WHO Technical Report Series

1011

WHO Expert Committee on Biological Standardization

Sixty-eighth report

World Health Organization

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WHO Expert Committee on Biological Standardization

17 to 20 October 2017

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Dr S. Hill, Director, EMP

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[8] A maximum of two representatives of the DCVMN and two representatives of the IFPMA were present in the meeting room during discussion of any one agenda item.

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Abbreviations

Ad	human adenovirus
Ag	antigen
anti-CCP	anti-cyclic citrullinated peptide
anti-dsDNA	antibody to double-stranded DNA
APTT	activated partial thromboplastin time
ASTM	ASTM International
BDBV	Bundibugyo ebolavirus
BRN	WHO Blood Regulators Network
BSE	bovine spongiform encephalopathy
CBER	Center for Biologics Evaluation and Research
ChAd3	chimpanzee adenovirus type 3
CHO	Chinese hamster ovary
CEG	Core Expert Group
CHIKV	chikungunya virus
CLSI	Clinical and Laboratory Standards Institute
CMV	cytomegalovirus
CRF	circulating recombinant form
CTP	cell therapy product
CV	coefficient of variation
CVV	candidate vaccine virus
DCVMN	Developing Countries Vaccine Manufacturers Network
DNA	deoxyribonucleic acid
EBOV	Ebola virus
ECSPP	WHO Expert Committee on Specifications for Pharmaceutical Preparations
EDQM	European Directorate for the Quality of Medicines & HealthCare
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay

EUAL	WHO emergency use assessment and listing (procedure)
EVD	Ebola virus disease
FIXa	activated blood coagulation factor IX
FXa	activated blood coagulation factor X
FXII	blood coagulation factor XII
FXII:Ag	blood coagulation factor XII (antigen value)
FXII:C	blood coagulation factor XII (functional activity)
GCP	good clinical practice
GCV	geometric coefficient of variation
GLP	good laboratory practice(s)
GMP	good manufacturing practice(s)
GP	glycoprotein
HA	haemagglutinin
HAV	hepatitis A virus
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HHV	human herpes virus
HIST	histamine sensitization test
HIV	human immunodeficiency virus
HPA	human platelet antigens
HPLC	high-performance liquid chromatography
HPV	human papillomavirus
HRP2	histidine-rich protein 2
ICDRA	International Conference of Drug Regulatory Authorities
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP	immune correlate of protection
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations

IFU	instructions for use
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
ISO	International Organization for Standardization
IU	International Unit(s)
IVD	in vitro diagnostic
KRAS	Kirsten rat sarcoma viral oncogene homolog
LMIC	low- and middle-income countries
LPS	lipopolysaccharide(s)
LVV	lentiviral vector
mAb	monoclonal antibody
MARV	Marburg virus
MCB	master cell bank
MERS-CoV	Middle East respiratory syndrome coronavirus
MVA	modified vaccinia Ankara
NAT	nucleic acid amplification technique
NCL	national control laboratory
NGS	next-generation sequencing
NIBSC	National Institute for Biological Standards and Control
NRA	national regulatory authority
OCV	oral cholera vaccine
OPV	oral poliomyelitis vaccine
PAS	prior approval supplement
PCR	polymerase chain reaction
PEI	Paul-Ehrlich-Institut
PKA	prekallikrein activator
pLDH	plasmodial lactate dehydrogenase
PT	prothrombin time

PTx	pertussis toxin
QA	quality assurance
QC	quality control
RDT	rapid diagnostic test
RESTV	Reston ebolavirus
RF	rheumatoid factor
rhPTH1-34	parathyroid hormone 1-34 (recombinant, human)
RNA	ribonucleic acid
RSV	respiratory syncytial virus
rVSV	recombinant vesicular stomatitis virus
SAGE	WHO Strategic Advisory Group of Experts
SBP	similar biotherapeutic product
SLE	systemic lupus erythematosus
SoGAT	Standardisation of Genome Amplification Techniques (group)
SUDV	Sudan ebolavirus
TAFV	Tai Forest ebolavirus
TGT	thrombin generation test
TGS	WHO Technical Guidance Series
TP	*Treponema pallidum*
TSE	transmissible spongiform encephalopathy
TSS	WHO Technical Specifications Series
Vi PS	Vi polysaccharide
VLP	virus-like particle
VSV	vesicular stomatitis virus
WCB	working cell bank
WHOCC	WHO collaborating centre
ZEBOV	Zaire ebolavirus
ZIKV	Zika virus

1. Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 17 to 20 October 2017. The meeting was opened on behalf of the Director-General of WHO by Dr Suzanne Hill, Director of Essential Medicines and Health Products (EMP) and currently Acting Assistant Director-General for the Health Systems and Innovations Cluster. Dr Hill welcomed the Committee, other meeting participants and observers, and briefly outlined several key changes in WHO staff and structures that had occurred since the previous meeting of the Committee. The election of the new Director-General, Dr Tedros Adhanom Ghebreyesus, in May 2017 meant that this was a time of transition for the Organization.

Three new clusters had now been formed from the previous Health Systems and Innovations Cluster, and senior management posts filled. One of the new clusters – Access to Medicines, Vaccines and Pharmaceuticals – would be led by a new Assistant Director-General, Dr Mariângela Batista Galvão Simão. Dr Hill also announced that Dr David Wood, Coordinator, Technologies, Standards and Norms – and Secretary to the Committee – had retired in April, and that Dr Francois-Xavier Lery, previously of the European Directorate for the Quality of Medicines & HealthCare, had been appointed as his successor.

Dr Hill highlighted the commitment of the Director-General to champion universal health coverage, including through improved access to medicinal products of assured quality, safety and efficacy. It was envisaged that this would be achieved through a coherent country ownership approach backed up by the strong normative efforts of WHO. As the longest-standing WHO Expert Committee, the WHO Expert Committee on Biological Standardization has long emphasized the importance of pursuing a coherent approach to improving access to medicines and strengthening WHO's normative work in this area. There would now be an opportunity to review the ways in which the Committee functioned and to decide how best to prioritize its substantial workload. On behalf of WHO, Dr Hill expressed her thanks to the Committee, to WHO Collaborating Centres, and to all the experts, institutions and professional societies working in this field, whose efforts provided vital support to WHO programmes in global public health. She concluded by reminding participants that Committee members acted in their personal capacities as experts and not on behalf of their organizations or countries.

Dr Ivana Knezevic, Acting Secretary to the Committee, outlined the working arrangements of the meeting before moving on to the election of the meeting officials. Professor Klaus Cichutek was elected as Chair and Dr Elwyn Griffiths as Rapporteurs for the plenary sessions and for the track considering vaccines and biotherapeutics. Mrs Teeranart Jivapaisarnpong was also Rapporteur for the vaccines and biotherapeutic track. Dr Harvey Klein

was elected as Chair and Dr Clare Morris and Dr Jens Reinhardt as Rapporteurs for the track considering blood products and in vitro diagnostics. Dr Klein was also elected as Vice-Chair for the plenary sessions of the Committee.

Dr Knezevic then gave a brief overview of WHO Expert Committees and of their important and greatly valued role in providing assistance to WHO Member States. She noted that the meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations meant that two WHO Expert Committees were meeting concurrently. Also meeting during the same week were the WHO Strategic Advisory Group of Experts (SAGE) on Immunization and the annual Consultation on International Nonproprietary Names (INN) for Pharmaceutical Substances. Dr Knezevic introduced the members of the 2017 Expert Committee on Biological Standardization and presented the declarations of interests made by Committee members, Temporary advisers and participants. After evaluation, WHO had concluded that none of the declarations made constituted a significant conflict of interest and that the individuals concerned would be allowed to participate fully in the meeting.

Following participant introductions, the Committee adopted the proposed agenda (WHO/BS/2017.2331).

2. General

2.1 Current directions

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

Ms Emer Cooke, newly appointed Head of Regulation of Medicines and other Health Technologies (RHT), presented an overview of WHO strategic directions in the regulation of medicines and other health technologies, with a particular focus placed on biologicals. After outlining the overall vision of the new Director-General, Ms Cooke provided more details of the new WHO Cluster on Access to Medicines, Vaccines and Pharmaceuticals. The cluster would comprise EMP and the two underlying groups, Innovation, Access and Use (IAU) led by Dr Sarah Garner and RHT. RHT would consist of Technologies, Standards and Norms, Regulatory System Strengthening, the WHO Prequalification Team and Safety and Vigilance. Mention was made of the fact that the WHO Prequalification Team now had responsibility for medical devices and vector-control products in addition to vaccines, medicines and diagnostics. Ms Cooke then expressed her thanks to Dr Knezevic, who had been the Acting Secretary to the Committee since the retirement of Dr Wood in May 2017, for her invaluable support and that of her team.

Ms Cooke noted that two strategic aspects of EMP activities which particularly impacted upon biologicals were access to medicines and public health emergencies. Since the previous meeting of the Committee, an Expert Consultation on improving access to and use of similar biotherapeutic products (SBPs)[1] had been held in Geneva, as had a WHO Informal Consultation on options to improve regulatory preparedness to address public health emergencies.[2] In addition, an EMP strategic framework entitled "Towards Access 2030"[3] had been published, with a supporting RHT strategy now under development. Furthermore, a new Strategic Advisory Group of Experts on In Vitro Diagnostics (SAGE IVD) had been established. The SAGE IVD would act as an advisory body to WHO on matters of global policies and strategies related to IVDs.

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[1] Report on the Expert Consultation on improving access to and use of similar biotherapeutic products. Geneva, 2–3 May 2017. Geneva: World Health Organization; 2017 (http://www.who.int/medicines/access/biotherapeutics/FINAL_Report-improving-access-to-and-use-of-biotherapeutics_October2017.pdf, accessed 2 April 2018).

[2] WHO Informal Consultation on options to improve regulatory preparedness to address public health emergencies. Geneva, 17–19 May 2018. Geneva: World Health Organization; 2017 (http://www.who.int/medicines/news/2017/PHEmeeting-reportIK-EG16_Nov_2017.pdf, accessed 2 April 2018).

[3] Towards Access 2030. WHO medicines and health products programme strategic framework 2016–2030. Geneva: World Health Organization; 2017 (WHO/EMP/2017.01; http://www.who.int/medicines/publications/Towards_Access_2030_Final.pdf?ua=1, accessed 2 April 2018).

A wide range of stakeholders had participated in the Expert Consultation on improving access to and use of SBPs, including clinicians, regulators, health economists, experts in public health and from academia, members of WHO Expert Committees and advisory panels, manufacturers, and patient organizations and professional societies. Recurrent themes which emerged included the need to ensure SBP quality, safety and efficacy (notably through robust regulation and guidance), the need for clarity regarding nomenclature and other aspects of terminology, and the need for education and effective communication regarding SBPs. The need for strengthened pharmacovigilance was also emphasized. Ms Cooke informed the Committee that no consensus had been reached on whether WHO should continue with plans for a Biological Qualifier and so WHO had decided not to proceed with this nomenclature. However, it was agreed that WHO would review and provide clarification of its 2009 Guidelines on evaluation of SBPs in order to reflect, where necessary, technological and analytical advances. WHO would also pilot the prequalification of two SBPs – trastuzumab and rituximab – in an attempt to extend the considerable experience of stringent regulatory authorities (SRAs) in this area to national regulatory authorities (NRAs) in low- and middle-income countries (LMIC). Manufacturers of rituximab and trastuzumab would be invited to submit expressions of interest to WHO.

