intermediate containers.

Final lot: a number of sealed, final containers that are equivalent with respect to the risk of contamination that may have occurred during filling and, when it is performed, freeze-drying. A final lot must therefore have been filled from a single container and if freeze-dried this should be completed in one continuous working session.

Master seed lot: bacterial suspensions for the production of Vi polysaccharide or the carrier protein should be derived from a strain that has been processed as a single lot and is of uniform composition. The master seed lot is used to prepare the **working seed lots**. Master seed lots should be maintained in the freeze-dried form or be frozen at or below -45°C .

Purified bulk conjugate: a purified bulk conjugate is prepared by the covalent bonding of activated Vi polysaccharide to the carrier protein, followed by the removal of residual reagents and reaction by-products. This is the parent material from which the **final bulk** is prepared.

Purified polysaccharide: the material obtained after final purification of polysaccharide. The lot of purified polysaccharide may be derived from a **single harvest** or a pool of single harvests that have been processed together.

Single harvest: the material obtained from one batch of culture that has been inoculated with the **working seed** lot (or with the inoculum derived from it), harvested and processed during one production run.

Working seed lot: a quantity of bacterial suspension for the production of Vi polysaccharide or the carrier protein that is of uniform composition and that has been derived from the **master seed lot** by growing the organisms and maintaining them in freeze-dried aliquots or frozen at or below -45°C . The working seed lot is used to inoculate the production medium.

General considerations

Typhoid fever is an acute generalized infection of the mononuclear phagocyte system (previously known as the reticuloendothelial system), intestinal lymphoid tissue and gall bladder caused by *Salmonella enterica* serovar Typhi (*S. Typhi*). Paratyphoid fever is a clinically indistinguishable illness caused by *S. enterica* serovar Paratyphi A or B (or, more rarely, C) (4–6). Typhoid and paratyphoid fevers are referred to collectively as enteric fever. In most endemic areas, typhoid accounts for approximately 75–80% of cases of enteric fever. However, in some regions, particularly in some parts of Asia, *S. Paratyphi A* accounts for a relatively larger proportion of all enteric fevers (7–9). Prospective vaccines against *S. Paratyphi* are not included in the scope of the current document.

Pathogen

S. Typhi is a member of the family *Enterobacteriaceae*. It is a Gram-negative, non-lactose fermenting bacillus that produces trace amounts of hydrogen sulfide. Its antigens include an immunodominant lipopolysaccharide (LPS) O9, flagellar H phase 1 antigen “d” and capsular polysaccharide Vi.

Vi acts as a virulence factor by preventing anti-O antibody from binding to the O antigen (10) and inhibits the C3 component of the complement system from fixing to the surface of *S. Typhi* (11). The Vi antigen is not unique to *S. Typhi* – it is also expressed by *S. Paratyphi C*, *Citrobacter freundii* sensu lato (*C. freundii* s.l.) and some clades of *S. enterica* serovar Dublin. The genes responsible for the biosynthesis of Vi polysaccharide are located in a locus (*viaB*) within the *Salmonella* pathogenicity island 7 (SPI-7) in the *S. Typhi* chromosome. Several other loci participate in the complex regulation of Vi expression. Almost all *S. Typhi* isolates from blood cultures express Vi. Nevertheless, Vi-negative strains have been identified occasionally, both in sporadic cases as well as during outbreaks (12). Some of these strains are regulatory mutants that can revert to a Vi-positive state (13). However, some Vi-negative isolates from blood have been shown to harbour deletion mutations in critical genes (for example, *tviB*) within the *viaB* locus that render the strains unable to synthesize Vi. This raises the theoretical concern that large-scale usage of Vi-containing vaccines (either polysaccharide or conjugate) could lead

to selective pressure that creates a biological advantage for the emergence of Vi-negative strains (14).

Pathogenesis

Typhoid infection begins with ingestion of *S. Typhi* in contaminated food or water. In the small intestine, the bacteria penetrate the mucosal layer and ultimately reach the lamina propria. Translocation from the intestinal lumen mainly occurs through *S. Typhi* targeting M cells overlying gut-associated lymphoid tissue. Within this lymphoid tissue and in the lamina propria, *S. Typhi* invokes an influx of macrophages and dendritic cells that ingest the bacteria but fail to destroy them. Thus, some bacteria remain within macrophages in the lymphoid tissue of the small intestine and flow into the mesenteric lymph nodes where there is an inflammatory response mediated by the release of various cytokines. Bacteria enter the bloodstream via lymphatic drainage, thereby seeding organs of the mononuclear phagocyte system (such as the spleen, liver and bone marrow) and gall bladder by means of a silent primary bacteraemia. After a typical incubation period of 8–14 days the clinical illness begins, usually with the onset of fever, abdominal discomfort and headache. An accompanying low-level secondary bacteraemia occurs.

Prior to the availability of fluoroquinolone antibiotics, clinical relapses were observed in 5–30% of patients treated with antibacterial agents such as chloramphenicol and sulfamethoxazole/trimethoprim. These post-treatment relapses occurred when typhoid bacilli re-emerged from their protected intracellular niches within the macrophages of the mononuclear phagocyte system, where the antibacterial agents could not penetrate.

Several lines of investigation indicate that in a small proportion of patients infected with *S. Typhi* who may have premorbid abnormalities of the gall bladder mucosa (such as occurs consequent to gallstones) gall bladder infection becomes chronic (that is, excretion lasts for longer than 12 months) (15). Such chronic carriers, who are themselves not clinically affected by the presence of typhoid bacilli in their system, may excrete the pathogen in their faeces for decades (16). They are thought to serve as a long-term epidemiological reservoir in the community, and to foster the transmission of typhoid wherever there is inadequate sanitation, untreated water supplies and/or improper food handling.

Epidemiology

Typhoid fever is restricted to human hosts and in the late nineteenth and early twentieth century was endemic in virtually all countries in Europe and the Americas. Subsequently, the widespread use of chlorination, sand filtration and other means of water treatment drastically reduced the incidence of typhoid fever despite the high prevalence of chronic carriers (15). Typhoid

remains endemic in most developing countries and is an important public health problem mainly because large segments of the population lack access to safe water and basic sanitation services (17). In addition, there are limited programmes for detecting carriers and restricting them from handling food.

Disease burden

Varied estimates of the annual epidemiological burden (incidence and total number of cases) of typhoid fever have been published in the scientific literature based on the extrapolation of data from various sources. The true incidence of typhoid fever in most regions of developing countries is not known. One study published in 2004 estimated that ~22 million cases occur each year causing 216 000 deaths, predominantly in school-age children and young adults; annual incidence was estimated to be 10–100 per 100 000 population (18). A systematic review of population-based studies published between 1984 and 2005 indicated an annual incidence of 13–976 per 100 000 population each year based on diagnosis by blood culture (19).

More recent analysis has shown that typhoid fever remains a major cause of enteric disease of children in low- and middle-income countries, with global estimates of disease burden ranging from 11 to 21 million typhoid fever cases and approximately 145 000 to 161 000 deaths annually (20). The majority of cases occur in Asia and sub-Saharan Africa but many of the island nations of Oceania also experience a moderate to high incidence of typhoid fever and large outbreaks (21).

Several factors affect the calculation of the burden of typhoid disease, including the way in which the information is obtained. For example, data on the incidence of typhoid, its age-specific distribution and the severity of clinical disease obtained from passive surveillance implemented at health facilities can differ from data acquired through active surveillance. During active surveillance, households are visited systematically once or twice a week to detect fever among household members, and mild or early clinical illness can be detected. One of the most important factors however is how to confirm that a patient with acute febrile illness has typhoid fever. Unfortunately, there is no rapid, affordable and accurate point-of-care or laboratory diagnostic test to confirm a case of acute typhoid fever. Bone marrow culture is widely recognized as the gold standard but is impractical for widespread use. Blood culture is the most practical accurate confirmatory test but its use alone identifies only 40–80% of the cases that are detectable using bone marrow culture (22–24). Cultures of bile containing duodenal fluid and of skin snips of rose spots can be positive when blood cultures are negative (19). Prior patient treatment with antibacterial agents and the volume of blood cultured also affect the yield of cultures. Reliance on clinical diagnosis alone is not advisable because several

other febrile syndromes caused by other microorganisms, such as malaria, dengue, brucellosis and leptospirosis, can be confused with typhoid in both adults and children.

A 2008 study reported on the incidence of typhoid fever detected through passive surveillance (and through modified passive surveillance in two countries where additional health clinics were introduced into the community) in five Asian countries (25). The incidence of typhoid fever ranged from 15.3 per 100 000 person-years among people aged 5–60 years in China to 451.7 per 100 000 person-years among children aged 2–15 years in Pakistan (25). More recently, its incidence in Nepal has been estimated to range from 297 to 449 per 100 000 person-years, with greater incidence occurring during the summer months (26). Incidence data from the placebo control groups in vaccine trials also provide information on the incidence of typhoid fever in multiple geographical areas and locations. However, because vaccine efficacy trials are typically carried out in areas with high endemicity, caution must be exercised when extrapolating these incidence rates to other populations. New data on age-specific occurrence in certain geographical regions, as in some sites in South Asia, confirm that typhoid fever of sufficient severity to seek medical care is common in the 1–4 year-old age group, with a large proportion of disease occurring in children between 6 months and 2 years of age (17).

There has also been an increasing number of major outbreaks associated with antimicrobial-resistant *S. Typhi* (17, 27–31), with the increased occurrence of outbreaks due to multidrug-resistant typhoidal *Salmonella* serovars being of particular concern. Extensively drug-resistant variants of *S. Typhi* have also emerged in India, Bangladesh and Pakistan that severely limit treatment options and are therefore becoming increasingly difficult to treat (32, 33). The *S. Typhi* H58 clade, with IncHII plasmids that carry multidrug-resistance genes and target site mutations mediating fluoroquinolone resistance, is responsible for much of the recent and dramatic spread of resistant strains in countries, such as occurred in Pakistan in 2018 (33, 34). This clade is believed to have emerged on the Indian subcontinent about 30 years ago and then spread to South-East Asia and more recently to sub-Saharan Africa (33). The emergence of extensively antibiotic-resistant *S. Typhi* (resistant to first- and second-line antibiotics) and the implications of this for disease control were reviewed in 2017 (20). The global pattern of drug-resistant *S. Typhi* is dynamic and changing in each location and over time. For example, in Ho Chi Minh City, Viet Nam the proportion of strains with a diminished susceptibility to fluoroquinolones increased from less than 5% to 80% within a few months in 1998 (35). A large-scale outbreak of extensively drug-resistant typhoid in Pakistan further demonstrates the importance of understanding local resistance patterns to enable the selection of appropriate antibiotics for the management of typhoid fever cases (33).

Prior to the availability of antibacterial agents, typhoid resulted in a case-fatality rate of approximately 10–20% (36). Current estimates covering the post-antibiotic era range from 1% to 4% of those who receive adequate therapy (37). Most of the mortality occurs in developing countries, predominantly in Asia. A 2008 review (19) reported community-based mortality ranging from 0% to 1.8% across five studies in developing countries; hospital-based mortality ranged from 0% to 13.9% across all ages in 12 studies; and in children younger than 15 years, mortality ranged from 0% to 14.8% across 13 studies. Hospitalization rates of 2–40% have also been reported (25) indicating that the disease can be severe in a considerable proportion of patients. The evolution and spread of multiple antibiotic resistant *S. Typhi* described above further complicates the situation and leads to an increased proportion of patients experiencing clinical treatment failure and complications, increasing hospital admission and prolonged hospital stay (20).

Few studies have estimated the prevalence of chronic carriers of typhoid and paratyphoid in developing countries. One survey in Santiago, Chile, conducted when typhoid fever was highly endemic there in the 1970s, estimated a crude prevalence of 694 typhoid carriers per 100 000 population (38). In Kathmandu, Nepal, among 404 patients (316 females and 88 males) with gall bladder disease undergoing cholecystectomy, *S. Typhi* was isolated from 3.0% of bile cultures and *S. Paratyphi A* from 2.2% (39). Since the overall prevalence of cholelithiasis in the population of Kathmandu was not known, the overall prevalence of chronic carriage in that population could not be calculated. The role of chronic carriers in the transmission of typhoid fever is still unclear (17) but is thought to vary between settings of high, medium and low disease incidence (18, 40). However, chronic carriers may represent a long-term reservoir of infection and contribute to the persistence of typhoid fever through ongoing shedding of *S. Typhi* and *S. Paratyphi* into the environment, possibly contaminating water supplies.

Clinical features

S. Typhi infection results in a wide spectrum of clinical features, most often characterized by persisting high-grade fever, abdominal discomfort, malaise and headache. Important clinical signs in hospitalized patients include hepatomegaly (41%), toxicity (33%), splenomegaly (20%), obtundation (2%) and ileus (1%) (41). Before antibacterial agents became available, gross bleeding from the gastrointestinal tract and perforations occurred in 1–3% of untreated patients and hospital-based reports suggest that more than 50% of patients may have serious complications. In one 2005 study (42), numerous extra-intestinal complications were reported on involving the central nervous system (3–55%), the hepatobiliary system (1–26%), the cardiovascular system (1–5%),

the pulmonary system (1–6%), bones and joints (less than 1%) and the haematological system (rarely). Intestinal perforations leading to peritonitis and death continue to be reported, albeit rarely, in some settings. Interestingly, the emergence of multidrug-resistant strains has been associated not only with failure to respond to antibiotic treatment but also with changes in the severity and clinical profile of enteric fever (5, 43).

Immune responses to natural infection

Natural typhoid infection is usually associated with detectable serum antibodies and mucosal secretory immunoglobulin A (IgA) intestinal antibody against various *S. Typhi* antigens. However, cell-mediated immune responses are also measurable (44–48). In areas where typhoid is endemic, there is an age-related increase in the prevalence and geometric mean titre of anti-Vi antibodies (49). Anti-flagella (H antigen) serum IgG antibodies following natural infection are long lived and have been studied for seroepidemiological surveys (50).

While serological responses to LPS and flagella antigens tend to be quite strong and are commonly found in patients with culture-confirmed acute typhoid fever, only about 20% of such patients exhibit significant levels of anti-Vi antibody (51, 52). In contrast, high concentrations of anti-Vi serum IgG antibody are detected in 80–90% of chronic carriers (51, 52).

Cell-mediated immunity also appears to play a part in protection – it has been observed that peripheral blood mononuclear leukocytes of healthy adults residing in typhoid-endemic areas, and who have no history of typhoid, proliferate upon exposure to *S. Typhi* antigens (53).

Disease control

As with other enteric and diarrhoeal diseases, typhoid fever occurs predominantly in populations with inadequate access to safe water and basic sanitation. Effective typhoid control requires a comprehensive approach that combines immediate measures, such as accurate and rapid diagnostic confirmation of infection and timely administration of appropriate antibiotic treatment, with sustainable longer-term solutions such as providing access to safe water and basic sanitation services, health education, appropriate hygiene among food handlers and typhoid vaccination. There is evidence that vaccination against typhoid can substantially reduce typhoid fever burden when targeted towards high-risk age groups and geographical areas, and when combined with improved sanitation (54). The most recent WHO position paper on the use of typhoid vaccines was published in 2018 (17).

Typhoid vaccines

Inactivated whole-cell vaccine

Inactivated *S. Typhi* bacteria (heat inactivated and phenol preserved) were first used to prepare parenteral vaccines more than 100 years ago. In the 1960s, WHO sponsored field trials that evaluated the efficacy of inactivated parenteral whole-cell vaccines in several countries (55, 56) and documented a moderate level of efficacy lasting up to 7 years (57). Data from studies of human immune responses and immunogenicity studies in rabbits suggested that anti-H antibodies might represent an immune correlate of protection (58); later extrapolation from the results of mouse protection studies suggested that responses to Vi antigen may also correlate with protection (59, 60). However, these vaccines were associated with considerable rates of systemic adverse reactions (61) and never became widely accepted public health tools, and are thus no longer produced.

Live-attenuated Ty21a oral vaccine

In the early 1970s, an attenuated strain of *S. Typhi* was developed through chemically induced mutagenesis of pathogenic *S. Typhi* strain Ty2 (59). The resultant mutant strain lost the activity of the epimerase enzyme encoded by the *galE* gene and no longer expressed the Vi antigen. The vaccine was found to be stable, safe and efficacious in adults as well as children (62–66). The level of protective immunity achieved varied according to the formulation of the vaccine, the number of doses administered and the interval between doses. For example, three doses of a provisional formulation of vaccine administered to around 32 000 children (aged 6–7 years) in Alexandria, Egypt gave a point estimate of efficacy of 95% (95% confidence interval (CI) = 77–99%) during 3 years of follow-up (67). Three doses of enteric-coated capsules administered to Chilean schoolchildren (aged 6–19 years) using two different dose intervals (either alternate days or 21 days between doses) gave a point estimate of efficacy of 67% (95% CI = 47–79%) during 3 years of follow-up. For the group receiving doses on alternate days, the point estimate of protection over 7 years was 62% (95% CI = 48–73%) (56, 68). For the group receiving each dose after a 21-day interval, the corresponding point estimate of protection was 49% (95% CI = 24–66%). Another trial among Chilean schoolchildren involved the administration of four doses within 7 days and demonstrated even greater protection (69). Human challenge studies showed that 5–8 doses of Ty21a oral vaccine resulted in high rates of anti-LPS antibody seroconversion and 87% protective efficacy (70). However, more recent human challenge studies showed that a three-dose Ty21a immunization schedule resulted in a protective efficacy of only 35% after challenge when using the end-points of fever and/or bacteraemia as a diagnosis of typhoid (71). When efficacy was recalculated using the same definition for typhoid diagnosis used in the original vaccine/

challenge studies (fever with subsequent microbiological confirmation) then Ty21a efficacy reached 80% (71), which is similar to that reported in the older challenge studies.

Two field trials in Chile (66) and Indonesia (65) compared the use of enteric-coated capsules with three doses of the liquid formulation. In both trials, the liquid formulation was associated with greater efficacy than the capsules. In Chile, where the doses were given on alternate days, the results for the liquid formulation were superior to those obtained in Indonesia, where the doses were administered 1 week apart (point estimate of efficacy = 77% in Chile and 53% in Indonesia). In Chile, 78% protection was documented up to 5 years following vaccination with the liquid formulation (68). However, the previously marketed liquid formulation is no longer produced, and only enteric-coated capsules are currently available (17).

All countries in which Ty21a is licensed utilize a three-dose course of enteric-coated capsules taken on alternate days, with the exception of the United States of America (USA) and Canada, which both use a four-dose course. This vaccine was first licensed in Europe in 1983 and in the USA in 1989, and is approved for use in individuals older than 6 years. Because the vaccine is highly acid labile, stomach acidity must be neutralized or bypassed when Ty21a is fed orally. There is indirect evidence that large-scale vaccination with Ty21a may provide some degree of protection against typhoid to people who have not been vaccinated through the mechanism of herd protection.

Vi polysaccharide vaccine (unconjugated)

Technological advances in the late 1960s made it possible to purify Vi polysaccharide without damaging its antigenic properties and to prepare vaccines that are almost totally free of contaminating LPS (72); these vaccines are associated with low rates of febrile reactions (1–2%). Vi polysaccharide vaccine was first licensed in the USA in 1994 and since then several products have been licensed for use in individuals aged 2 years and older. One such product (Typhim-Vi) has been prequalified by WHO.¹⁵

The immunological basis of protection by purified Vi polysaccharide parenteral vaccines is the generation of serum anti-Vi IgG antibodies in 85–90% of vaccine recipients older than 2 years.

Clinical trials with these vaccines showed a rise in anti-Vi antibody titres in adults and children (73–75). However, subsequent inoculations with Vi did not boost the antibody response. Although a single dose has been associated with the persistence of antibodies for up to 3 years in some recipients, many adult

¹⁵ See: WHO Prequalified Vaccines at: https://extranet.who.int/gavi/PQ_Web/

recipients in non-endemic areas showed a marked drop in antibody levels after 2 years (76, 77). An outbreak of typhoid fever among French soldiers deployed in Côte d'Ivoire indicated that the risk of typhoid fever was significantly higher in those who had been vaccinated more than 3 years previously compared to those who had been vaccinated in the 3 years prior to the outbreak (78).

Field trials in children and adults in Nepal given a single 25 µg dose showed 72% vaccine efficacy during 17 months of follow-up (73) and a field trial in schoolchildren in South Africa (also using a single 25 µg dose) showed 60% protection during 21 months of follow-up (74). In South Africa, protection was found to decline to 55% at 3 years (79). Another field trial in China in people aged 3–50 years given a single 30 µg dose showed 69% efficacy during 19 months of follow-up (80). Thus, while a single dose of an unconjugated Vi polysaccharide vaccine provides moderate protection, the available data suggest that protective efficacy does not last beyond 3 years and revaccination is necessary within that time.

Most data suggest that children younger than 5 years respond poorly to unconjugated Vi polysaccharide vaccines (81). However, one cluster-randomized trial in Kolkata, India (82) found that protective efficacy among young children (aged 2–4 years) was 80%, which was higher than that observed in children aged 5–14 years (56%) and in older persons (46%). In contrast, a cluster-randomized field trial of similar design and using the same Vi polysaccharide vaccine in Karachi, Pakistan reported an adjusted total protective effectiveness of –38% (95% CI = minus 192–35%) for children aged 2–5 years compared with 57% (95% CI = 6–81%) for children aged 5–16 years (81).

Thus, a single dose of unconjugated Vi polysaccharide vaccine can provide moderate protection for a limited duration, but such vaccines have the usual limitations associated with polysaccharide vaccines, including poor immunogenicity in infants and young children, short-lived immunity and lack of anamnestic immune responses to subsequent doses (76, 82, 83).

Vi polysaccharide–protein conjugate vaccine

Experience with other polysaccharide–protein conjugate vaccines (such as *Haemophilus influenzae* type b, meningococcal and pneumococcal vaccines) has shown that conjugation to a carrier protein overcomes many of the limitations associated with unconjugated bacterial polysaccharides. On the basis of this, several Vi polysaccharide–protein conjugate vaccines have been developed or are under development. These include vaccines based on Vi polysaccharide conjugated to tetanus toxoid (TT), diphtheria toxoid (DT), the nontoxic mutant of diphtheria toxin cross-reactive material 197 (CRM₁₉₇) as well as on the prototype Vi polysaccharide conjugated to nontoxic recombinant exoprotein A of *Pseudomonas aeruginosa* (Vi-rEPA) (84). One TCV that uses Vi prepared

from *C. freundii* s.l. and CRM₁₉₇ as the carrier protein has been shown to elicit a higher level of anti-Vi IgG compared to an unconjugated Vi polysaccharide vaccine in European adults who have never been exposed to typhoid fever (85). Vi preparations from *C. freundii* s.l. are immunologically indistinguishable from and structurally similar to those from *S. Typhi* (85–87), though size and viscosity differences have been observed for Vi polysaccharide from *S. Typhi* and *C. freundii* s.l. using high-performance size-exclusion chromatography (HPSEC) (87).

Four TCVs have been licensed in India since 2008; three consisting of Vi polysaccharide conjugated to TT and one to CRM₁₉₇. Other TCVs are in late-stage development in some Asian countries. Typbar-TCV (a Vi–TT conjugate vaccine) was licensed in India in 2013 for use in children aged 6 months or older and in adults up to 45 years of age on the basis of immunogenicity and safety demonstrated in a Phase III study in an endemic setting (17, 88). The results showed that anti-Vi antibody titres were significantly higher among recipients of Typbar-TCV than those vaccinated with the unconjugated Vi polysaccharide vaccine. Furthermore, the high geometric mean titres of IgG anti-Vi antibodies elicited by a single dose of Typbar-TCV persisted for up to 5 years in approximately 84% of children. The vaccine was prequalified by WHO in December 2017. A protective efficacy of 87.1% (95% CI = 47.2–96.9%) against persistent fever associated with positive blood culture for *S. Typhi* was subsequently demonstrated in human challenge studies (89). Interim data on the efficacy of Typbar-TCV in an endemic population in Nepal have also recently been published (26). These data, from a Phase III participant-observer blinded randomized study in children aged 9 months to 16 years of age, confirmed that a single dose of Typbar-TCV was safe, immunogenic and effective in this field setting, with an efficacy of 81.6% (95% CI = 58.8–91.8%) (26). This conclusion is supported by new data from Pakistan (vaccine efficacy = ~89%) and India (vaccine efficacy = 82%). In view of the improved immunological properties of TCVs compared to the other available typhoid vaccine types, their suitability for use in young children and longer expected duration of protection, the WHO SAGE recommended such vaccines as the preferred type for use in individuals from 6 months to 45 years of age, and that the introduction of TCVs be prioritized in countries with the highest burden of typhoid fever or with a high burden of antimicrobial-resistant *S. Typhi* (17).

Although no internationally agreed correlates or surrogates of protection have yet been identified for Vi conjugate vaccines, a number of suggested correlates have been proposed. Based on the assay used to measure anti-Vi IgG serum antibodies generated in response to the prototype United States National Institutes of Health Vi-rEPA conjugate vaccine in Viet Nam, a threshold value of 4.3 µg/mL anti-Vi antibody measured by enzyme-linked immunosorbent assay

(ELISA) was found to be associated with a high level of sustained protection lasting 4 years after vaccination (90, 91). A placebo-controlled randomized double-blind study in Vietnamese children aged 2–5 years in a highly endemic area produced an estimated efficacy of 89% for the Vi-rEPA vaccine over 46 months of follow-up (92, 93). However, although a study to evaluate Vi-TT in Nepal (94) found that higher anti-Vi IgG levels are associated with greater protection against typhoid infection, no threshold level could be identified at which the probability of infection becomes negligible within the range of antibody levels induced by vaccination.

It is acknowledged that there are difficulties in comparing any immunogenicity data generated using new TCVs and current ELISA protocols to the data generated in the original Vi-rEPA trial in Viet Nam (20). However, the inclusion of a working reference serum calibrated against the First WHO International Standard for anti-typhoid capsular Vi polysaccharide immunoglobulin G (see **International reference materials** below) can improve the interpretation of data from clinical trials (95, 96). The use of this WHO international standard will ensure consistency in determining serum titres and thus allow for the comparison of data generated by different assays and/or different laboratories.

It has been suggested that variability in the biophysical properties of antibodies induced by Vi polysaccharide and Vi-TT conjugated vaccines (such as antibody subclass distribution and avidity) may also impact protective outcomes. One recent study (97) identified serum Vi IgA as a biomarker of protective immunity against typhoid fever and quantified the concentration of Vi IgA in vaccine recipients. However, no correlate of protection was identified and it was concluded that further work was needed to determine whether IgA represents a true correlate of protection or a surrogate marker of another underlying immune response.

Challenge studies

The development of vaccines against typhoid fever has been complicated by the human host restriction of *S. Typhi*, the lack of clear correlates of protection, the required scale of field trials of efficacy and uncertainty about the estimation of vaccine impact. Historically, only the chimpanzee model of the 1960s demonstrated a pathogenesis and clinical illness somewhat resembling typhoid fever in humans (98–101). However, the chimpanzee model is no longer permissible and recent animal models (including ones based on “humanized” small animals) have not been able to mimic the disease process of human typhoid, despite many attempts (102–107). Instead, a human challenge model has been used to overcome some of these difficulties and to provide direct estimation of efficacy in vaccine recipients who are deliberately challenged with

the pathogen in a controlled setting (108, 109). The first setting during the 1950s to early 1970s involved volunteers in a penal institution (70, 110–112) whereas more recent studies have involved community volunteers (108, 109).

International reference materials

Two WHO international standards for Vi polysaccharide have been developed to measure the polysaccharide content of typhoid vaccines (113–115). The Vi polysaccharide content of these two standards was assessed using quantitative nuclear magnetic resonance (qNMR). The First WHO International Standard for *Salmonella* Typhi Vi polysaccharide (NIBSC code 16/126) has a content of 2.03 ± 0.10 mg Vi polysaccharide/ampoule. The First WHO International Standard for *Citrobacter freundii* Vi polysaccharide (NIBSC code 12/244) has a content of 1.94 ± 0.12 mg Vi polysaccharide/ampoule (113–115). Both standards can be used in physicochemical assays, for example, high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) or in immunoassays such as rocket immunoelectrophoresis to measure the amount of Vi polysaccharide in final product, bulk conjugate and process intermediates. In addition, these Vi polysaccharide standards can be used as coating antigens for in-house ELISAs (115–117). When analyzing the content of Vi polysaccharide vaccines the homologous Vi polysaccharide standard should be used. For example, if the conjugate has been made with *Citrobacter* Vi polysaccharide then the *Citrobacter* Vi polysaccharide standard would be the more appropriate standard to use. The use of these WHO standards decreases the variability of in-house analytical assays (114, 115).

In addition, a First WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (NIBSC code 16/138) is available and consists of pooled post-immunization sera obtained following vaccination with plain Vi polysaccharide or conjugated Vi polysaccharide according to the immunization schedule of Jin et al. (89). This international standard was evaluated in both commercial and in-house ELISAs, and assigned a concentration of 100 International Units (IU)/ampoule (95, 116, 117). This primary reference standard should be used as a calibrant for in-house and working standards that are used to evaluate the immunogenicity of licensed vaccines and vaccine candidates in clinical studies (116, 117). The American national reference reagent Vi-IgG_{R1,2011}, distributed by the United States Food and Drug Administration, was also included in the collaborative study to establish the standard material 16/138 and was determined to contain 163 IU/mL (116). As Vi-IgG_{R1,2011} had been established as containing 33 µg anti-Vi IgG/mL (90) (equivalent to 33 µg/vial) it can be assumed that 1 µg anti-Vi IgG/mL is equivalent to 4.94 IU/mL.

A further collaborative study was then conducted in which the standard materials 16/138 and 12/244 were used to evaluate a standardized in-house ELISA based on a co-coating of Vi polysaccharide and poly-L-lysine. The results obtained indicate that this generic assay would be a suitable alternative to the commercial Vi polysaccharide ELISA (96).

All of the above WHO international standards are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom.¹⁶ For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations should be consulted.¹⁷

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

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

The use of the international name should be limited to vaccines that meet the specifications given below.

A.1.2 Descriptive definition

A typhoid conjugate vaccine (TCV) is a preparation of *S. Typhi* or *C. freundii* s.l. Vi polysaccharide covalently linked to a carrier protein. It may be formulated with a suitable adjuvant and/or a preservative. It should be presented as a sterile aqueous suspension or as freeze-dried material. The preparation should meet all of the specifications given below.