The Committee was also informed that the WHO Informal Consultation on options to improve regulatory preparedness to address public health emergencies had been well attended by regulators from high-income countries and from LMIC, as well as by manufacturers, subject matter experts and other stakeholders. Pertinent issues relating to in vitro diagnostics, therapeutics and vaccines had been discussed, and available regulatory tools and pathways – including the WHO Emergency Use Assessment and Listing (EUAL) procedure – were reviewed, along with regulatory collaboration arrangements between countries, and capacity-building activities. Preliminary outcomes highlighted the need to map out current emergency provisions in LMIC and to address legal or regulatory deficiencies, to clarify and revise the current EUAL procedure based on experience to date, and to consider a possible "pre-EUAL" submission process for priority pathogens. Clarification was also needed of what happens following a EUAL procedure regarding, for example, national licensing, product procurement, importation and liability issues. It had been proposed that WHO guidance be developed on procedures and pathways to enable the use of unlicensed products during a public health emergency, including guidance on the minimum competencies required by NRAs to deal with such situations. The possibility of a diagnostics preparedness consortium was raised, as was the use of "mock-up" exercises in expediting the review of submissions or clinical trials in emergency contexts. A trial tabletop exercise was scheduled to take place at the end of November 2017. The Committee was informed that WHO would now

reflect on all the recommendations made for moving this agenda forward, with a view to developing an action plan based on priorities and available resources.

The Committee was then reminded of the two main strategic roles of EMP – namely, to act as a facilitator, supporting innovation and promoting access to health products, and as a guardian in efforts to strengthen regulatory capacity and practices and thus ensure the quality, safety and efficacy of products in order to secure health gains. An integrated approach across all RHT activities would be needed to achieve these goals. This would include strengthening the ability of NRAs to effectively regulate medicines, and promoting, where appropriate, the concept of regulatory reliance. Ms Cooke emphasized that the work of the Committee was a fundamental enabler of many of the normative activities of WHO, and noted its strong links with other WHO activities and entities such as the SAGE on Immunization. In addition, WHO initiatives in the area of public health emergencies – including the WHO Blueprint for Research and Development: Responding to Public Health Emergencies of International Concern (R&D Blueprint) and collaboration with other international initiatives such as the Coalition for Epidemic Preparedness Innovations – would continue to depend upon sound regulatory science, standards and norms.

The Committee thanked Ms Cooke for sharing this helpful overview of current WHO developments in the regulation of medicines and other health technologies and raised a number of issues for clarification. In particular, clarification was sought of the remit of the newly established SAGE IVD and of how its work would complement the longstanding responsibilities of the Committee in this area. Ms Cooke indicated that this was not yet clear but agreed that it would be very important to avoid both excessive workload and the overlapping of responsibilities. It was also noted that the Committee would normally provide the SAGE on Immunization with a report of its work on vaccine standardization and related issues. However, this year both groups were meeting in parallel and so representatives of the Committee were only able to participate in parts of the SAGE on Immunization meeting. A full report of the work of the Committee in this regard would be presented at the next SAGE on Immunization meeting. The Committee requested that consideration be given by WHO to improving the coordination of meetings at WHO headquarters of all the various advisory and other groups working in this area.

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

Dr Knezevic reported on recent and planned activities in the area of standardization and regulatory evaluation of vaccines and biotherapeutics. Current WHO Recommendations, Guidelines and guidance documents ("written standards") in the area of biological standardization are primarily vaccine

specific (or relevant to all vaccines), a number specifically cover biotherapeutic products, while others apply to both vaccines and biotherapeutic products.

Two new WHO Guidelines – one on Ebola vaccines and the other on post-approval changes to biotherapeutic products, including similar biotherapeutic products (SBPs) – were being considered for adoption at the present meeting (see sections 3.1.1 and 3.4.1 respectively). Dr Knezevic reminded the Committee that written standards relating specifically to blood products are listed on the separate WHO Blood Products website (http://www.who.int/bloodproducts/en/) and suggested that the coordination between the two sites could be improved.

Dr Knezevic then outlined the range of recently adopted WHO measurement standards in this area. Such standards are crucial elements in the development, licensing and ongoing oversight of biological medicines. Nine new WHO international standards for vaccines and related substances were being considered for establishment at the present meeting (see sections 8.1.1–8.1.5). Ideally, measurement standards and written standards should be developed simultaneously but this is not always feasible, and in cases where a written standard is adopted before the establishment of the international measurement standard there may be a need to update the former. Currently, there are nine WHOCCs contributing to this work and their evolving role in the development of measurement standards was under discussion.

Dr Knezevic informed the Committee of the progress being made in the development of guidelines on the quality, safety and efficacy of respiratory syncytial virus (RSV) vaccines. A WHO consultation on RSV vaccines held in September 2017 had highlighted a significant surge in RSV vaccine and monoclonal antibody (mAb) development, with 42 candidate vaccines and four mAbs in development, targeting different populations (paediatric, pregnant women and the elderly). Although a WHO international standard for antiserum to RSV for standardizing RSV neutralization assays was being submitted for establishment at the present meeting, its suitability as a standard in the mAb competition assay was still being assessed and additional international reference materials for such products may be required. Consideration was also being given to the development of a manual on the standardization of such assays with subsequent evaluation of training needs.

The Committee was also informed that WHO Recommendations to assure the quality, safety and efficacy of hepatitis E vaccines were also under development. Such vaccines had already been developed, licensed and used in China but several more were in development. A range of issues had been discussed at a WHO meeting in May 2017 and a drafting group established. A first round of public consultation on the draft Recommendations document was scheduled for late 2017 and a second meeting of the drafting group planned for April 2018.

An update was then provided on the revision of the WHO Guidelines on the safe production and quality control of inactivated poliomyelitis vaccines manufactured from wild polioviruses in the context of the biocontainment needs described in the *WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use* (GAPIII). The Committee was reminded that the focus of the WHO Guidelines on production safety and product quality was complementary to the focus of GAP III on environmental safety and biocontainment following the eradication of wild-type polioviruses. A first round of public consultation on the draft Guidelines document was scheduled for late 2017 and a third meeting of the working group planned for April 2018.

The Committee was also reminded of the crucial importance of harmonizing international biosafety expectations for both pilot- and large-scale production of human pandemic influenza vaccines. Such harmonization was a key element in facilitating influenza vaccine development during the interpandemic period and thus in ensuring the timely availability of vaccine in the event of an influenza pandemic. The current WHO document on biosafety risk assessment and the production and quality control of human influenza pandemic vaccines had been adopted by the Committee in 2007. Following an expert group review of the document in the light of existing biosafety guidance, new knowledge and other inputs, it had been concluded that the text required updating in a number of key areas and expansion of its scope to cover all influenza A virus subtypes. A first draft of the revised guidance was expected to be ready for public consultation in late 2017 with further rounds of revision and public consultation scheduled for 2018, with the aim of submitting the final revised document for consideration by the Committee in October 2018.

Dr Knezevic then reported on the outcomes of a 2017 WHO consultation on improving access to SBPs. One of the proposals made was for WHO to review and revise its 2009 Guidelines on evaluation of similar biotherapeutic products to reflect recent technological and analytical advances. However, during a previous WHO implementation workshop it had been concluded that the principles set out in the current Guidelines were still relevant and it had instead been suggested that a Questions & Answers (Q&A) document might better clarify and inform the current understanding of the principles outlined. An early draft Q&A text was now undergoing public consultation with the intention of publishing the resulting document on the WHO website in 2018.

The vital role played by implementation workshops in promoting and clarifying WHO written standards, and in moving the biologicals field forward, was highlighted. During 2016, three such WHO workshops had been held – two (in China and Thailand) on human papillomavirus (HPV) vaccines, and one (in Indonesia) on typhoid conjugate vaccines. In July 2017, an implementation workshop had been held on biotherapeutics (including SBPs) for Russian-

speaking countries, organized by the WHO Regional Office for Europe in Copenhagen. Further implementation workshops were scheduled for late 2017 on good manufacturing practices (GMP) for biologicals (in Thailand) and on typhoid conjugate vaccines (in the Republic of Korea).

Dr Knezevic concluded by outlining the ways in which WHO written and measurement standards contribute to the process of regulatory convergence by promoting a common understanding in all WHO Member States of the complex issues involved and by providing international standards for the regulatory evaluation of medicinal products. Implementation workshops then provide valuable educational and training tools for improving the expertise of NRAs. After noting that many challenges remain, Dr Knezevic outlined a number of successful collaborations that had been undertaken, and the numerous opportunities that exist for further collaboration with other organizations and networks in promoting the use of WHO standards and thus regulatory convergence.

The Committee thanked Dr Knezevic for her informative overview and expressed its support for the proposed initiatives. In particular, the Committee agreed that a Q&A document for SBPs should be developed. The Committee also reiterated the view that the conducting of technically detailed implementation workshops, including through the use of case studies, will continue to be an important element in promoting regulatory convergence.

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

Dr Micha Nübling presented an overview of activities in the areas of blood products and in vitro diagnostics over the past 12 months, highlighting five main topics: (a) the recommendations of a workshop on blood products held during the 17th International Conference of Drug Regulatory Authorities (ICDRA) in South Africa in December 2016; (b) progress in the assessment of snake antivenoms; (c) blood regulation in Zambia; (d) the impact of emerging infections on blood supply; and (e) issues related to the First WHO International Standard for anti-rubella immunoglobulin.

The ICDRA blood products workshop recommendations emphasized the need for countries to implement blood regulations, to regulate reagents and devices associated with the use of blood, to model new regulations on already-existing regulations in other countries and to regulate snake antivenoms. In support of this, WHO was urged to provide assistance in the assessment of national blood regulation, provide training for inspectors and assessors with a focus on regional networks, update the global database on snakes and antivenoms, develop regional reference standards for venoms, and continue in its assessment and listing of snake antivenoms.

The Committee was informed that snake-bites lead to more than 100 000 fatalities each year, with women and children in rural areas being the

most affected. However, at present, antivenoms are still widely unregulated and often of unknown quality. WHO had therefore implemented its programme of assessment and listing of snake antivenoms in response to the need for antivenoms of assured quality. Manufacturers of antivenoms are invited to submit a dossier for evaluation along with product samples for laboratory testing of their efficacy and other characteristics. GMP inspections are also carried out. Following a call to manufacturers of antivenoms specifically intended for use in sub-Saharan Africa, seven dossiers had been submitted for evaluation and five product samples for laboratory testing. Antivenoms meeting WHO requirements and with a favourable risk–benefit ratio will be listed on the WHO website for easy access by procurement agencies and other relevant parties. Dr Nübling indicated that a WHO prequalification process for antivenoms may be developed if the conducting of the risk–benefit assessments indicates that this would generate significant public health benefits.

The Committee was informed that an invited assessment of the Zambia Medicines Regulatory Agency (ZAMRA) by staff from the WHO Regional Office for Africa and WHO headquarters, together with a member of the BRN, had taken place in 2017. The assessment indicated that although legal preconditions and medicines regulation is in place, blood regulation in Zambia is still at a very early stage. The Zambia National Blood Transfusion Service (ZNBTS) was not overseen by ZAMRA but instead was auto-regulated. It was considered that the current medicines regulations should be used as a blueprint for blood regulation in the country. Advancing blood regulation would require both training – which could be delivered using ZNBTS expertise – and twinning with (or having greater reliance on) mature NRAs in other countries.