A.2 General manufacturing recommendations

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (118) and WHO good manufacturing practices for biological products (119) should be followed at establishments manufacturing Vi conjugate vaccines.

The production method should be shown to consistently yield Vi conjugate vaccines of satisfactory quality as outlined in these WHO Recommendations. All assay procedures used for quality control of the

¹⁶ See: www.nibsc.org/products

¹⁷ See: www.who.int/bloodproducts/catalogue

conjugate vaccine and vaccine intermediates should be validated. Post-licensure changes to the manufacturing process and quality control methods should also be validated and may require approval from the NRA prior to implementation (120–122).

Production strains for Vi polysaccharide and the carrier proteins may represent a hazard to human health and should be handled under appropriate containment conditions based on risk assessment and applicable national and local regulations (123). Standard operating procedures should be developed to deal with emergencies arising from accidental spills, leaks or other accidents. Personnel employed by the production and control facilities should be adequately trained. Appropriate protective measures, including vaccination, should be implemented if available.

If raw materials used in the culture media or in subsequent manufacturing steps contain materials of animal origin, they should comply with the current *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (124).

A.3 Control of source materials

A.3.1 Bacterial strains

The bacterial strain used for preparing Vi polysaccharide or carrier protein should be from a single well-characterized stock that can be identified by a record of its history, including the source from which it was obtained, number of passages and the tests used to determine the characteristics of the strain. Information regarding materials of animal origin used during passages of the bacterial strain should be provided, such as compliance with the current *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (124) or a statement of risk assessment.

A.3.1.1 Bacterial strain for preparing Vi polysaccharide

The strain used should be capable of stably producing Vi polysaccharide. *S. Typhi* and *C. freundii* s.l. have been shown to be suitable sources of Vi polysaccharide. Proton nuclear magnetic resonance (^1H NMR) spectroscopy, immunochemical tests or any other suitable method may be used for confirming the identity of the polysaccharide.

A.3.2 Bacterial seed lot system

The production of both Vi polysaccharide and the carrier protein should be based on a seed lot system consisting of a master seed and a working seed. Cultures derived from the working seed should have the same characteristics as the cultures of the strain from which the master seed lot was derived.

Each new seed lot prepared should be characterized using appropriate control tests to ensure comparable quality attributes to those of the previous seed lot. New seed lots should also be shown to have comparable Vi polysaccharide or carrier protein yields in routine manufacturing prior to their use.

The control tests for master and working seed lots may include culture purity, strain identity, Vi polysaccharide immunoassay or any other method(s) suitable for the characterization of Vi polysaccharide or carrier protein.

A.3.3 Bacterial culture media

Manufacturers are encouraged to avoid the use of materials of animal origin. However, if the culture medium does contain materials of animal origin, these should comply with the current *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (124)*. The use of materials of animal origin should be discussed with and approved by the NRA.

The culture medium used to prepare bacterial seed lots and commercial vaccine lots should also be free from substances likely to cause toxic or allergic reactions in humans. Additionally, the liquid culture medium used to produce polysaccharide intermediate should be free from ingredients that will form a precipitate upon addition of chemical compounds, such as hexadecyltrimethylammonium bromide (CTAB), used for the purification of the Vi polysaccharide.

A.4 Control of vaccine production

A.4.1 Control of polysaccharide antigen production

The Vi polysaccharides that are used in licensed vaccines are defined chemical substances if they are prepared to similar specifications, for example as described in the WHO Requirements for Vi polysaccharide typhoid vaccine (125) and the requirements set out in the following sections of the current document. As a result, it is expected that they will be suitable for the preparation of TCVs.

A.4.1.1 Single harvests for preparing Vi polysaccharide antigen

The consistency of the production process should be demonstrated by monitoring the growth of the organisms and the yield of Vi polysaccharide.

A.4.1.1.1 Consistency of microbial growth for antigen production

The consistency of the growth of production strains should be demonstrated by monitoring the growth rate, pH, pO₂ and the final yield of Vi polysaccharide – though monitoring should not be limited to these parameters.

A.4.1.1.2 *Bacterial purity*

Samples of the culture should be taken before inactivation and examined for microbial contamination. The purity of the culture should be verified using suitable methods, such as inoculation on appropriate culture media. If contamination is found, the culture and any product derived from it should be discarded.

A.4.1.2 *Bacterial inactivation and antigen purification*

Generally, the production culture is inactivated using a suitable method such as chemical treatment (for example, with formaldehyde), heating or other alternative methods prior to purification. The inactivation process should be validated and monitored using a validated test during routine manufacturing. If a chemical agent is used for inactivation, its residual level should be controlled as described in section A.4.1.3.10 below.

After inactivation where appropriate, the biomass of *S. Typhi* or *C. freundii* s.l. is removed using an appropriate method such as centrifugation or tangential flow filtration. The Vi polysaccharide may be then purified from the supernatant by precipitation with CTAB or by other suitable methods approved by the NRA. Bioburden should be monitored during purification. The purified Vi polysaccharide should be stored under appropriate conditions that have been shown to retain the integrity of the Vi polysaccharide (for example, powder at 2–8 °C or lower and solution at –20 °C or lower). Hold times should be based on the results of stability studies and approved by the NRA.

A.4.1.3 *Control of purified Vi polysaccharide antigen*

Each lot of purified Vi polysaccharide should be tested for identity and purity, as well as the additional parameters described below. All tests should be validated and any test limits or ranges not defined by a pharmacopoeia should be agreed with the NRA.

A.4.1.3.1 *Identity*

Vi polysaccharide is a linear homopolymer composed of (1 →4)-2-acetamido-2-deoxy- α -D-galacturonic acid that is *O*-acetylated at carbon-3 (126).

A test should be performed on the purified polysaccharide to verify its identity, such as NMR spectroscopy (127) or a suitable immunoassay, as appropriate and convenient.

A.4.1.3.2 *Molecular size or mass distribution*

The molecular size or mass distribution of each lot of purified polysaccharide should be estimated to assess the consistency of each batch. The distribution constant (K_D) should be determined by measuring the molecular size

distribution of the polysaccharide at the main peak of the elution curve obtained by a suitable chromatographic method. The K_D value or the mass distribution limits, or both, should be established and shown to be consistent from lot to lot for a given product. To ensure consistency and a defined proportion of high molecular size polysaccharide for gel filtration using HPSEC, typically at least 50% of the Vi polysaccharide should elute at a K_D value less than a predefined value, depending on the chromatographic method used. However, if molecular weight (MW) is determined by static light scattering then there is no need for a K_D value since it is a coefficient that is dependent on the column used. As an alternative, polysaccharide MW distribution can be determined by gel permeation chromatography using a MW standard calibration curve (that is, dextran, pullulans or polyethylene oxide standards) – the number average molecular weight (M_n), the weight average molecular weight (M_w) and the size average molecular weight (M_z) should be determined to describe the distribution.

An acceptable level of consistency should be agreed with the NRA. Alternatively, calculation of the peak width at the 50% level can be used to analyse the distribution of MW. Suitable detectors for this purpose include a refractive index detector (128), alone or in combination with a static light scattering detector (for example, multi-angle laser light scattering detector) (87) and/or a viscometer. The methodology used should be validated to demonstrate sufficient resolution in the appropriate MW range. Manufacturers are encouraged to produce Vi polysaccharide that has a consistent distribution of molecular size.

Due to its high viscosity on molecular sizing columns, the Vi polysaccharide does not behave the same as other polysaccharides; therefore, column matrices and eluents should be carefully chosen to ensure a representative recovery (87, 129).

A.4.1.3.3 Polysaccharide content

The concentration of the Vi polysaccharide in its *O*-acetylated acid form can be measured using NMR (127) or HPAEC-PAD (131, 132), while methods such as the Hestrin method (114, 130) or the acridine orange method (126, 131) are also acceptable, and a suitable immunoassay, for example rocket immunoelectrophoresis or ELISA, may be considered. A suitable reference preparation of Vi polysaccharide calibrated against the First WHO International Standard for *Citrobacter freundii* Vi polysaccharide (NIBSC code 12/244) or against the First WHO International Standard for *Salmonella* Typhi Vi polysaccharide (NIBSC code 16/126) should be used where appropriate (see **International reference materials** above). These methods should be validated and agreed with the NRA.

A.4.1.3.4 *O*-acetyl content

The *O*-acetyl content of the purified Vi polysaccharide is important for the immunogenicity of Vi (87, 126, 133) and should be at least 2.0 mmol/g polysaccharide (52% *O*-acetylation), unless justified. The Hestrin method (130), NMR (127, 134), high-performance anion exchange chromatography with conductivity detection (HPAEC-CD) (135) or other suitable methods may be used to quantitatively determine *O*-acetylation. The methods and acceptance criteria used should be agreed with the NRA.

A.4.1.3.5 *Moisture content*

If the purified polysaccharide is to be stored as a dried form, the moisture content should be determined using suitable validated methods, and the results should be within established limits. The methods and acceptable limits used should be agreed with the NRA.

A.4.1.3.6 *Protein impurity*

The protein content should be determined using a suitable validated method, such as that of Lowry et al. (136), and using bovine serum albumin as a reference. Sufficient polysaccharide should be assayed to accurately detect protein contamination. Each lot of purified polysaccharide should typically contain no more than 1% (weight/weight) of protein.

A.4.1.3.7 *Nucleic acid impurity*

Each lot of purified polysaccharide should contain no more than 2% nucleic acid by weight as determined by ultraviolet spectroscopy on the assumption that the absorbance of a 10 g/L nucleic acid solution contained in a cuvette of 1 cm path length at 260 nm is 200 (137). Other validated methods may be used. Sufficient polysaccharide should be assayed to accurately determine nucleic acid contamination.

A.4.1.3.8 *Phenol content*

If phenol has been used to prepare the Vi polysaccharide antigen, each lot should be tested for phenol content using a validated method approved by the NRA. The phenol content should be expressed in µg/mg of purified Vi antigen and shown to be consistent and within the limits approved by the NRA.

A.4.1.3.9 *Endotoxin*

The endotoxin content of each lot of purified Vi polysaccharide should be determined and shown to be within limits agreed with the NRA. Suitable *in vitro* methods include the *Limulus* amoebocyte lysate (LAL) test.

A.4.1.3.10 *Residual process-related contaminants*

The levels of residual process-related contaminants in the purified polysaccharide (for example, CTAB, formaldehyde or other bacterial inactivating agent, and antifoaming agents) should be determined, and shown to be below the limits agreed with the NRA. The routine testing of each lot before release for residual process-related contaminants may be omitted once consistency of production has been demonstrated on a number of lots; this number should be agreed with the NRA.

A.4.1.4 **Activated polysaccharide**

Purified Vi polysaccharide is usually activated to enable conjugation; it may also be partially depolymerized or fragmented, either before or during the activation process.

A.4.1.4.1 *Chemical activation*

Several methods are satisfactory for the chemical activation modification of Vi polysaccharides prior to conjugation. The method that is chosen should be approved by the NRA. As part of the in-process control procedures, the processed Vi polysaccharide that will be used in the conjugation reaction should be assessed to determine the number of functional groups introduced.

A.4.1.4.2 *Molecular size or mass distribution*

If any size-reduction (138, 139) or activation steps are performed, the average size or mass distribution (that is, the degree of polymerization) of the processed Vi polysaccharide should be measured using a suitable method. The size or mass distribution should be controlled using appropriate limits as these may affect the reproducibility of the conjugation process as well as the immunogenicity of the conjugate.

A.4.2 **Control of carrier protein production**

A protein that is safe and, when covalently linked with polysaccharide, elicits a T-cell-dependent immune response against polysaccharide could potentially be used as a carrier protein. Suitable carrier proteins include, but are not limited to, TT, DT, CRM₁₉₇ and rEPA.

A.4.2.1 **Consistency of production of the carrier protein**

The manufacturing process for a carrier protein should be shown to consistently yield batches that are suitable for the conjugation process. Adequate in-process control should be implemented to monitor critical process parameters, such as

the growth rate of the microorganism, pH of production culture and the final yield of the carrier protein.

A.4.2.2 Characterization and purity of the carrier protein

Carrier proteins should be assayed for purity and concentration and tested to ensure they are nontoxic and appropriately immunogenic. All tests used to control the carrier protein should be approved by the NRA.

Preparations of TT and DT should satisfy the relevant WHO recommendations (140, 141). CRM₁₉₇ can be obtained from cultures of *Corynebacterium diphtheriae* C7/β197 (142) or by expression in other genetically modified microorganisms (143). CRM₁₉₇ with a purity of not less than 90% as determined by high-performance liquid chromatography (HPLC) should be prepared by column chromatographic methods. Residual host cell DNA content should be determined and results should be within the limits approved by the NRA for the particular product. Testing for residual host cell DNA content may be omitted if adequate validation data are available. When CRM₁₉₇ is produced in the same facility as DT, tests should be carried out to distinguish the CRM₁₉₇ protein from the active toxin.

A test should be performed on the purified carrier protein to verify its identity. Mass spectrometry or a suitable immunoassay or physicochemical assay could be performed as appropriate and convenient.

Additionally, the carrier protein should be further characterized using appropriate physicochemical methods, such as: (a) sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE); (b) isoelectric focusing; (c) HPLC; (d) amino acid analysis; (e) amino acid sequencing; (f) circular dichroism; (g) fluorescence spectroscopy; (h) peptide mapping; or (i) mass spectrometry (144). Outcomes should be within the specifications of the carrier protein that was used to prepare the TCV lots evaluated in the definitive clinical studies used for licensing.

A.4.2.3 Degree of activation of the carrier protein

Adipic acid dihydrazide (ADH) or other appropriate linkers, such as N-succinimidyl-3-(2-pyridyldithio)-propionate, can be used to modify the carrier protein. The level of protein modification should be monitored, quantified and be consistent. The use of an in-process control may be required. The reproducibility of the method used for modification should be validated.

The level of modification of the carrier protein by ADH can be assessed by determining the amount of hydrazide; this can be achieved by using colorimetric reactions with 2,4,6-trinitrobenzenesulfonic acid using ADH as a standard (145–147). Other suitable methods include fluorescent tagging followed by HPLC or quadrupole time-of-flight mass spectrometry.

A.4.3 Conjugation and purification of the conjugate

A number of methods of conjugation are in use and all involve multistep processes (131, 138, 145–147). Prior to demonstrating the immunogenicity of the Vi conjugate vaccine in clinical trials, both the method of conjugation and the control procedures should be established to ensure the reproducibility, stability and safety of the conjugate.

The derivatization and conjugation processes should be monitored and analyzed for unique reaction products. Residual unreacted functional groups or their derivatives are potentially capable of reacting *in vivo* and may be present following the conjugation process. The manufacturing process should be validated and the limits for unreacted activated functional groups (those that are known to be clinically relevant) at the conclusion of the conjugation process should be agreed with the NRA.

After the conjugate has been purified, the tests described below should be performed to assess the consistency of the production process. These tests are critical for ensuring consistency from lot to lot.

A.4.4 Control of the purified bulk conjugate

Tests for releasing purified bulk conjugate should be validated.

A.4.4.1 Identity

A suitable immunoassay or other method should be performed on the purified bulk conjugate to verify the identity of the Vi polysaccharide. Depending on the buffer used, NMR spectroscopy may be used to confirm the identity and integrity of the polysaccharide in the purified bulk conjugate (134, 148–150). The identity of the carrier protein should also be verified using an immunoassay or other suitable method.

A.4.4.2 Endotoxin

The endotoxin content of the purified bulk conjugate should be determined using a suitable *in vitro* method such as a LAL test and shown to be within limits agreed with the NRA.

A.4.4.3 O-acetyl content

The O-acetyl content of the purified bulk conjugate should be determined by NMR, Hestrin method or other appropriate methods. The specification for the O-acetyl content of the purified bulk conjugate should be agreed with the NRA. The specification for O-acetyl content of the conjugate bulk should not be higher than that set for the purified Vi polysaccharide.

A.4.4.4 Process- and product-related impurities

The purification procedures for the conjugate should remove any residual reagents that were used for conjugation and capping. The removal of reagents, their derivatives and reaction by-products such as ADH, phenol and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (known as EDC, EDAC or EDCI) should be confirmed using suitable tests or by validation of the purification process. The routine testing of each lot may be omitted once consistency of production has been demonstrated on a number of lots; this number should be agreed with the NRA.

The specifications of the residual reagents and the quantifiable methods to be used should be agreed upon in consultation with the NRA.

Process validation should also demonstrate that no significant covalent modification of the Vi polysaccharide itself has occurred, and the percentage of modified Vi monosaccharides should not exceed what was shown to be safe and immunogenic in clinical studies. An example of this is the use of EDC, which leads to N-acylurea modifications. The N-acylurea content can be readily measured using NMR.

A.4.4.5 Polysaccharide content

The content of Vi polysaccharide should be determined using an appropriate validated assay such as HPAEC-PAD (90, 113, 131, 132) or immunological methods (for example, rate nephelometry, rocket electrophoresis). For recommendations on suitable reference materials to use see **International reference materials** above.

A.4.4.6 Conjugated and unbound (free) polysaccharide

A limit for the presence of unbound (free) Vi polysaccharide relative to total Vi polysaccharide should be set for the purified bulk conjugate; this limit should be agreed with the NRA. Methods that have been used to assay unbound polysaccharide include size-exclusion chromatography–reverse phase liquid chromatography (151), Capto Adhere anion-exchange resin binding (129) and deoxycholate precipitation (152) followed by HPAEC-PAD or other method listed in section A 4.4.5 above. Other suitable methods may be developed and validated.

A.4.4.7 Total protein and unbound (free) protein

The protein content of the purified bulk conjugate should be determined using an appropriate validated assay. Each batch should be tested for conjugated and unbound protein. The unbound protein content of the purified bulk conjugate should comply with the limit for the product that has been agreed with the NRA. Appropriate methods for determining unbound protein include HPLC and capillary electrophoresis.

A.4.4.8 Conjugation markers

The success of the conjugation process can be assessed by characterizing the conjugate using suitable methods. For example, an increase in the MW of the protein component of the conjugate compared with the carrier protein can be demonstrated using the Coomassie blue stain with SDS-PAGE; an increase in the MW of the conjugate compared with both the Vi polysaccharide and the protein components should be demonstrated by the gel filtration profile, HPSEC, capillary electrophoresis or other suitable method.

Where the chemistry of the conjugation reaction results in the creation of a unique linkage marker (for example, a unique amino acid, a linker or other measurable marker of conjugation) this should be quantified for each conjugate batch to assess the extent of the covalent reaction between the Vi polysaccharide and the carrier protein (138). This assessment of the unique linkage marker may be omitted once the consistency of conjugation is established, with the agreement of the NRA.

A.4.4.9 Absence of reactive functional groups

The validation batches should be shown to be free of reactive functional groups or their derivatives that are suspected to be clinically relevant on the polysaccharide and the carrier protein.

Where possible, the presence of reactive functional groups (for example, those derived by ADH treatment) should be assessed for each batch. Alternatively, the product of the capping reaction may be monitored, or the capping reaction can be validated to show that reactive functional groups have been removed.

A.4.4.10 Ratio of polysaccharide to carrier protein

The ratio of polysaccharide to carrier protein (expressed, for example, as g/g or mol/mol) in the purified bulk conjugate should be calculated. For this ratio to be a suitable marker of conjugation, the content of each of the conjugate components prior to their use should be known. For each purified bulk conjugate, the ratio should be within the range approved by the NRA for that particular conjugate and should be consistent with the ratio in vaccine that has been shown to be effective in clinical trials.

A.4.4.11 Molecular size or mass distribution

It is important to evaluate the molecular size or mass of the polysaccharide-protein conjugate to establish the consistency of production, product homogeneity and stability during storage.

The relative molecular size of the polysaccharide–protein conjugate should be determined for each purified bulk conjugate using a gel matrix appropriate for the size of the conjugate (87). The method used should be validated and should have the specificity required to distinguish the polysaccharide–protein conjugate from other components that may be present (for example, unbound protein or polysaccharide). The specification of molecular size or mass distribution should be vaccine specific and consistent with that of lots shown to be immunogenic in clinical trials.

Typically, the size of the polysaccharide–protein conjugate may be examined by methods such as gel filtration using HPSEC on an appropriate column. Since the ratio of polysaccharide to protein is an average value, characterization of this ratio over the molecular size or mass distribution (for example, by using dual monitoring of the column eluent) can provide further proof of the consistency of production (144, 153).

A.4.4.12 Bacterial and fungal sterility

The purified bulk conjugate should be tested for bacterial and fungal sterility according to the methods described in Part A, sections 5.1 and 5.2 of the WHO General requirements for the sterility of biological substances (154), or using methods approved by the NRA. If a preservative has been added then appropriate measures should be taken to prevent it from interfering with the tests.

A.4.4.13 Specific toxicity of the carrier protein

The purified bulk conjugate should be tested to confirm the absence of toxicity specific to the carrier protein where appropriate (for example, when DT or TT is used as the carrier protein). Alternatively, the absence of specific toxicity of the carrier protein may be demonstrated at the purified carrier protein stage if agreed with the NRA.

A.4.4.14 pH

The pH of each batch should be tested, and the results should be within the established range and compatible with stability data.

A.4.4.15 Appearance

The appearance of the purified bulk conjugate solution, with respect to its form and colour, should be examined by a suitable method and should meet the established specifications. For a Vi polysaccharide conjugated to a toxoid, the appearance is typically clear to moderately turbid, and colourless to pale yellow.

For a dried or lyophilized preparation, the appearance should be checked after reconstitution with the appropriate diluent and should meet the established specifications.

A.4.5 Preparation and control of the final bulk

A.4.5.1 Preparation

The final bulk is prepared by mixing a suitable quantity of the purified bulk conjugate with all the other vaccine constituents, which may include stabilizer, preservative and/or adjuvant. The final bulk should be prepared using a validated process and should meet the specifications based on the quality attributes of vaccine lots that have been shown to be safe and efficacious in clinical trials. If an adjuvant is used, it should be mixed with the final bulk at this stage. The use of a preservative in either single-dose or multi-dose vaccine vials is optional. If a preservative has been added, its effect on antigenicity and immunogenicity must be assessed to ensure that the preservative does not affect the immune response.

A.4.5.2 Test for bacterial and fungal sterility

Each final bulk should be tested for bacterial and fungal sterility according to the methods described in Part A, sections 5.1 and 5.2 of the WHO General requirements for the sterility of biological substances (154), or using methods approved by the NRA. If a preservative has been added to the final bulk, appropriate measures should be taken to prevent it from interfering with the tests.

A.5 Filling and containers

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (118) and WHO good manufacturing practices for biological products (119) should be followed.

A.6 Control of the final product

A.6.1 Inspection of the final containers

All filled final containers should be inspected as part of the routine manufacturing process. Those containers showing abnormalities – such as vial defects, improper sealing, clumping or the presence of endogenous or exogenous particles – should be discarded. The test should be performed against a black, and a white, background, and according to pharmacopoeial specifications.

A.6.2 Control tests on the final lot

The following tests should be performed on each final lot of vaccine (that is, in the final container) and the tests used should be validated and approved by the NRA. The permissible limits for tests listed under this section should be justified and approved by the NRA.

A.6.2.1 Appearance

The appearance of the final container and its contents should be verified using a suitable method and should meet the established criteria with respect to form and colour. For freeze-dried vaccines, their appearance should be verified before and after reconstitution, and should meet the established criteria.

A.6.2.2 Identity

Identity tests on the Vi polysaccharide and the carrier protein should be performed on each final lot. An immunological test or a physicochemical assay may be used for the Vi polysaccharide and the carrier protein.

A.6.2.3 Bacterial and fungal sterility

The contents of the final containers should be tested for bacterial and fungal sterility according to the methods described in Part A, sections 5.1 and 5.2 of the WHO General requirements for the sterility of biological substances (154), or using a method approved by the NRA.

A.6.2.4 Polysaccharide content

The amount of Vi polysaccharide conjugate in the final containers should be determined and shown to be within the specifications agreed with the NRA.

The formulations of conjugate vaccines produced by different manufacturers may differ. A quantitative assay for the Vi polysaccharide should be carried out. The specification should be justified based on the clinical lots shown to be safe and immunogenic, and approved by the NRA. Examples of tests that may be used include: (a) colorimetric methods; (b) chromatographic methods (including HPLC and HPAEC-PAD); and (c) immunological methods (including rate nephelometry and rocket immunoelectrophoresis) as discussed in sections A.4.1.3.3 and A.4.4.5 of these Recommendations.

A.6.2.5 Unbound (free) polysaccharide

A limit for free Vi polysaccharide content should be set for each conjugate vaccine as discussed in section A.4.4.6 above. Assessing the level of unconjugated polysaccharide in the final lot may be technically demanding (129); as an alternative, the molecular size of the conjugate could be determined for the final lot to confirm the integrity of the conjugate. A more-quantitative assessment of free Vi in solution can be performed by HPAEC-PAD following separation of the intact conjugate. An acceptable value should be consistent with the value seen in batches used for clinical trials that showed adequate immunogenicity and should be approved by the NRA.

A.6.2.6 *O*-acetyl content

The *O*-acetyl content of the Vi polysaccharide conjugate in the final container should be determined for each final lot by NMR (127) or by other appropriate methods, such as the Hestrin method (130). Routine release testing of each lot for *O*-acetyl content in the final product may be omitted if:

- the NRA agrees; and
- the *O*-acetyl content is measured at the level of conjugate bulk; and
- process-validation data obtained during the product's development confirm that formulation and filling do not alter the integrity of the functional groups.

The specification for the *O*-acetyl content of the final lot should not be higher than that set for the conjugate bulk. A limit for the *O*-acetyl content of the Vi polysaccharide conjugate should be approved by the NRA (133).

A.6.2.7 Molecular size or mass distribution

The molecular size or mass distribution of the polysaccharide–protein conjugate should be determined for each final lot using a gel matrix appropriate for the size of the conjugate. The analysis of molecular size or mass distribution for each final lot may be omitted provided that the NRA agrees and the test has been performed on the conjugate bulk (see section A.4.4.11 above).

A.6.2.8 Endotoxin or pyrogen content

The endotoxin content of the final product should be determined using a suitable *in vitro* assay such as a LAL test. The endotoxin content should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and within the limits agreed with the NRA.

The need for pyrogenicity testing should be determined during the manufacturing development process. It should also be evaluated following any changes in the production process or relevant reported production inconsistencies that could influence the quality of the product with regard to its pyrogenicity. When required, the monocyte activation test (MAT) or rabbit pyrogenicity test may be used for monitoring potential pyrogenic activity subject to the agreement of the NRA.

A.6.2.9 Adjuvant content and degree of adsorption

If an adjuvant has been added to the vaccine, its content should be determined using a method approved by the NRA. The amount of the adjuvant should also be agreed with the NRA. If aluminium compounds are used as adjuvants, the aluminium content should not exceed 1.25 mg per single human dose.

The consistency of adsorption of the antigen to the adjuvant is important; the degree of adsorption should be tested in each final lot and should be within the range of values measured in vaccine lots shown to be clinically effective. The methods and specifications used should be approved by the NRA.

A.6.2.10 Preservative content

If a preservative has been added to the vaccine, its content should be determined using a method approved by the NRA.

The amount of preservative in each dose of the vaccine should be shown not to have any deleterious effect on the antigen or to impair the safety of the product in humans. The effectiveness of the preservative should be demonstrated, and the concentration used should be approved by the NRA.

A.6.2.11 pH

If the vaccine is a liquid preparation, the pH of each final lot should be tested, and the results should be within the range of values approved by the NRA. For a lyophilized preparation, the pH should also be measured after reconstitution with the appropriate diluent.

A.6.2.12 Moisture content

If the vaccine is a lyophilized preparation, the level of residual moisture should be determined, and the results should be within the limit agreed with the NRA.

A.6.2.13 Osmolality

The osmolality of the final lots should be determined and shown to be within the range considered to be safe for intramuscular administration to humans and agreed with the NRA. The test for osmolality may be omitted once consistency of production is demonstrated or justification is provided, with the agreement of the NRA.

A.6.2.14 Protein content

The protein content should be determined using an appropriate and validated assay. Routine release testing of each lot for protein content in the final product may be omitted if the NRA agrees.

A.6.3 Control of diluents

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (118) should be followed during the manufacture and quality control of the diluents used to reconstitute TCVs. An expiry date should be established for the diluents based upon stability data.

For lot release of the diluent, tests should be done to assess its appearance, identity, volume and sterility, and the concentrations of its key components.

A.7 **Records**

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (118) and WHO good manufacturing practices for biological products (119) should be followed as appropriate for the level of development of the vaccine.

A.8 **Retained samples**

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

A.9 **Labelling**

The guidance on labelling provided in WHO good manufacturing practices for pharmaceutical products: main principles (118) and WHO good manufacturing practices for biological products (119) should be followed as appropriate and the label on the cartons enclosing one or more final containers, or the leaflet accompanying each container, should include:

- a statement that the vaccine fulfils Part A of these WHO Recommendations;
- the instruction that any vaccine in a lyophilized form should be used immediately after reconstitution; if data have been provided to the licensing authority indicating that the reconstituted vaccine may be stored for a limited time then the length of time should be specified;
- where needed, information on the volume and nature of the diluent to be added to reconstitute the lyophilized vaccine; this information should specify that the diluent approved by the NRA should be supplied by the vaccine manufacturer; and
- for multi-dose vials, the storage conditions and shelf-life after opening.

A.10 **Distribution and transport**

The guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (118) and WHO good manufacturing practices for biological products (119) should be followed.

Shipments should be maintained within specified temperature ranges, and packages should contain cold-chain monitors. Further guidance on these and related issues is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (155).

A.11 **Stability testing, storage and expiry date**

The relevant guidance provided in WHO good manufacturing practices for biological products (119) should be followed. Any statements concerning storage temperature and expiry date that appear on primary or secondary packaging should be based on experimental evidence and should be approved by the NRA.

A.11.1 **Stability testing**

Adequate stability studies form an essential part of vaccine development. These studies should follow the general principles outlined in the WHO Guidelines on stability evaluation of vaccines (156) and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (157). The shelf-life of the final product and the hold time of each process intermediate (such as the purified polysaccharide, the carrier protein and the purified bulk conjugate) should be established based on the results of real-time, real-condition stability studies, and approved by the NRA.