Dr Nübling reported that a WHO Global Technical Expert Consultation on estimating the impact of emerging infections on the blood supply: requirements for risk estimation and decision-making support had been held in Geneva on 14–15 June 2017. Consultation participants had noted that regulatory decisions on the protection of the blood supply are often taken on an ad hoc basis when confronted with the emergence of a pathogen. Such decisions may include the introduction of a new test for screening blood donors, deferral of certain donors from blood donation or quarantining of blood components. In these situations, promoting the public perception of "safe blood" or political expectations were sometimes more dominant factors than scientific considerations. There was thus a recognized need for consistency in estimating threats to the blood supply and making subsequent regulatory decisions. It was proposed that a prototype guidance tool could be developed covering different aspects (such as quantitative risk estimates, potential interventions and the evaluation of risk outcomes in terms of benefits and costs) in order to guide the potential scientific, epidemiological and regulatory considerations involved. Such considerations would include whether or not the pathogen is novel and previously unknown,

and the extent to which the precautionary principle may therefore prevail. In cases where an infection is re-emerging there would be far fewer uncertainties in relation to potential interventions and outcomes. Since considerable differences exist between low-, middle- and high-income countries not only in the experience of using decision tools but also in the considerations to be taken into account, the pilot testing of such a tool should include users in a range of countries.

Dr Nübling then informed the Committee of the outcomes of a WHO Consultation on the First WHO International Standard for anti-rubella immunoglobulin held in Geneva in June 2017. The current international standard was comprised of polyclonal antibodies and was used in standardizing the different results of assays of different design and purpose. This raises issues when working with the immunity threshold of 10 IU/ml and questions have been raised as to the commutability of this International Standard. It was concluded that the International Standard is well characterized and should continue to be used but that the lack of commutability must be clearly communicated to users in the instructions for use (IFU). However, uncertainty remained as to whether the immunity threshold was still appropriate and whether this standard should still be used in qualitative assays of high specificity. Further work would be necessary to resolve the outstanding issues and, depending on the conclusions reached, the revision of WHO guidelines and regulatory requirements may need to be considered.

Dr Nübling concluded by outlining the documents and measurement standards in the areas of blood products and in vitro diagnostics to be considered for adoption and establishment respectively by the Committee this year. These consisted of two WHO prequalification guidance documents, one on the performance evaluation of HIV rapid diagnostic tests (RDTs) and another on establishing the stability of in vitro diagnostic medical devices (see sections 3.3.2 and 3.3.3 respectively); three measurement standards for blood products and related substances (see sections 6.1.1–6.1.3) and 10 measurement standards for in vitro diagnostics (see sections 7.1.1–7.1.10).

The Committee thanked Dr Nübling for his wide-ranging report and after making a number of observations and comments looked forward to being updated on the progress made in these important areas of public health at its next meeting.

2.2 Reports

2.2.1 Report from the WHO Blood Regulators Network

Dr Christian Schärer began by reminding the Committee that the objectives of the BRN were to identify issues and share expertise and information, to promote the science-based convergence of regulatory policy (including through fostering the development of international consensus on regulatory

approaches) and to propose solutions to specific blood-related issues. After presenting an overview of the current BRN membership, Dr Schärer informed the Committee that a new BRN Chair, Dr Anneliese Hilger (PEI, Germany), had recently been elected.

Following its face-to-face meeting during the previous Committee meeting in October 2016, four teleconferences and several further meetings had been held. At these meetings, discussions had been held on a wide range of topics, including the sharing of information on the hepatitis A and Zika virus situations, the storage of red blood cells, follow-up of the results of Ebola virus (EBOV) clinical trials using convalescent plasma in Guinea, national decision-making on donor deferral for men who have sex with men, and raising awareness of the need to promote the topic of blood products.

Dr Schärer then outlined a number of BRN work products which included a BRN Position Statement on *Collection of blood for transfusion in the setting of a vaccination campaign against yellow fever*, and a BRN Position Paper on *Use of convalescent plasma, serum or immune globuline concentrates as an element in response to an emerging virus*. The latter paper was a follow-up to previous BRN position papers on the use of convalescent plasma in specific situations, and takes a more generic approach. Both papers had been published on the WHO BRN website. Other work products included efforts made to strengthen stakeholder involvement in BRN activities.

During this period BRN had also provided support for the integration of the BRN assessment criteria for a national blood regulatory system into the WHO global benchmarking tool (GBT) for evaluation of national regulatory systems (see section 2.4.2). During a workshop held in Geneva in August 2017, BRN representatives from the Food and Drug Administration (the USA), PEI (Germany), the Ministry of Health, Labour and Welfare (Japan) and Swissmedic (Switzerland) and other workshop participants reviewed a large number of datasets and identified a number of gaps in the current version of the BRN assessment criteria. The integration of BRN criteria into the WHO GBT is intended to be completed by the end of 2017. BRN had also organized a workshop on blood products at the ICDRA meeting held in South Africa in December 2016 and was involved in the WHO assessment of the blood regulatory system in Zambia using the BRN assessment criteria (see section 2.1.3 above). A number of additional BRN work products and engagements were then outlined.

Dr Schärer concluded by informing the Committee that this year the BRN face-to-face meeting had been held immediately prior to the meeting of the Committee. Among the main outcomes of this meeting had been the election of the new BRN Chair and development of the 2017–2018 BRN workplan, which would incorporate the provision of specific BRN support to the WHO Regional Office for Africa.

The Committee thanked Dr Schärer and noted his report.

2.2.2 Report from the WHO network of collaborating centres on standardization and regulatory evaluation of vaccines

Work of the Core Expert Group

Dr Lindsay Elmgren presented a progress report on the work of the network's Core Expert Group (CEG). The Committee was reminded that the original rationale for the establishment of the CEG was to reduce and streamline the workload of the Committee in the vaccines area. At present, the reviewing of large numbers of proposals for new or replacement measurement standards consumed a significant amount of Committee time and it had been suggested that relevant expertise within the network could be utilized to expedite both the review and priority-setting processes. At its previous meeting, the Committee had agreed that as a first step the CEG could pre-review selected measurement standards.

Dr Elmgren reported that six CEG WebEx meetings had been held at regular intervals between September 2016 and September 2017. The specific issues discussed included the structure of the CEG, the feasibility of expanding its scope to include written standards and biotherapeutic products, and the likely timeframe available for pre-review. During discussions, it became clear that the proposed timeframe might be problematic and that expanding the scope of the CEG might conflict with the Terms of Reference of individual WHOCCs. Dr Elmgren concluded by outlining some of the options for the next steps, along with the potential advantages and disadvantages of each option, and closed by presenting an overview schematic of the envisaged CEG process.

The Committee thanked Dr Elmgren for his report and provided a number of inputs in relation to the role of the CEG process in facilitating its work.

Proposal to simplify the structure of type-specific vaccine written standards

Dr Elmgren reported on discussions which had been undertaken by the network regarding the most practical way of developing and structuring new type-specific vaccine guidelines. It had been concluded that a case could be made for simplifying guidelines on the quality, safety and efficacy of specific vaccine types by focussing only on the major points to be considered for such vaccines. A proposal was therefore made to change and simplify the present structure of product-specific vaccine guidelines – a structure which could be considered duplicative and which had now been in use for many years. It was proposed that a pilot guidelines document be developed for group B meningococcal vaccines focussing only on the key points to consider, and possibly taking the form of a discussion paper.

Although the Committee was sympathetic to this suggestion, and considered that drafting groups might indeed welcome a reduction in workload when preparing such guidelines, little support was expressed for this proposal. Although the idea might seem to have merit in principle, there were concerns

that in practice such a document would not meet the needs of users of WHO guidelines, who often required a comprehensive document – and one which could potentially be adopted as national guidelines. In addition, WHO guidelines were also used to inform decisions on the WHO prequalification of vaccines and thus on United Nations procurement.

2.2.3 Report from the WHO network of collaborating centres for blood products and in vitro diagnostics

Dr Clare Morris reported on a number of recent activities of the network. Three WebEx meetings on blood products and IVDs had been held to address issues prior to the 2017 meeting of the Committee, help set the meeting agenda and share practices between network member WHOCCs. Several of the issues covered would be discussed in detail during the upcoming Blood Products and IVD track sessions. However, Dr Morris raised the concern that although some standards (such as anti-rubella serum and new diagnostic reagents for HPV) had been established in the Vaccines track, the use of these materials also had implications for the diagnosis of infectious disease (see section 3.3.4 below). There was therefore a need to ensure awareness of such potential overlaps between the diagnostics and vaccines fields, which are currently dealt with independently in the different tracks of Committee meetings.

Dr Morris then outlined a number of issues discussed at the 2017 meeting of the Standardisation of Genome Amplification Techniques (SoGAT) group with particular relevance to the current meeting.

The Committee thanked Dr Morris for her report and requested clarification on one specific point raised at the SoGAT group meeting in relation to the need for standardization in the expanding area of next generation sequencing. Discussion also took place on the need for improved interaction between the currently separate tracks of Committee meetings and on the network's role in assisting the work of the Committee by means of the preparatory WebEx meetings.

2.3 Feedback from custodian laboratories

2.3.1 Developments and scientific issues highlighted by custodians of WHO biological reference preparations

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

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

Dr Christian Schneider informed the Committee that NIBSC currently holds more than 800 000 ampoules/vials of 354 WHO international standards. During the period October 2016 to October 2017, 351 different standards were

distributed to 75 different countries. Of the standards distributed, approximately 66% went to commercial organizations and the rest to other organizations, including national control authorities and universities. On the assumption that the projects to be submitted to the Committee at this meeting are endorsed NIBSC will have 83 active projects under way for new or replacement WHO international standards. Twelve of these projects involve biotherapeutic products, eight involve blood products, 20 involve IVDs and 22 involve vaccines – with 21 projects awaiting confirmation and assignment.

Among the issues raised by Dr Schneider was the difficulty sometimes experienced in meeting the current deadlines for establishment by the Committee of WHO standards for emerging/priority pathogens. In the past, the Committee had demonstrated flexibility and had accepted the late submission of study reports (for example, in establishing interim EBOV standards). Dr Schneider suggested that, going forward, consideration should be given to developing alternatives to the current fixed cycle of submission/establishment of WHO international standards.

Dr Schneider also highlighted the increasing difficulty of sourcing biological materials for the development of candidate standards and/or for inclusion as samples in collaborative studies. Such difficulties can delay the development and availability of both new and replacement standards, which in turn can negatively impact upon public health worldwide. Despite a number of concerted efforts made by NIBSC, accessing suitable biological materials would remain an ongoing issue and the support of the Committee, regional centres and other WHOCCs would be vital. Dr Schneider also pointed out that publishing the collaborative study reports in high-impact scientific journals targeted at a relevant audience was one of the best ways to promote the availability and use of WHO international standards. Dr Schneider enquired about the feasibility of WHO waiving copyright to allow for such publication.

The Committee thanked Dr Schneider and noted his report.

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

Dr Karl-Heinz Buchheit outlined a number of recent EDQM activity areas in biological standardization, including the European Pharmacopoeia standards, international standards for antibiotics and the biological standardization programme, in which WHO has Observer status. The Committee was reminded that EDQM is the custodian centre for international standards for antibiotics – a responsibility it took over from NIBSC in 2006. At present, 23 such standards are available for "old" antibiotics, with several of these antibiotics being on the WHO Essential Medicines List. Since 2006, eight replacement standards had been established, and in 2017 the Committee was being asked to endorse a proposal

to establish the Third WHO International Standard for erythromycin due to low stocks of the existing standard.

Dr Buchheit then discussed a number of recent activities of the EDQM biological standardization programme – the goal of which is to establish European Pharmacopoeia biological reference preparations and to standardize methods. The programme of work is established by a Steering Committee and, whenever possible, collaboration and common projects were undertaken with WHO and non-European partners. Current EDQM projects of potential interest to the Committee included the ongoing development of an ELISA to replace the current in vivo potency test for human rabies vaccines.