The stability of the vaccine in its final container and at the recommended storage temperature should be demonstrated to the satisfaction of the NRA on at least three lots of the final product manufactured from different bulk conjugates. In addition, a real-time real-condition stability study should be conducted on at least one final container lot produced each year.

A protocol should be established and followed for each stability study which specifies the stability-indicating parameters to be monitored, as well as the applicable specifications. Some stability-indicating parameters may change over the shelf-life as discussed below. The specifications should take into consideration the expected quality of the vaccine at the end of shelf-life and should be linked to lots demonstrated to be safe and effective/immunogenic in clinical trials. For vaccines intended for use under extended controlled temperature conditions, the manufacturer should refer to the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (157).

The polysaccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate that may vary depending upon the type of conjugate, the formulation or adjuvant, the excipient and the conditions of storage. The hydrolysis may result in reduced molecular size of the Vi polysaccharide component, a reduction in O-acetyl content, a reduction in the amount of polysaccharide bound to the carrier protein, a change in pH, reduced molecular size of the conjugate, or some combination of these.

If applicable, the residual moisture should be monitored as part of stability testing and release testing.

Where applicable, the level of adsorption of the conjugate to the adjuvant should be shown to be within the limits agreed with the NRA, unless data show that the immunogenicity of the final product does not depend on the adsorption of the antigen to the adjuvant.

Accelerated stability studies may provide additional supporting evidence of the stability of the product or other product characteristics, or both, but are not recommended for establishing the shelf-life of the vaccine under a defined storage condition.

When any changes are made in the production process that may affect the stability of the product, the vaccine produced by the new method should be shown to be stable.

A.11.2 **Storage conditions**

The recommended long-term storage conditions should be based on the findings of the stability studies and should ensure that all stability-indicating parameters of the conjugate vaccine (for example, free saccharide) meet the required specifications at the end of the shelf-life. The labelled and packaged vaccine products should be stored at the recommended long-term storage conditions.

If approved by the NRA, the use of a vaccine under extended controlled temperature conditions requires specific monitoring as described in the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (157).

A.11.3 **Expiry date**

The expiry date should be based on the shelf-life as supported by stability studies and approved by the NRA. The start of the dating period (for example, the date of formulation of final bulk or the date of filling) should be agreed with the NRA. The expiry dates for vaccine and diluent may be different from one another. If the vaccine and diluent are packaged together, the expiry date for the package should be that of the component with the earliest expiry date.

A.11.4 **Expiry of reconstituted vaccine (if applicable)**

For single-dose containers the reconstituted vaccine should be used immediately. For multi-dose containers the use of the reconstituted container should follow the WHO multi-dose vial policy¹⁸, and this should be reflected in the package insert and supplied instructions.

¹⁸ See: https://apps.who.int/iris/bitstream/handle/10665/135972/WHO_IVB_14.07_eng.pdf;sequence=1

Part B. Nonclinical evaluation of typhoid conjugate vaccines

B.1 General principles

Detailed WHO guidelines on the design, conduct, analysis and evaluation of nonclinical studies of vaccines are available separately (158) and should be read in conjunction with Part B of these WHO Recommendations. Plans for nonclinical studies of candidate vaccines should be discussed with the NRA prior to the review process.

B.2 Product characterization and process development

It is critical that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing and the collection of nonclinical data that may suggest safety and efficacy in humans.

Candidate formulations of Vi conjugate vaccines should be characterized to define the critical structural and chemical attributes that indicate that the polysaccharide, the conjugating protein and the conjugate product are sufficiently pure and stable, and their properties are consistent. The extent of product characterization may vary depending on the stage of vaccine development. Vaccine lots used in nonclinical studies should be adequately representative of those intended for use in clinical investigations. Ideally, the lots used should be the same as those used in the clinical studies. If this is not feasible then the lots should be comparable with respect to physicochemical data, stability and formulation.

B.3 Nonclinical immunogenicity studies

The immunogenicity of glycoconjugate vaccines can vary greatly between different animal species and between strains within a species. Therefore, animal models used for immunogenicity studies during glycoconjugate vaccine development programmes should be selected with care as they may be poorly predictive of efficacy in humans. Animal studies should only be conducted when they provide proof-of-concept information in support of a clinical development plan, and any animal testing plan used in vaccine development should incorporate 3Rs (Replace, Reduce, Refine) best practices.

Immunogenicity data derived from appropriately selected animal models may be useful in establishing the immunological characteristics of the Vi polysaccharide conjugate product, and may guide the selection of doses, schedules and routes of administration to be evaluated in clinical trials. When animal models are used for the preclinical testing of vaccine immunogenicity, they should elicit an anti-Vi IgG response that is significantly greater than that of the control group (for example, a group that receives unconjugated Vi

polysaccharide vaccine). It should be noted that a booster response may not be observed following a second dose if the priming dose induced a maximal response. Therefore, a good understanding of the dose-immunogenic response should be established prior to evaluating any booster effect.

Immunogenicity studies have demonstrated that Vi polysaccharide conjugates induce anti-Vi IgG in mice (102, 131, 138, 159–161). In humans, high levels of anti-Vi IgG are associated with greater levels of protection against typhoid infection (90, 91, 94) – although there is no agreement that this may be considered a true correlate or surrogate of protection. Based on these observations, the level of anti-Vi IgG elicited in mice may be considered as a primary end-point for nonclinical studies of the immunogenicity of Vi conjugate vaccines.

Nonclinical studies of immunogenicity may include an evaluation of seroconversion rates or geometric mean antibody titres, or both. When possible, nonclinical studies should be designed to assess relevant immune responses, including functional immune response (for example, by evaluating serum bactericidal antibodies, opsonophagocytic activity and serum-dependent opsonophagocytic killing). These studies may also address the interference that can occur among antigens when multi-antigen vaccines are used. In such cases, the response to each antigen should be evaluated.

Although there have been advances in the use of animal models, no ideal animal model exists that establishes direct serological or immunological correlates of clinical protection. In the absence of such a model, it is important to ensure consistency of production using modern physical, chemical and immuno-based quality control methods as described in Part A of these WHO Recommendations. Additionally, any changes in critical quality attributes should be assessed for their impact on immunogenicity. Once the physicochemical tests are validated, these non-animal methods are considered more appropriate for use in lot release processes than animal models.

B.4 Nonclinical toxicity and safety studies

The WHO guidelines on nonclinical evaluation of vaccines (158) should be followed when assessing toxicity and safety in an appropriate animal model. These studies should entail careful analysis of all major organs, as well as of tissues proximal to and distal from the site of administration, to detect unanticipated direct toxic effects. If the target population for the vaccine includes pregnant women, or women of childbearing age, developmental toxicity studies should also be considered unless there is a scientific and clinically sound justification showing that conducting such studies is unnecessary (158).

Dose-response studies may not be necessary as the nonclinical evaluation of potential toxicity can be performed at a dose that maximizes both the exposure of the animal and the subsequent immune response (such as

antibody titre). This dose can be determined during pilot dose-response and/or immunogenicity studies. The dosing frequency should be the same as, or greater than, the number of administrations intended in clinical studies, but the interval between doses should not be longer (158).

Requirements for the toxicity testing of individual vaccine components or any novel proteins may vary between regulatory jurisdictions. These requirements should be scientifically justified as individual vaccine components may have different toxicity and safety profiles when present in the formulated product. Therefore, manufacturers are encouraged to discuss these testing requirements with the NRA prior to commencing nonclinical studies.

Nonclinical safety studies should be conducted in accordance with the good laboratory practices described elsewhere (162, 163). For ethical reasons, it is desirable to apply the 3Rs concept of “Replace, Reduce, Refine” to minimize the use of animals where scientifically appropriate.

Part C. Clinical evaluation of typhoid conjugate vaccines

C.1 General considerations

The general principles described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (2) apply to Vi conjugate vaccines and should be followed. In addition, a number of issues specific to the clinical development programme for Vi conjugate vaccines are discussed below. The WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (164) are also available and should be consulted.

Vi conjugate vaccines have now been licensed in some countries for use in children aged 6 months or older and in adults up to 45 years of age, with one such vaccine having been prequalified by WHO (17). The licensure of effective Vi conjugate vaccines in some countries and their availability through the WHO prequalification programme have implications for the pathway to approval and design of clinical studies in children above the age of 6 months and adults up to 45 years old. Information supporting the safety, immunogenicity, efficacy and effectiveness of Vi conjugate vaccines in typhoid endemic regions, as well as insights into putative immune correlates of protection, are continually emerging (26, 88, 165–168). The principles for clinical evaluation outlined below are based on the current situation and should be read in light of the specific circumstances in the jurisdiction of the individual NRA.

C.2 Outline of the clinical development programme

It is recommended that the major part of the pre-licensure clinical development programme is conducted in subjects who are representative of the intended target population.

C.2.1 Dose and schedule

The early clinical development programme should provide a preliminary assessment of safety and should be suitable for identifying an appropriate dose of conjugated Vi antigen and dose regimen(s) for the target age group(s). Such studies are necessary for each candidate Vi conjugate vaccine that is developed since it is not possible to extrapolate the dose and schedule identified for one conjugate vaccine to another. This consideration applies even if the same carrier protein is used for two different Vi conjugate vaccines as experience with other conjugated polysaccharide vaccines has indicated that differences in conjugation chemistry can affect immune responses to the polysaccharide(s).

C.2.2 Demonstrating or inferring vaccine efficacy

In the absence of a pre-licensure efficacy study, pathways to approval of a candidate Vi conjugate vaccine in the jurisdiction of any one NRA may depend on the following:

- If there is a licensed Vi conjugate vaccine for which protective efficacy has been documented (the data may come from pre- and/or post-licensure efficacy studies and/or from post-licensure studies of effectiveness), and subject to any pertinent national legislation, the efficacy of a candidate Vi conjugate vaccine may be inferred based on adequately designed comparative immunogenicity studies to bridge to the efficacy data for the licensed vaccine.
- If there are data that point to a specific anti-Vi antibody concentration that strongly correlates with efficacy, the efficacy of a candidate Vi conjugate vaccine may be inferred by estimating the proportion of baseline seronegative subjects with post-vaccination immune responses that exceed the concentration identified. In this situation, it may still be appropriate for an NRA to request that the sponsor compares the immune response to the candidate vaccine with the immune response to a licensed Vi conjugate vaccine for which protective efficacy has been demonstrated.
- If there is no widely accepted antibody concentration that strongly correlates with efficacy and no licensed Vi conjugate vaccine for which protective efficacy has been documented, it may be appropriate to infer the efficacy of a candidate Vi conjugate vaccine by comparing the immune response with a licensed unconjugated Vi polysaccharide vaccine in subjects aged 2 years and above. For further details see section C.4 below.

C.3 Assessment of the immune response

C.3.1 Immune parameters of interest

There are no well-established or standardized assays for assessing functional antibody responses to Vi-containing vaccines, and it is not known how the results of such assays correlate with vaccine efficacy.

Assessment of the immune response to licensed unconjugated (82, 169, 170) and conjugated (26, 89, 90, 171) Vi polysaccharide vaccines has predominantly relied upon ELISA methods to measure total anti-Vi IgG in serum. For unconjugated Vi polysaccharide vaccines, approval has often been based on directly comparing the proportion of subjects that achieves anti-Vi IgG of at least 1 µg/mL and/or the proportion that achieves at least a 4-fold increase in anti-Vi IgG from pre- to post-vaccination. A regional or in-house working reference serum preparation calibrated against the First WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (see **International reference materials** above) should be used in the interpretation of immunogenicity data from clinical trials. The use of this WHO international standard improves consistency in the determination of serum titres and provides a basis for the comparison of data generated by different assays and/or different laboratories.

At present, there is no established or widely agreed immune correlate of protection for typhoid vaccines – though correlations between total serum antibody (79), total anti-Vi IgG (81, 92, 93, 172, 173) or anti-Vi IgA (97) in serum and protection against typhoid have been described. A putative immune correlate of protection based on anti-Vi IgG has been proposed based on long-term follow-up of Vietnamese children who received a candidate Vi conjugate vaccine in a large efficacy trial. However, the value reported is specific to the assay that was applied during that study and it is not yet clear what the corresponding values may be when using alternative assays.

C.3.2 Considerations regarding the carrier protein

Proteins such as CRM₁₉₇, DT, TT and rEPA have been used in the production of various Vi conjugate vaccines. Based on experience with other types of conjugate vaccines that use CRM₁₉₇, DT or TT as the carrier protein, there is a possibility that the immune response to the Vi conjugated antigen may be reduced or enhanced in subjects who have pre-existing high levels of tetanus or diphtheria antitoxin before vaccination. This phenomenon should be explored during the development of Vi conjugate vaccines; this may be accomplished by analysing post-vaccination responses and comparing these with pre-vaccination antibody concentrations to the protein carrier. The potential clinical significance of any effect requires careful consideration.

Depending on the target age range, it may be important to assess the effects of co-administering Vi conjugate vaccines with other routine vaccinations. Guidance on such studies, including instances in which co-administered vaccines contain the carrier protein, may be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (2).

C.3.3 Immune memory

Vi conjugate vaccines are expected to elicit T-cell-dependent immune responses, which can be assessed by administration of a post-priming Vi conjugate dose after an interval of at least 6–12 months. Details of the clinical assessment of priming may be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (2). Whether or not booster doses will be needed to maintain protection after successful priming with Vi conjugate vaccines is not yet known. Until this issue is resolved, plans should be put in place to document antibody persistence and to evaluate vaccine effectiveness.

C.4 Immunogenicity

This section should be read in conjunction with the guidance on comparative immunogenicity trials provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (2). The selection of the most appropriate licensed vaccine for use as a comparator in clinical studies must be agreed between the sponsor and the NRA.

C.4.1 Studies that compare conjugated Vi polysaccharide vaccines

If the aim of the study is to immunobridge efficacy documented with a licensed Vi conjugate vaccine to a candidate vaccine, the study should be designed to demonstrate the non-inferiority of the immune response elicited by the candidate vaccine when compared with a licensed Vi conjugate vaccine. The primary immune parameter for the purposes of immunobridging and the acceptance criteria for concluding that the candidate vaccine will have at least similar efficacy to the licensed vaccine should be predefined and agreed between the sponsor and the NRA.

If efficacy data have supported the derivation of an immune correlate of protection, the proportion of subjects that achieves at least this concentration after vaccination with the candidate vaccine should be the primary immune parameter. In this case, a direct comparison with a licensed Vi conjugate vaccine would not be essential though some NRAs may request that a comparison is made with a licensed Vi conjugate vaccine for which vaccine efficacy has been documented to provide a comparison of safety.

If the sponsor wishes, or is requested, to conduct a comparative study against a licensed Vi conjugate vaccine for which efficacy is not documented then demonstrating non-inferiority for the candidate versus licensed vaccine does not evidence the potential efficacy of the candidate vaccine. Therefore, either the immune responses to the candidate vaccine should be interpreted against an immune correlate of protection or threshold value or, if neither exists, consideration should be given to alternative study designs as described below.

C.4.2 **Studies that compare Vi conjugate vaccines with unconjugated Vi polysaccharide vaccines**

Studies that compare candidate Vi conjugate vaccines with licensed unconjugated Vi polysaccharide vaccines should only be conducted in subjects who are at least 2 years of age. It is recommended that such studies are conducted only if a licensed Vi conjugate vaccine comparator is not available and it is considered important to generate comparative safety and immunogenicity data versus a licensed vaccine (see section C.2 above). If such studies are to be the basis for approval, data should be generated for the age range for which a claim for use of the candidate vaccine will be sought. Studies should stratify subjects by appropriate age subgroups, or separate studies should be conducted in different age groups.

For potential approaches to the primary comparison of immune responses see section C.3 above.

The immune responses should be measured in samples collected at day 28 after the initial vaccination series has been completed (that is, after a single dose or after the last assigned dose of the primary series) or in samples collected at an alternative time point if this is justified by data on antibody kinetics.

C.4.3 **Studies that compare a group vaccinated with a Vi conjugate vaccine with a control group that does not receive a vaccine containing Vi**

There are two situations in which such studies may be considered:

- As explained in section C.2 above, if there is an established immune correlate of protection, a direct comparison of immune responses with a licensed vaccine is not necessary. However, such a comparison may still be useful for interpreting the safety data and for putting the immune responses to the candidate vaccine into context.
- In the absence of an immune correlate of protection or the possibility of immunobridging the candidate Vi conjugate vaccine to the protective efficacy of a licensed Vi conjugate vaccine, a study that compares a candidate Vi conjugate vaccine with an unvaccinated group could be considered for subjects under 2 years of age.

A comparison between a candidate Vi conjugate vaccine and a licensed unconjugated Vi polysaccharide vaccine would not be appropriate due to lack of reliable protective immune responses to the latter in children under 2 years of age. In this situation, it is recommended that studies are based upon randomized allocation to the candidate Vi conjugate vaccine (that is, the vaccinated group) or to a licensed non-typhoid vaccine from which study subjects may derive some benefit (that is, the control group). To put the immune responses observed into context, the anti-Vi titres elicited by the candidate Vi conjugate vaccine in children under 2 years of age may be compared (either directly or in a cross-study comparison) with one or both of:

- the immune response to an unconjugated Vi polysaccharide vaccine in subjects ≥ 2 years of age;
- the immune response to the candidate Vi conjugate vaccine in subjects ≥ 2 years of age.

C.5 Efficacy

This section should be read in conjunction with the guidance on efficacy trials and effectiveness studies provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (2).

Protective efficacy studies against typhoid can be conducted only in endemic areas with relatively high rates of disease. If a protective efficacy study is conducted, it should compare the rates of febrile illnesses associated with a positive blood culture for *S. Typhi* between a group that receives the candidate Vi conjugate vaccine and an appropriate control group.

Successful typhoid challenge studies conducted in healthy adults using an appropriate and validated model (that is, one in which some protective efficacy of unconjugated Vi polysaccharide vaccines is detectable) could provide considerable supporting evidence of the efficacy of a Vi conjugate vaccine. Human challenge studies may provide information on the relationship between the immune response and various efficacy parameters. If, in consultation with the NRA, sponsors decide to conduct typhoid challenge studies in humans, they should be undertaken only by physicians with appropriate expertise, and in a carefully controlled setting, to ensure the safety of the volunteers (109). Healthy adults that are expected or known to be naive to typhoid and typhoid vaccines should be screened to detect any underlying pre-existing conditions that could impact on safety. In particular, subjects who might be at risk of complications of typhoid should be excluded, including any subject with gall bladder disease. The challenge strain should be well characterized and there should be complete information available on its susceptibility to antibacterial agents.

An issue to consider after initial licensure is the possibility that widespread use of a Vi conjugate vaccine and high vaccination coverage in a population in which typhoid fever is endemic may lead to the emergence of otherwise rare Vi-negative variants of *S. Typhi* (174–177); such variants exist and can cause typhoid fever, albeit at lower attack rates (110, 111).

C.6 Safety

Current evidence suggests there are no major specific safety issues for Vi conjugate vaccines (178) and that reports of adverse events are similar to those of other polysaccharide–protein conjugate vaccines. It is recommended that the assessment of safety in pre-licensure studies should follow the usual approaches to ensure comprehensive monitoring and data collection (2). When considering the pre-licensure safety database, the need for a sufficient sample size to estimate adverse event rates with precision is an important factor. For example, a total database of 3000 subjects across all trials and populations provides a 95% chance of observing one instance of an adverse event that occurs on average in 1 in every 1000 subjects (179). Other considerations include the type of carrier protein used in the candidate Vi conjugate vaccine and the extent of clinical experience with similar conjugated vaccines.

Part D. Recommendations for NRAs

D.1 General recommendations

The guidance for NRAs and national control laboratories (NCLs) given in the WHO Guidelines for national authorities on quality assurance for biological products (120) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (180) should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and quality has been established and demonstrated by the manufacturer.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety or efficacy of the Vi conjugate vaccine, should be discussed with and approved by the NRA. For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards as appropriate. The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of Vi conjugate vaccines. 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 final bulk and final product.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (180).

A summary protocol for the manufacturing and control of typhoid conjugate vaccines, based on the model summary protocol provided below in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA/NCL in support of a request for the release of a vaccine for use. This protocol may also be referred to as the Product Specification File.

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review or independent laboratory testing). The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines. A model NRA/NCL Lot Release Certificate for typhoid conjugate vaccines is provided below in Appendix 2.

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The draft document was then posted on the WHO Biologicals website for public consultation from 6 April 2020 to 6 May 2020. Comments were received from: Dr V. Bansal, Central Drugs Laboratory, India; Dr B. Bolgiano, Dr F. Gao and Dr S. Rijpkema, National Institute for Biological Standards and Control, the United Kingdom; Dr Z. Diaz, Dr L-F. Ma, Dr C. MacLennon and Dr D. Steele, Bill and Melinda Gates Foundation, Seattle, WA, the USA; Dr S. Gairola, Serum Institute of India PVT. Ltd, India; Dr K.P. Gonzalez, Dr L.R. Ariza and Dr S.M.M. Sanchez, Instituto Nacional de Vigilancia de Medicamentos y Alimentos, Colombia; Dr J. Lim, National Institute of Food and Drug Safety Evaluation, Republic of Korea; Dr K. Maithal, Cadila Healthcare Ltd, India; Mr S.D. Prasad, Bharat Biotech International Ltd, India; Mr D. Ugiyadi and Mr Y. Sofyan, PT Bio Farma (Persero), Indonesia; and Dr M. Alali and Dr A. Fauconnier, World Health Organization, Switzerland. Consolidated comments were also submitted by Ms P. Barbosa on behalf of members of the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), Switzerland.

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Further changes were made to document WHO/BS/2020.2387 by the WHO Expert Committee on Biological Standardization.

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Appendix 1

Model summary protocol for the manufacturing and control of typhoid conjugate vaccines

The following protocol is intended for guidance and indicates the minimum information that should be provided by the manufacturer to the NRA or NCL. Information and tests may be added or omitted as necessary with the approval of the NRA or NCL.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations for a particular product should be provided in the protocol submitted.

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

1. Summary information on final lot

International name of product: _____

Commercial name: _____

Product licence (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Nature of final product: _____

Final packaging lot number: _____

Type of container: _____

Final container lot number: _____

Number of containers in this final lot: _____

Number of doses per final container: _____

Volume of each recommended single human dose: _____

Preservative used and nominal concentration: _____

Summary of composition (*include a summary of the qualitative and quantitative composition of the vaccine per single human dose; including the conjugate, any adjuvant used and other excipients*):

Shelf-life approved (months): _____

Date of manufacture: _____

Expiry date: _____

Storage conditions: _____

2. Detailed information on manufacture and control

The following sections are intended for reporting the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. If any test had to be repeated, this information must be indicated. Any abnormal results must be recorded on a separate sheet.

Summary of source materials

It is possible that a number of bulk lots may be used to produce a single final lot. A summary of the bulk polysaccharide, activated saccharide, bulk carrier protein and bulk conjugate lots that contributed to the final lot should be provided.

Control of typhoid Vi polysaccharide

Bacterial strain

Identity of bacterial strain used

(e.g. *Salmonella* Typhi Ty2 or *Citrobacter freundii*): _____

Origin and short history: _____

Authority that approved the strain: _____

Date approved: _____

Bacterial culture media for seed-lot preparation and Vi production

Free from ingredients that form precipitate when CTAB

is added: _____

Free from toxic or allergenic substances: _____

Any components of animal origin (list): _____

Certified as TSE-free: _____

Master seed lot

Lot number: _____

Date master seed lot established: _____

Working seed lot

Lot number: _____

Date working seed lot established: _____

Type of control tests used on working seed lot: _____

Date seed lot reconstituted: _____

Control of single harvests

For each single harvest, indicate the medium used; the dates of inoculation; the temperature of incubation; the dates of harvests and harvest volumes; the results of tests for bacterial growth rate, pH, purity and identity; the method and date of inactivation if used; the method of purification; and the yield of purified polysaccharide.

Control of purified typhoid Vi polysaccharide

Lot number: _____

Date of manufacture: _____

Volume: _____

Identity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Molecular size or mass distribution

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Polysaccharide content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

O-acetyl content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Moisture content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Protein impurity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Nucleic acid impurity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Phenol content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Endotoxin content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Residues of process-related contaminants

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Appearance

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Control of modified polysaccharide

Lot number: _____

Method of chemical modification: _____

Extent of activation for conjugation

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Molecular size or mass distribution

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Control of carrier protein

Microorganisms used

Identity of strain used to produce carrier protein: _____

Origin and short history: _____

Authority that approved the strain: _____

Date approved: _____

Bacterial culture media for seed-lot preparation and carrier-protein production

Free from ingredients that form precipitate when CTAB is added: _____

Free from toxic or allergenic substances: _____

Any components of animal origin (list): _____

Certified as TSE free: _____

Master-seed lot

Lot number: _____

Date master-seed lot established: _____

Working-seed lot

Lot number: _____

Date established: _____

Type of control tests used on working-seed lot: _____

Date seed lot reconstituted: _____

Control of carrier-protein production

List the lot numbers of harvests; indicate the medium used; the dates of inoculation; the temperature of incubation; the dates of harvests and harvest volumes; the results of tests for bacterial growth rate, pH, purity and identity; the method and date of inactivation; the method of purification; and the yield of purified carrier protein. Provide evidence that the carrier protein is nontoxic.

Purified carrier protein

Lot number: _____

Date produced: _____

Identity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Protein impurity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Nucleic acid impurity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Modified carrier protein

Lot number: _____

Date produced: _____

Method of modification: _____

Extent of activation

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Control of purified bulk conjugate

Production details of bulk conjugate

List the lot numbers of the saccharide and carrier protein used to manufacture the conjugate vaccines, the production procedure used, the date of manufacture and the yield.

Tests on purified bulk conjugate

Identity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Endotoxin content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

O-acetyl content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Residual reagents

Date tested: _____

Method used: _____
Specification: _____
Result: _____

Vi polysaccharide content

Date tested: _____
Method used: _____
Specification: _____
Result: _____

Conjugated and unbound (free) polysaccharide

Date tested: _____
Method used: _____
Specification: _____
Result: _____

Protein content

Date tested: _____
Method used: _____
Specification: _____
Result: _____

Conjugation markers

Date tested: _____
Method used: _____
Specification: _____
Result: _____

Absence of reactive functional groups (capping markers)

Date tested: _____
Method used: _____
Specification: _____
Result: _____

Ratio of polysaccharide to protein

Date tested: _____
Method used: _____

Specification: _____

Result: _____

Molecular size or mass distribution

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Bacterial and fungal sterility

Method used: _____

Media: _____

Volume tested: _____

Date of inoculation: _____

Date of end of test: _____

Specification: _____

Result: _____

Specific toxicity of carrier protein (where appropriate)

Method used: _____

Strain and type of animals: _____

Number of animals: _____

Route of injection: _____

Volume of injection: _____

Quantity of protein injected: _____

Date of start of test: _____

Date of end of test: _____

Specification: _____

Result: _____

pH

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Appearance

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Depending on the conjugation chemistry used to produce the vaccine, tests should also be included to demonstrate that amounts of residual reagents and reaction by-products are below a specified level.

Control of final bulk

Lot number: _____

Date prepared: _____

Preservative (if used)

Name and nature: _____

Lot number: _____

Final concentration in the final bulk: _____

Stabilizer (if used)

Name and nature: _____

Lot number: _____

Final concentration in the final bulk: _____

Adjuvant (if used)

Name and nature: _____

Lot number: _____

Final concentration in the final bulk: _____

Tests on final bulk

Bacterial and fungal sterility

Method used: _____

Media: _____

Volume tested: _____

Date of inoculation: _____

Date of end of test: _____

Specification: _____

Result: _____

Filling and containers

Lot number: _____

Date of sterile filtration: _____

Date of filling: _____

Volume of final bulk: _____

Volume per container: _____

Number of containers filled (gross): _____

Date of lyophilization (if applicable): _____

Number of containers rejected during inspection: _____

Number of containers sampled: _____

Total number of containers (net): _____

Maximum duration approved for storage: _____

Storage temperature and duration: _____

Control tests on final lot

Inspection of final containers

Date tested: _____

Method used: _____

Specification: _____

Results: _____

Appearance before reconstitution:¹⁹ _____

Appearance after reconstitution: _____

Diluent used: _____

Lot number of diluent used: _____

Tests on final lot

Identity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Sterility

Method used: _____

Media: _____

¹⁹ This applies to lyophilized vaccines.

Number of containers tested: _____

Date of inoculation: _____

Date of end of test: _____

Specification: _____

Result: _____

Polysaccharide content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Unbound (free) polysaccharide

Date tested: _____

Method used: _____

Specification: _____

Result: _____

O-acetyl content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Molecular size or mass distribution

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Endotoxin content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Adjuvant content and degree of adsorption (if applicable)

Date tested: _____

Nature and concentration of adjuvant per single

human dose: _____

Method used: _____

Specification: _____

Result: _____

Preservative content (if applicable)

Date tested: _____

Method used: _____

Specification: _____

Result: _____

pH

Date tested: _____

Method used: _____

Specification: _____

Result: _____

***Moisture content*²⁰**

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Osmolality

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Control of diluent (if applicable)

Name and composition of diluent: _____

Lot number: _____

Date of filling: _____

Type of diluent container: _____

²⁰ This applies only to lyophilized vaccines.

Appearance: _____
 Filling volume per container: _____
 Maximum duration approved for storage: _____
 Storage temperature and duration: _____
 Other specifications: _____

Control of adjuvant²¹

Summary of production details for the adjuvant

When an adjuvant suspension is provided to reconstitute a lyophilized vaccine, a summary of the production and control processes should be provided. The information provided and the tests performed depend on the adjuvant used.

Summary information for the adjuvant

Name and address of manufacturer: _____

 Nature of the adjuvant: _____
 Lot number: _____
 Date of manufacture: _____
 Expiry date: _____

Tests on the adjuvant

Adjuvant content

Date tested: _____
 Method used: _____
 Specification: _____
 Result: _____

Appearance

Date tested: _____
 Method used: _____
 Specification: _____
 Result: _____

²¹ This section is required only when an adjuvant is provided separately to reconstitute a lyophilized vaccine.

Purity or impurity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

pH

Date tested: _____

Method used: _____

Specification: _____

Result: _____

***Pyrogenicity*²²**

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Sterility

Method used: _____

Media: _____

Number of containers used: _____

Date of inoculation: _____

Date of end of test: _____

Specification: _____

Result: _____

²² A pyrogen test of the adjuvant is not needed if a pyrogen test was performed on the adjuvanted reconstituted vaccine.

3. Certification by the manufacturer

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

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

I certify that lot no. _____ of typhoid conjugate vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A²³ of the WHO Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines.²⁴

Signature _____

Name (typed) _____

Date _____

4. Certification by the NRA/NCL

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

²³ With the exception of provisions on distribution and transport, which the NRA may not be in a position to assess.

²⁴ WHO Technical Report Series, No. 1030, Annex 2.

Appendix 2

Model NRA/NCL Lot Release Certificate for typhoid conjugate vaccines

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

Certificate no. _____

The following lot(s) of typhoid conjugate vaccine produced by

_____ ²⁵
in _____, ²⁶ whose numbers appear on the labels of the final containers, meet all national requirements²⁷ and Part A²⁸ of the WHO Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines,²⁹ and comply with WHO good manufacturing practices for pharmaceutical products: main principles;³⁰ WHO good manufacturing practices for biological products;³¹ and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.³²

The release decision is based on _____ ³³

Final lot number _____

Number of human doses released in this final lot _____

Expiry date _____

²⁵ Name of manufacturer.

²⁶ Country of origin.

²⁷ 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 or NCL.

²⁸ With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.

²⁹ WHO Technical Report Series, No. 1030, Annex 2.

³⁰ WHO Technical Report Series, No. 986, Annex 2.

³¹ WHO Technical Report Series, No. 999, Annex 2.

³² WHO Technical Report Series, No. 978, Annex 2.

³³ 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.

The certificate may also include the following information:

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

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

Signature _____

Name (typed) _____

Date _____

Annex 3

Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated)

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Recommendations published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations 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 recommendations set out below. The parts of each section printed in small type are comments or examples intended to provide additional guidance to manufacturers and NRAs.

Abbreviations

BPL	beta-propiolactone
ELISA	enzyme-linked immunosorbent assay
EV71	enterovirus 71
HFMD	hand, foot and mouth disease
ICP	immune correlate of protection
Ig	immunoglobulin
IPV	inactivated poliomyelitis vaccine
MCB	master cell bank
NAT	nucleic acid amplification technique
NCL	national control laboratory
NIBSC	National Institute for Biological Standards and Control
NIFDC	National Institutes for Food and Drug Control
NRA	national regulatory authority
PCR	polymerase chain reaction
PDL	population doubling level
PSGL-1	P-selectin glycoprotein ligand-1
RD	rhabdomyosarcoma
SCARB2	scavenger receptor class B member 2
SCARB2	scavenger receptor class B member 2 (gene)
WCB	working cell bank

Introduction

Enterovirus 71 (EV71) was first isolated from the faeces of a female suffering from encephalitis in 1969 in California (1). However, a retrospective study conducted in the Netherlands indicated that the virus could have emerged as early as 1963 (2), a finding consistent with reports of possible worldwide EV71 epidemics in the late twentieth century (3). The virus is associated with hand, foot and mouth disease (HFMD) throughout the world and has caused epidemics in Asia, Europe and North America. Manifestations of the disease range from asymptomatic infection to mild HFMD to neurological disease with severe central nervous system complications and cardiopulmonary failure. In severe cases mortality rates can be high, especially in children aged 5 years and younger. EV71 is considered to be the most severe neurotoxic enterovirus and severe EV71 disease has become a major public health problem in China. In 2011, the WHO Regional Office for the Western Pacific issued A guide to clinical management and public health response for hand, foot and mouth disease (HFMD) (4) to support the treatment, prevention and control of HFMD.

Several vaccines against EV71 are currently under development and three inactivated EV71 vaccines have been licensed in China (5–10). The WHO Expert Committee on Biological Standardization discussed the EV71 situation at its 67th meeting in 2016 and considered it to be of major regional significance (11). The Committee noted that the joint efforts of the National Institutes for Food and Drug Control (NIFDC) and the National Institute for Biological Standards and Control (NIBSC) had resulted in the development of the First WHO International Standard for anti-EV71 serum (human), and recommended that consideration also be given to the development of a WHO written standard for EV71 vaccines. In addition, the First WHO International Standard for EV71 inactivated vaccine was established by the Committee in October 2019 following a collaborative study led by NIBSC and NIFDC (12, 13). National standards for antigen content and neutralizing antibody responses for evaluating EV71 vaccines are also available in China (14) where they have supported the development and clinical assessment of such vaccines. In 2018, the Committee also endorsed a proposal to develop WHO international standards for enterovirus RNA for nucleic acid amplification technique (NAT)-based assays for EV71 (15, 16).

Following requests from regulators and other stakeholders for WHO to develop recommendations to assure the quality, safety and efficacy of EV71 vaccines, a series of meetings was convened by WHO to review the current status of their development and licensure (17). These meetings were attended by experts from around the world involved in the research, manufacture, regulatory assessment and approval, and control testing and release of EV71 vaccines.

Participants were drawn from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry. The recommendations provided in the current document for the production, quality control and evaluation of inactivated EV71 vaccines have been based upon the experiences gained during the development and production of the first three licensed EV71 vaccines in China, the candidate EV71 vaccines now under development (5–8, 18–22) and other inactivated viral vaccines, such as inactivated poliomyelitis vaccines (IPVs) and hepatitis A vaccines (23, 24).

Purpose and scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the manufacturing processes, quality control and nonclinical and clinical evaluations needed to assure the quality, safety and efficacy of inactivated EV71 vaccines.

The guidance applies to EV71 vaccines prepared by the inactivation of whole EV71 virus for prophylactic use, grown in mammalian cells in culture, and using formaldehyde or other chemical inactivation procedures.

The document does not cover recombinant and other forms of subunit vaccines, vectored vaccines, virus-like particle vaccines or bivalent EV71-CA16 vaccines, which are at an early stage of development. However, some aspects outlined in this document may be relevant and may be taken into consideration during the development of such vaccines.

These WHO Recommendations should be read in conjunction with current WHO guidance documents on the nonclinical (25) and clinical (26) evaluation of vaccines, good manufacturing practices for biological products (27), characterization of cell banks (28), nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (29) and lot release (30).

Manufacturers and regulators should also take note of the decision of the Committee in 2018 to discontinue the inclusion of the innocuity test (also referred to as the abnormal toxicity test or general safety test) in routine lot release testing requirements for all vaccines in WHO Recommendations, Guidelines and other guidance documents for biological products (31, 32). As this test is no longer recommended, it is not included in the routine testing requirements for EV71 vaccines provided in the current document.

Terminology

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

Adjuvant: a vaccine adjuvant is a substance, or combination of substances, that is 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.

Adventitious agents: contaminating microorganisms of the cell culture, or source materials used in its culture, that may include bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, Rickettsia, protozoa, parasites, transmissible spongiform encephalopathy agents and endogenous/exogenous viruses that have been unintentionally introduced into the manufacturing process of a biological product.

Cell bank: a collection of vials of cells of uniform composition (though not necessarily clonal) derived from a single tissue or cell and used for the production of a vaccine, either directly or via a cell bank system.

Cell seed: a quantity of well-characterized cells that are frozen and stored under defined conditions, such as in the vapour or liquid phase of liquid nitrogen, in aliquots of uniform composition derived from a single tissue or cell, one or more of which would be used for the production of a **master cell bank (MCB)**. Cell seed is also referred to as a pre-MCB or seed stock. It may be made under conditions of good manufacturing practices or under the manufacturer's research and development conditions.

EV71 antigen: the virus-specific antigen produced in infected cell cultures and purified from such cultures. It can be assayed by methods such as enzyme-linked immunosorbent assay (ELISA) using EV71-specific antibodies. The antigen may consist of empty or full virus particles or both. The full and empty particles differ in their antigenic reactivity and both may be present in the final vaccine.

Final bulk: a formulated vaccine preparation from which the final containers are filled. The final bulk may be prepared from one or more purified antigen bulks formulated to contain all excipients and homogenous with respect to composition.

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

Immunogenicity: the capacity of a vaccine to induce antibody-mediated and/or cell-mediated immunity and/or immunological memory.

Master cell bank (MCB): a quantity of well-characterized cells of human or animal origin derived from a **cell seed** at a specific population doubling level (PDL) 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. The MCB is prepared from a single homogeneously mixed pool of cells and is used to derive all **working**

cell banks. The testing performed on a replacement MCB (derived from the same clone or from an existing master or working cell bank) is the same as for the initial MCB, unless a justified exception is made.

Master seed lot: a quantity of virus suspension that has been processed at the same time to ensure a uniform composition, and passaged for a specific number of times that does not exceed the maximum approved by the NRA. It is characterized to the extent necessary to support development of the **working seed lot**.

Purified inactivated bulk: a purified pool of virus harvests in which the virus has been inactivated through the use of a validated method either before or after purification. It may be prepared from one **single harvest** or a number of single harvests and may yield one or more **final bulks**.

Seed lot: a preparation of live viruses constituting the starting material for the vaccine antigen. A seed lot is of uniform composition (though not necessarily clonal), is derived from a single culture process and is aliquoted into appropriate storage containers, from which all future vaccine production will be derived either directly or via a seed lot system.

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.

Working cell bank (WCB): a quantity of well-characterized cells of animal or other origin, derived from the MCB at a specific PDL or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions, such as in the vapour or liquid phase of liquid nitrogen, in aliquots of uniform composition. The WCB is prepared from a single homogeneously mixed pool of cells. One or more of the WCB containers is used for each production culture. All containers are treated identically and once removed from storage are not returned to stock.

Working seed lot: a quantity of virus of uniform composition derived from the **master seed lot** used at a passage level approved by the NRA for the manufacturing of vaccine.

General considerations

Clinical disease

HFMD was first reported in New Zealand in 1957 (33) and occurs mostly in young children, with a peak incidence at about 2 years of age. The common mild disease involves lesions on the mucosal surfaces of the mouth and spots on the palms of the hands and soles of the feet which resolve in a few days; this is not life threatening. However, a more severe and potentially fatal form of the disease was reported in 1969 (1) which is now recognized to encompass meningitis/

encephalitis, autonomic nervous system dysregulation, cardiovascular collapse and pulmonary oedema. The overall mortality rate of HFMD is of the order of one per 1000 to 10 000 cases.

The frequency of reported HFMD cases is geographically highly variable with most cases occurring in East Asian countries, particularly China but including Viet Nam, Thailand, Singapore, Malaysia and the Republic of Korea. Normally, only few cases of severe disease are reported in Europe or the USA with reports of mild HFMD also being less common – though underreporting of the latter is very likely. Typically, the total combined number of cases in Europe and the USA is of the order of several hundred per year, whereas in 2008 there were 488 955 cases and 128 deaths in China alone (4, 34, 35). HFMD is now a reportable disease in China (in contrast to Europe and the USA) and during the period 2013–2018 between 300 000 and 400 000 cases occurred in May–June each year, with a small number of deaths (4, 34, 35). The reason for the differences in disease burden in different geographical areas is not yet clear.

Enteroviruses and their epidemiology

The causative agents of HFMD are picornaviruses, most often of human enterovirus species A. The picornaviruses belong to the Picornaviridae, a family of small non-enveloped viruses with a single-stranded positive-sense RNA genome of about 7500 nucleotides. The enteroviruses are a recognized taxon by the International Committee on Taxonomy of Viruses and are sub-classified into numerous species including the four human enteroviruses A, B, C and D (36). The archetypal human picornavirus is poliovirus (belonging to species C) but the most common cause of HFMD is species A enteroviruses, chiefly the Coxsackie A viruses and EV71. The relative frequency of different enterovirus species found varies from year to year when clinical isolates or environmental samples such as sewage are examined.

In recent years, Coxsackie viruses such as A16, A10 and A6 have caused most of the HFMD cases in Asia. However, other human enteroviruses of the Picornaviridae family have also been implicated and as the different serotypes are antigenically distinct, the development of a vaccine based on a single serotype to protect against all mild HFMD cases is unlikely using existing technologies. There is therefore interest in the development of combination vaccines containing several serotypes. However, while mild HFMD is caused by many strains of enterovirus, the great majority of severe disease in recent years has been caused by EV71 which accounts for 70% of severe HFMD cases and 90% of HFMD-related deaths in China (35). As a result, EV71 has been the main focus of vaccine development.

EV71 isolates can be clustered according to their genomic sequence into at least eight genogroups (A–H) (36, 37) but belong to one serotype. Genogroups

B and C have been of greatest interest because of their frequency of isolation and implication in disease in Eastern Asia, and can each be sub-classified into five subgenogroups (C1–C5 and B1–B5) (36, 37). C4 is by far the major genogroup circulating in China, while B4, B5 and C5 are found in other Asian countries. In contrast, strains of genogroups C1 and C2 are predominantly found in Europe where severe disease is uncommon. It is possible that this has some effect on the disease burden, with C4 being particularly virulent. However, an outbreak of HFMD with severe disease caused by a C1 genogroup strain occurred in Spain in 2016 (38, 39). At the time of development of the current document, little information was available on the situation in South America, possibly due to inadequate surveillance or because EV71 infection is less common in this region (40–42). A recent study showing that the EV71 genotype C exists in Peru was the first report of this lineage circulating in South America (42). Previously, low levels of EV71 genotype B had been identified in the State of Para, Brazil (40).

Animal models

A valid animal model would be useful in vaccine development to measure protective efficacy and potency, as well as to resolve issues related to virulence. As yet, the available models are imperfect. Neonatal mice are susceptible to EV71 by intracerebral inoculation and neonatal (but not adult) rhesus monkeys develop symptoms of HFMD on infection. Adult or infant mice are not susceptible to infection. Infant rhesus monkeys have been demonstrated to develop HFMD symptoms upon inoculation with the virus and could therefore be used as a model of protection (43, 44). The neurovirulence of EV71 was demonstrated in cynomolgus monkeys and this model would be useful for challenge-protection studies for candidate EV71 vaccines (45, 46). Picornaviruses are believed to use specific receptors to infect human cells. Human P-selectin glycoprotein ligand-1 (PSGL-1) is expressed in leukocytes and involved in their binding to endothelial cells in the early stages of inflammation, and has been identified as a receptor for EV71. However, the disease produced by clinical EV71 strains in transgenic mice carrying PSGL-1 was not enhanced compared to non-transgenic strains. Human scavenger receptor class B member 2 (SCARB2) has also been identified as a receptor for EV71. Transgenic mice carrying the *SCARB2* gene are more susceptible to infection and disease than non-transgenic controls but the effect is not dramatic – two-week old transgenic mice develop mild symptoms and then recover (47–50).

Vaccines against EV71

Three vaccines against EV71 have been licensed in China, all using C4 genogroup strains. Candidate vaccines containing B4 and B5 genogroups are in development elsewhere but have not yet reached the licensing stage. In addition,

the development of vaccines against Coxsackie A16, A6 and A10 is being considered with a view to developing combination/multivalent vaccines. The efficacy of the three licensed EV71 vaccines after two doses ranges from 90.0% to 97.4% after one year of surveillance (5–7) to 95.1% after two year follow-up (8).

Licensed EV71 vaccines are produced from virus grown on mammalian cells and inactivated by validated methods – similar to the approach used for IPVs and hepatitis A vaccines. Tissue culture grown virus harvests comprise two types of particle forms; one containing the RNA genome (full) and one that does not (empty). In the case of both polioviruses and EV71 viruses, the two particle forms have different antigenic and immunogenic properties; poliomyelitis vaccines are purified so that they contain little if any empty virus particles but EV71 vaccines contain both types; potentially complicating potency assays. The atomic structures of both full and empty particles of EV71 viruses and polioviruses have been resolved by X-ray crystallography and cryogenic electron microscopy (51, 52).

Specific issues in the development of inactivated EV71 vaccines include:

- The degree to which a vaccine based on one genogroup will protect against the others is not established. Although there is good cross-neutralization between genogroups, including by sera induced by vaccination (53, 54), it has not been established that this translates into good cross-protection in humans. One recent collaborative study indicated that assays of antigen content work acceptably on all genogroups tested. However clinical cross-protection has not been demonstrated. Thus, the C4 genogroup vaccines may or may not protect against other genogroups.
- There is a lack of a convenient and convincing animal model, with the model most accurately reflecting human disease at present being infant rhesus monkeys. This makes the study of protective efficacy and immunogenic potency difficult other than by clinical trial. Neonatal mice are susceptible to disease and transgenic mice carrying the *SCARB2* gene which encodes EV71 receptors have been developed and can prove useful without fully imitating human pathogenesis.
- Virological issues include the existence of full and empty particle forms in the licensed products. These two particle forms differ in their antigenic and immunogenic properties, thus complicating potency assays. It is not clear whether current national and international vaccine reference standards and antigen potency assays are suitable for specifically detecting one or other of the particle forms (55).

International reference materials

Subsequent sections of this document refer to WHO reference materials that may be used in laboratory or clinical evaluations. The following key standards are currently used in the control of EV71 vaccines.

- A WHO international standard for anti-EV71 serum is available for the standardization of diagnostic tests for use in seroprevalence studies and for assessing immunity. This standard was established by the WHO Expert Committee on Biological Standardization in 2015 as the First WHO International Standard for anti-EV71 serum (human) (NIBSC code 14/140) with an assigned value of 1000 IU/ampoule (56, 57). The preparation is held and distributed by NIBSC and NIFDC.
- A WHO international reference reagent for EV71 neutralization assays (low-titre) is also available for use in the standardization of virus neutralization assays. This reference reagent was established by the Committee in 2015 as the First WHO International Reference Reagent for EV71 neutralization assays (NIBSC code 13/238) with an assigned value of 300 IU/ampoule (56, 57). The preparation is held and distributed by NIBSC and NIFDC.
- In 2019, the First WHO International Standard for EV71 inactivated vaccine (NIBSC code 18/116) was established by the Committee with an assigned unitage of 3625 IU/ampoule (12, 13). In addition, WHO international reference reagents for EV71 genogroups C4 and B4 inactivated vaccine (NIBSC codes 18/120 and 18/156 respectively) were established with assigned unitages of 300 and 250 IU/ampoule respectively (12, 13). These preparations are held and distributed by NIBSC. The First WHO International Standard for EV71 inactivated vaccine is intended for use in *in vitro* assays to measure the antigen content of vaccine products through the calibration of secondary reference preparations. However, it is known that full and empty virus particles (known to be present in EV71 vaccine preparations from all manufacturers) differ in their antigenicity and immunogenicity. The proportion of empty/full virus particles in the WHO international standard is not known. It is also not known whether this matters for the overall assessment of vaccines or if the current international standard is suitable for accurately measuring antigen content across manufacturers. International standards and reference reagents for the control of *in vivo* potency assays are under investigation (13).

- Product-specific national standards for EV71 neutralizing antibody and EV71 antigen were established by NIFDC following collaborative studies conducted by NIFDC and the three main vaccine manufacturers in China (14). This has helped to ensure the accuracy, comparability and repeatability of anti-EV71 neutralizing antibody and EV71 antigen detection assays, and hence improve EV71 vaccine standardization. In addition, NIFDC has also developed a new national reference for in vivo vaccine potency. These preparations are held and distributed by NIFDC.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name of the vaccine should be “enterovirus 71 vaccine (inactivated)”. The proper name should be the equivalent of the international name in the language of the country in which the vaccine is licensed.

The use of the international name should be limited to vaccines that meet the specifications given below.

A.1.2 Descriptive definition

An EV71 vaccine (inactivated) consists of a sterile suspension of EV71 grown in cell cultures, concentrated, purified and inactivated. The antigen may be formulated for delivery with a suitable adjuvant. The preparation should meet all of the specifications given below.

A.2 General manufacturing recommendations

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (58) and WHO good manufacturing practices for biological products (27) should be applied to the design, establishment, operation, control and maintenance of manufacturing facilities for EV71 vaccine.

Staff involved in the production and quality control of inactivated EV71 vaccine should be shown to be immune to EV71.

A.3 Control of source materials

A.3.1 Virus strains and seed lot system

A.3.1.1 Virus strains

Strains of EV71 used in the production of EV71 vaccine should be identified by historical records, which should include information on their origin

and subsequent manipulation or passage (for example, the genogroup and subgenogroup of EV71). Strain identity should be determined by infectivity tests and immunological methods, and by full or partial genomic sequencing.

Only virus strains that are approved by the NRA and that yield a vaccine complying with the recommendations set out in these WHO Recommendations should be used.

A.3.1.2 **Virus seed lot system**

Vaccine production should be based on the virus seed lot system. Unless otherwise justified and authorized, the virus in the final vaccine should not have undergone more passages from the virus master seed lot than were used to prepare a vaccine shown to be satisfactory with respect to safety and efficacy.

Virus master and working seed lots should be stored in a dedicated temperature-monitored system that ensures stability during storage (for example, at or below -60°C).

A.3.1.3 **Tests on virus master and working seed lots**

Each virus master and working seed lot used for the production of vaccine lots should be subjected to the tests listed in this section.

Each virus master and working seed lot should have been derived from materials that comply with the recommendations made in sections A.3.2 and A.3.3 below and should be approved by the NRA.

A.3.1.3.1 *Tests for adventitious agents*

The virus master and working seed lots used for the production of vaccine lots should be free from adventitious agents.

A sample of at least 20 mL of each virus master seed lot should be tested for the presence of adventitious agents. The sample should be neutralized by a high-titred antiserum against EV71. If the virus cannot be completely neutralized, alternative testing methods should be explored. Any alternative method used should be validated and approved by the NRA.

If polyclonal antisera are used, the immunizing antigen used for the preparation of the antiserum should not be the same as the production seed.

The immunizing antigen should be shown to be free from adventitious agents and should be grown in cell cultures free from adventitious microbial agents that might elicit antibodies that could inhibit the growth of any adventitious agents present.

The sample should be tested in susceptible cells such as Vero, human rhabdomyosarcoma (RD) or human diploid cells. The tissue cultures should be incubated at 37 °C and observed for 2 weeks. Within this observation period, at least one subculture of supernatant fluid should be made in the same tissue culture system. The sample should be inoculated in such a way that the dilution of the supernatant fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm² per mL of supernatant fluid. At least one culture vessel of the cell cultures should remain uninoculated and should serve as a control. The cells inoculated with the supernatant fluid and the uninoculated control cultures should be incubated at 37 °C and observed at appropriate intervals for an additional 2 weeks.

The virus master seed lot passes the test if there is no evidence of the presence of adventitious agents. For the test to be valid, not more than 20% of the culture vessels should have been discarded for any reason by the end of the observation period.

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. 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 micro-arrays of conserved viral sequencing, or digital subtraction of expressed sequences; and (c) high-throughput sequencing. These methods might be used in the future to supplement existing methods or as alternative methods to both *in vivo* and *in vitro* tests after appropriate validation and with the approval of the NRA (28).

The theoretical risk of the presence of potential human, simian, bovine or porcine adventitious agents in the seed lots, which may be derived from the use of bovine serum or porcine trypsin, should be assessed. If necessary, viruses such as bovine polyomavirus, porcine parvovirus or porcine circovirus may be screened for using specific assays, such as molecular NAT-based assays (28).

The need for adventitious virus testing on working seed lot should be based on risk assessment. However, sterility testing for bacteria, fungi and mycoplasmas should be conducted.

A.3.1.3.2 *Identity test*

The strain identity of the seed lot should be determined by infectivity tests. The test for antigen content described in section A.4.4.2.2 below can be used to identify the seed lot.

A.3.1.3.3 *Virus titration*

The virus concentration of the seed lot should be determined by titration of infectious virus using validated tissue culture methods. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of human RD, human diploid or Vero cells in microtitre plates is suitable for this purpose (28).

A.3.2 **Cell lines**

The general production precautions, as formulated in WHO good manufacturing practices for biological products (27), should apply to the manufacture of EV71 vaccine, with the additional requirement that, during production, only one type of cell should be introduced or handled in the production area at any one time. Vaccines may be produced in a human diploid cell line or in a continuous cell line.

A.3.2.1 **Master cell bank (MCB) and working cell bank (WCB)**

The use of a cell line for the manufacture of EV71 vaccine should be based on the cell bank system. The cell seed and cell banks should conform to 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 (28). The MCB should be approved by the NRA. The maximum number of passages (or population doublings) by which the WCB is derived from the MCB and the maximum number of passages of the production cultures should be established and confirmed through process validation and characterization of end-of-production cell culture by the manufacturer and approved by the NRA.

The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB (59) and is available to manufacturers on application to the Group Lead, Norms and Standards for Biologicals, Technical Specifications and Standards, Department of Health Product Policy and Standards, Access to Medicines and Health Products Division, World Health Organization, Geneva, Switzerland.

A.3.2.2 **Identity test**

Identity tests on the MCB and WCB should be performed in accordance with 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 (28) and should be approved by the NRA.

The cell banks should be identified by means of tests such as biochemical tests (for example, isoenzyme analysis), immunological tests, cytogenetic marker

tests and DNA fingerprinting or sequencing. The tests used should be approved by the NRA.

A.3.3 Cell culture medium

Where serum is used for the propagation of cells it should be tested to demonstrate freedom from bacterial, fungal and mycoplasmal contamination – as specified in Part A, sections 5.2 (60) and 5.3 (61) of the WHO General requirements for the sterility of biological substances – as well as freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum 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 (28).

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera if approved by the NRA. As an additional monitor of quality, sera may be examined for freedom from bacteriophage and endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation.

The source(s) of animal components used in the culture medium should be approved by the NRA. The components should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (62). The serum protein concentration should be reduced by rinsing the cell cultures with serum-free medium and/or by purification of the virus harvests.

In some countries, control tests are carried out to detect the residual animal serum content in the final vaccine.

Human serum should not be used. If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the 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* (63). In addition, human albumin, as with all materials of animal origin, should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (62).

Manufacturers are encouraged to explore the possibilities of using serum-free media for the production of EV71 vaccine.

Penicillin and other beta-lactams 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. Clearance should be demonstrated through a residual removal study (or studies) and acceptable levels should be approved by the NRA.

Bovine or porcine trypsin used for preparing cell cultures should be tested and found to be free of cultivatable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate (28). The methods used to ensure this should be approved by the NRA.

In some countries, irradiation is used to inactivate potential contaminant viruses. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all lots and to the component units of each lot. The irradiation dose must be low enough for the biological properties of the reagents to be retained but also high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process (28).

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free from risk of contamination and should be subject to the usual considerations for any reagent of biological origin (28).

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* (62).

A.4 Control of vaccine production

A.4.1 Control cell cultures

A fraction of the production cell culture equivalent to at least 5% of the total or 500 mL of cell suspension, or 100 million cells, at the concentration and cell passage level employed for seeding vaccine production cultures, should be used to prepare control cultures.

If bioreactor technology is used, the NRA should determine the size and treatment of the cell sample to be examined.

A.4.1.1 Tests of control cell cultures

The treatment of the cells set aside as control material should be similar to that of the production cell cultures but they should remain uninoculated for use as control cultures for the detection of any adventitious agents.

These control cell cultures should be incubated under conditions as similar as possible to the inoculated cultures for at least 2 weeks, and should be tested for the presence of adventitious agents as described below. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for any reason by the end of the test period.

At the end of the observation period, the control cell cultures should be examined for evidence of degeneration caused by an adventitious agent. If this examination or any of the tests specified in this section shows evidence of the presence of any adventitious agent in the control culture, the EV71 grown in the corresponding inoculated cultures should not be used for vaccine production.

If not tested immediately, samples should be stored at -60°C or below.

A.4.1.2 Tests for haemadsorbing viruses

At the end of the observation period, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded 7 days and the storage temperature should have been in the range of $2-8^{\circ}\text{C}$. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

Some NRAs require, as a test for haemadsorbing viruses, that other types of red blood cells, including cells from humans, monkeys and chickens (or other avian species), are also used instead of guinea-pig cells alone.

A reading should be taken after incubation at $2-8^{\circ}\text{C}$ for 30 minutes, and again after a further incubation for 30 minutes at $20-25^{\circ}\text{C}$.

If a test with monkey red blood cells is performed, readings should also be taken after a final incubation for 30 minutes at $34-37^{\circ}\text{C}$.

In some countries the sensitivity of each new lot of red blood cells is demonstrated by titration against a haemagglutinin antigen before use in the test for haemadsorbing viruses.

A.4.1.3 Tests for other adventitious agents in cell supernatant fluid

At the end of the observation period, a sample of the pooled supernatant fluid from each group of control cultures should be tested for adventitious agents. For this purpose, 10 mL of each pool should be tested in the same cells, but not the same lot of cells, as those used for the production of vaccine.

A second indicator cell line should be used to test an additional 10 mL sample of each pool. When a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line (28).

The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per

mL of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated cultures should be incubated at the same temperature as that of the production of virus antigen and observed at appropriate intervals for 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 14 days. Furthermore, some NRAs require that these cells should be tested for the presence of haemadsorbing viruses.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for any reason by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvests produced from the lot of cells from which the control cells were taken should be discarded.

Some selected viruses may be screened for using specific validated assays which are approved by the NRA, such as molecular NAT-based assays (28).

If these tests are not performed immediately, the samples should be kept at a temperature of -60°C or below.

A.4.1.4 Identity tests

At the production level, the control cells should be identified using tests approved by the NRA.

Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), immunological tests, cytogenetic tests (for example, for chromosomal markers), morphological identification and tests for genetic markers (for example, DNA fingerprinting or sequencing).

A.4.2 Cell cultures for vaccine production

A.4.2.1 Observation of cultures for adventitious agents

On the day of inoculation with the virus working seed lot, each cell culture or a sample from each culture vessel should be examined visually for degeneration caused by infective agents. If this examination shows evidence of the presence of any adventitious agent in a cell culture then the culture should not be used for vaccine production.

A.4.3 Control of single harvests

After inoculation of the production cells with virus, the culture conditions of inoculated and control cell cultures should be standardized and kept within limits agreed with the NRA.

Samples required for the testing of single harvests should be taken immediately on harvesting.

Samples may be taken after storage and filtration with the agreement of the NRA.

A.4.3.1 Identity test

The single harvests or pool of single harvests should be identified as EV71 by serum neutralization in cell culture infectivity assays using specific antibodies, or by molecular methods such as NAT-based assays. The test for antigen content described in section A.4.4.2.2 below can be used to identify the single harvest.