Dr Buchheit reminded the Committee that the development of alternatives to animal experiments remained a major EDQM commitment in line with European Union directives. WHO was once again strongly urged to consider the incorporation of the 3Rs principles (Replacement, Reduction, Refinement) into its written standards and other guidance where appropriate. Such a step would be key to the global acceptance of these principles. The Committee was also reminded that one of the main outcomes of a 2015 International Alliance for Biological Standardization meeting on the 3Rs concept was a formal request to WHO to initiate steps to delete the abnormal toxicity test from all its written standards.

Dr Buchheit concluded by proposing that the Committee evaluate the possibility of its more active involvement in the validation of alternative quality control assays aligned with the 3Rs principles. In addition, there was need for the Committee to reflect upon the consequences of replacing in vivo potency assays with in vitro assays.

The Committee thanked Dr Buchheit and noted his report.

Paul-Ehrlich-Institut (PEI), Langen, Germany

Dr Heidi Meyer provided the Committee with an update on the activities of PEI in the development of WHO international standards. This year, two candidate standards would be proposed for establishment by the Committee. One of these was a proposed First WHO International Standard for anti-cytomegalovirus immunoglobulin G for serological assays, which had been developed to improve the comparability of the divergent results generated by current assays. PEI was also proposing the establishment of a First WHO International Standard for chikungunya virus RNA for NAT-based assays. Dr Meyer outlined to the Committee the main steps and timeframe involved for each of these standards.

In addition, a number of other standards projects were ongoing at PEI. These included the development of a WHO reference panel for hepatitis E virus (HEV) antibodies, a WHO reference reagent for anti-chikungunya virus (CHIKV) immunoglobulin M (IgM) and immunoglobulin G (IgG), and the

further extension of the First WHO Repository of platelet transfusion relevant bacterial strains.

The Committee thanked Dr Meyer and noted her report.

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

Dr Jay Epstein informed the Committee that CBER was currently involved in several international projects evaluating new vaccine-related standards. These included a study into the potential use of a pneumococcal reference serum as a standard in the pneumococcal opsonophagocytosis assay (OPA), the international collaborative study to establish the First WHO International Standard for Zika virus antibodies (see section 7.1.10) and the international collaborative study to establish the First WHO International Standard for antiserum to respiratory syncytial virus (see section 8.1.5). CBER had also provided support for the development of new vaccine technologies, including the development of a sequence database to facilitate detection of novel adventitious viruses using next generation sequencing (NGS). CBER had also participated in a series of projects on the standardization of influenza vaccines.

In the area of blood products, CBER had distributed a number of both European and WHO international standards. The limited supply and restricted distribution of one such standard – the Second WHO International Standard for thrombin – was highlighted and a recommendation made to develop a replacement standard. CBER also recommended developing an international standard for human activated factor X (FXa) to support the development of genetically modified FXa therapies.

CBER was also actively involved in developing reference preparations for a range of different IVDs. FDA support had also been provided in the development of technical guidance documents on the WHO prequalification of IVDs, including one on HIV rapid diagnostic tests (RDTs) scheduled for consideration by the Committee this year (see section 3.3.2). In addition, funding provided through a CBER-WHO Cooperative Agreement was being used to support the revision of the current WHO EUAL procedures introduced as part of strengthening regulatory preparedness for public health emergencies of international concern.

The Committee thanked Dr Epstein for his report and noted the potential importance of NGS as a tool for regulatory evaluation in the future.

2.4 Cross-cutting activities of other WHO committees and groups

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

Dr Sabine Kopp presented an update of the work of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP). The scope of ECSPP activities covers the life-cycle of medicines from development

to delivery to the patient. Within this overall context, the ECSPP has to date actively overseen the development and publication of 85 official WHO guidance texts and guidelines on medicines, quality assurance and related regulatory standards. A new CD-ROM including all guidelines and the International Pharmacopoeia had been produced. In 2017, seven new or revised annexes were to be proposed for adoption by the ECSPP, which meets each year during the same week as the Committee.

Dr Kopp outlined a number of cross-cutting issues across the two Expert Committees, including: (a) good regulatory practices (GRP); (b) the definition of a stringent regulatory authority (SRA); (c) the use of a collaborative procedure for medical products; (d) aspects of GMP; (e) issues related to the transition from microbiological to physicochemical assays in monographs on capreomycin API and products; (f) the need for additional data for products already on the market; and (g) comparison between microbiological and chemical methods of analysis. Dr Kopp indicated that some of these issues could appear on the agenda of the Expert Committee on Biological Standardization in future and that a working group might be established to deal with some of them.

Dr Kopp then informed the Committee that the ECSPP also oversees the revision of the International Pharmacopoeia and the establishment of International Chemical Reference Substances (ICRS). In 2017, 17 new specifications and general texts were adopted for inclusion in the International Pharmacopoeia and four new ICRS established.

The Committee thanked Dr Kopp for the update on the activities of the ECSPP. During subsequent discussion it was noted that several of the above issues of common interest to the two Expert Committees will require proactive coordination, and the example of the WHO Global Benchmarking Tool was given as an issue that was to be discussed this year at both Expert Committees.

2.4.2 WHO Global Benchmarking Tool

Dr Alireza Khadem informed the Committee of the progress made in the development of the unified WHO Global Benchmarking Tool (GBT) for assessing national regulatory systems. The aim of the project was to align all of the various benchmarking tools developed by different WHO programmes and by other agencies. It is anticipated that this will allow for greater alignment of policy and scope, and greater consistency in standards and approach. This will lead to improved outcomes and impact, and to a reduced burden of assessments, costs and duplication for WHO Member States, as well as for WHO and its partners working in regulatory systems strengthening.

The project started in 2013 with the unification of the WHO vaccines and medicines benchmarking tools and is now progressing towards the integration of the Pan American Health Organization (PAHO) assessment tools, medical devices assessment tool and blood products assessment criteria. The Committee

heard that there had been several revisions of the proposed tool, extensive global discussion and a number of pilot studies, with work still ongoing. The integration of the blood products assessment criteria into the WHO GBT had involved support from the WHO BRN.

The Committee thanked Dr Khadem for his report and raised a number of points. The value of a harmonized tool was acknowledged and it was suggested that linking NRA assessment to the WHO prequalification scheme may provide an additional incentive for countries to undertake an NRA assessment. The Committee was informed that internal WHO discussions along these lines had already been scheduled. It was further suggested that reliance between regulatory agencies might become less of an issue once the results of assessment are widely accepted. A number of other important points were then raised in relation to the use of the unified tool. Topics discussed included the need for transparency in the criteria used to define a stringent NRA, whether the definition of blood included plasma and plasma-derived products, and the selecting of countries for piloting of the tool. The need to ensure precision in the questions asked was also highlighted, for example to ascertain whether all relevant guidance from WHO and ICH was being implemented rather than simply being in place.

2.4.3 Development of WHO guidelines on good regulatory practices

Dr Mike Ward reported on the progress being made in the development of WHO GRP guidelines for NRAs. This initiative had been undertaken in response to requests from WHO Member States through ICDRA and at various WHO consultations for guidance on best practices for collaboration and cooperation between NRAs – in areas such as information exchange, joint assessments and inspections, and activities aimed at reducing duplication. This foundational document would apply internationally accepted GRP principles to the regulation of all medical products, and was intended for a range of audiences, including senior policy-makers responsible for the formulation of health policies, laws and regulations, NRAs and other interested parties. Dr Ward then outlined the concept of GRP as:

> *Internationally recognised processes, systems, tools and methods for improving the quality of regulations. GRP systematically implements public consultation and stakeholder engagement as well as impact analysis of government proposals, before they are implemented to make sure they are fit for purpose and will deliver what they are set out to achieve.*[4]

[4] See: http://www.oecd.org/gov/regulatory-policy/asean-oecd-good-regulatory-practice-conference-2015.htm

The Committee was informed that a draft document had been developed and subjected to public consultation. The document included sections on background and scope, on the principles of GRP and on the implementation of regulations. Three appendices cover the details of regulatory impact assessment, legal instruments and international regulatory cooperation. Although the draft document was well received, and considered to be helpful for regulatory convergence, public consultation indicated a need for streamlining and other refinements of the content. Further inputs from a consultation held in July 2017 included requests for the text to be made more understandable and usable by its intended readers, and to become more guidance-like in nature, while ensuring that key messages were both clear and relevant to LMIC. Other suggestions included the addition of practical tools such as examples and checklists to assist in the implementation of the guidance provided. Revision was now underway and it was expected that a final draft would be available for presentation to the ECSPP for endorsement in 2018. It had been suggested that the path forward should also include exploration of a pilot phase that would serve to validate the relevance and usability of the guideline in LMIC.

The Committee thanked Dr Ward for his report and raised a number of points in relation to the language used in the document, its content and the applicability of some of the specific guidance given, especially in LMIC. A number of potential challenges in implementation were also raised, including the need to avoid damaging existing national systems, and the need for high-level advocacy and political engagement in this area.

2.4.4 Snake-bite envenoming

Dr David Williams reminded the Committee that antivenoms are immunoglobulin preparations manufactured from equine (or ovine) plasma and can be either monospecific to individual snake species or polyspecific. Currently, antivenoms are the only effective therapy against snake-bite envenoming but because of regulatory deficiencies in affected regions, falling numbers of antivenom producers and the fragility of current production systems, envenoming had become a crucial global health issue. Following international advocacy efforts, snake-bite envenoming became a category one neglected tropical disease (NTD) in May 2017.

There was now an ongoing initiative to address the situation and the Committee was provided with an update of current WHO activities in this area. The period 2016–2017 had been one of rapid progress, new opportunities and key challenges. The Committee was reminded of its adoption of the updated WHO Guidelines for the production, control and regulation of snake antivenom immunoglobulins at its meeting in 2016. Other activities had included the

updating of the WHO antivenoms website[5] and the revision and expansion of antivenom manufacturer data, which now included package inserts and published literature citations for each product. In addition, the names and photographs of snake species are being updated along with information on their geographical distribution. Links to clinical treatment information resources are also now being incorporated into the WHO website.

A WHO assessment of antivenoms intended for use in sub-Saharan Africa had also been initiated. A call for applications for the evaluation of products suitable for use in this region resulted in the submission of nine applications. Following an initial assessment and selection process, five products are now undergoing laboratory evaluation, including physicochemical characterization, specific venom immune-recognition and potency testing. Corresponding GMP inspections of production facilities in Costa Rica, India, Mexico, South Africa and the United Kingdom are also ongoing. Once these studies and inspections are completed, WHO will be in a position to recommend suitable products to procurement agencies.

Where GMP deficiencies have been found, manufacturers had demonstrated a willingness to respond but resources are very limited and further support from WHO and other stakeholders is needed. Dr Williams indicated that a roadmap for addressing snake-bite envenoming issues is being developed and will be supported by a WHO technical working group. A stakeholders meeting is planned for 2018 prior to the publication of the roadmap but resources and funding will be required if the plan is to be implemented. There is however growing political will to address this issue and a draft World Health Assembly resolution has been developed, led by Costa Rica and supported by 30 other WHO Member States. It is expected that the resolution will go to the WHO Executive Board in January 2018 and to the World Health Assembly in May 2018.