A.4.3.2 Sterility tests for bacteria, fungi and mycoplasmas

A volume of at least 10 mL of each single harvest should be tested for bacterial, fungal and mycoplasmal contamination using appropriate tests, as specified in Part A, sections 5.2 (60) and 5.3 (61) of the WHO General requirements for the sterility of biological substances, or by methods approved by the NRA.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and the agreement of the NRA (28).

In some countries this test is performed on the purified virus harvest instead of on the single harvest.

A.4.3.3 Virus titration

The virus concentration of each single harvest should be determined by titration of infectious virus using validated tissue culture methods to monitor production consistency and as a starting point for monitoring the inactivation curve. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of human RD, human diploid or Vero cells in microtitre plates is suitable for this purpose (28). The same cells should be used for virus titrations throughout the production process.

Information on virus titre will help in selecting single harvests that can be expected to meet potency requirements after inactivation.

The virus titration may be carried out on the pooled harvest after demonstration of consistency of production at the stage of the single harvest.

A.4.4 Control of virus pools

Several single harvests may be mixed to prepare a pool of virus before inactivation. The order in which the purification, filtration and inactivation of virus pools is conducted should be carefully established by the manufacturer to ensure consistent full virus inactivation and absence of residual infectivity. Based on experience of the production of IPVs, the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (23) recommends purification, filtration and inactivation steps in that order.

The requirement for filtration before and during inactivation was introduced into the IPV production process following the Cutter incident during which a number of paralytic polio cases occurred in children following vaccination with a defective IPV (23). The vaccine used was later found to contain aggregates which led to incomplete virus inactivation likely due to formaldehyde not accessing some virus particles inside the aggregates.

Any deviation from the production sequence shown to be acceptable for IPV for a vaccine against a virus similar to poliovirus (such as EV71) should be fully validated and justified in terms of yielding a product of equivalent safety.

Inactivation of virus may be performed before or after purification according to current approved procedures for the production of licensed vaccines. The method of purification and inactivation as well as the agent used for inactivation should be appropriately validated and should be approved by the NRA.

If inactivation of the virus pool is conducted after purification, please see section A.4.4.1 below. If inactivation of the virus pool is conducted before purification please see section A.4.4.2 below.

A.4.4.1 Purification of virus pools

Each pool of virus should be purified. Removal of host cell protein should be assessed during process validation (28).

An acceptable method is to clarify the virus suspension by filtration, to concentrate the virus by ultrafiltration and, thereafter, to collect the virus peak after passing it through a gel-filtration column. Further purification is achieved by passing the virus through an ion-exchange column. Other purification procedures resulting in acceptable release criteria may be used.

A.4.4.2 Tests on virus pools (purified or not) before inactivation

A.4.4.2.1 *Virus titration*

The virus concentration of each virus pool should be determined by titration of infectious virus using validated tissue culture methods. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of human RD, human diploid or Vero cells in microtitre plates is suitable for this purpose (28). The same cells should be used for virus titrations throughout the production process.

Information on virus titre will help in selecting pools that can be expected to meet potency requirements following inactivation.

A.4.4.2.2 *Virus antigen content*

The antigen content of each virus pool should be determined using a validated immunochemical method and should be calculated using a reference vaccine calibrated against the First WHO International Standard for EV71 inactivated vaccine (see **International reference materials** above) and expressed in IU.

A.4.4.2.3 *Specific activity*

The ratio of virus concentration or antigen content to the total protein content (specific activity) of the virus pool before inactivation should be within the limits of material shown to be safe and effective in clinical trials and approved by the NRA. This would ensure a consistent ratio of chemical agent to the viral protein, and thus a consistent inactivation process.

A.4.4.3 Filtration before inactivation (purified or not)

In order to avoid interference with the inactivation process, virus aggregation should be prevented or aggregates should be removed immediately before and during the inactivation process. For this reason, each virus pool should be filtered before inactivation.

Satisfactory results have been reported with several filter types but a final filtration using a 0.22 µm filter should be used.

Inactivation should be initiated as soon as possible and, in any case, not later than 72 hours after filtration.

It is preferable to start inactivation within 24 hours of filtration. Since the purpose of the filtration step is to remove particulate matter and other interfering substances that may diminish the effectiveness of the inactivation process and since aggregates tend to increase on standing after filtration, efforts should be made to keep within this time limit.

A sample of the filtered virus pool should be retained and its virus titre determined as described in section A.4.4.2.1 above.

The main purpose of determining the titre of filtered virus pools destined for inactivation is to provide the starting titre to monitor the kinetics of inactivation.

A.4.5 **Control of inactivated bulk**

A.4.5.1 **Inactivation procedure**

The virus in the filtered pools should be inactivated using a validated method approved by the NRA. Prior to inactivation, the concentration of the filtered pool (based on viral titre, virus antigen or protein content) should be adjusted to the acceptable range established during the process validation.

Most manufacturers currently use formaldehyde as the method for inactivation but at least one manufacturer is using other inactivating agents such as beta-propiolactone (BPL).

The method of inactivation should have been shown to consistently inactivate EV71 virus without destroying the antigenic and immunogenic activity. Inactivation of the virus pool may take place before or after purification depending on the approved production process. The progress of inactivation should be monitored by suitably spaced determinations of virus titres. The inactivation period should usually exceed the time taken to reduce the titre of live virus to undetectable amounts by a factor of at least 2 and be agreed to by the NRA.

A second filtration should be carried out during the process of inactivation. This step is taken after the virus titre has fallen below detectable levels but before the first sample for the safety test is taken. Following this filtration step, the inactivation process should continue.

The kinetics of viral inactivation should be established by each manufacturer and approved by the NRA. During the validation studies, an inactivation curve should be established with at least four time points showing the decrease in live virus concentration with time. The consistency of the inactivation process should be monitored; the virus titre and antigen content of each pool before, during and at the end of inactivation should be determined.

A record of consistency in the effectiveness and kinetics of inactivation should be established by the production of at least five consecutive lots and, if broken, a root-cause analysis should be performed and a further five consecutive filtered purified virus pools should be prepared and shown to be satisfactory for establishing this record.

A.4.5.2 Purification of inactivated virus pool

If inactivation is conducted using a non-purified virus pool, the inactivated pool should be purified as described in section A.4.4.1 above.

A.4.5.3 Tests on purified inactivated bulk

A.4.5.3.1 *Test for effective inactivation*

After removal or neutralization (as appropriate) of the inactivating agent, the absence of residual live EV71 virus should be verified by inoculating a quantity of the inactivated virus bulk equivalent to 5% of the lot (or not less than 1000 doses of vaccine) into sensitive cell cultures of the same type as those used for vaccine production.

The virus sample should be incubated for no less than 21 days making no fewer than two cell passages during that period. The dilution of the sample in the nutrient fluid should not exceed 1 in 4 and the area of the cell sheet should be at least 3 cm² per mL of sample. One or more culture vessels of each lot of cultures should be set aside to serve as uninoculated control culture vessels with the same medium. The sensitivity of the assay should be demonstrated.

If formaldehyde has been used as the inactivating agent, samples of vaccine for tissue culture tests are generally neutralized at the time of sampling by the addition of bisulfite. Usually, the samples are subsequently dialysed or another validated method used.

It is possible to conduct tissue culture tests on non-dialysed material. However, this is often found to be toxic to cells, even with a dilution of 1 in 4. If in such tests, nonspecific degeneration of cells occurs, or if the sensitivity of the tissue culture system is reduced, the test should be repeated on dialysed material. The virus antigen content after dialysis should be determined to ascertain whether the viral antigen was lost during the dialysis process.

If infectious virus is detected, the bulk should not be used for further processing. The isolation of live virus from an inactivated bulk should be regarded as a break in the manufacturing consistency record and a production process review and revalidation should be undertaken.

It is important to demonstrate that each test retains sensitivity to detect partially inactivated EV71 virus. At the end of the observation period, the cell culture used for the detection of residual live virus should be challenged with a validated amount of live EV71 virus of the same strain as that of the inactivated virus bulk. The details of the challenge procedure should be approved by the NRA. It is recommended that the ability to detect infectious virus is checked concurrently for each test by including a positive control at the beginning of

each test. Positive control flasks should be inoculated with a low quantity of virus close to the detection limit of the method.

The problem of detecting residual active virus in an inactivated vaccine is not the same as that of measuring infective virus in untreated suspensions. Other similar viruses that have been exposed to the action of formaldehyde without becoming inactivated have been shown to require a much longer period to produce cytopathic changes than untreated virus. For this reason, it is desirable that tissue cultures in tests for the presence of residual active virus are observed for as long a time as is technically possible. A satisfactory tissue culture system for this purpose depends, therefore, not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

Serum added to the nutrient fluid should be tested for inhibitors to EV71 at serum concentrations up to 50%. Only serum free from inhibitors should be used.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that early changes of fluid may result in unadsorbed virus being removed and the validity of the test would thus be impaired. Therefore, the fluid should be changed no earlier than 5–7 days after inoculation.

A.4.5.3.2 *Sterility tests for bacteria and fungi*

Each purified inactivated bulk should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (60), or by methods approved by the NRA.

A.4.5.3.3 *Antigen content*

The EV71 antigen content of each purified inactivated bulk should be determined by the use of a validated immunochemical method and should be calculated using a reference vaccine calibrated against the First WHO International Standard for EV71 inactivated vaccine (see **International reference materials** above). The results obtained should be expressed in IU and be within the required limits established by the NRA.

A.4.5.3.4 *Residual inactivating agent*

The concentration of free residual formaldehyde or any other chemical used for inactivating the virus should be determined by a method approved by the NRA. The maximum acceptable limit should be approved by the NRA.

If BPL is used then its hydrolysis kinetics should be determined during the validation of the inactivation process to ensure that no residual BPL is present in the inactivated bulk at the end of the inactivation step.

A.4.5.3.5 *Residual cellular DNA*

If continuous cell lines are used for production, the purification procedure should have been shown to consistently reduce the level of residual host cell DNA (28). The amount and size of residual host cell DNA should not exceed the maximum levels 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 (28). Human diploid cell lines have been used successfully for many years for the production of viral vaccines and the residual cellular DNA deriving from these cells is not considered to pose any significant risk (28).

This test can be performed on the purified virus bulk and may be omitted from routine testing, with the agreement of the NRA, if the manufacturing process is validated to achieve this specification (28).

If assessed, the size distribution of the DNA may be considered as a characterization test, taking into account the amount of DNA detectable using appropriate methods, as approved by the NRA (28).

A.4.5.3.6 *Residual chemicals*

If chemical substances are used during the purification process, tests for these substances should be carried out. Their concentration should not exceed the limits approved for the particular product.

A.4.5.3.7 *Test for residual animal serum protein*

If animal serum has been used in the cell culture system, a sample of purified bulk should be tested. The residual amount of serum albumin should be less than 50 ng per single human dose.

A.4.6 **Control of final bulk**

Preservatives, excipients or other substances that might be added to form the final bulk should have been shown, to the satisfaction of the NRA, to have no deleterious effect on the immunizing potency and safety profile of the EV71 antigens. Preservative efficacy should be demonstrated during product development using a method approved by the NRA.

The operations necessary for preparing the final bulk from the purified inactivated bulk should be conducted in such a manner as to avoid contamination of the product. In preparing the final vaccine bulk, any substances that are added to the product (such as diluents, stabilizers or adjuvants) should have been shown, to the satisfaction of the NRA, not to impair the safety and efficacy of the vaccine in the concentrations used. Until the final bulk is filled into containers,

the final vaccine bulk suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity.

A.4.6.1 Sterility tests for bacteria and fungi

The final bulk should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (60), or by methods approved by the NRA.

A.4.6.2 Potency tests

Each final bulk should be tested using an *in vivo* assay for immunogenicity and *in vitro* antigen content assay approved by the NRA, unless this is to be performed on final product. Product-specific reference preparations may be used in these tests.

The EV71 antigen content of each final bulk should be determined using a validated immunochemical method and calculated using a reference vaccine calibrated against the First WHO International Standard for EV71 inactivated vaccine (see **International reference materials** above). The *in vitro* assay found to be the most suitable for measuring the antigen content is the EV71 antigen ELISA. The results obtained should be within the required limits established by the NRA.

Once consistency of production has been established for a suitable number of consecutive final bulks, the *in vivo* assay may be omitted for the purpose of routine lot release, with the agreement of the NRA. This can occur once it has been demonstrated that the acceptance criteria for the EV71 antigen determination are such that the *in vitro* test yields a comparable result to the *in vivo* assay in terms of acceptance or rejection of a lot. This demonstration should include testing of sub-potent lots, produced experimentally if necessary by heat treatment or other means of diminishing the immunogenic activity.

If an adjuvant is used in the final bulk, a desorption or treatment step may be necessary before performing the EV71 antigen ELISA.

If the final bulk is formulated with other antigens into a combination vaccine, the suitability of performing the EV71 antigen ELISA on the final bulk will have to be determined. If the EV71 antigen ELISA is not suitable for a particular combination, an *in vivo* assay should be used.

The potency of the final bulk should be approved by the NRA.

A.4.6.3 Preservative content (if applicable)

If preservative is added, its concentration in the final bulk should be determined by a method approved by the NRA. The preservative used and concentration permitted should be approved by the NRA. The preservative should not adversely affect the quality of the antigens.

A.4.6.4 Adjuvant (if applicable)

Each final vaccine bulk should be assayed for adjuvant content. This test may be omitted if it is to be performed on the final lot. Where aluminium compounds are used, the aluminium content should not exceed 1.25 mg per single human dose.

A.5 Filling and containers

The requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles (58) and WHO good manufacturing practices for biological products (27) should apply to vaccine filled in the final form. Single- and multi-dose containers may be used.

Care should be taken to ensure that the materials of which the container and (if applicable) the transference devices and closure are made do not adversely affect the quality of the vaccine.

Manufacturers should provide the NRA with adequate data to prove the stability of the product under appropriate conditions of storage and transport.

A.6 Control tests on the final lot

Samples should be taken from each final lot for the tests described in the following sections. The following tests should be performed on each final lot of vaccine (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. All tests and specifications, including methods used and permitted concentrations, should be approved by the NRA.

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded and recorded for each relevant abnormality. A limit should be established for the percentage of rejections permitted before triggering an investigation of the cause, potentially resulting in lot failure.

A.6.1.1 Appearance

The appearance of the vaccine should be described with respect to its form and colour.

A.6.2 Identity test

An identity test should be performed on at least one labelled container from each final lot using an appropriate method. The potency test described in section A.6.4 below may serve as the identity test.

A.6.3 Sterility tests for bacteria and fungi

Each final lot should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (60), or by methods approved by the NRA.

A.6.4 Potency test

The potency of each final lot should be determined using an in vivo assay and a validated in vitro antigen content assay (see section A.4.6.2 above) if such a test has not been performed on the final bulk. Potency should be calculated using a reference vaccine calibrated against the First WHO International Standard for EV71 inactivated vaccine (see **International reference materials** above) or other reference preparation.

If the use of an adjuvant in the final bulk interferes with the assay, a desorption or treatment step may be necessary. If treatment/desorption is not possible, the interference of the adjuvant should be documented and an in vivo assay should be performed (see section A.4.6.2 above).

The potency of the vaccine should be approved by the NRA.

A.6.5 Preservative content

Where appropriate, the preservative content of each final lot should be determined. The method used and content permitted should be approved by the NRA. This test may be omitted if conducted on the final bulk.

A.6.6 Endotoxin content

The endotoxin content of each final lot should be determined using a method approved by the NRA. Endotoxin levels should be consistent with levels found to be acceptable in vaccine lots used in pre-licensure clinical trials and should be approved by the NRA.

A.6.7 Residual formaldehyde

The concentration of free residual formaldehyde in each final lot should be determined using a method approved by the NRA. The acceptable maximum limit should be approved by the NRA. This test may be omitted if performed on the final bulk.

A.6.8 pH

The pH of each final lot should be determined and should be within limits approved by the NRA.

A.6.9 Adjuvant and degree of adsorption (if applicable)

If an adjuvant is used in the formulation, each final lot should be assayed for adjuvant content. Where aluminium compounds are used, the aluminium content should not exceed 1.25 mg per single human dose.

The degree of adsorption of the antigen to the aluminium compounds (aluminium hydroxide or hydrated aluminium phosphate) in each final lot should be assessed.

Both tests may be omitted for routine lot release upon demonstration of consistency of production, subject to the agreement of the NRA.

A.6.10 Residual antibiotics (if applicable)

If any antibiotics are added during vaccine production, the residual antibiotic content should be determined and should be within limits approved by the NRA. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

If aluminium adsorption has an impact on the test, then testing for antibiotic content may be done at the purified inactivated bulk stage.

A.6.11 Extractable volume

For vaccines filled into single-dose containers, the extractable content should be checked and shown to be not less than the intended dose.

For vaccines filled into multi-dose containers, the extractable content should be checked and should be shown to be sufficient for the intended number of doses.

A.7 Records

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (58) and WHO good manufacturing practices for biological products (27) should apply.

A.8 Retained samples

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (58) and WHO good manufacturing practices for biological products (27) should apply.

A.9 Labelling

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (58) and WHO good manufacturing practices for biological products (27) should apply, and additionally the label

on the container or package, or the leaflet accompanying each container, should include the following information:

- the designation(s) of the strain(s) of EV71 contained in the vaccine
- the cell substrate used for the preparation of vaccine
- the antigen content
- the method and inactivating agent used to inactivate the virus
- the nature and amount of any stabilizer and preservative present in the vaccine
- the nature and amount of adjuvant, if applicable.

It is desirable for the label to carry the names both of the producer and of the source of the bulk material if the producer of the final vaccine did not prepare it. The nature and amount of antibiotics present in the vaccine, if any, may also be included.

A.10 **Distribution and transport**

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (58) and WHO good manufacturing practices for biological products (27) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (64).

A.11 **Stability testing, storage and expiry date**

A.11.1 **Stability testing**

Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (65). Stability testing should be performed at different stages of production when intermediate product is stored, namely on single harvests, purified inactivated bulk, final bulk and final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. During vaccine production a shelf-life should be assigned to all in-process materials – particularly intermediates such as single harvests, purified inactivated bulk and final bulk.

The stability of the vaccine in its final containers, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA. As a guide, containers from at least three consecutive final lots derived from different bulks may be tested.

Accelerated stability tests may be undertaken to provide additional information on the overall characteristics of the vaccine and may also aid in

assessing comparability should the manufacturer decide to change any aspect of manufacturing.

The formulation of the vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (65). Data should be provided to the NRA in accordance with 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 vaccine(s).

A.11.2 Storage conditions

EV71 vaccine (inactivated) should be stored at all times at a temperature of 2–8 °C.

If a vaccine has been shown to be stable at temperature ranges higher than the approved 2–8 °C range, it may be stored under extended controlled temperature conditions for a defined period, subject to the agreement of the NRA (66).

A.11.3 Expiry date

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

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals were inoculated with the final bulk.

Part B. Nonclinical evaluation of enterovirus 71 vaccines (inactivated)

Nonclinical evaluation of a new EV71 vaccine should follow the principles outlined in the WHO guidelines on nonclinical evaluation of vaccines (25) which provide details on the design, conducting, analysis and evaluation of nonclinical studies. Further guidance on the general principles for the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines can be found in separate WHO Guidelines (29). The following sections B.1–B.3 provide specific guidance on addressing important issues related to the nonclinical development of a new inactivated whole EV71 virus vaccine.

B.1 Product characterization and process development

The vaccine lots used in nonclinical studies should be adequately characterized as described in Part A of these WHO Recommendations, taking into consideration the stage of product development. Both the antigen(s) of the vaccine and the end product need to be clearly defined, and the manufacturing process carefully monitored for all crucial steps so as to confirm the consistency of production. It is essential that sufficient data are generated to confirm the full inactivation of vaccine virus and the absence of virulent virus in the end product. Furthermore, sufficient vaccine stability data are necessary to support its suitability for use in the nonclinical studies.

It is crucially important that vaccine manufacturing processes are appropriately standardized and controlled to ensure consistency of production. The extent of product characterization may vary according to the stage of development. To support their validity, nonclinical studies should be carried out on vaccine lots that are adequately representative of the concurrent clinical lots in terms of their physicochemical data, stability, qualitative and quantitative impurity profiles, and formulation.

B.2 Nonclinical immunogenicity and protection studies

B.2.1 Evaluation of immunogenicity in animal models

Unless otherwise justified, the immunogenicity of any new EV71 vaccine needs to be characterized in relevant animal models (for example, mice, rats or rabbits) before proceeding to human trials. These proof-of-concept nonclinical studies should reflect the clinically proposed use of the vaccine, including the administration route, and should include an evaluation of serum neutralizing antibody response against isolated virus strains or pseudoviruses (5–7, 67, 68), and dose-range testing of the antigen. The immune response to the candidate vaccine should ideally be assessed after each dose of vaccine, and whenever possible against a licensed EV71 vaccine used as an active control. Data on cross-neutralizing antibodies should be obtained from nonclinical immunogenicity studies using a range of isolated heterologous viruses or pseudoviruses of different subgenogroups. These data may guide selection of the doses, dosing regimen and administration route to be evaluated in clinical trials.

When a candidate EV71 vaccine is formulated with a new adjuvant, a rationale for the selection of the adjuvant should be provided and the benefit of its inclusion in the vaccine formulation should be demonstrated by the immunogenicity data.

The immunogenicity studies in animals may additionally be considered, when appropriate, as part of a comparability exercise to demonstrate the reproducibility of the manufacturing process whenever major changes are

introduced during the different stages of process development or during the validation phase of a new candidate EV71 vaccine manufacturing process.

B.2.2 Challenge-protection studies

Current evidence suggests that serological immune responses play an essential role in mediating protection by formalin-inactivated whole EV71 virus vaccines. Animal studies conducted in newborn mice, transgenic mice and nonhuman primates have demonstrated that vaccination with inactivated EV71 vaccines induces protective immunity against EV71 and that protection in challenged animals is primarily mediated by neutralizing antibodies (44, 50, 53, 69). Importantly, human efficacy trials conducted with several formalin-inactivated EV71 vaccines have shown a strong correlation between vaccine-induced serum neutralizing antibodies and protection against EV71-associated diseases (5–7). Based on these observations, it is considered that, for a similarly manufactured candidate EV71 vaccine, no further challenge-protection studies in animal models need to be performed.

However, challenge-protection studies may be useful for any candidate EV71 vaccine based on a novel production process or intended to have novel mechanisms of action.

Since evidence from epidemiological studies and clinical trials for cross-protection against EV71 disease has thus far been limited, any claims of cross-protection should in general be supported by appropriate animal data. Specifically, challenge-protection studies should be conducted in appropriate animal models to evaluate the potential for protection against heterologous viruses of different genogroups, as this could indicate the breadth of protection.

B.3 Nonclinical safety studies

For a new EV71 vaccine based on inactivated whole EV71 virus, a repeat-dose toxicity study in a relevant animal species is generally needed to assess potential local and systemic toxicity and any other undesirable effects. Omission of standalone local tolerance and single-dose toxicity studies is possible if assessment of acute toxic effects and local tolerance has been incorporated into the repeat-dose toxicity study.

If the candidate vaccine contains a novel adjuvant, the principles set out in the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (29) should be followed. For example, consideration should be given to assessing the toxicity of the adjuvant alone.

For candidate EV71 vaccines manufactured using novel cell substrates, efforts should be made during the nonclinical safety study to explore biomarkers indicative of potential allergic reactions – for example, by measuring type 2 CD4+ T-helper cell responses.

Part C. Clinical evaluation of enterovirus 71 vaccines (inactivated)

C.1 Introduction

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (70) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (26). This section focuses only on issues relevant or specific to the clinical development of inactivated EV71 vaccines.

At present, no efficacy data on cross-protection are available from completed clinical trials with inactivated EV71 vaccines due to the distinct regional circulation of specific subgenogroups. In addition, no internationally recognized immune correlate of protection (ICP) or surrogate marker of protection has been established. Although immunogenicity results from clinical trials suggest that a neutralizing antibody titre of 1:16 to 1:32 might be related to protection, further analysis based upon the use of a scaled logit model has indicated that significantly higher levels of neutralizing antibodies might be needed to achieve protection (5–7, 54, 67).

If cross-protection against heterologous EV71 viruses is to be claimed then appropriate nonclinical and/or clinical studies should be conducted to evaluate the potential for such cross-protection (see section B.2.1 above). In addition, data demonstrating the ability of antibodies obtained from vaccinated individuals to neutralize various subgenogroups of EV71 viruses *in vitro*, including recently circulating isolates, are expected to be provided. Continuous evaluation of protective vaccine efficacy post licensure is encouraged due to the evolution of new EV71 strains or a rapid change of subgenogroups in different countries and regions, which may result in outbreaks. Should new subgenogroups emerge in a country in which the vaccine is licensed and used, further clinical investigation of potential cross-protection may be needed.

C.2 Assays

General guidance on the use and validation of assays for the evaluation of immune responses is provided in section 5.3.3 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (26).

Sections C.2.1 and C.2.2 below provide specific guidance on the following assays relevant to the investigation of immune responses to inactivated human EV71 vaccines in clinical trials and to the confirmation of vaccine efficacy in pivotal studies respectively:

- serological assays for establishing the baseline serostatus of trial subjects and evaluating the humoral immune response to vaccination (see also section C.3); and
- detection assays for laboratory confirmation of HFMD and herpangina caused by EV71 infection, in vaccine efficacy trials (see also section C.4).

C.2.1 Serological assays

C.2.1.1 Functional antibody

The direct measurement of anti-EV71 neutralizing antibody is well established. Neutralizing antibody has been estimated using methods such as plaque reduction neutralization assays employing either isolated virus strains or pseudoviruses (5–7, 67, 68). Sponsors are encouraged to develop high-throughput assays for anti-EV71 neutralizing antibody. These assays should be standardized using the First WHO International Standard for anti-EV71 serum (human) and the First WHO International Reference Reagent for EV71 neutralization assays (see **International reference materials** above). In addition, a reference neutralizing antibody panel for the evaluation of neutralizing antibody responses has been established in China (14).

C.2.2 Virus detection assays

Since HFMD and herpangina can be caused by different human enteroviruses, including EV71, Coxsackie and echoviruses, appropriate RNA or virus detection assays are required to confirm the presence of EV71 in throat and vesicle swabs and/or stool samples (see section C.4.2 below). International guidance on enterovirus diagnostics and characterization should be taken into consideration (4).

For other enterovirus infections, laboratory confirmation of diagnosis based on cell culture, virus isolation and virus identification remains a standard approach. The use of established cell lines such as human RD or Vero cells is recommended for virus isolation.

Various quantitative polymerase chain reaction (PCR) assays are commercially available. Although several EV71-specific PCR systems have been described, the ability of an assay to reliably detect EV71 RNA from specific subgenogroups should be taken into account when selecting the method to be used in clinical trials. In general, it is recommended that the subgenogroup be determined based upon VP1 gene sequences.

Sponsors should provide full details of the methodology applied, and appropriate controls should be used.

In addition, EV71 infection can be confirmed by the use of anti-EV71 immunoglobulin M (IgM) assays.

C.3 Immunogenicity

C.3.1 Formulation, dose and regimen

C.3.1.1 Primary series

EV71 vaccines will be used mainly or exclusively in regions with relatively high rates of clinically apparent infections. In naturally primed individuals the first dose of EV71 vaccine may elicit large increments in antibody due to an anamnestic response. In contrast, multiple doses of the same vaccine may be required to achieve similar antibody levels in EV71-naive subjects. Since pre-vaccination testing for EV71 serostatus will not be practical in routine use, it is important that the primary series should be selected on the basis of the immune responses observed in subjects who were seronegative prior to vaccination.

In the absence of an internationally established ICP for EV71 – that is, one meeting the WHO definition of an ICP (26) – the selection of the vaccine dose and regimen may be based on the reaching of a plateau antibody response unless this is precluded by concerns over reactogenicity. It is desirable that immunogenicity studies should explore the minimum number of doses and the shortest dose interval(s) required to achieve a plateau immune response.

C.3.1.2 Cross-protection

The ability of a candidate EV71 vaccine to protect against a range of wild-type strains covering the main EV71 genogroups may vary according to the vaccine strain used. For example, lower cross-neutralization against an atypical C2-like strain was observed in naturally infected EV71 patients (71) and in clinical trials using B4-based vaccine strains (22).

In clinical trials in which vaccine-elicited antibody is determined against the antigen in the vaccine, it is recommended that neutralizing activity is also measured using antigens derived from a range of circulating wild-type EV71 strains from different (sub)genogroups. If marked differences are observed in measured antibody levels using vaccine versus non-vaccine strains, and/or by EV71 subgenogroup, it would be of particular interest to assess whether a similar effect is observed for functional antibody levels in naturally infected individuals.

C.4 Efficacy

C.4.1 Requirement for a demonstration of vaccine efficacy

It is currently recommended that licensure of a candidate EV71 vaccine should be based on evidence of its protective efficacy against clinically apparent HFMD and herpangina. The following considerations apply:

- At the time of preparing these WHO Recommendations, three vaccines against human EV71 had been licensed in one country (see **General considerations** above) (4, 9, 17).
- These licensed vaccines are not yet widely used internationally. As a result, the use of a control group that does not receive vaccination against EV71 is possible.
- In jurisdictions in which a licensed vaccine is available, it is possible that individual NRAs may consider that licensure can be based on a trial that evaluates the efficacy of the candidate vaccine relative to that of the licensed vaccine in a population similar to that in which the efficacy of the licensed vaccine was established.
- The lack of an established ICP against EV71 does not rule out immunobridging a candidate vaccine to a licensed vaccine that has been shown to be efficacious. However, this approach is only possible if both vaccines contain the same antigen(s) so that anti-EV71 neutralizing antibody immune responses can be compared directly. In addition, the demonstration of efficacy of all three licensed vaccines was confined to EV71 subgenogroup C4 and it is not known whether protective efficacy may vary between genogroups circulating in different regions.

Taking these considerations into account, the focus of the following sections is on clinical development programmes that include vaccine efficacy trials in which the control group does not receive vaccination against EV71. However, most of the recommendations are also applicable to trials in which the control group receives a licensed vaccine against EV71. Clinical programmes leading to licensure based on immunobridging are not addressed in the following guidance. The general principles to be considered are discussed in sections 5.6.2 and 6.3.3 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (26).

C.4.2 Considerations for efficacy trial design

C.4.2.1 Primary objective

The primary objective will be to demonstrate that the candidate vaccine protects against clinically apparent (that is, symptomatic) HFMD and herpangina caused by EV71 infection regardless of the genogroup (see section C.4.2.4).

- It is not required for efficacy to be shown against asymptomatic EV71 infection as such infections are of no clinical significance.
- It is not required for vaccine efficacy trials to be powered to demonstrate genogroup-specific efficacy (see section C.4.2.2).

C.4.2.2 Trial sites

Efficacy trials will be conducted in endemic areas in which the estimated attack rate for HFMD and herpangina due to EV71 infection is sufficient to complete enrolment into an adequately powered vaccine efficacy trial within a reasonable time frame. Sites may be chosen on the basis of available public health disease-surveillance data and/or pre-trial evaluations of epidemiology conducted by the sponsor. In three prior efficacy trials (6–9) the EV71 viruses that caused clinically apparent HFMD and herpangina were limited to strains of the C4 subgenogroup circulating at the trial sites in the years in which they were conducted. Sponsors are encouraged to consider selecting sites in a range of geographical areas in which strains of different genogroups are circulating and/or to conduct separate vaccine efficacy trials in regions with different genogroup distributions.

C.4.2.3 Subject selection criteria

Because of the age-dependent incidence and severity of EV71 infections it is likely that vaccine efficacy trials will target infants and children. An upper age limit may be set depending on the age-specific attack rates.

C.4.2.4 Primary end-point

In accordance with the recommended primary objective above, the primary end-point should be clinically apparent HFMD or herpangina that is confirmed to be due to EV71 infection. Sponsors could consider appointing an independent data-monitoring committee to review the data and determine which subjects meet the case definition to be counted in the primary analysis.

C.4.2.4.1 *Clinical features for the case definition*

The clinical features that cause subjects to present to study site staff or to a local designated health care facility for laboratory investigations for acute HFMD or herpangina should be identified with the aim of capturing as many cases as possible while limiting unnecessary investigations. On this basis, it is reasonable to define a possible case of HFMD or herpangina requiring laboratory investigation as an illness presenting with febrile illness accompanied by a papular or vesicular rash in the characteristic distribution on the oral mucosa, hands, feet or buttocks. A severe case of HFMD should be defined as associated with neurological, respiratory or circulatory complications as described by WHO (4).

C.4.2.4.2 *Laboratory confirmation of HFMD or herpangina caused by EV71 infection*

It is recommended that laboratory confirmation of HFMD and herpangina cases should be conducted in a designated qualified central laboratory. If more than one central laboratory is necessary for practical reasons, it is essential that all

of the laboratories use identical methodologies, and consideration should be given to testing a randomly selected subset of samples at each laboratory to assess concordance. The laboratory methods used should be validated.

The confirmation of EV71 as causative of the clinical picture should be based on any of the following:

- detection of EV71 RNA in vesicle/throat swabs or in stool;
- virus isolation and analysis of VP1 sequence;
- detection of IgM against EV71 – which is often detectable at the time of onset of clinical symptoms but which may peak after 1–2 weeks.

To avoid cases being missed, protocols should plan for appropriately timed repeat specimens to be collected from individuals with a first negative test for EV71 RNA (for example, 3–7 days after the first sample).

Samples obtained at first presentation and repeat specimens should also be tested to detect infection with other enteroviruses, such as Coxsackie and echoviruses, that can also cause HFMD and which regularly co-circulate with EV71 in affected countries.

C.4.2.5 Primary, secondary and other analyses

In a vaccine efficacy trial, it may be permissible for the primary analysis to include only confirmed cases of HFMD and herpangina caused by EV71 as follows:

- in subjects who completed the vaccination series within predetermined visit windows, if more than one dose is required; and
- with symptom onset occurring more than a defined period after the only or final dose of the series, taking into account what is known about the timing of the post-dose anti-EV71 IgG peak.

This approach gives the most optimistic estimation of vaccine efficacy.

If the primary analysis is confined to cases counted as described above, it is essential that predefined secondary analyses are carried out to estimate vaccine efficacy based on confirmed cases of clinically apparent HFMD and herpangina caused by EV71 infection, defined and counted as follows:

- all cases in subjects who received at least one assigned dose as randomized, and regardless of adherence to study visit windows;
- cases that occurred at any time after the last dose received (that is, counted from the day of dosing) in those who completed the assigned number of doses;
- cases that occurred after each sequential dose, depending on the number of doses in the series and counted from the day of dosing.

Vaccine efficacy should be explored according to EV71 genogroup if this is feasible, depending on the numbers of cases that occur due to individual genogroups.

It is recommended that an additional analysis should explore any differences in clinical or laboratory features (including severity) between cases that occur in the candidate vaccine group and the control group (whether the control group receives placebo or a licensed vaccine against EV71). The analysis should take into account whether the severity observed in individual subjects could reflect coinfection with other enteroviruses.

C.4.2.6 Case ascertainment

It is recommended that an active case-ascertainment strategy is used throughout the time frame of a vaccine efficacy trial. This is essential at least up to the time of the primary analysis, which may be conducted after a specific number of total cases has been accumulated or after a predefined period in which a sufficient number of cases are expected to occur to estimate vaccine efficacy.

C.4.2.7 Duration of protection

While the primary analysis may lead to licensure, it is recommended that trials continue to use active case ascertainment to follow up subjects for several years to provide data on waning vaccine protection, without un-blinding of treatment assignment at the level of the individual. These data can then be reported at some point after licensure of the vaccine and may point to a need for further doses to be administered at intervals to maintain protection, or a need to change the vaccine strains used.

C.4.2.8 Vaccine effectiveness

The need for vaccine effectiveness studies should be established at the time of licensure.

If longer-term follow-up within a pre-licensure trial is not considered to be feasible, the duration of vaccine protection should be investigated within a vaccine effectiveness study and/or as part of routine disease surveillance conducted by public health authorities. Furthermore, the efficacy of the vaccine against individual subgenogroups should be explored as part of a vaccine effectiveness study and/or during routine disease surveillance.

C.5 Safety

Evaluation of the safety of candidate EV71 vaccines should be undertaken in accordance with the recommendations made in section 7 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (26). If

the primary series consists of several vaccine doses it is important to document whether reactogenicity increases with sequential doses. Additionally, the safety of post-primary doses should be evaluated. There may be special considerations for vaccine safety depending on the vaccine construct and the intended target population.

If a candidate vaccine is evaluated in a large pre-licensure trial, and if the safety profile documented during immunogenicity trials did not give rise to any major concerns, it may be acceptable for a full assessment of safety (that is, including detailed documentation of local and systemic reactogenicity, as well as all unsolicited adverse events) to be confined to a randomized subset of the total subjects. Any serious adverse event occurring in any subject enrolled at any of the trial sites should be documented.

Part D. Recommendations for NRAs

D.1 General recommendations

The guidance for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (72) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (30) should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and quality has been established and demonstrated by the manufacturer.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety or efficacy of inactivated EV71 vaccines (73), should be discussed with and approved by the NRA. For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards as appropriate (74). The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of inactivated EV71 vaccines. 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 final product.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (30).

A summary protocol for the manufacturing and control of enterovirus 71 vaccines (inactivated), based on the model summary protocol provided below in Appendix 1 and signed by the responsible official of the manufacturing

establishment, should be prepared and submitted to the NRA/NCL in support of a request for the release of a vaccine for use.

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review or independent laboratory testing). The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines. A model NRA/NCL Lot Release Certificate for enterovirus 71 vaccines (inactivated) is provided below in Appendix 2.

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Appendix 1

Model summary protocol for the manufacturing and control of enterovirus 71 vaccines (inactivated)

The following protocol is intended for guidance and indicates the minimum information that should be provided by the manufacturer to the NRA or NCL. Information and tests may be added or omitted as necessary with the approval of the NRA or NCL.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations for a particular product should be provided in the protocol submitted.

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

1. Summary information on final lot

International name of product: _____

Commercial/trade name: _____

Product licence (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Name and address of licence holder, if different: _____

Final packaging lot number: _____

Type of container: _____

Number of containers in this final lot: _____

Final container lot number: _____

Nature of final product (adsorbed): _____

Preservative and nominal concentration: _____

Volume of each single human dose: _____

Number of doses per final container: _____

Virus strain: _____

Cell substrate used for production: _____

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

Shelf-life approved (months): _____

Date of manufacture: _____

Expiry date: _____

Storage conditions: _____

2. Detailed information on manufacture and control

The following sections are intended for reporting the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. If any test had to be repeated, this must be indicated. Any abnormal results must be recorded on a separate sheet.

Summary of source materials

Identity of seed lot strain used for vaccine production

Reference number of seed lot: _____

Date(s) of reconstitution (or opening) of seed lot container(s): _____

Test for adventitious agents

Methods: _____

Result: _____

Date: _____

Identity of cell bank used for vaccine production

Reference number of cell bank: _____

Date(s) of reconstitution (or opening) of cell bank container(s): _____

Identity test

Methods: _____

Result: _____

Date: _____

Control of vaccine production

Control cell cultures

Tests on control cell cultures

Ratio of control to production cell cultures: _____

Incubation conditions: _____

Period of observation of cultures: _____

Dates observation started/ended: _____

Ratio or proportion of cultures discarded: _____

Results of observation: _____

Tests for haemadsorbing viruses

Quantity of cell tested: _____

Method used: _____

Date of start of test-1: _____

Date of end of test-1: _____

Results: _____

Date of start of test-2: _____

Date of end of test-2: _____

Results: _____

Tests for adventitious agents on supernatant culture fluids

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Control of single harvests

Name of the culture medium: _____

Date of inoculation: _____

Temperature of incubation: _____

Microscopic observation

Result: _____

Date: _____

Date of harvest: _____

Volume of harvest: _____

Yield (mg/mL): _____

Sterility tests for bacteria, fungi and mycoplasmas**Test for bacteria and fungi**

Method: _____

Media: _____

Volume inoculated: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Test for mycoplasmas (if applicable)

Method: _____

Volume inoculated: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Virus titration

Method: _____

Reference lot no. _____

Date: _____

Result: _____

Control of virus pool

Lot number of virus pool: _____

Date of pooling: _____

Number of harvests: _____

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

Purification of virus pool (may be performed after inactivation)

Purification methods: _____

Volume before purification: _____

Volume after purification: _____

Date: _____

Tests on virus pool

Virus titration

Method: _____

Reference lot no. _____

Date: _____

Result: _____

Virus antigen content

Method: _____

Reference lot no. _____

Date: _____

Result: _____

Specific activity

Virus antigen content: _____

Total protein content: _____

Specification: _____

Date: _____

Result: _____

Inactivation of harvest pool (may be performed before purification)

Filtration before inactivation

Filtration method: _____

Date: _____

Time of start of filtration: _____

Time of end of filtration: _____

Inactivation

Agent(s) and concentration of inactivation agent: _____

Temperature of inactivation: _____

Date of start of inactivation: _____

Virus antigen units at start of inactivation: _____

Date of taking first samples: _____

Date of completion of inactivation: _____

Virus antigen units at end of inactivation: _____

Filtration during inactivation

Filtration method: _____

Date: _____

Time of start of filtration: _____

Time of end of filtration: _____

Control of inactivated bulk

Test for effective inactivation (after removal/neutralization of inactivating agent)

Sample size tested: _____

Dates of sampling (1–4): _____

Test method: _____

Period of observation of cell cultures: _____

Period of observation of subcultures: _____

Period of observation of subcultures: _____

Period of observation of subcultures: _____

Result: _____

Sterility tests for bacteria and fungi

Method: _____

Media: _____

Volume inoculated: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Antigen content

Method: _____

Specification: _____

Date: _____

Result: _____

Test for residual inactivating agent

Method: _____

Specification: _____

Date: _____

Result: _____

Test for residual host-cell DNA (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Test for residual chemicals (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Test for residual animal serum protein (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Control of final bulk

Identification (lot number): _____

Date of manufacture/blending: _____

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

Blending:	Prescription (per dose)	Added
Virus antigen (IU or unit):	_____	_____
Adjuvant:	_____	_____
Preservative (specify):	_____	_____
Others (chemicals):	_____	_____
Final volume (mL):	_____	_____

Sterility tests for bacteria and fungi

Method: _____

Media: _____

Volume inoculated: _____
 Date of start of test: _____
 Date of end of test: _____
 Result: _____

Potency test

In vivo assay (may be performed at final bulk stage)

Species, strain, sex and weight specifications: _____
 Number of mice tested: _____
 Dates of vaccination, bleeding: _____
 Date of assay: _____
 Lot number of reference vaccine and assigned potency: _____
 Vaccine doses (dilutions) and number of animals responding at each dose:

 ED₅₀ of reference and test vaccine: _____
 Potency of test vaccine (with 95% fiducial limits): _____

If an in vitro assay is used

Method: _____
 Specification: _____
 Date: _____
 Result: _____

Adjuvant content

Method: _____
 Specification: _____
 Date: _____
 Result: _____

Preservative content (if applicable)

Method: _____
 Specification: _____
 Date: _____
 Result: _____

Control of final lot

Lot number: _____
 Date of filling: _____

Type of container: _____

Filling volume: _____

Number of containers after inspection: _____

Number and percentage of containers rejected: _____

Appearance

Method: _____

Specification: _____

Date: _____

Result: _____

Identity test

Method: _____

Specification: _____

Date: _____

Result: _____

Sterility tests for bacteria and fungi

Method: _____

Media: _____

Volume inoculated: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Potency test

In vivo assay (may be performed at final bulk stage)

Species, strain, sex and weight specifications: _____

Number of mice tested: _____

Dates of vaccination, bleeding: _____

Date of assay: _____

Lot number of reference vaccine and assigned potency: _____

Vaccine doses (dilutions) and number of animals responding at each dose:

ED₅₀ of reference and test vaccine: _____

Potency of test vaccine (with 95% fiducial limits): _____

If an in vitro assay is used

Method: _____

Lot number of reference and assigned potency: _____

Specification: _____

Date: _____

Result: _____

Preservative content (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Endotoxin content

Method: _____

Specification: _____

Date: _____

Result: _____

pH

Method: _____

Specification: _____

Date: _____

Result: _____

Adjuvant content

Method: _____

Specification: _____

Date: _____

Result: _____

Degree of adsorption

Method: _____

Specification: _____

Date: _____

Result: _____

Residual antibiotics (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Extractable volume

Method: _____

Specification: _____

Date: _____

Result: _____

3. Certification by the manufacturer

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

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

I certify that lot no. _____ of enterovirus 71 vaccine (inactivated), whose number appears on the label of the final containers, meets all national requirements and satisfies Part A³⁴ of the WHO Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated).³⁵

Signature _____

Name (typed) _____

Date _____

4. Certification by the NRA/NCL

If the vaccine is to be exported, attach the model NRA/NCL Lot Release Certificate for enterovirus 71 vaccines (inactivated) (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

³⁴ With the exception of provisions on distribution and transport, which the NRA may not be in a position to assess.

³⁵ WHO Technical Report Series, No. 1030, Annex 3.

Appendix 2

Model NRA/NCL Lot Release Certificate for enterovirus 71 vaccines (inactivated)

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

Certificate no. _____

The following lot(s) of enterovirus 71 vaccine (inactivated) produced by

_____ ³⁶
 in _____, ³⁷ whose numbers appear on the labels of the final containers, meet all national requirements³⁸ and Part A³⁹ of the WHO Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated),⁴⁰ and comply with WHO good manufacturing practices for pharmaceutical products: main principles;⁴¹ WHO good manufacturing practices for biological products;⁴² and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.⁴³

The release decision is based on _____ ⁴⁴

Final lot number _____

Number of human doses released in this final lot _____

Expiry date _____

³⁶ Name of manufacturer.

³⁷ Country of origin.

³⁸ 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 or NCL.

³⁹ With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.

⁴⁰ WHO Technical Report Series, No. 1030, Annex 3.

⁴¹ WHO Technical Report Series, No. 986, Annex 2.

⁴² WHO Technical Report Series, No. 999, Annex 2.

⁴³ WHO Technical Report Series, No. 978, Annex 2.

⁴⁴ 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.

The certificate may also include the following information:

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

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

Signature _____

Name (typed) _____

Date _____

Annex 4

Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics

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Information note to applicants and NRAs wishing to participate in this Procedure

This Annex is provided for information only as a reproduction of the actual online application form to be electronically completed by applicants and participating NRAs. No attempt should be made to complete the facsimile appendices provided at the end of the current document. Instead, interested parties should download and complete the latest electronic version of the relevant document and associated appendices which can be obtained at: https://www.who.int/diagnostics_laboratory/evaluations/en/.

1. Introduction

National assessment of applications for registration (marketing authorization) of in vitro diagnostics (IVDs) is a key regulatory process that enables national regulatory authorities (NRAs) to evaluate and monitor the quality, safety and performance of IVDs.

Consideration of the outcomes/results of WHO prequalification dossier assessments, performance evaluations and manufacturing site inspections by NRAs during the national decision-making process is an example of a regulatory approach based on reliance. Such reliance on WHO prequalification outcomes/results contributes substantially to savings in regulatory resources and improvements in the quality of regulatory decisions, while retaining the prerogative of NRAs to conclude their assessment with sovereign decisions that reflect their own judgement of the risk–benefit balance in terms of their specific country situation and legislation. Consideration of the WHO prequalification outcomes or results requires a system that will permit:

- assurance that the product for which the WHO prequalification decision was taken is the same as the product being assessed (see section 4.2) or, if it is not the same, that a clear understanding exists of the differences between the products subjected to assessment in the two regulatory environments;
- efficient use of available scientific expertise and human and financial resources to determine, with reasonable certainty, the risk–benefit profile of an evaluated product when used in a given country; and
- selection by each NRA of the approach that will make the best use of their resources, workload and competencies.

Approaches could range from completely independent data reviews and inspections to adoption of the prequalification outcomes/results without further review. One pragmatic approach is to verify whether the product submitted for registration is the same as the product already prequalified by WHO (see section 4.2) and to assess only those areas relating to the use of the product in the country concerned, and where failure to comply with regulatory standards could pose health risks. In the remaining areas, the WHO prequalification outcomes may be adopted.

Collaborative procedures have been developed and implemented with a view to accelerating the national registration and regulatory life-cycle of pharmaceutical products and vaccines prequalified by WHO or approved by reference authorities (1, 2). On the basis of experience with the WHO Collaborative procedure between the World Health Organization (WHO) Prequalification Team and national regulatory authorities in the assessment and

accelerated national registration of WHO-prequalified pharmaceutical products and vaccines (1) the procedure set out in the current document is intended to facilitate and accelerate national registration processes and post-registration regulatory life-cycles of WHO-prequalified IVDs by enabling participating NRAs to take advantage of the expertise and outcomes of WHO assessment.

This collaborative procedure (hereafter referred to as “the Procedure”) has been developed on the basis of the above considerations to promote timely access to WHO-prequalified products in countries, to ensure that “sameness” can be demonstrated (that is, that the product to be used in countries is the same as that prequalified by WHO) and to provide a model for the exchange of regulatory information between countries.

2. Purpose and scope of the Procedure

The Procedure aims to provide a convenient tool for NRAs wishing to enhance their pre marketing evaluation and registration system by taking advantage of WHO prequalification assessment, in accordance with the *Overview of the WHO prequalification of in vitro diagnostics assessment: WHO prequalification of in vitro diagnostics* (3) and the *Essential principles of safety and performance of medical devices and IVD medical devices* (4).

The objectives of this document are:

- to describe the Procedure for accelerating the national registration of WHO-prequalified IVDs based on exchange of dossier assessment, manufacturing site inspection and performance evaluation outcomes between WHO and participating NRAs; and
- to provide a resource for manufacturers or applicants and participating NRAs to implement facilitated national registration of WHO-prequalified IVDs.

Enhanced collaboration and information exchange between participating NRAs and WHO benefits all partners. Subject to the agreement of the concerned applicant or manufacturer of a WHO-prequalified IVD, participating NRAs will be able to access assessment, manufacturing site inspection and performance evaluation outcomes that are not in the public domain and that have been prepared in conformity with WHO guidance (3). Such reports and relevant WHO documents will help participating NRAs make their decisions, and will assist in the training of national regulatory staff. At the same time, feedback from participating NRAs on the information and documentation received from WHO under the Procedure will allow WHO to improve its activities in this area and ensure that the outcomes of its dossier assessments are relevant to NRAs. Consequently, patients will benefit from this collaboration through faster

access to IVDs found to be acceptable in principle for procurement by United Nations agencies and WHO Member States.

The Procedure will also benefit manufacturers of WHO-prequalified IVDs through faster and better harmonized regulatory approvals in participating countries, thus contributing to a reduction in the burden of additional assessments such as on-site inspections and performance evaluations.

The Procedure is applicable to IVDs that have been prequalified by WHO in line with its current procedures and standards⁴⁵ and that have been found to be acceptable in principle for procurement by United Nations agencies and WHO Member States as shown in the WHO list of prequalified IVD products.⁴⁶

In addition to accelerating the assessment and registration of IVDs prequalified by WHO, the Procedure also covers the national registration and management of post-approval changes.

3. Terminology

For the purposes of the Procedure, the following definitions and descriptions apply. These terms may have different meanings in other contexts.

Abridged assessment: a limited independent assessment of specific parts of the dossier, or regulatory submission of data for suitability of use under local conditions and regulatory requirements, taking into account prior assessment (including dossier review and/or independent performance evaluation) and inspection outcomes from WHO prequalification to inform the NRA decision.

Applicant: the legal person or institution that applies for registration of a product on behalf of the manufacturer.

Collaborative procedure (Procedure): procedure for collaboration between WHO and participating NRAs in the assessment and accelerated national registration of WHO-prequalified IVDs.

In vitro diagnostic (IVD) medical device:⁴⁷ a medical device, used alone or in combination, intended by the manufacturer for the in vitro examination of specimens derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes.

⁴⁵ See: https://www.who.int/diagnostics_laboratory/evaluations/en/

⁴⁶ See: https://www.who.int/diagnostics_laboratory/evaluations/201013_prequalified_product_list.pdf?ua=1

⁴⁷ IVD medical devices include reagents, calibrators, control materials, specimen receptacles, software and related instruments or apparatus or other articles, and are used, for example, for the following test purposes: diagnosis, aid to diagnosis, screening, monitoring, predisposition, prognosis, prediction and determination of physiological status.

Manufacturer: any natural or legal person⁴⁸ with responsibility for the design and/or manufacture of a medical device with the intention of making the medical device available for use, under their name, whether or not such a medical device is designed and/or manufactured by that person themselves or on their behalf by another person(s).

Participating authorities or participating NRAs: NRAs that voluntarily agree to implement this Procedure and accept the task of processing applications for registration of WHO-prequalified IVDs in accordance with its terms.

Performance evaluation: assessment and analysis of data to establish or verify the scientific validity, and the analytical and (where applicable) clinical performance of an IVD.

4. Principles and general considerations

4.1 Participating parties

The Procedure has three major stakeholders: participating NRAs, WHO and interested applicants or manufacturers who agree that the Procedure can be used for applications submitted to participating NRAs for national registration of their WHO-prequalified IVD. The marketing authorization in a given country will be issued by the NRA. Although institutions may be commissioned by NRAs to carry out performance evaluation as part of the overall marketing authorization assessment, this does not change the fact that the main stakeholder with regard to the Procedure is the NRA itself.

4.2 Sameness of the WHO-prequalified and nationally registered IVD

WHO and participating NRAs receive applications for the same IVD product. Within the context of the Procedure, the same product is characterized by:

- the same product name;
- the same regulatory version;
- the same product code(s);
- the same site of manufacture and quality management system;
- the same data on quality, safety and performance;

⁴⁸ This natural or legal person has ultimate legal responsibility for ensuring compliance with all applicable regulatory requirements for medical devices in the countries or jurisdictions in which the devices are intended to be made available or sold, unless this responsibility is specifically imposed on another person by the regulatory authority within that jurisdiction.

- the same design, with the same components from the same suppliers; and
- the same information, labelling⁴⁹ and packaging, including instructions for use and intended use.⁵⁰

4.3 **Submissions format and content of product dossiers for NRAs**

4.3.1 The technical data included in the dossier should be essentially the same as that approved for the WHO prequalification of the product.

In exceptional circumstances, data may be organized differently in line with specific national requirements.

4.3.2 However, participating authorities may require applicants to comply with specific additional national requirements or may accept abbreviated dossier submissions. Each participating authority is encouraged to reduce the scope of specific national requirements in order to align them with the Procedure and to harmonize the requirements with the international format and content of a regulatory dossier. Specific national requirements should be made public.

4.3.3 Advantages of a harmonized format include enabling the same dossier to be submitted across several participating NRAs, thus facilitating comparison, reliance, optimal utilization of assessment resources and reduced workload for participating NRAs and manufacturers or other applicants.

4.3.4 As a minimum, the technical documentation in the submission should be sufficient to enable a participating NRA to verify and ensure the sameness of the product, as defined above in section 4.2 of this Procedure, and to meet existing technical requirements for a specific country.

4.3.5 Should the applicant for national registration be a person or legal entity other than the manufacturer of the WHO-prequalified product, the relationship between the two parties must be clearly stated and agreement must be reached to the effect that the information requested by the participating NRA will be passed from the manufacturer via the applicant.

⁴⁹ Labelling includes labels and the instructions for use.

⁵⁰ The product information may be translated into another language provided the information content is the same as that approved by WHO.

4.3.6 The translation of documents required in the national language is the responsibility of the manufacturer. The method and extent of verification of translation accuracy required are matters for decision by the individual participating NRAs.

4.4 Information shared under the Procedure

4.4.1 WHO, with the agreement of the applicant/manufacturer of the WHO-prequalified product (see Appendix 2), shares the full outcome of prequalification assessment – including dossier review, manufacturing site inspections and performance evaluation reports – with participating authorities under appropriate obligations of confidentiality and restrictions on use.

4.4.2 With regard to sharing the outcomes/results of the dossier review, manufacturing site inspections and performance evaluations, only data owned by the applicant/manufacturer of the WHO-prequalified product and/or by WHO are shared. Sharing of any other data (for example, related to third parties) is subject to the additional agreement of the data owners concerned.

4.4.3 For the purpose of the Procedure, participating authorities accept the product documentation and reports in the formats in which they are routinely prepared by WHO as specified in *Overview of the WHO prequalification of in vitro diagnostics assessment: WHO prequalification of in vitro diagnostics* (3). It should be noted, however, that participating authorities may require applicants to comply with specific requirements for local regulatory review. Each participating authority should make such specific requirements public.

4.4.4 WHO encourages participating NRAs not to perform repetitive dossier assessment of thoroughly assessed data but rather to focus on data verification so that they can be assured that the product submitted for registration is the same as the WHO-prequalified product. Nor is it recommended that participating NRAs re-inspect sites that have already been inspected and found to be compliant with WHO requirements. It is also not recommended to repeat any performance evaluations to determine sensitivity/specificity if such evaluations were carried out as part of the WHO prequalification assessment. Efforts should instead focus on market surveillance.

Results from the performance evaluation organized in the course of the WHO prequalification assessment will be included in the information package available to each participating authority.

4.4.5 The sharing of information related to the Procedure between WHO, applicants/manufacturers of WHO-prequalified products and participating NRAs is governed by Appendices 1–4 of this document. Completed Appendices 1 and 2 must be submitted to WHO without any change in their content. Provision of Appendices 3 and 4 can be substituted by the provision of the same information by other means.

4.5 **Applicable national registration fees**

The payment of fees by the applicants to participating authorities continues to follow standard national procedures. Similarly, the submission by manufacturers of product samples (if required or applicable) continues to follow the standard operating procedures defined in national legislation and/or as defined by the NRAs.

4.6 **Participating authority commitments**

4.6.1 Consistent with the terms of Appendix 1: Part A and Appendix 3: Part B, each participating authority commits itself:

- to treat any information and documentation provided to it by WHO pursuant to this Procedure as confidential in accordance with the terms of Appendix 1: Part A, and to allow access to such information and documentation only to persons:⁵¹
 - who have a need to know for the purposes of the dossier assessment, manufacturing site inspections, performance evaluation and accelerated registration of the product in question in the country, and any post-registration processes that may be required; and
 - who are bound by confidentiality undertakings in respect of such information and documentation which are no less stringent than those set out in Appendix 1: Part A;

⁵¹ This includes the focal point(s) and all other persons in the NRA who have access to any information and documentation provided by WHO.

- to issue its national regulatory decision on registration of a given WHO-prequalified product (whether positive or negative) within 90 calendar days⁵² of regulatory time.⁵³

If the applicant:

- fails to reply within a reasonable time frame (for example, 90 days);
- fails to outline a plan to obtain and provide the requested information; and/or
- fails to provide additional data, or respond to other queries raised by NRAs, and delays the completion of missing parts of the documentation without any justification then the participating NRA is entitled to terminate the Procedure for that specific product and switch to the normal registration process. Such termination is communicated to the applicant and to WHO using Appendix 3: Part C or by providing the same information in another format.

4.6.2 These commitments are agreed to by each participating authority in writing to WHO by entering into the agreement for participation in the Procedure reproduced in Appendix 1: Part A and are reconfirmed for each IVD for which collaboration is being sought (see Appendix 3: Part B).

4.6.3 Each participating NRA nominates a maximum of three focal points and specifies their areas of responsibility (for example, manufacturing site inspections, dossier assessment or performance evaluation). These focal points will access the restricted-access website through which WHO will communicate all confidential information and documentation. The number of focal points can be increased upon a justified request by the participating NRA to WHO.

⁵² Participating authorities should issue their national regulatory decision at the earliest opportunity after being given access to the confidential information and documentation on a given WHO-prequalified product. If a participating authority does not issue its decision within 90 calendar days of regulatory time and does not communicate valid reasons for the delay to WHO, WHO may follow up with the head of the NRA to clarify the situation. In emergency situations, the timeline should be reduced as much as possible to facilitate access to urgently needed products.

⁵³ Regulatory time starts after a valid application for the registration according to the Procedure has been received and access to the confidential information has been granted (whichever is the later) and continues until the date of decision on registration. The regulatory time does not include the time granted to the applicant to complete missing parts of the documentation, provide additional data or respond to queries raised by NRAs.