The Committee thanked Dr Williams for his report and discussed some of the issues raised. In response to one query, Dr Williams pointed out that although there are around 260 medically relevant snake species there was also some degree of commonality of toxins. The production of antisera against the venoms of around 100 different snake species might allow for the treatment of most snake-bites. It should also be possible to prepare polyvalent sera that can neutralize several different snake venoms. However, the characterization of antivenoms based on ED_{50} was challenging. It was further pointed out that the standardization of venom would be crucial, with the importance of reference venoms having been identified at the previous ICDRA meeting and efforts already under way in some countries to produce reference standards. Both the improved characterization of venoms to help assure the quality of the raw

[5] See: http://www.who.int/bloodproducts/snake_antivenoms/en/

material used to raise animal antisera and the development of in vitro assays for potency testing to replace ED_{50} testing would likely result in significant improvements in future. One other issue of concern was that animal welfare standards in some countries were not always sufficiently addressed which had led to legitimate concerns by animal-rights campaigners and which potentially jeopardized antivenom manufacture.

2.4.5 Update from the WHO Product Development for Vaccines Advisory Committee

Dr David Kaslow provided the Committee with a brief overview of the outcomes of the 2017 WHO Product Development for Vaccines Advisory Committee (PDVAC). A range of vaccine development and related issues across a wide range of pathogen areas had been addressed. This had involved discussion of current developments in the areas of HIV, tuberculosis, malaria, influenza, gonorrhoea, RSV, Group B streptococci, enteropathogenic Escherichia coli, shigella, herpes simplex virus (HSV) and CMV. In addition, following the publication by WHO in 2017 of the first-ever list of antibiotic-resistant pathogens that pose the greatest threat to human health, Dr Kaslow reported that PDVAC had recommended the development of a quantitative framework through which the public health impact of vaccines in combating antimicrobial resistance (AMR) could be evaluated.

A number of cross-cutting issues were then identified by Dr Kaslow with particular relevance to the activities of the Committee. For example, PDVAC had expressed an interest in the development of several candidate vaccines designed to elicit antibodies to conserved epitopes on the haemagglutinin head or stem of influenza viruses. If successful, these would become the next generation of influenza vaccines. It was also noted that the concept of heterologous prime-boost was under consideration for a number of candidate vaccines, including HIV vaccines. The question had therefore been raised as to whether the Committee might need to provide guidance on the testing or licensing strategies for vaccines using such regimes. PDVAC had also discussed the progress made in developing the new generation of DNA and RNA-based vaccines, a number of which are now in pre-clinical and early clinical development, including candidate vaccines for Zika and influenza viruses. In relation to this, PDVAC had asked if the Committee needed to update current guidelines on nucleic acid based vaccines to include RNA and considerations for maternal immunization. Among the other main topics discussed were developments in passive immunization using mAbs. It was noted that a number of such products were in development against an increasing number of pathogens, including HIV, RSV, *Staphylococcus aureus* and rabies virus. As PDVAC had recommended evaluation of the technical, regulatory and commercial barriers to the development, licensure and availability of mAbs specifically for use in LMIC, the need for guidance from the Committee on the testing and licensing of mAbs for use in neonates was also raised.

The Committee thanked Dr Kaslow for his helpful update and noted the specific points raised for its consideration. In particular, the interest expressed by PDVAC in the new projects outlined complemented that of the Committee. An international standard for RSV antiserum along with proposed new projects on international standards for candidate influenza vaccines based on conserved antigens were scheduled for consideration by the Committee this year (see sections 8.1.5 and 8.2.4/5). It was also pointed out that WHO guidance is already available on the clinical evaluation of vaccines to be used in heterologous prime-boost regimens.[6] The issue of recent developments in nucleic acid based vaccines was another topic of common interest to both PDVAC and the Committee, with a WHO consultation on this subject planned for 2018.

2.4.6 Pilot WHO prequalification of biosimilar monoclonal antibodies

Dr Deus Mubangizi reminded the Committee that, as outlined in an earlier presentation (see section 2.1.1 above), WHO was intending to pilot the prequalification of two SBPs as part of efforts to improve access to biotherapeutics by extending the considerable experience of SRAs to NRAs in LMIC. The two SBPs selected for the pilot project – trastuzumab and rituximab – were now included in the WHO Model List of Essential Medicines and manufacturers would be invited to send expressions of interest.

Dr Mubangizi went on to provide the Committee with further details of the project. The pilot prequalification would assess SRA-approved originator products, SRA-approved SBPs and SBPs approved by non SRAs, using one SRA-approved reference biotherapeutic product as a comparator. Project procedures had been developed and were now undergoing public review and comment. For WHO prequalification purposes, candidate products to be provided through the United Nations for use in different countries must meet the quality, safety and efficacy criteria set out in relevant WHO guidelines,[7, 8] including compliance with GMP, GCP and good distribution practices.

[6] Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization: sixty-seventh report. Geneva: World Health Organization; 2017: Annex 9 (WHO Technical Report Series, No. 1004; http://www.who.int/entity/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf?ua=1, accessed 6 April 2018).

[7] Guidelines on evaluation of similar biotherapeutic products (SBPs). In: WHO Expert Committee on Biological Standardization: sixtieth report. Geneva: World Health Organization; 2013: Annex 2 (WHO Technical Report Series, No. 977; http://who.int/biologicals/publications/trs/areas/biological_therapeutics/TRS_977_Annex_2.pdf, accessed 6 April 2018).

[8] Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products (SBPs). In: WHO Expert Committee on Biological Standardization: sixty-seventh report. Geneva: World Health Organization; 2017: Annex 2 (WHO Technical Report Series, No. 1004; http://who.int/biologicals/biotherapeutics/WHO_TRS_1004_web_Annex_2.pdf?ua=1, accessed 6 April 2018).

Two pathways had been proposed for the pilot project – an abridged assessment of SRA-approved innovator products or SBPs (which may lead to waivers for requirements) and full assessment of SBPs already registered by non-SRAs using the SRA-approved reference biotherapeutic product as a comparator and marketed in the authorized country. Dr Mubangizi provided details of the proposed process, which would involve the concept of reliance and the exchange of relevant information between WHO and the SRA or applicant. One major issue arising from the public consultation process was the need to better distinguish between the two assessment pathways – that is, for applicants with products approved by an SRA and those with products approved by other NRAs.

The Committee thanked Dr Mubangizi for his presentation and looked forward to being updated on the outcome of this project.

2.4.7 Model NRA Lot Release Certificate for prequalified vaccines

Dr Ute Rosskopf informed the Committee of a proposal, presently under discussion, to develop a unified certificate for the lot release by NRAs of WHO prequalified vaccines. At present, NRA lot release certificates vary in their content depending on the vaccine, and different certificates may be in use for vaccine released onto domestic or export markets. The objective of developing a common lot release certificate would be to harmonize release practices and to increase acceptance of vaccine lot release certificates by recipient countries.

Dr Rosskopf highlighted that vaccine-specific guidance and model lot release certificates were typically provided by WHO written standards for vaccines, with specific advice on certificate issuance also available.[9] In addition, the WHO National Control Laboratory (NCL) Network for Biologicals (WHO-NNB) had been established in 2016 (see section 2.4.9 below), the objectives of which included harmonizing lot release standards and practices, and fostering reliance on member NCL lot release to reduce redundant testing in recipient countries. The Committee was informed that in collaboration with a WHO-NNB working group, WHO had now developed a proposed template for a model lot release certificate for prequalified vaccines. Dr Rosskopf outlined the format and content of the template certificate which had been provided to the Committee for its consideration.

The Committee thanked Dr Rosskopf for drawing its attention to this development. The view was expressed that numerous issues remained to be resolved in moving towards a harmonized model lot release certificate for

[9] Guidelines for independent lot release of vaccines by regulatory authorities. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 2 (WHO Technical Report Series, No. 978; http://www.who.int/biologicals/TRS_978_Annex_2.pdf?ua=1, accessed 8 April 2018).

prequalified vaccines. The Committee also noted that lot release certificates needed to convey relevant information to the recipient on the vaccine lot released which may not be covered in the general format presented. One point to consider would be whether lot release was on the basis of independent testing by the releasing NCL or on the basis of testing by the manufacturer followed by review of the lot summary protocol by the releasing NRA. The latter procedure is considered to be the minimum basis for vaccine lot release and this may be an important consideration for some NRAs. There was also the question of the impact of using a harmonized model lot release certificate on the product-specific model lot release certificates usually provided in WHO Recommendations and Guidelines for vaccines.

2.4.8 Planned proficiency testing study of a standardized method for determining total and free saccharide content of Hib liquid combined vaccines

Dr Rosskopf reminded the Committee that a key test in the quality control of *Haemophilus influenzae* type b (Hib) conjugate vaccines is measurement of the quantities of conjugated and unconjugated polyribosyl-ribitol-phosphate (PRP) present to ensure that these are within the approved specifications. Only PRP that is covalently bound to the carrier protein – that is conjugated PRP – is immunologically important for clinical protection. However, testing liquid-formulated combination vaccines containing Hib conjugate is challenging and different protocols are used by different manufacturers and different control laboratories.

The Committee was informed of plans by WHO to undertake a proficiency testing study on the use of High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) for the determination of total and free saccharide content of Hib vaccines. This study will aim to evaluate the performance of laboratories in applying a previously identified standardized HPAEC-PAD test protocol that is applicable to all eight WHO prequalified vaccine combinations containing the whole cell pertussis component. The collaborative study is planned to start in the first quarter of 2018 and will involve around 25–30 participating laboratories.

The Committee thanked Dr Rosskopf for her presentation and looked forward to being updated on the outcome of the study in due course.

2.4.9 Vaccine prequalification – establishment of the WHO-NNB

Dr Rosskopf provided the Committee with an update on recent developments in the area of WHO vaccine prequalification activities. To date, the independent laboratory testing of vaccines both pre- and post-prequalification had been undertaken by a limited number of WHO-contracted NCLs. However, the present

system faced a number of challenges, including: (a) the increasing number of sophisticated and complex vaccines; (b) the globalization of the vaccine industry (with an increasing number of production sites); (c) the limited capacities of regulatory authorities in both developed and developing countries; and (d) redundant testing by multiple countries leading to delays in vaccine supply and subsequent shortages. In addition, there number of applications for vaccine prequalification is increasing and pre- and post-prequalification quality control testing is both costly and demanding.

In response to these challenges, and in an effort to improve the efficiency of the WHO prequalification process and utilization of resources, the WHO Vaccine Prequalification Team was now working on a number of initiatives. These included efforts to: (a) harmonize test methods; (b) provide hands-on training in quality control methods; (c) reach agreements with manufacturers of prequalified vaccines to enable confidential reporting of lot release data by NCLs to WHO; (d) use the NCL of the country of production for quality control testing; and (e) shortening lead times for vaccine shipments.

Against this backdrop, the need for a WHO network of NCLs involved in prequalification testing had been raised by WHO laboratories and other key stakeholders. At a 2016 meeting, attended by representatives from 21 NCLs, manufacturers' associations and EDQM, it was agreed that a WHO National Control Laboratory Network for Biologicals (WHO-NNB) should be established. This proposal then received subsequent support in the form of a recommendation adopted by the 17th ICDRA held in Cape Town, South Africa in late 2016. Dr Rosskopf reported that the WHO-NNB terms of reference had now been developed and information-sharing agreements were being worked out. The first meeting of the new network was scheduled to take place in India in late 2017.

The Committee thanked Dr Rosskopf for her presentation and noted these developments.

2.5 Strategic issues

2.5.1 Standards for priority pathogens for public health emergencies

Dr Martin Friede reminded the Committee of the new WHO initiative – the Blueprint for Research and Development: Responding to Public Health Emergencies of International Concern (R&D Blueprint) – which had been brought to the attention of the Committee at its previous meeting. The R&D Blueprint had been developed in the light of previous epidemics, particularly the 2014–2016 Ebola epidemic, in order to accelerate R&D preparedness and effective collaboration in advance of any new epidemic. A list of priority pathogens had been developed and roadmaps constructed, with target product profiles for vaccines and diagnostics now under development.

A number of gaps in regulatory preparedness had also been identified at a recent WHO Informal Consultation.[10] These included a lack of coordinated emergency regulatory processes, weaknesses and lack of capacity in drug regulatory systems, limited capacity and experience in stakeholder communication, poor engagement of product developers with affected regulators and weakness in the regulation of supply chains. Meeting participants had also reviewed the WHO EUAL procedure developed to provide regulatory decision-making support to impacted countries and United Nations procurement. Details of the range of outcomes and proposed actions resulting from the review process can be found in section 2.1.1 above.

The Committee was informed that the R&D Blueprint also covers the development of tools used to evaluate vaccines, as well as tools for collaboration, data exchange and sample sharing. Of particular relevance to the work of the Committee was the development of reference reagents for priority pathogens and the potential need for new norms and standards tailored to the epidemic context.

The Committee thanked Dr Friede for the update provided and discussed a number of the issues raised. It was pointed out that international standards and reference reagents were now available in the case of both Zika and Ebola but were seemingly not well used. There was thus a need to consider how best to advertise their availability and encourage their implementation. The importance of having reference materials available during early vaccine development and evaluation was also highlighted, and could be one of the goals of the Coalition on Epidemic Preparedness Innovations (CEPI). The Committee heard that CEPI activities would indeed involve the promoting and funding of the development of standards, reagents and assays, initially for a limited number of priority pathogens. It was considered that collaboration between CEPI and the Committee would be vital in achieving a coordinated and timely outcome at the global level. The need for developing WHO guidance documents for priority pathogen vaccines or further guidance on prime-boost vaccines should also be kept under review as the R&D Blueprint evolves. In addition, consideration should be given to the development of more flexible and dynamic approaches for developing and establishing standards for the quality, safety and efficacy of products intended for use in public health emergencies.

[10] WHO Informal Consultation on options to improve regulatory preparedness to address public health emergencies. Geneva, 17–19 May 2017. Meeting report. Geneva: World Health Organization; 2017 (http://www.who.int/medicines/news/2017/PHEmeeting-reportIK-EG16_Nov_2017.pdf, accessed 8 April 2018).

2.5.2 International standards and reference preparations – revision of TRS 932 Annex 2

Dr Clare Morris informed the Committee that discussions held during the 2017 meeting of the Standardisation of Genomic Amplification Techniques (SoGAT) group had highlighted a number of issues relevant to the ways in which WHOCCs produce, evaluate and distribute international standards and reference preparations. Current approaches to developing and replacing WHO international standards are based upon guidance provided by WHO in 2004.[11] Dr Morris indicated that the points raised could impact upon both the content of the current guidance and the procedures established in different WHOCCs.

The question had been raised as to whether manufacturers have to recalibrate their (otherwise unchanged) systems when a replacement international standard becomes available. Current approaches vary, with some manufacturers keeping their system calibrated to previous versions of the standard while others recalibrate to the most current. However, WHO assures the "continuity of unitage" and further guidance in this area is sought. Another point raised was whether replacement standards should be calibrated against a stockpile of the first international standard or against the most recent batch of international standard. Although calibrating against the first (or other early) standard would likely prevent potential drift of the IU, current practice is to assess candidates against the current standard, potentially favouring drift in the IU across batches. One implication of recommending the first option would be the need for the long-term storage of ampoules of the first international standard at low temperature (for example, at −80 °C) for future replacement studies. This requirement would need to be communicated in a guidance document and implemented.

The proposal was also made that that when a standard is replaced, only commercial assays should be included in the replacement collaborative study on the basis of their assumed higher consistency, and the possibility of a drift in IU introduced by "less sensitive" laboratory-developed tests. The argument against this approach is that it would not allow for a check to be made on the harmonization of locally developed tests, nor on assays that may have been developed after the assessment of the previous standard.

The Committee thanked Dr Morris for her report, following which there was considerable discussion of these and related issues. It was widely agreed that the current WHO Recommendations required revision and updating to include

[11] 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%202_Inter%20_biol%20ef%20standards%20rev2004.pdf?ua=1, accessed 8 April 2018).

more relevant examples. Currently the document makes reference only to the development of reference standards for vaccine potency assessment, and no reference to topics such as commutability assessment and calibration of secondary standards. It was urged that any revision process take into consideration all the different target audiences and seek their views and inputs.

After highly detailed and wide-ranging technical discussion, the Committee concluded that further guidance was evidently needed on all of the many issues raised. It would be very timely to now review in detail the current WHO Recommendations and to develop up-to-date guidance on the production and evaluation of international standards for IVDs, blood products, biotherapeutics and vaccines. The Committee also considered it worthwhile to explore the need for a companion document primarily directed towards the users of international standards and other reference preparations which could include guidance on the calibration of secondary standards, as well as broader metrological considerations. The Committee requested that the broad range of issues raised be discussed by WHOCCs and that specific proposals be presented to the Committee for consideration at its next meeting.

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

3.1 Biotherapeutics other than blood products

3.1.1 Guidelines on procedures and data requirements for changes to approved biotherapeutic products

Changes are essential for the continual improvement of the manufacturing process and for maintaining state-of-the-art controls on biotherapeutic products, and such changes often need to be implemented after the product has been approved (that is, when it has been licensed or when marketing authorization has been received). Changes may be made for a variety of reasons including: (a) to maintain routine production (for example, replenishment of reference standards or change of raw materials); (b) to improve product quality, or the efficiency and consistency of manufacture (for example, changes in the manufacturing process, equipment or facility, or adding a new manufacturing site). Changes may also need to be made to the product labelling information to reflect, for example, a new indication, a change in the dosage regimen, information on co-administration with other medicines or improvement in the management of risk by the addition of a warning statement for a particular target population.

Biotherapeutic products are an increasingly important component of global health care and several WHO guidelines on their regulatory evaluation are available. During international consultations on the development of these WHO guidelines, and during their implementation, it became clear that there was a need to develop specific WHO guidelines on changes to approved biotherapeutic products in order to help address the complexity and other challenges associated with the global life-cycle management of such products. In May 2014, the 67th World Health Assembly adopted two relevant resolutions: one on promoting access to biotherapeutic products and ensuring their quality, safety and efficacy (WHA67.21) and the other on regulatory systems strengthening (WHA.67.20). In support of these resolutions, WHO had been requested to provide guidance, particularly on how to deal with increasingly complex biotherapeutic products, including SBPs. In addition, it had been recommended during the 16th ICDRA that WHO assist Member States in ensuring regulatory oversight throughout the life-cycle of biotherapeutic products.

A WHO Guidelines document had therefore been prepared to provide guidance to NRAs and manufacturers on the regulation of changes to already licensed biotherapeutic products, including SBPs, in order to assure their continued quality, safety and efficacy, as well as continuity of supply and access. These WHO Guidelines note that the implementation of new regulations should not adversely affect product supply and accessibility. Therefore, NRAs are

strongly encouraged to establish requirements that are commensurate with their own regulatory capacity, experience and resources, and to apply the concepts of reliance or work sharing, or to use collaborative approaches, when reviewing post-approval changes. The NRAs of procuring countries are encouraged to consider the establishment of procedures for the expedited approval of changes based on previous expert review and approval of the same changes by the NRAs of the countries in which the products are licensed, or based on the decision of a recognized regional regulatory authority.

The Committee was informed that the latest version of the proposed WHO Guidelines (WHO/BS/2017.2311) was the result of three rounds of international consultations during 2016–2017 and one informal consultation. Although no major issues had been raised during the final public consultation (since these had already been addressed) a number of points for clarification had been identified and addressed. The view expressed by industry was that these WHO Guidelines would be extremely valuable at the global level and were very much welcomed.

The Committee reviewed the document WHO/BS/2017.2311 and reflected upon the points raised in the final public consultation. Following discussion, the Committee concluded that no major issues remained to be resolved but indicated that a number of minor amendments to the document be made as these were considered to be helpful clarifications. Subject to these changes being made to the text, the Committee recommended that the guidelines be adopted and attached to its report (Annex 3).

3.2 Cellular and gene therapies

3.2.1 Global activities in cell therapy products

The Committee was reminded of the outcome of its discussions on this topic during its previous meeting and provided with an update on the progress made since then. At that time, the Committee had recognized that new cell-based medicinal products – referred to as cell therapy products (CTPs) – have great potential in the treatment of various diseases and would become important future public health interventions. There was also a clear consensus within the Committee that global harmonization in the cell therapy field is needed and that WHO should become engaged in this area. The Committee had recommended that WHO collaborate with a range of international groups active in cell therapy, with the goal of providing a common guideline document.

It was felt that the document should focus on somatic and not stem cell therapy and should include quality considerations. An agreed definition of cell therapy would also be helpful, along with clarification of whether genetically modified cells should be included or considered under gene therapy. At the 16th ICDRA in 2014, it had been concluded that products containing genetically

modified viable cells should be considered CTPs. However, products containing viable cells which are used in transfusion medicine (for example, thrombocyte, erythrocyte or granulocyte concentrates) or for haematopoietic reconstitution should not be considered to be CTPs. Conversely, there were many cases where genetically modified cell therapy and tissue-engineered products had been excluded from the area of CTPs. In this context, the Committee considered that the development of harmonized definitions and terminology would be particularly helpful for countries now setting their own national requirements in this area. Although deliberations on the development of measurement standards for CTPs was considered to be premature at this time, an analysis of licensed and clinical trials of CTPs in various countries since the previous meeting of the Committee showed the field to be extremely active worldwide.

The Committee also heard that WHO had been involved in discussions organized by the International Pharmaceutical Regulatory Forum Cell Therapy Working Group on the preparation of a draft reflection paper entitled *General principles to address the nature and duration of follow-up for subjects of clinical trials using cell therapy products*. Furthermore, an overview of the current regulatory landscape, along with an outline of the common principles that may facilitate future discussions on the regulatory evaluation of these products, had been developed at a 2016 meeting organized by the International Alliance for Biologicals. Many experts saw no reason to exclude stem cell therapy from standardization activities and had proposed that WHO include both stem cell and somatic cell therapies in future WHO standardization activities.

The Committee discussed these and other developments and agreed that WHO standardization activities should include stem cells. The Committee also recommended that WHO urgently establish a small working group of experts to consider further the most appropriate approach and timeframe for developing WHO guidelines for CTPs and to update the Committee on the further progress made in this complex and rapidly developing field at its next meeting.

3.3 In vitro diagnostics

3.3.1 WHO IVD prequalification: update report

The WHO prequalification (PQ) programme for IVD devices aims to promote and facilitate equitable access to safe, appropriate and affordable IVDs of good quality. WHO IVD PQ involves a comprehensive assessment of individual IVDs using a standardized procedure to determine whether or not a product meets the necessary requirements. This approach is based on international regulatory practice with a particular focus placed on IVDs for priority diseases. The Committee was informed that the activities of the IVD PQ group are coordinated through EMP, with its scope of work currently lying primarily in the area of rapid diagnostic tests (RDTs), enzyme immunoassays (EIAs), flow cytometry and NAT-

based assays for the management of a number of bloodborne diseases (including malaria), with IVDs for HPV having recently been added to the programme.

IVD manufacturers can submit a dossier to the PQ group at any time for either a full or abridged PQ assessment. The abridged protocol is used in cases where a stringently assessed version of the product is submitted for PQ or where a non-stringently assessed (rest-of-world) version is submitted but a stringently assessed version also exists that is not substantially different. Inspections of IVD manufacturers are performed, with the frequency and degree of scrutiny of inspections determined using a risk-based approach.