- 4.6.4 Focal points designated by the participating NRA must sign the undertaking reproduced in Appendix 1: Part B before they will be granted access to the restricted-access website. Any change in designated focal points must be communicated to WHO in writing without delay and must be accompanied by an undertaking (Appendix 1: Part B) signed by the new focal point(s).
- 4.6.5 To successfully operate the Procedure, it is important for participating authorities to establish a clear registration pathway for WHO-prequalified IVDs, including by making relevant information publicly available for applicants, and by developing and implementing standard operating procedures for internal use to facilitate regulatory decision-making based on available information from WHO.

4.7 **Regulatory decision(s) on a WHO-prequalified IVD**

The decision on whether to register a given product in a particular country remains the prerogative and responsibility of each participating authority. Accordingly, a participating authority may come to a different conclusion from that reached by WHO or can decide to terminate the Procedure for a specific product. Within 30 calendar days of having taken its decision, the participating authority reports this decision to WHO, together with the dates of submission and registration and, if applicable, any deviations from the WHO prequalification decision and the reasons for such deviations.⁵⁴ A decision to discontinue the Procedure for a specific product should also be reported to WHO. All decisions are reported through the restricted-access website by completing the form provided in Appendix 3: Part C or by providing the same information in another format. The participating NRA provides either a copy of the completed form or the corresponding information in the other format to the applicant.

4.8 **Manufacturer commitments**

- 4.8.1 Participation in the Procedure by a manufacturer of a WHO-prequalified IVD is voluntary and involves submission to a participating NRA of the expression of interest provided in Appendix 3: Part A. For each product, participation will be subject to acceptance by the manufacturer of the terms of the Procedure, including the confidential exchange of information and documentation between WHO and the participating NRA (see Appendix 2).

⁵⁴ This refers to a decision not to approve the registration of a WHO-prequalified product and to a decision to approve the registration, but with deviations.

- 4.8.2 The manufacturer of the WHO-prequalified product can end their participation in the Procedure at any time, provided they inform WHO and the participating NRAs in writing of their decision to do so. In such a case, the participating NRA shall stop using all of the information disclosed to it for the relevant product(s) as per the terms of the participation agreement (see Appendix 1).
- 4.8.3 Participation in this Procedure does not exempt applicants for national registration and/or holders of national registration from the respective national regulatory requirements.

5. Steps in the Procedure for market authorization of a WHO-prequalified IVD

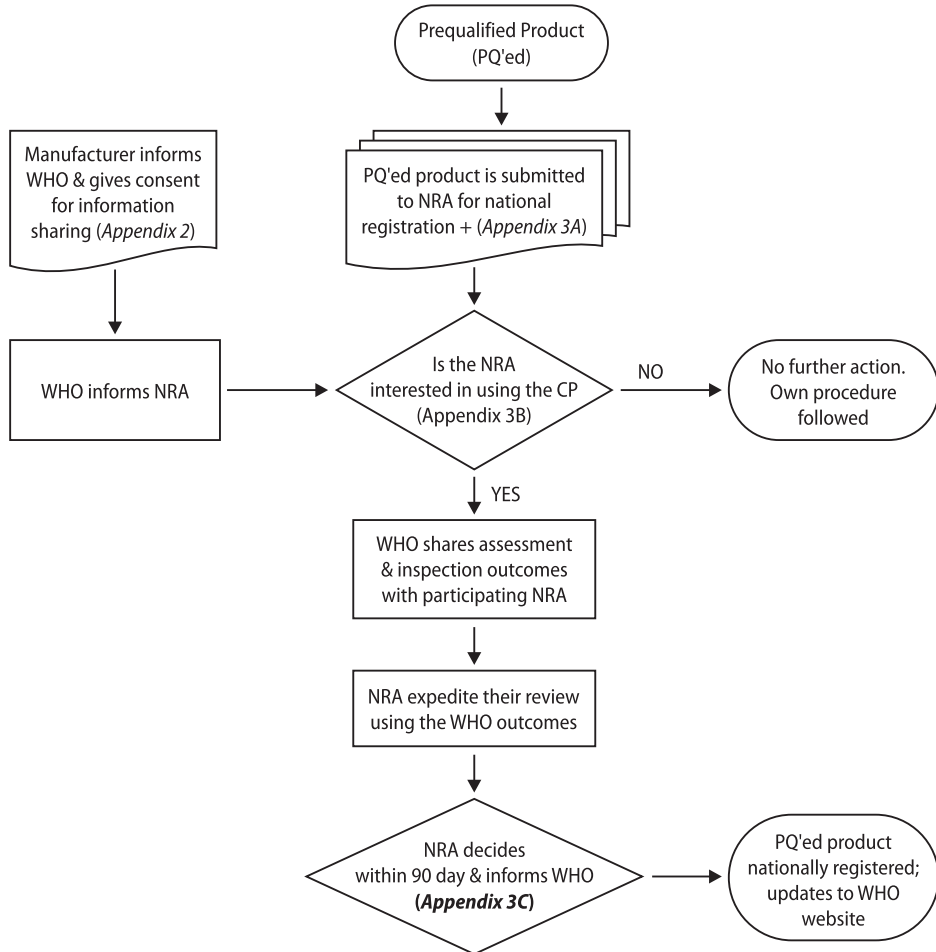
- 5.1 As a preliminary step, the NRA confirms its interest in participating in the Procedure by signing and submitting to WHO the agreement for participation in the Procedure provided in Appendix 1: Part A. The NRA also designates the focal points to be given access to the WHO restricted-access website. The designated focal points complete, sign and submit to WHO the confidentiality undertaking (Appendix 1: Part B). This step is updated as necessary (for example, if the NRA changes its designated focal points). Thereafter, WHO lists the participating NRAs on its public website.
- 5.2 The applicant submits the application for national registration of a WHO-prequalified IVD to a participating NRA. The technical data included in the dossier should be essentially the same as the data in the dossier approved for the prequalification of the product, including changes (where applicable). The submission should be consistent with section 4.3 above. The applicant must provide the participating authority with:
- a product dossier complying with established national requirements and in line with section 4.3 above:
 - to the extent that national regulatory requirements allow, the technical content of the dossier should be essentially the same as that of the WHO-prequalified product. In specific cases the NRA may prefer a dossier which is abbreviated in line with national requirements;
 - if acceptable to NRAs, not only should the technical content of the dossiers be essentially the same, but the format in which data are presented should also closely follow the format in which dossiers are submitted to WHO – that is, a “Table of Contents” format;

- the expression of interest provided in Appendix 3: Part A;
 - data according to country-specific requirements; and
 - any fees that may be payable to the NRA pursuant to national requirements.
- 5.3 The applicant informs the participating NRA of their interest in following this Procedure by completing the expression of interest provided in Appendix 3: Part A. If the applicant for national registration is not the same as the manufacturer/holder of the WHO-prequalified IVD, the manufacturer of the WHO-prequalified IVD must confirm to the participating NRA and to WHO by means of an authorization letter (as per the form annexed to Appendix 3: Part A) that the applicant is acting for, or pursuant to rights derived from, the manufacturer of the WHO-prequalified IVD, and that the manufacturer agrees with the application of this Procedure in the country concerned.
- 5.4 Wherever possible, to minimize the workload of the participating NRA and facilitate the process, applicants should ensure that they express their interest in using the Procedure (Appendix 3: Part A) to the participating NRA and to WHO before submitting a national application for registration. In situations where the applicant wishes to apply the Procedure to an application already pending within the NRA, the applicant should first update the dossier to ensure that the technical part of the information is essentially the same as that approved by WHO.
- 5.5 For each application under this Procedure, the manufacturer of the WHO-prequalified IVD informs WHO of the submission of its application to the participating NRA(s) by providing WHO with a completed copy of Appendix 3: Part A. For each product and country, the manufacturer also provides WHO with its written consent for WHO to provide the product-related information and documentation, in compliance with the applicable confidentiality requirements, to the participating NRA of the country concerned. To this end the manufacturer completes, signs and submits to WHO the consent form provided in Appendix 2.
- 5.6 For each application, the participating NRA notifies WHO and the relevant applicant of its decision to accept or decline to apply this Procedure to the application (Appendix 3: Part B). It is at the discretion of each participating NRA to decide whether to apply the Procedure to individual submissions. The Procedure applies only to applications that the participating NRA has accepted as complete.

- 5.7 Within 30 calendar days of receipt of the manufacturer's consent, WHO shares with the participating authority the most recent product-related information, and dossier assessment, manufacturing site inspection and performance evaluation outcomes, through the restricted-access website. This information is subject to the obligations of confidentiality and restrictions on use, and may include dossier assessment report(s), change assessment report(s) (if applicable), manufacturing site inspection report(s), performance evaluation results and the letter of WHO prequalification. At the request of the participating authority, WHO will provide explanations and/or more-detailed information. If participating NRAs have significant concerns or questions which would preclude the registration of the prequalified IVD in their country, questions may be sent to WHO – preferably within 60 calendar days of the first day of regulatory time. WHO will facilitate resolution of the problem in cooperation with relevant parties.
- 5.8 After receiving the information and documentation from WHO, the participating authority undertakes an assessment of the product in question within the agreed 90-day turnaround time. One pragmatic approach is to verify whether the product submitted for registration is the same as the product already prequalified (see section 4.2 above) and to assess only those areas which relate to the use of the product in the country concerned, and where failure to comply with regulatory standards could pose health risks. In the remaining areas, the WHO prequalification outcomes may be adopted.
- 5.9 For each application, the participating authority is required to issue the relevant national decision within 90 calendar days of regulatory time. Within 30 days of having taken its decision, the participating authority reports this decision, together with an indication of the dates of submission, registration and, if applicable, the length of the non-regulatory time. The participating authority also reports any deviations from the WHO prequalification conclusion and the reasons for such deviations or, if a decision has been made to discontinue the Procedure for a product, the reasons for such discontinuation, to WHO. All decisions are reported through the restricted-access website by completing the form provided in Appendix 3: Part C or by providing the same information in another format. The participating NRA provides either a copy of the completed form or the corresponding information in the other format to the applicant. WHO lists IVDs registered by participating NRAs pursuant to this Procedure on its public website.
- 5.10 The steps in the Procedure for national registration of a WHO-prequalified IVD product are summarized in Fig. 1.

Fig. 1

Steps in the Procedure for national registration of a WHO-prequalified IVD product



6. Collaboration mechanisms for post-prequalification and/or post-registration changes

6.1 The requirements and procedures in case of a change – as defined in applicable WHO guidance (5) – may differ between participating authorities and WHO. This Procedure includes a change procedure which aims to promote consistency between changes accepted by WHO and changes accepted by participating authorities. There could be situations in which a manufacturer of a WHO-prequalified product submits a change application to WHO but not to the participating NRA or vice versa.

In such a situation the conditions of the national registration, which were initially “harmonized” with the WHO decision, may become essentially different through the life-cycle of the product. In this case, a product registered and procured in a participating country would no longer be the same as the WHO-prequalified product because the specifications, manufacturing sites and/or other essential parameters would no longer be the ones accepted by WHO.

Manufacturers of WHO-prequalified products and participating NRAs are expected to inform WHO of the changes and the reasons for them if, due to inconsistencies in changes, the nationally registered product is no longer the same as the WHO-prequalified product. Similarly, WHO will inform participating NRAs when such changes are accepted for WHO-prequalified products. It is important for the manufacturer to evaluate the potential effect the change may have on the safety, quality and performance of the product. Certain changes – such as change of labelling into the local language with no impact on product quality, safety and performance – are not considered as reportable.

- 6.2 Applicants are required to submit to any relevant participating authorities without delay – at the latest 30 calendar days after acceptance of the changes by WHO – those changes which are subject to national regulatory requirements. Applicants should inform participating NRAs that the same application for change is being processed by WHO. The submission of changes to participating NRAs should be in line with national regulatory requirements.
- 6.3 WHO promptly shares with the relevant participating NRA (through the restricted-access website, and subject to the above-mentioned obligations of confidentiality and restrictions on use) the outcomes of change assessments and of manufacturing site inspections conducted subsequent to prequalification listing, as well as any field safety corrective action undertaken to maintain compliance with prequalification quality, safety and performance requirements. Participating authorities are encouraged to follow the outcomes of the WHO change assessments for nationally approved WHO-prequalified IVDs.

The obligations of manufacturers to report adverse events and other relevant post-marketing information to WHO are spelt out in WHO prequalification procedures. In addition to these procedures, manufacturers should follow the specific safety provisions of national regulations when reporting to NRAs.

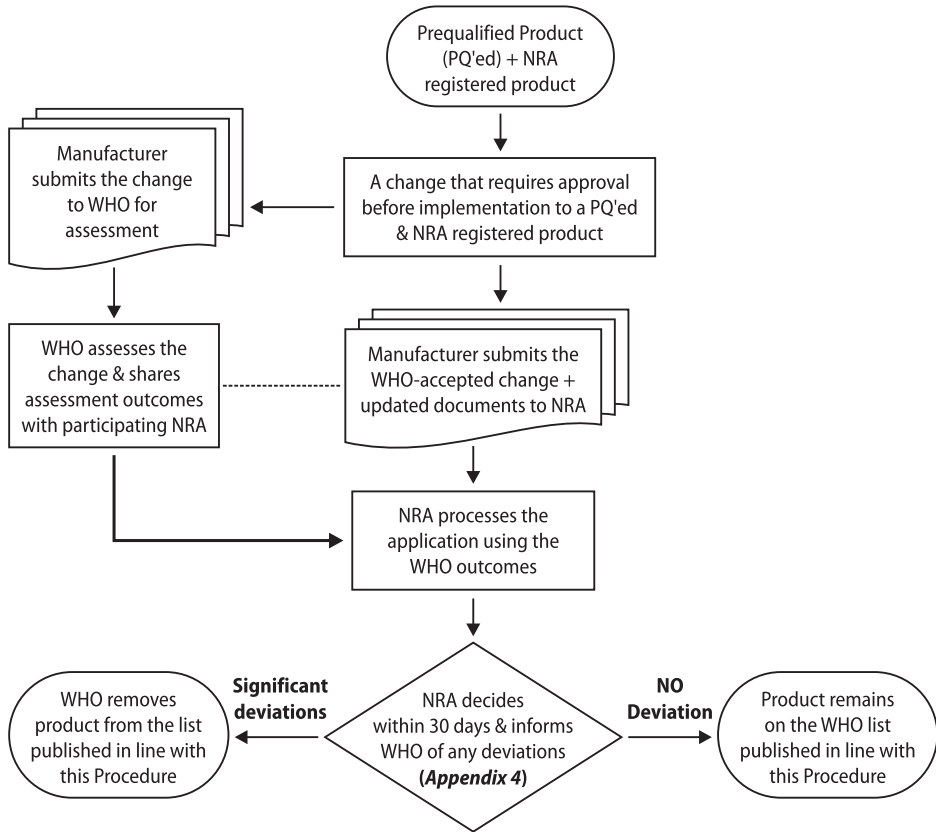
- 6.4 If a change approved by the participating NRA results in the nationally registered product no longer being the same as the WHO-prequalified

product (see section 4.2) or if a change to the WHO-prequalified product is not followed by the same change to the nationally registered product (in the event that the particular change is subject to national regulatory requirements) and, as a consequence, the nationally registered product is no longer the same, then:

- the manufacturer of the WHO-prequalified IVD informs WHO of the differences and the reasons for them; and
- the participating authority informs WHO of the situation by submitting the form provided in Appendix 4, clearly specifying the deviations.

- 6.5 Within 30 days of obtaining access to the relevant information and documentation from WHO, each participating authority will inform WHO, through the restricted-access website, if and to what extent changes to a WHO-prequalified product are not followed by the same accepted changes to the nationally registered product and that, as a consequence, the nationally registered product is no longer the same as the WHO-prequalified product (see section 4.2 above). Changes approved by WHO will be considered by WHO as accepted by the participating NRA on a non-objection basis 30 days after the information-sharing described in section 6.3 above, unless and until the participating NRA informs WHO otherwise. Other participating NRAs that have registered the prequalified product in question pursuant to this Procedure will be made aware of such deviations through the restricted-access website.
- 6.6 WHO shall remove a product from the list of products approved under the Procedure if the nationally registered product is no longer the same as the WHO-prequalified product (see section 4.2 above). In addition, if a WHO-prequalified product has been registered in a particular country pursuant to this Procedure and this has been made public by the NRA then any subsequent deviations should also be made public.
- 6.7 The steps for managing post-approval changes under this Procedure are summarized in Fig. 2.

Fig. 2
Managing post-approval changes under this Procedure



7. Withdrawals, suspensions or delisting of WHO-prequalified IVDs and national deregistration

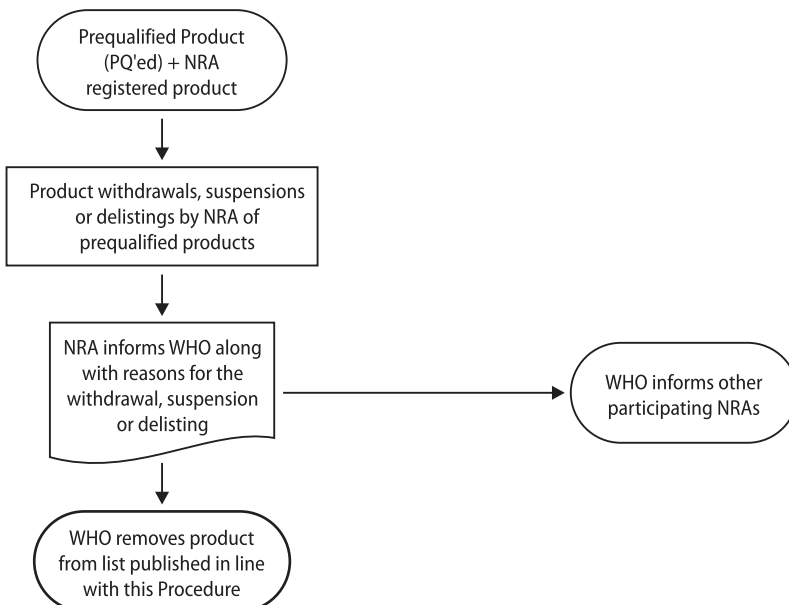
- 7.1 If a WHO-prequalified product is withdrawn by the manufacturer, or is suspended or delisted by WHO, then WHO will inform each participating authority that has approved the product or is in the process of reviewing the product pursuant to this Procedure of the withdrawal, suspension or delisting (together with the reasons for taking the action) through the restricted-access website, and subject to the obligations of confidentiality contained in Appendix 1: Part A.
- 7.2 In the case that a participating NRA deregisters or suspends the registration of a WHO-prequalified IVD for any reason, the participating authority will inform WHO of the decision (together with an indication of the reasons)

through the restricted-access website. This information should be provided promptly whenever there are concerns about product quality, safety or performance and in all other cases within 30 days of the decision being taken. A participating authority is encouraged to consult WHO before adopting a decision about deregistration or suspension of registration of a WHO-prequalified product. Other participating NRAs who have registered the WHO-prequalified product in question pursuant to this Procedure will be made aware of such national deregistration or suspension through the restricted-access website.

- 7.3 In the case that a participating NRA deregisters or suspends registration of a WHO-prequalified product at the national level, or in the case that WHO suspends or delists a prequalified product, WHO will adjust the information on the product given on its website accordingly.
- 7.4 Fig. 3 summarizes the maintenance of registration status of a WHO-prequalified product. The participating NRA should inform WHO of any regulatory action taken nationally for a product registered through the Procedure. WHO will update the list of nationally registered products accordingly and inform other participating NRAs, where applicable, in cases of quality- or safety-related regulatory action.

Fig. 3

Maintenance of registration status of a WHO-prequalified product



8. References

1. Collaborative procedure between the World Health Organization (WHO) Prequalification Team and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified pharmaceutical products and vaccines. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fiftieth report. Geneva: World Health Organization; 2016: Annex 8 (WHO Technical Report Series, No. 996; https://www.who.int/medicines/publications/pharmprep/WHO_TRS_996_annex08.pdf, accessed 14 December 2020).
2. Collaborative procedure in the assessment and accelerated national registration of pharmaceutical products and vaccines approved by stringent regulatory authorities. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-second report. Geneva: World Health Organization; 2018: Annex 11 (WHO Technical Report Series, No. 1010; <https://apps.who.int/iris/bitstream/handle/10665/272452/9789241210195-eng.pdf>, accessed 14 December 2020).
3. Overview of the WHO prequalification of in vitro diagnostics assessment: WHO prequalification of in vitro diagnostics; Version 8. Geneva: World Health Organization; 2018 (Document WHO/EMP/RHT/PQT/2017.02; <https://www.who.int/publications/i/item/overview-of-WHO-prequalification-of-in-vitro-diagnostics-assessment-v8-WHO-EMP-RHT-PQT-2017.02>, accessed 14 December 2020).
4. Essential principles of safety and performance of medical devices and IVD medical devices. IMDRF Good Regulatory Review Practices Group. International Medical Device Regulators Forum; 2018 (<http://www.imdrf.org/docs/imdrf/final/technical/imdrf-tech-181031-grpp-essential-principles-n47.pdf>, accessed 14 December 2020).
5. Reportable changes to a WHO prequalified in vitro diagnostic medical device. Geneva: World Health Organization; 2016 (Document WHO/EMP/RHT/PQT/2016.01; <https://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf>, accessed 14 December 2020).

Appendix 1

NRA participation agreement and undertaking for NRA focal point(s)

Appendix 1: Part A

Agreement to participate in the WHO Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics

1. Details of the NRA

Name of NRA:	Click or tap here to enter text (“the NRA”)
Postal address:	Click or tap here to enter text Click or tap here to enter text Click or tap here to enter text Click or tap here to enter text
Country:	Click or tap here to enter text (“the Country”)
Telephone number:	Click or tap here to enter text (please include codes)
Email:	Click or tap here to enter text

2. Scope of agreement

Applicants for national registration of a particular WHO-prequalified in vitro diagnostic product (hereafter referred to as “Applicants”) may express their interest to the participating NRA in the assessment and accelerated registration of this in vitro diagnostic product (“the Product”) in the Country under the Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics (hereafter referred to as “the Procedure”).⁵⁵

Subject to the NRA agreeing to conduct such assessment and consider such accelerated registration of the Product under the Procedure (by submitting

⁵⁵ If the applicant for national registration is not the same as the holder of the WHO prequalification (“WHO PQ holder”) then the WHO PQ holder must confirm to the NRA and to WHO via an authorization letter (as per the template annexed to Appendix 3: Part A) that the applicant is acting for, or pursuant to rights derived from, the WHO PQ holder, and that the WHO PQ holder agrees with the application of the Procedure in the country concerned.

the form provided in Appendix 3: Part B of the Procedure to WHO through the restricted-access website), the NRA hereby confirms that for each such Product it will adhere to the terms of the Procedure, and will collaborate with WHO and the Applicant in the registration of the Product.

3. Confidentiality of information

Information and documentation relating to the Product and provided by WHO to the NRA under the Procedure may include but shall not necessarily be limited to:

- the full WHO assessment, performance evaluation and inspection outcomes (reports); and
- information and documentation on changes (as defined in WHO guidance),⁵⁶ as well as information and documentation on any actions taken by WHO or participating NRAs or the manufacturer post-prequalification of the Product; and
- all such data, reports, information and documentation being hereinafter referred to as “the Information”.

As regards sharing the outcomes of dossier assessments, inspections and performance evaluation, only data owned by the manufacturer and WHO are shared. Sharing of any other data is subject to the additional agreement of the data owners concerned.

WHO agrees to make such information available to the NRA through a restricted-access website exclusively for the purpose of the assessment and accelerated registration of the Product in the Country, and any post-registration processes that may be required, in accordance with and subject to the terms of the Procedure (“the Purpose”). The NRA agrees to treat the aforesaid Information provided by WHO as strictly confidential and proprietary to WHO, the WHO PQ holder/Applicant and/or third parties collaborating with WHO and/or the WHO PQ holder/Applicant, as applicable. In this regard, the NRA agrees to use such Information only for the Purpose and to make no other use thereof. Thus, the NRA undertakes to maintain the Information received from WHO in strict confidence and to take all reasonable measures to ensure that:

- the Information received from WHO shall not be used for any purpose other than the Purpose; and

⁵⁶ Reportable changes to a WHO prequalified in vitro diagnostic medical device. Geneva: World Health Organization; 2016 (Document WHO/EMP/RHT/PQT/2016.01; <https://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf>, accessed 14 December 2020).

- the Information shall be disclosed only to persons who have a need to know for the aforesaid Purpose and are bound by confidentiality undertakings in respect of such information and documentation which are no less stringent than those contained herein.

The NRA warrants and represents that it has adequate procedures in place to ensure compliance with its aforesaid obligations.

The obligations of confidentiality and restrictions on use contained herein shall not cease on completion of the Purpose.

The obligations of confidentiality and restrictions on use contained herein shall not apply to any part of the Information which the NRA is clearly able to demonstrate:

- was in the public domain or the subject of public knowledge at the time of disclosure by WHO to the NRA under the Procedure; or
- becomes part of the public domain or the subject of public knowledge through no fault of the NRA; or
- is required to be disclosed by law, provided that the NRA shall in such event immediately notify WHO and the Applicant in writing of such obligation and shall provide adequate opportunity to WHO and/or the Applicant to object to such disclosure or request confidential treatment thereof (provided always, however, that nothing contained herein shall be construed as a waiver of the privileges and immunities enjoyed by WHO and/or as submitting WHO to any national court jurisdiction).

Upon completion of the Purpose, the NRA shall cease all use and make no further use of the Information disclosed to it under the Procedure, and shall promptly destroy all of the Information received from WHO which is in tangible or other form, except that the NRA may retain copies of the Information in accordance with its established archival procedures, subject always, however, to the above-mentioned obligations of confidentiality and restrictions on use. The Purpose for each product shall be deemed completed as soon as:

- the WHO PQ holder/Applicant discontinues participation in the Procedure for the particular product; or
- the Product is deregistered by the NRA and/or delisted by WHO.

The access right of the NRA focal point(s) to the restricted-access website will cease automatically upon the NRA ceasing to participate in the Procedure. If and as soon as an NRA focal point is replaced by a new focal point or ceases to be an employee of the NRA, such a focal point's access to the restricted-access website shall automatically terminate.

The NRA agrees that it has no right in or to the Information and that nothing contained herein shall be construed, by implication or otherwise, as the grant of a licence to the NRA to use the Information other than for the Purpose.

4. Timelines

In respect of each Product that the NRA agrees to assess and consider for accelerated registration under the Procedure, the NRA undertakes to abide by the terms of the Procedure, including but not limited to the following timelines for processing each application:

- the NRA undertakes to take a decision on the national registration of the Product within 90 calendar days of regulatory time⁵⁷ after obtaining access (through the WHO restricted-access website) to:
 - the data submitted to WHO for prequalification of the Product and owned by the WHO PQ holder; and
 - the full WHO dossier assessment, performance evaluation, and inspection outcomes (reports);
- within 30 working days of the NRA's decision on national registration of the Product, the NRA undertakes to inform WHO of this decision and of any deviations from WHO conclusions during prequalification (with an indication of the reasons for such deviations) by completing and submitting the form attached to the Procedure as Appendix 3: Part C to WHO through the restricted-access website;
- if a national change procedure results in the nationally registered product being no longer the same⁵⁸ as the WHO-prequalified product, or if and to the extent change to a WHO-prequalified product is not followed by a change to the nationally registered product and, as a consequence, the nationally registered product is no longer the same as the WHO-prequalified product, the NRA undertakes to inform WHO thereof (together with an indication of the reasons for such deviations) within 30 days of the conclusion of the national

⁵⁷ Regulatory time starts after a valid application for the registration according to the Procedure has been received and access to the confidential information has been granted (whichever is the later) and continues until the date of decision on registration. The regulatory time does not include the time granted to the applicant to complete missing parts of the documentation, provide additional data or respond to queries raised by NRAs.

⁵⁸ Within the context of this Procedure, the same *in vitro* diagnostic is characterized by the same name (including proprietary name), same information, same design with comparable components from the same suppliers, same specifications, same regulatory version code, same site of manufacturer and quality management system, same data on quality and performance, same intended use, same labelling and packaging, and same instructions for use.

change procedure or within 30 days of having received access to the information and documentation provided by WHO, as the case may be (that is, by completing and submitting the form attached to the Procedure as Appendix 4 to WHO through the restricted-access website);⁵⁹

- the NRA undertakes to inform WHO in the case that the NRA deregisters or suspends the registration of the Product in the Country by completing and submitting the form attached to the Procedure as Appendix 4 to WHO through the restricted-access website, and to do so promptly if this decision is based on quality, safety or efficacy concerns, and within 30 days if this decision is based on other reasons.

5. Focal points for access to the WHO restricted-access website

The NRA has designated the person(s) listed below to act as focal point(s) for access to the WHO restricted-access website. The undertaking(s) completed and signed by the focal point(s) is (are) attached hereto as an Appendix to this agreement.

Any change in designated focal points must be communicated to WHO in writing without delay and will be subject to the new focal point having signed and submitted to WHO the undertaking (“the Undertaking”) provided in Appendix 1: Part B to the Procedure. The NRA also undertakes to inform WHO if and as soon as a designated focal point ceases to be an employee of the NRA.

6. Focal point for inspections

If applicable, this should be the same focal point as for the WHO Collaborative procedure between the World Health Organization (WHO) and selected national medicines regulatory authorities (NMRAs) in inspection activities.⁶⁰ This same person should be designated for IVD-related inspections.

- Mr/Ms/Dr

First name (and initials): [Click or tap here to enter text](#)

Surname/family name: [Click or tap here to enter text](#)

Title in NRA: [Click or tap here to enter text](#)

Telephone number: [Click or tap here to enter text](#) (please include codes)

Email: [Click or tap here to enter text](#)

A signed Undertaking (see Appendix 1: Part B below) is attached

⁵⁹ If the fact that a WHO-prequalified product has been registered in a country pursuant to this Procedure has been made public, any subsequent deviations should also be made public.

⁶⁰ See: <https://extranet.who.int/pqweb/inspection-services>

7. Focal point(s) for dossier assessment

Different persons can be nominated for dossier assessment and performance evaluation. The same person may be nominated to be the focal point for inspections, performance evaluation and dossier assessment. If additional person(s) are nominated for dossier assessment, please complete the details below.