During 2016–2017 the IVD PQ group experienced a surge in demand for malaria RDT assessment, with the benefits of WHO PQ being increasingly recognized by manufacturers in light of changes in WHO procurement policy criteria. To date, the group had assessed 20 applications in 2017, with a clear trend towards submissions from new manufacturers.

The Committee was then provided with an outline of an alternative approval process designed to speed up PQ assessment. In this scenario, a manufacturer could select a laboratory from the list of Prequalification Evaluating Laboratories. The selected laboratory then informs the IVD PQ group that an evaluation has been commissioned. The manufacturer would then bear the cost of the evaluation and would be responsible for coordinating directly with the chosen laboratory. This process was expected to provide greater flexibility as manufacturers can choose between following the standard PQ pathway in which WHO mandates the evaluation or mandating a WHO-assessed laboratory to evaluate the product directly. It is envisaged that such an approach will save time in cases where dossier screening is straightforward, and will reduce the need for WHO coordination, bring evaluations closer to the countries of use, create a broader network of laboratories and reduce the overlapping and duplication of activities. As mechanisms for greater transparency are now in place, a number of IVD PQ group documents are being revised to clarify issues of eligibility, fees and scrutiny of assessment.

To assist manufacturers, the group also produces guidance documents that appear either in its Technical Guidance Series or Technical Specification Series. These documents are intended to provide clear directions on the extent of validation and verification required of IVD manufacturers, as well as guidance on the formulation of a suitable design dossier. Among its range of collaborative and other activities, the IVD PQ group provides inputs to a number of external agencies, including ISO and CLSI, in the development of standards and guidelines, evaluates new IT business solutions, participates in working groups and develops and expands assessor pools, including through the training of assessors. There were also plans to extend the scope of the work to include cholera IVDs with technical specifications and laboratory protocols having been developed and technical expert assessors identified.

During discussions the Committee raised the issue of the degree of awareness among new start-up companies of the activities of the WHO IVD PQ group. It was suggested that members of the group could attend relevant meetings in order to raise the profile of this work. Discussion then moved on to the proportion of IVDs that were rejected. The Committee was informed that this varied considerably according to the maturity of the testing method involved. For example, whereas most EIAs and NAT-based assays are approved, the rejection rate for RDTs, especially for malaria, was much higher. However, it was also noted that there has been an improvement in the quality of manufacturer assessments in this field as the commercial benefits of WHO PQ were increasingly being recognized.

3.3.2 Human immunodeficiency virus rapid diagnostic tests for professional use and/or self-testing

The Committee was provided with an overview of the main elements required in a technical dossier submitted by a manufacturer for the purpose of WHO IVD PQ, with particular reference to the performance studies required. Current WHO guidance[12] instructs that for each study the manufacturer should provide a study description, study identifier, product identifier, IFU version, study/report dates (and summary of findings), conclusions reached in regard to meeting the predefined objectives, a study protocol and a full report.

Examples were then given of the type of TSS and TGS documents available from the IVD PQ group. Each of these documents addresses specific aspects of IVD validation to help manufacturers improve the quality of their IVDs. It was intended that such documents would be read in conjunction with relevant international and national standards and guidance.

The presentation then focused on the details of the proposed new TSS document on HIV RDTs intended for professional use and/or self-testing. The need for such guidance was first identified in 2015 and a small drafting group consisting of PQ dossier assessors and external experts was assembled to produce a first draft. This draft was first published on the WHO website in September 2016 with comments invited over a 3-month period from regulatory agencies, manufacturers and professional societies. Following review of all comments received, a revised version was produced in December 2016 in order to elicit further comments prior to the presentation of the document for consideration by the Committee.

[12] Instructions for compilation of a product dossier. Prequalification of In Vitro Diagnostics Programme. Geneva: World Health Organization; 2014 (PQDx_018 v3, 27 August 2014; http://www.who.int/entity/diagnostics_laboratory/evaluations/141015_pqdx_018_dossier_instructions_v4.pdf?ua=1, accessed 7 April 2018).

The Committee was informed that most of the further comments received related to minor editorial changes which for the most part had been incorporated into the document WHO/BS/2017.2305. One comment questioning the necessity to validate kit stability once opened was not incorporated as the immediate use of kits could not always be assumed in field settings in LMIC. The Committee considered the document WHO/BS/2017.2305 and recommended that it be adopted and attached to its report (Annex 4). The question was then raised of whether the future oversight of such documents would fall to the newly established SAGE IVD. Clarification was provided that, although its precise remit had yet to be established, it was envisaged that SAGE IVD would oversee WHO PQ activities from a more strategic aspect.

3.3.3 Establishing stability of in vitro diagnostic medical devices

Although the stability of a diagnostic device is an essential characteristic, many RDT manufacturers were not sufficiently familiar with suitable procedures for assessing this. The lack of appropriate stability studies noted by the WHO IVD PQ group indicated that manufacturers were not taking into account the actual environmental and other conditions of use of products in LMIC. As a result, many stability studies had been undertaken under optimal conditions that had satisfied regulatory requirements in high-income countries but which did not reflect conditions in a field setting in LMIC, where the cold chain supply may be limited and where conditions such as dust and extreme humidity are a reality.

It was therefore proposed in 2015 that a WHO IVD PQ TGS document be developed that explicitly outlined the necessary requirements for establishing the stability of IVD medical devices. Although other guidelines and standards were available on this topic, it was felt that these were often written in language that was not well understood by manufacturers in LMIC. It was therefore intended that the TGS document would highlight the implicit principles required to address stability evaluation using examples relevant to the intended audience, such as assessing extremes of temperature, humidity and the effect of light on repeated opening. There was also a need to highlight the requirement to generate stability data for each critical component, and to provide greater clarity on the minimum number of lots that should be tested. Guidance was also to be given on assessing the suitability of specimens used for stability assessment.

Guided by these considerations, a first draft was produced in August 2015 and a round of public comments invited in December 2015. A version of the document incorporating all comments (WHO/BS/2017.2304) was then produced. The Committee was informed that most comments received during the consultation phase were editorial and had been accepted and incorporated. It was indicated that although the document was being submitted for consideration by The Committee, an additional annex was to be finalized over the coming

months comprising case studies for IVD medical devices. Clarification was sought and confirmation received that the additional annex would constitute a "how to" guide complementing the principles provided in the main document. The possibility of separately reviewing and adopting the additional annex at a later date via a WebEx meeting of the WHO network of collaborating centres for blood products and in vitro diagnostics was raised and it was agreed that this suggestion would be presented to the Committee during its closed session.

The Committee considered the document WHO/BS/2017.2304 and recommended that it be adopted and attached to its report (Annex 5). Consideration of the additional annex with a view to its adoption would be undertaken prior to the next meeting of the Committee in 2018.

3.3.4 WHO consultation on the First WHO International Standard for anti-rubella immunoglobulin

The Committee was informed that a WHO consultation had been held in June 2017 to discuss a number of issues associated with the use of the First WHO International Standard for anti-rubella immunoglobulin. Since 1966, a sequence of three WHO measurement standards for anti-rubella had been used, with the current material (RUBI-1-94) having been in use since 1996. This material had been derived from human normal immunoglobulin obtained from healthy Danish volunteers attending blood donation centres.

Although data from the establishment study are limited, it appears that the current standard gives different results across laboratories using a range of methodologies. It is also widely acknowledged that the use of the material had evolved over time from its initial established purpose in therapeutic monitoring to its use now in calibrating diagnostics to establish vaccine-mediated protection. In essence, a shift has occurred away from the measurement of functional antibody activity to evaluation of their binding ability in high-throughput assays. Problems in the standardization of such assays are widely acknowledged.

Furthermore, since the 1980s, the protective cut-off for immune protection has been changed from 15 IU/ml to 10 IU/ml. This figure was largely derived from values close to the limit of detection in neutralization assays. However, it has been demonstrated that once vaccinated, individuals often have titres < 10 IU/ml and yet are known to be protected due to the absence of reported rubella cases amongst vaccinated individuals. Additionally, epidemiological studies have demonstrated that there have been no cases of congenital rubella in individuals with values < 10 IU/ml, suggesting that values < 10 IU may be protective.

The Committee was provided with a summary of the main outcomes of the consultation. There was agreement that the current international standard should continue to be made available as a well-characterized reference material.

However, IVD manufacturers, regulators and assay users should be made aware (through the amending of the IFU) of its potential lack of commutability when used as a calibrant. At the same time, diagnostic expert committees, vaccine efficacy evaluators and regulators should be encouraged to reconsider the appropriateness of quantitative anti-rubella determinations. Other discussion points raised included the continuing relevance of using 10 IU/ml as an assumed immunity threshold and the potential need for further studies to resolve this and other issues. In general, the question remains of whether the current standard should continue to be made available following changes to the IFU and if so what mechanisms should be used to ensure that stakeholders are made aware of the relevant issues and WHO recommendations.

The Committee agreed that the First WHO International Standard for anti-rubella immunoglobulin should continue to be made available as a well-characterized reference material. The Committee further agreed that manufacturers, regulators and assay users should be made aware of its potential lack of commutability and other limitations in the IFU, and that stakeholders in the diagnostic field should be encouraged to reconsider both the appropriateness of quantitative anti-rubella measurement for the determination of immune status and the use of 10 IU/ml as a cut-off point for assessing immune protection. To establish the protection status of individuals, anti-rubella determination using high-specificity qualitative assays should be considered as an alternative approach to antibody quantitation. In conclusion, the Committee proposed that the outcomes of the consultation and its own subsequent conclusions should be disseminated to technical and relevant clinical audiences, for example in a scientific publication in a high-impact journal and through targeted distribution of this Committee report.

3.4 Vaccines and related substances

3.4.1 Guidelines on the quality, safety and efficacy of Ebola vaccines

The Committee was reminded that as part of ongoing WHO efforts to support the development of Ebola vaccines, draft WHO Guidelines had been prepared on the scientific and regulatory considerations relating to their quality, safety and efficacy. The proposal to develop such Guidelines had been endorsed by the Committee in 2014 and various drafts had now been prepared and reviewed during a series of WHO informal consultations and public consultation, and by the Committee itself. The Committee was reminded that work had started on the Guidelines during the evolving Ebola epidemic. With the end of the large-scale outbreak in Africa in 2016, Ebola disease had returned to its previous sporadic pattern. This epidemiological situation made the evaluation of Ebola vaccine efficacy and licensing more challenging. Interest also shifted away from monovalent Ebola Zaire vaccines to multivalent preparations directed

against more than one Ebola strain as well as the Marburg virus. Developing the Guidelines had therefore presented many challenges, not least in keeping up to date in a rapidly evolving situation.

At its meeting in 2016, the Committee had considered a version of the WHO Guidelines for adoption but after extensive discussion agreed that the guidance given on multivalent Ebola vaccines and on the clinical evaluation of vaccine candidates using innovative clinical trial designs would benefit from expansion. There was also a need to provide guidance on how to evaluate and license Ebola vaccines subsequent to the potential licensure of one of the advanced vectored vaccines. Further revision of the document was therefore undertaken to address the comments received from the Committee and other experts. These revisions were reviewed through a process of international public consultation through the WHO website in 2017 and no further key issues were identified. The majority of comments received concerned improvements in clarity or the updating of cited publications. Respondents generally considered the document to be very comprehensive and of value in the evaluation of vaccines other than Ebola vaccines.