- Mr/Ms/Dr as a focal point for

Dossier assessment only

Dossier assessment and performance evaluation

First name (and initials): [Click or tap here to enter text](#)

Surname/family name: [Click or tap here to enter text](#)

Title in NRA: [Click or tap here to enter text](#)

Telephone number: [Click or tap here to enter text](#) (please include codes)

Email: [Click or tap here to enter text](#)

A signed Undertaking is attached

- Mr/Ms/Dr as a focal point for performance evaluation

First name (and initials): [Click or tap here to enter text](#)

Surname/family name: [Click or tap here to enter text](#)

Title in NRA: [Click or tap here to enter text](#)

Telephone number: [Click or tap here to enter text](#) (please include codes)

Email: [Click or tap here to enter text](#)

A signed Undertaking is attached

8. Miscellaneous

The NRA agrees that WHO may list its name on the WHO website as a participant in the Procedure. Except as provided hereinbefore, neither party shall, without the prior written consent of the other party, refer to the relationship of the parties under this agreement (“the Agreement”) and/or to the relationship of the other party to the Product, the Information and/or the Purpose in any statement or material of an advertising or promotional nature.

This Agreement shall not be modified except with the mutual agreement of WHO and the NRA in writing. The NRA furthermore undertakes to promptly inform WHO of any circumstances or change in circumstances that may affect the implementation of this Agreement.

The parties shall use their best efforts to settle amicably any dispute relating to the interpretation or execution of this Agreement. In the event of failure of the latter, the dispute shall be settled by arbitration. The arbitration shall be conducted in accordance with the modalities to be agreed upon by the parties or in the absence of agreement, with the UNCITRAL Arbitration Rules in effect on the date of this Agreement. The parties shall accept the arbitral award as final.

It is agreed furthermore that nothing contained in or relating to the Procedure or this Agreement shall be construed as a waiver of any of the privileges and immunities enjoyed by WHO under national and international law, and/or as submitting WHO to any national court jurisdiction.

Agreed and accepted for IVDs.

For the NRA

Signature: [Click or tap here to enter text](#)

Name: [Click or tap here to enter text](#)

Title: [Click or tap here to enter text](#)

Place: [Click or tap here to enter text](#)

Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Attachments:

- Signed Undertaking(s) of NRA focal point(s) (see Appendix 1: Part B below)

Appendix 1: Part B

Undertaking for national regulatory authority (NRA) focal point(s)

The undersigned:

- Mr/Ms/Dr

First name (and initials): [Click or tap here to enter text](#)

Surname/family name: [Click or tap here to enter text](#)

Title in NRA: [Click or tap here to enter text](#)

Name of NRA: [Click or tap here to enter text](#) (“the NRA”)

Country: [Click or tap here to enter text](#) (“the Country”)

Telephone number: [Click or tap here to enter text](#) (please include codes)

Email: [Click or tap here to enter text](#)

Applicants for the national registration of WHO-prequalified in vitro diagnostics (hereafter referred to as “Applicants”) may express to the NRA

their interest in the assessment and accelerated national registration of such products under the WHO Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics (hereafter referred to as “the Procedure”).⁶¹ Subject to the NRA agreeing to conduct such assessment and consider such accelerated registration of a WHO-prequalified product under the Procedure, WHO will communicate confidential Information (as hereinafter defined) relating to each such product to the NRA – and the NRA will communicate the outcomes of the national registration procedure and post-registration actions in respect of such products to WHO – through a restricted-access website which can be accessed only by the focal points designated by the NRA. For the purpose of accessing the restricted-access website and downloading the Information, and uploading reports in accordance with and subject to the terms of the Procedure, WHO will provide the undersigned with a secret access code. The undersigned undertakes to treat this access code as strictly confidential and not to disclose it to any other person whatsoever. The undersigned furthermore undertakes to take all precautionary measures that may be needed to prevent any other person whatsoever from obtaining the aforesaid secret access code and from accessing the restricted-access website (that is, except for other designated NRA focal points who have signed this Undertaking).

The aforesaid “Information” comprises any information and documentation relating to a WHO-prequalified product to be provided by WHO to the NRA under the Procedure, including but not necessarily limited to:

- the full WHO assessment and inspection outcomes (reports) and the results of performance evaluation;
- information and documentation on subsequent changes (as defined in WHO guidance),⁶² as well as information and documentation on any actions taken by WHO or NRAs post-prequalification of the Product.

As regards sharing the outcomes of dossier assessment, inspections and performance evaluation, only data owned by the WHO PQ holder and WHO

⁶¹ If the applicant for national registration is not the same as the holder of the WHO prequalification (“WHO PQ holder”) then the WHO PQ holder must confirm to the NRA and to WHO via an authorization letter (as per the template annexed to Appendix 3: Part A) that the applicant is acting for, or pursuant to rights derived from, the WHO PQ holder, and that the WHO PQ holder agrees with the application of the Procedure in the country concerned.

⁶² Reportable changes to a WHO prequalified in vitro diagnostic medical device. Geneva: World Health Organization; 2016 (Document WHO/EMP/RHT/PQT/2016.01; <https://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf>, accessed 14 December 2020).

are shared. Sharing of any other data is subject to the additional agreement of the data owners concerned.

The undersigned confirms that:

1. the NRA has bound them to obligations of confidentiality and restrictions on use no less stringent than those contained in Appendix 1: Part A to the Procedure; and
2. the aforesaid obligations of confidentiality and restrictions on use shall not cease on completion of the assessment and accelerated registration of any Product in the Country, or on completion of any post-registration processes that may be required, or on the undersigned ceasing to be an employee of (or ceasing to have another relationship with) the NRA.

The undersigned shall automatically cease to have the right to access the restricted-access website when the NRA designates a new focal point to replace the undersigned or when the undersigned ceases to be an employee of the NRA.

This Undertaking shall not be modified except with the mutual agreement of WHO and the undersigned in writing. The undersigned furthermore undertakes to promptly inform WHO of any circumstances or changes in circumstances that may affect the implementation of this Undertaking.

The parties shall use their best efforts to settle amicably any dispute relating to the interpretation or execution of this Undertaking. In the event of failure of the latter, the dispute shall be settled by arbitration. The arbitration shall be conducted in accordance with the modalities to be agreed upon by the parties or in the absence of agreement, with the UNCITRAL Arbitration Rules in effect on the date of this Undertaking. The parties shall accept the arbitral award as final.

It is agreed furthermore that nothing contained in or relating to the Procedure or this Undertaking shall be construed as a waiver of any of the privileges and immunities enjoyed by WHO under national and international law, and/or as submitting WHO to any national court jurisdiction.

Agreed and accepted by the undersigned:

Signature: [Click or tap here to enter text](#)
 Name: [Click or tap here to enter text](#)
 Title: [Click or tap here to enter text](#)
 Place: [Click or tap here to enter text](#)
 Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Appendix 2

Consent of WHO prequalification holder for WHO to confidentially share information with the NRA under the Procedure

Reference is made to the attached expression of interest in the assessment and accelerated national registration under the WHO Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics (hereafter referred to as “the Procedure”) of the following WHO-prequalified IVD (hereafter referred to as “the Product”) in:

Country: [Click or tap here to enter text](#) (“the Country”).⁶³

IVD

WHO prequalification details

WHO prequalification (PQ) reference number: [Click or tap here to enter text](#)

Date of prequalification (dd/mm/yyyy): [Click or tap here to enter text](#)

Name of WHO PQ holder:⁶⁴ [Click or tap here to enter text](#)

Application details

Name of entity: [Click or tap here to enter text](#) (“the Applicant”)

Street: [Click or tap here to enter text](#)

City and country: [Click or tap here to enter text](#)

Telephone number: [Click or tap here to enter text](#) (please include codes)

Email: [Click or tap here to enter text](#)

The WHO PQ holder hereby consents to WHO providing the following information and documentation to the national regulatory authority (NRA) of [Click or tap here to enter text](#) [country] (“the NRA”) for the assessment and

⁶³ Please complete a separate copy of this Appendix for each country.

⁶⁴ If the applicant for national registration is not the same as the holder of the WHO prequalification (“WHO PQ holder”) then the WHO PQ holder must confirm to the NRA and to WHO via an authorization letter (as per the template annexed to Appendix 3: Part A) that the applicant is acting for, or pursuant to rights derived from, the WHO PQ holder, and that the WHO PQ holder agrees with the application of the Procedure in the country concerned.

accelerated registration of the Product in the country under the Procedure and to freely discuss the same with the aforesaid NRA for this purpose:

- the full WHO assessment and inspection outcomes (reports), results of performance evaluation and, if relevant, dossier assessment and inspection reports of other regulatory bodies, provided that these bodies gave their written consent to the use of such reports for the purpose of the Procedure;
- information and documentation on subsequent changes (as defined in WHO guidance),⁶⁵ as well as information and documentation on any actions taken by WHO post-prequalification of the Product; and
- all such data, reports, information and documentation being hereinafter referred to as “the Information”.

As regards sharing the outcomes of dossier assessment, inspections and performance evaluations, only data owned by the WHO PQ holder and WHO are shared. Sharing of any other data is subject to the additional agreement of the data owners concerned.⁶⁶ Such consent is subject to the NRA having entered into an agreement with WHO as per Appendix 1: Part A to the Procedure and having agreed to conduct the assessment and consider the accelerated registration of the Product under the Procedure, by having submitted the form reproduced in Appendix 3: Part B to the Procedure to WHO.

The WHO PQ holder/Applicant commits to submit post-prequalification changes to WHO and any relevant participating authorities, respecting national regulatory requirements. Changes should be submitted to participating authorities at the latest 30 calendar days after acceptance of the changes by WHO. Participating authorities should be informed of the fact that the same application for a change is being processed by WHO. If a national change procedure results in the nationally registered product being no longer the same⁶⁷ as the WHO-prequalified product, or if a change to the WHO-prequalified product is not followed by a change to the nationally registered product and, as a consequence,

⁶⁵ Reportable changes to a WHO prequalified in vitro diagnostic medical device. Geneva: World Health Organization; 2016 (Document WHO/EMP/RHT/PQT/2016.01; <https://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf>, accessed 14 December 2020).

⁶⁶ In the case that certain data submitted to WHO by the WHO PQ holder in relation to the prequalification of the Product are not in their ownership, the WHO PQ holder specifies such data in an annex to this declaration of consent.

⁶⁷ Within the context of this Procedure, the same in vitro diagnostic is characterized by the same name (including proprietary name), same information, same design with comparable components from the same suppliers, same specifications, same regulatory version code, same site of manufacturer and quality management system, same data on quality and performance, same intended use, same labelling and packaging, and same instructions for use.

the nationally registered product is no longer the same, the WHO PQ holder/Applicant will inform WHO of the differences and the reasons for them.

For the WHO PQ holder

Signature: [Click or tap here to enter text](#)

Name: [Click or tap here to enter text](#)

Title: [Click or tap here to enter text](#)

Place: [Click or tap here to enter text](#)

Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Appendix 3

Expression of interest to NRA in the assessment and accelerated national registration, acceptance by NRA and notification of Procedure outcomes

Appendix 3: Part A

Expression of interest to NRA in the assessment and accelerated national registration of a WHO-prequalified in vitro diagnostic

In line with the Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics (hereafter referred to as “the Procedure”) the undersigned Applicant⁶⁸ expresses its interest in the application of the Procedure by the NRA of [Click or tap here to enter text](#) [country] (“the NRA”) in respect of the following submission for national registration:

IVD

Application details:

Name of entity: [Click or tap here to enter text](#) (“the Applicant”)
 Street: [Click or tap here to enter text](#)
 City and country: [Click or tap here to enter text](#)
 Telephone number: [Click or tap here to enter text](#) (please include codes)
 Email: [Click or tap here to enter text](#)
 Date of application (dd/mm/yyyy): [Click or tap here to enter text](#)
 Product name in national system (if known): [Click or tap here to enter text](#)
 National reference number (if known): [Click or tap here to enter text](#)

⁶⁸ If the applicant for national registration is not the same as the holder of the WHO prequalification (“WHO PQ holder”) then the WHO PQ holder must confirm to the NRA and to WHO via an authorization letter (as per the template annexed to Appendix 3: Part A) that the applicant is acting for, or pursuant to rights derived from, the WHO PQ holder, and that the WHO PQ holder agrees with the application of the Procedure in the country concerned.

Product details for IVD

Product name: [Click or tap here to enter text](#)

Product code(s): [Click or tap here to enter text](#)

Regulatory version: [Click or tap here to enter text](#)

Manufacturer: [Click or tap here to enter text](#)

Manufacturing site(s): [Click or tap here to enter text](#)

Packaging: [Click or tap here to enter text](#)

WHO prequalification details

WHO PQ reference number: [Click or tap here to enter text](#)

Date of prequalification (dd/mm/yyyy): [Click or tap here to enter text](#)

Name of WHO PQ holder: [Click or tap here to enter text](#)

The Applicant confirms that the information and documentation provided in support of the above-mentioned submission for national registration is true and correct, that the product submitted for national registration is the same⁶⁹ as the WHO-prequalified product and that the technical information in the registration dossier is the same⁷⁰ as that approved by WHO during the initial prequalification procedure, and any subsequent change procedures. Minor differences⁷¹ from the information submitted to WHO are as follows:

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

Subject to the NRA agreeing to conduct the assessment and consider the accelerated registration of the Product under the Procedure, the Applicant:

1. undertakes to adhere to, and collaborate with the NRA and WHO in accordance with, the terms of the Procedure; and

⁶⁹ Within the context of this Procedure, the same in vitro diagnostic is characterized by the same name (including proprietary name), same information, same design with comparable components from the same suppliers, same specifications, same regulatory version code, same site of manufacturer and quality management system, same data on quality and performance, same intended use, same labelling and packaging, and same instructions for use.

⁷⁰ Only the technical data included in the dossier must be the same. There may be country-specific differences in administrative data or, if required by NRAs under exceptional circumstances, additional technical data can be provided.

⁷¹ As defined in section 4.2 of the Procedure, examples of minor differences which are not considered essential may include differences in administrative information, name of applicant (provided that the applicant is acting for, and has the authority to represent, the WHO PQ holder) and the language of product information.

2. will authorize WHO⁷² to provide the NRA with confidential access to the following information and documentation and to freely discuss the same with the aforesaid NRA for the above-mentioned Purpose:
 - the full WHO dossier assessment and inspection outcomes (reports), results of performance evaluation and, if relevant, the dossier assessment and inspection reports of other regulatory bodies, provided that these bodies gave their written consent to the use of such reports for the purpose of the Procedure; and
 - information and documentation on subsequent changes (as defined in WHO guidance),⁷³ as well as information and documentation on any actions taken by WHO post-qualification of the Product.

As regards sharing the outcomes of dossier assessments, inspections and performance evaluations, only data owned by the WHO PQ holder and WHO are shared. Sharing of any other data is subject to the additional agreement of the data owners concerned.

3. authorizes the NRA to freely share and discuss with WHO all registration-related and Product-related information provided by the Applicant to the NRA, subject to the obligations of confidentiality and restrictions on use as contained in the NRA's participation agreement and focal point undertakings.
- The application for national registration was submitted before the Applicant decided to apply the Procedure to the Product and therefore at the time of submission the registration dossier did not respect the conditions of the Procedure. Steps taken to update the submission to the NRA to make the dossier “the same” as required by the Procedure are listed and referenced in the attached letter.
- The Applicant is not the WHO PQ holder. An authorization letter from the WHO PQ holder is attached.

⁷² If the applicant for national registration is not the same as the WHO PQ holder then the authorization to WHO must be provided by the WHO PQ holder or their legal representative.

⁷³ Reportable changes to a WHO prequalified in vitro diagnostic medical device. Geneva: World Health Organization; 2016 (Document WHO/EMP/RHT/PQT/2016.01; <https://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf>, accessed 14 December 2020).

For the Applicant

Signature: [Click or tap here to enter text](#)
Name: [Click or tap here to enter text](#)
Title: [Click or tap here to enter text](#)
Place: [Click or tap here to enter text](#)
Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Template for authorization letter

[To be provided if the applicant is not the WHO PQ holder. Please provide a separate letter for each NRA concerned, with a copy to WHO]

This is to confirm that [Click or tap here to enter text](#) (name of applicant) seeking registration for the WHO-prequalified in vitro diagnostic product number [Click or tap here to enter text](#) (WHO prequalification number) in [Click or tap here to enter text](#) (name of country) under the Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics (“the Procedure”) is acting for, or pursuant to rights derived from, [Click or tap here to enter text](#) (name of WHO PQ holder) and that [Click or tap here to enter text](#) (name of WHO PQ holder) agrees with the application of the Procedure in the country concerned.

For [Click or tap here to enter text](#) (name of WHO PQ holder)

Signature: [Click or tap here to enter text](#)
Name: [Click or tap here to enter text](#)
Title: [Click or tap here to enter text](#)
Place: [Click or tap here to enter text](#)
Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Appendix 3: Part B

Decision on acceptance by the NRA to apply the Procedure to a specified WHO-prequalified in vitro diagnostic product and request for access to product-specific information and documentation

Please complete all fields marked with an *. For other fields, if there have been changes to the details provided in Part A above please also complete the relevant fields below. Where fields below are left blank, the data in Part A are considered to be valid.

Application details

- Name of entity: [Click or tap here to enter text](#) (“the Applicant”)
- Street: [Click or tap here to enter text](#)
- City and country: [Click or tap here to enter text](#)
- Telephone number: [Click or tap here to enter text](#) (please include codes)
- Email: [Click or tap here to enter text](#)
- *Date of receipt of submission (dd/mm/yyyy): [Click or tap here to enter text](#)
- Product name in national system (if known): [Click or tap here to enter text](#)
- *National reference number (if known): [Click or tap here to enter text](#)

Product details for IVD

- Product name: [Click or tap here to enter text](#)
- Product code(s): [Click or tap here to enter text](#)
- Regulatory version: [Click or tap here to enter text](#)
- Manufacturer: [Click or tap here to enter text](#)
- Manufacturing site(s): [Click or tap here to enter text](#)
- Packaging: [Click or tap here to enter text](#)

WHO prequalification details

- *WHO PQ reference number: [Click or tap here to enter text](#)
- Date of prequalification (dd/mm/yyyy): [Click or tap here to enter text](#)
- Name of WHO PQ holder: [Click or tap here to enter text](#)

Please complete either section A or section B below.

Section A

The NRA agrees to conduct the assessment for accelerated registration of the above-mentioned product (“the Product”) under the Procedure and requests access to product-specific information, in accordance with and subject to the terms of the Procedure and the Agreement between WHO and the NRA dated [Click or tap here to enter text](#) (dd/mm/yyyy).

Section B

The NRA has decided not to apply the Procedure to the above-mentioned Product for the following reasons:

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

***For the NRA of** [Click or tap here to enter text](#) **(indicate country)**

Signature: [Click or tap here to enter text](#)

Name: [Click or tap here to enter text](#)

Title: [Click or tap here to enter text](#)

Place: [Click or tap here to enter text](#)

*Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Appendix 3: Part C

Notification of outcomes of national registration procedure by the NRA

Product and application details as completed in Parts A and B above apply unless otherwise indicated below.

Please complete either section A or section B below.

Section A

Registration has been granted under the terms of the Procedure, and the above-mentioned product (“the Product”) is identified as follows in the national medicines register:

Name of the Product: [Click or tap here to enter text](#)

National registration number: [Click or tap here to enter text](#)

Date of registration (dd/mm/yyyy): [Click or tap here to enter text](#)

Non-regulatory time (days): [Click or tap here to enter text](#)

Product details (if different from those specified in Parts A and B)

Product name: [Click or tap here to enter text](#)

Product code(s): [Click or tap here to enter text](#)

Regulatory version: [Click or tap here to enter text](#)

Manufacturer: [Click or tap here to enter text](#)

Manufacturing site(s): [Click or tap here to enter text](#)

Packaging: [Click or tap here to enter text](#)

Registration holder (if different from the Applicant as specified in Parts A and B)

Name of entity: [Click or tap here to enter text](#)
 Street: [Click or tap here to enter text](#)
 City and country: [Click or tap here to enter text](#)
 Telephone number: [Click or tap here to enter text](#) (please include codes)
 Email: [Click or tap here to enter text](#)

Are the national registration conclusions different from the prequalification outcomes?⁷⁴

Yes No

If you answered yes to the above question, please specify:

Deviation	Reason
Click or tap here to enter text	Click or tap here to enter text
Click or tap here to enter text	Click or tap here to enter text

Please specify whether registration is subject to specific commitments, the registration is provisional or conditional, use of the Product is limited by specific restrictions, or additional trials or additional data are required:

[Click or tap here to enter text](#)
[Click or tap here to enter text](#)
[Click or tap here to enter text](#)
[Click or tap here to enter text](#)

Section B

Please complete as appropriate.

The application for registration of the Product was rejected for the following reasons:

[Click or tap here to enter text](#)
[Click or tap here to enter text](#)
[Click or tap here to enter text](#)

⁷⁴ This refers to deviations in indications, contraindications, intended use, special warnings and precautions for use, storage conditions and shelf-life.

The Procedure was discontinued for this application for the following reasons:

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

For the NRA

Signature: [Click or tap here to enter text](#)

Name: [Click or tap here to enter text](#)

Title: [Click or tap here to enter text](#)

Place: [Click or tap here to enter text](#)

Date (dd/mm/yyyy): [Click or tap here to enter text](#)

Appendix 4

Report on post-registration actions in respect of a product registered under the Procedure

- Change of the national registration resulting in the national registration conditions being inconsistent with the WHO prequalification conclusions
- Deregistration or suspension of the registration of the product
- Field Safety Corrective Action (FSCA) issued on the product

Product details

Product name in national system: [Click or tap here to enter text](#) (“the Product”)

National registration number: [Click or tap here to enter text](#)

Date of registration (dd/mm/yyyy): [Click or tap here to enter text](#)

WHO prequalification details

WHO PQ reference number: [Click or tap here to enter text](#)

Date of prequalification (dd/mm/yyyy): [Click or tap here to enter text](#)

Name of WHO PQ holder: [Click or tap here to enter text](#)

- The national changes procedure has resulted in the nationally registered Product being no longer the same⁷⁵ as the WHO-prequalified product

Deviation	Reason
Click or tap here to enter text	Click or tap here to enter text
Click or tap here to enter text	Click or tap here to enter text

⁷⁵ Within the context of this Procedure, the same in vitro diagnostic is characterized by the same name (including proprietary name), same information, same design with comparable components from the same suppliers, same specifications, same regulatory version code, same site of manufacturer and quality management system, same data on quality and performance, same intended use, same labelling and packaging, and same instructions for use.

- The changes notified to the NRA by WHO have not been followed by a change to the nationally registered Product and, as a consequence, the nationally registered Product is no longer the same⁷⁶ as the WHO-prequalified product

Deviation	Reason
Click or tap here to enter text	Click or tap here to enter text
Click or tap here to enter text	Click or tap here to enter text

- The Product has been deregistered or the registration of the Product has been suspended

Deregistration: Yes No

Suspension of registration: Yes No

Effective date (dd/mm/yyyy): [Click or tap here to enter text](#)

Reasons:

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

[Click or tap here to enter text](#)

For the NRA

Signature: [Click or tap here to enter text](#)

Name: [Click or tap here to enter text](#)

Title: [Click or tap here to enter text](#)

Place: [Click or tap here to enter text](#)

Date (dd/mm/yyyy): [Click or tap here to enter text](#)

⁷⁶ Within the context of this Procedure, the same in vitro diagnostic is characterized by the same name (including proprietary name), same information, same design with comparable components from the same suppliers, same specifications, same regulatory version code, same site of manufacturer and quality management system, same data on quality and performance, same intended use, same labelling and packaging, and same instructions for use.

Annex 5

New and replacement WHO international reference standards for biological products

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

The considerations and guiding principles used to assign priorities and develop the programme of work in this area have previously been set out as WHO Recommendations.⁷⁷ In order to facilitate and improve transparency in the priority-setting process, a simple tool was developed as Appendix 1 of these WHO Recommendations. This tool describes the key considerations taken into account when assigning priorities, and allows stakeholders to review and comment on any new proposals being considered for endorsement by the WHO Expert Committee on Biological Standardization.

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

At its meetings held via WebEx on 19–23 October 2020 and via Zoom video conferencing on 9 and 10 December 2020, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list. Each of the WHO international reference standards shown in this table should be used in accordance with their instructions for use (IFU).

⁷⁷ Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO Expert Committee on Biological Standardization: fifty-fifth report. Geneva: World Health Organization; 2006: Annex 2 (WHO Technical Report Series, No. 932; http://www.who.int/immunization_standards/vaccine_reference_preparations/TRS932Annex%20Inter%20biol%20ef%20standards%20rev2004.pdf?ua=1, accessed 9 November 2019).

Additions⁷⁸

Material	Unitage	Status
Biotherapeutics other than blood products		
Interferon alpha 2b	24 000 IU/ampoule	Third WHO International Standard
Bevacizumab	1000 IU/ampoule for VEGF165 neutralizing activity; 1000 IU/ampoule for VEGF165 binding activity	First WHO International Standard
Trastuzumab	1000 IU/ampoule (IOP activity) 1000 IU/ampoule (ADCC activity) 1000 IU/ampoule (HER2 binding activity) 1000 IU/ampoule (ADCP) 1000 IU/ampoule (FcγRIIIa binding activity)	First WHO International Standard
Chorionic gonadotrophin (human)	159 IU/ampoule for bioassay; 186 IU/ampoule for immunoassay; corresponding to 0.41 nmol/ampoule (expanded uncertainty 0.40-0.43 nmol/ampoule; k = 2.12)	Sixth WHO International Standard
Blood products and related substances		
Anti-human platelet antigen-15b immunoglobulin G (human)	[No assigned unit] Detection at 1 in 8 dilution validates assay	WHO International Reference Reagent
In vitro diagnostics		
Insulin-like growth factor 1 (recombinant, human)	33.0 µg/ampoule (expanded uncertainty = 30.5–35.6 µg/ampoule; k = 2.36)	Second WHO International Standard

⁷⁸ 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 * are held and distributed by the Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, the USA.

Material	Unitage	Status
Herpes simplex virus type 1 DNA for NAT-based assays	7.19 log ₁₀ IU/vial	First WHO International Standard
Herpes simplex virus type 2 DNA for NAT-based assays	7.31 log ₁₀ IU/vial	First WHO International Standard
West Nile virus lineage 1 RNA for NAT-based assays	7.20 log ₁₀ IU/vial	First WHO International Standard
West Nile virus lineage 2 RNA for NAT-based assays	[No assigned unitage]	WHO International Reference Reagent
Standards for use in high-throughput sequencing technologies		
Porcine circovirus type 1 (CBER code: SC-VR-6000P)*	2.7 x 10 ¹¹ genome copies/mL	WHO International Reference Reagent
Mammalian orthoreovirus type 1 (CBER code: SC-VR-6001P)*	1.4 x 10 ¹⁰ genome copies/mL	WHO International Reference Reagent
Feline leukaemia virus (CBER code: SC-VR-6002P)*	5.3 x 10 ¹⁰ genome copies/mL	WHO International Reference Reagent
Human respiratory syncytial virus (CBER code: SC-VR-6003P)*	1.0 x 10 ⁹ genome copies/mL	WHO International Reference Reagent
Epstein-Barr virus (CBER code: SC-VR-6004P)*	3.7 x 10 ⁸ genome copies/mL	WHO International Reference Reagent
Standards for use in public health emergencies		
SARS-CoV-2 RNA for NAT-based assays	7.40 log ₁₀ IU/ampoule	First WHO International Standard
Anti-SARS-CoV-2 immunoglobulin	250 IU/ampoule (neutralizing antibody activity)	First WHO International Standard
Anti-SARS-CoV-2 immunoglobulin panel	[no assigned unitage]	First WHO International Reference Panel
Vaccines and related substances		
Anti-MERS-CoV immunoglobulin G (human)	250 IU/ampoule	First WHO International Standard

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

WHO Expert Committee on Biological Standardization

Seventy-first report.

WHO Technical Report Series, 1028, 2021 (xii + 102 pages)

WHO Expert Committee on Biological Standardization

Seventieth report.

WHO Technical Report Series, No. 1024, 2020 (xvi + 227 pages)

WHO Expert Committee on Biological Standardization

Sixty-ninth report.

WHO Technical Report Series, No. 1016, 2019 (xv + 251 pages)

WHO Expert Committee on Biological Standardization

Sixty-eighth report.

WHO Technical Report Series, No. 1011, 2018 (xvi + 380 pages)

WHO Expert Committee on Biological Standardization

Sixty-seventh report.

WHO Technical Report Series, No. 1004, 2017 (xviii + 591 pages)

WHO Expert Committee on Biological Standardization

Sixty-sixth report.

WHO Technical Report Series, No. 999, 2016 (xix + 267 pages)

WHO Expert Committee on Biological Standardization

Sixty-fifth report.

WHO Technical Report Series, No. 993, 2015 (xvi + 262 pages)

WHO Expert Committee on Biological Standardization

Sixty-fourth report.

WHO Technical Report Series, No. 987, 2014 (xviii + 266 pages)

WHO Expert Committee on Biological Standardization

Sixty-third report.

WHO Technical Report Series, No. 980, 2014 (xv + 489 pages)

Website: <http://www.who.int/biologicals>

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization, 1211 Geneva 27, Switzerland
(tel.: +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int;
order online: 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 products used in medicine, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of issues brought to the attention of the Committee at its meetings held in October 2020 (via WebEx video conferencing) and December 2020 (via Zoom video conferencing) during the COVID-19 outbreak. Of particular relevance to manufacturers and national regulatory authorities are the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, the following three documents were adopted on the recommendation of the Committee: (a) WHO Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines; (b) WHO Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated); and (c) Collaborative procedure between the World Health Organization and national regulatory authorities in the assessment and accelerated national registration of WHO-prequalified in vitro diagnostics.

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

A series of annexes is then presented which includes an updated list of all WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products (Annex 1). The above three WHO documents adopted on the advice of the Committee are then presented as part of this report (Annexes 2–4). Finally, all new and replacement WHO international reference standards for biological products established during the October 2020 and December 2020 meetings are summarized in Annex 5. The updated full catalogue of WHO international reference standards is available at: <http://www.who.int/bloodproducts/catalogue/en/>.

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