The Committee was reminded of the overall structure and content of the Guidelines which include guidance on regulatory expectations in relation to the quality, nonclinical and clinical aspects of vaccines submitted for full licensure. Additional text considers those aspects of Ebola vaccine development which might be accelerated during a public health emergency. Particular attention is given to viral-vectored vaccines as these are currently the most advanced vaccine candidates. The latest revision of the Guidelines (WHO/BS/2017.2327) also takes into account the fact that Ebola vaccine development had been discussed by the WHO SAGE on Immunization. This allowed for a number of streamlining enhancements, notably the replacement of an earlier appendix on Ebola vaccines currently in clinical trials with a reference to the report of the SAGE Ebola Working Group which includes a detailed listing of such vaccines.

The Committee noted the comments and suggestions which had been received by WHO and expressed its agreement on the way in which these had been addressed. After making a number of further clarifications to the text, the Committee recommended that the document WHO/BS/201.2327 be adopted and attached to its report (Annex 2). The Committee also commended the WHO Secretariat and the drafting group for all their efforts in developing the document under such difficult and rapidly changing circumstances.

4. International reference materials – antibiotics

All reference materials established at the meeting are listed in Annex 6.

4.1 Proposed new projects and updates – antibiotics

4.1.1 Proposed Third WHO International Standard for erythromycin

Erythromycin is used globally as an antibiotic and is listed as a "watch group antibiotic" in the WHO Model List of Essential Medicines (March 2017). The international standard for erythromycin is used to calibrate regional and national secondary standards, as well as manufacturers' in-house standards, all of which are routinely used to guarantee the appropriate filling and dosing of erythromycin preparations. There is therefore a global need for this standard.

The Committee was informed that stocks of the Second WHO International Standard for erythromycin were running low and a replacement standard was now required. Bulk material would be obtained from a major manufacturer and then suitably formulated and processed by EDQM. The collaborative study required to calibrate the replacement would involve pharmacopoeias, NCLs and manufacturers, and would be followed by appropriate statistical evaluation using the current international standard as the material against which the replacement standard would be calibrated. Around 12 laboratories from different regions of the world were expected to participate in the study.

Mindful of the issue of widespread and increasing resistance to antibiotics worldwide, the Committee endorsed the proposal (WHO/BS/2017.2328) to establish a Third WHO International Standard for erythromycin. In addition, the Committee recommended that the current WHO listing of international standards for antibiotics be reviewed and stocks updated as required to ensure their ready availability.

5. International reference materials – biotherapeutics other than blood products

All reference materials established at the meeting are listed in Annex 6.

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

5.1.1 Second WHO International Standard for parathyroid hormone 1-34 (recombinant, human)

Human parathyroid hormone 1-34 is the N-terminal biologically active fragment of parathyroid hormone. The recombinant form of this peptide (rhPTH1-34), expressed in *Escherichia coli* cells, is commonly known as teriparatide and is prescribed in the USA and Europe as a treatment for osteoporosis. The product is under patent protection until 2019, after which it is anticipated that teriparatide SBPs will reach the market.

The First WHO International Standard for parathyroid hormone 1-34 (recombinant, human) was established in 2007 for the calibration of therapeutic teriparatide preparations. This material contained rhPTH 1-34 donated by Eli Lilly & Co. The Committee was informed that this same manufacturer had again donated rhPTH1-34 for the purpose of producing a replacement for the current standard, stocks of which were now running low.

The candidate material (NIBSC code 15/304) had been formulated and distributed into ampoules for evaluation in an international collaborative study. Twelve participating laboratories in eight countries were asked to determine the mass content of the candidate standard using high-performance liquid chromatography (HPLC) in order to calibrate the candidate material against the same primary calibrant used to calibrate the first international standard. Mean estimated rhPTH1-34 content of the candidate standard was 0.914 mg/ampoule (CV = 2.16%; n = 28; 95% confidence interval of 0.902–0.926 mg/ampoule). Assessments were also made of the purity, bioactivity and stability of the candidate standard. Mean estimated purity was 99.08% (CV = 0.59%, n=11) with bioassay data showing good agreement between the potencies of the candidate material and the current international standard. Results also indicated that the candidate material was sufficiently stable to serve as an international standard and plans were in place to assess its stability again in 12 months.

However, the Committee was also informed that the estimated content of the candidate material in terms of the current international standard was 0.936 mg/ampoule – 2.4% higher than the estimate of 0.914 mg/ampoule obtained using the primary calibrant. HPLC assays of the current international standard suggested a lower content than had been assigned on establishment, almost certainly due to lower precision and greater uncertainty during content

determination compared to current assays. Furthermore, to maintain continuity with the historical unitage it was proposed that the candidate material 15/304 also be assigned a content of 9140 IU (1 IU = 100 ng).

The Committee considered the report of the study (WHO/BS/2017.2312) and recommended that the candidate material 15/304 be established as the Second WHO International Standard for parathyroid hormone 1-34 (recombinant, human) with an assigned content of 0.914 mg/ampoule and 9140 IU/ampoule.

5.1.2 First WHO International Standard for rituximab

Rituximab is a chimeric mouse-human mAb used in the treatment of CD20-positive B-cell lymphoproliferative malignancies, transplant rejection and autoimmune disorders. Rituximab is administered as a monotherapy or in combination with chemotherapy regimens. The exact anti-tumour mechanism of rituximab remains unclear. However, it is assumed that it exerts its effects by various mechanisms involving the binding of its Fab domain to CD20-positive B lymphocytes and the induction of apoptosis – either directly or by the immune effector functions of its Fc domain.

Rituximab appears on the WHO Model List of Essential Medicines for a basic health-care system. The Committee was informed that large sales of the innovator product had driven the rapid growth of SBP development which is expected to widen market competition and increase patient access worldwide. Numerous rituximab SBPs were now under development, with some being at late stages of development. A number of non-originator versions had also been approved in some countries under local regulatory pathways that did not appear to involve the rigorous comparability exercise required.

Because mAbs derived by recombinant DNA technology are structurally complex molecules sensitive to small changes in the manufacturing process there is a recognized global need for their standardization to ensure the quality, safety and efficacy of such products. A proposal to develop an international standard for rituximab had been endorsed by the Committee in 2014. The Committee was reminded that WHO international standards for the biological activity of therapeutic mAbs were intended for the evaluation of bioassay performance, including the calibration and validation of potency assays and must be clearly differentiated from the reference product mAb used to determine biosimilarity. In the case of rituximab, the proposed WHO international standard would be expected to facilitate assessment of the biological activities of products by different stakeholders and thus enable the development of rituximab SBPs that are consistent in terms of quality and efficacy. The proposed standard would define bioactivity units for rituximab but would not define specific activity (IU/mg) requirements.

A preparation of recombinant chimeric rituximab expressed in Chinese hamster ovary (CHO) cells had been donated by Sandoz GmbH. The material had been formulated and lyophilized at NIBSC prior to evaluation in an international collaborative study of its suitability to serve as an international standard. The candidate material (NIBSC code 14/210) was tested in 16 laboratories in nine countries alongside a coded duplicate, a second rituximab lyophilized preparation and an in-house reference standard where available. Comparator rituximab had been purchased and reformulated by NIBSC. All preparations were tested for their complement dependent cytotoxic activity (CDC) with 11 laboratories also testing for their antibody-dependent cytotoxic activity (ADCC). A limited number of laboratories also performed cell-based antibody binding and apoptosis assays. Stability monitoring the candidate material 14/210 showed no loss of CDC or ADCC activity upon storage at the recommended storage temperature of –20 °C. Nevertheless, stability monitoring and prediction studies over a further extended period were ongoing. The study results indicated that the candidate preparation would be suitable to serve as an international potency standard for rituximab and that its use would help harmonize the reporting of rituximab bioactivities by different laboratories using their in-house potency assays.

The Committee considered the report of study (WHO/BS/2017.2309) and following discussion and clarification of a number of points recommended that the candidate material 14/210 be established as the First WHO International Standard for rituximab with the following assigned values:

- 1000 IU of CDC activity per ampoule
- 1000 IU of ADCC activity per ampoule
- 1000 IU of cell-binding activity per ampoule
- 1000 IU of apoptotic activity per ampoule.

The Committee emphasized the importance of explaining very clearly in the IFU that this international standard was to be used solely to standardize bioassays. It is not intended to form the basis of any revised product labelling or dosing requirements as any decisions regarding the use of the IU for specific activity specifications are solely the responsibility of the relevant competent regulatory authorities. The international standard should also be very clearly distinguished from the reference product mAb to be used in the comparability studies of SBPs.

5.1.3 First WHO International Standard for infliximab

Infliximab, the first anti-TNF-α mAb to be developed, is a chimeric mAb consisting of human IgG1 heavy chain and kappa light chain constant regions with fused mouse variable regions. Infliximab was first approved in the USA in

1998 and has been extremely successful in the treatment of various autoimmune diseases or disorders associated with increased TNF-α and resultant excess inflammation. Current therapeutic indications include rheumatoid arthritis (in combination with methotrexate), Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriatic arthritis and psoriasis.

Following recent patent expiration in Europe and imminent expiry in the USA, infliximab is an important target for SBP manufacturers with several such products already approved in the European Union, the USA and several other countries worldwide. The availability of a WHO international standard for infliximab with a bioactivity expressed in IU would facilitate determination of the biological activity of infliximab products and enable its harmonization worldwide, thus ensuring patient access to products which are consistent in quality and effectiveness.

The Committee was informed that a proposed WHO international standard had now been developed in collaboration with the European Pharmacopoeia following endorsement of the project by the Committee in 2012. The Committee was further informed that despite its clinical and commercial success, there are a number of safety and efficacy issues surrounding its use and that monitoring to rationalize treatment strategies was now being considered. Studies had shown that monitoring infliximab serum trough levels as a basis for clinical decision-making had increased both therapeutic and cost effectiveness in a number of indications. Commercially available ELISAs or newly developed mass spectrometry methods were currently used to monitor serum drug trough levels. For each of these methods the availability of an international standard would serve to qualify the in-house reference standards used thus globally harmonizing therapeutic infliximab monitoring.

A preparation of recombinant infliximab expressed in SP2/0 cells had been donated by Celltrion. This was then filled at NIBSC following standardized procedures to produce the candidate material (NIBSC code 16/170). Commercial infliximab had also being purchased and reformulated for use as a comparator product. An international collaborative study was then conducted to evaluate the suitability of candidate material 16/170 to serve as an international standard. Twenty eight laboratories in 16 countries participated in the study using in vitro cell-based bioassays and binding assays. Human serum samples, spiked with differing amounts of the two infliximab preparations, were also assessed to evaluate the suitability of the candidate material in harmonizing currently used methods for determining serum trough levels of infliximab. Stability studies over 9.5 months indicated that the candidate material 16/170 was stable during long-term storage at −20 °C. Furthermore, potency was not diminished after 1 week of storage at either 4 °C or 20 °C following reconstitution, or after repeated freeze-thaw cycles. As no loss in activity was detected at any of the

elevated temperatures, no predicted loss in activity could be calculated. Stability monitoring was reported to be ongoing.

Study results indicated that the candidate material 16/170 was suitable to serve as an international standard for the in vitro determination of infliximab potency. No overall consensus regarding unitage assignment for either ADCC or CDC was reached. In addition, and on the basis of limited data, the candidate material 16/170 also appeared suitable for use in the qualification of in-house standards for tests used in therapeutic drug monitoring of infliximab. However, a further study may be required to facilitate harmonization in clinical practice.

The Committee considered the report of the study (WHO/BS/2017.2323) and following discussion recommended that the candidate material 16/170 be established as the First WHO International Standard for infliximab, with assigned values per ampoule of 500 IU of TNF-neutralizing activity and 500 IU of binding activity. In addition, the candidate material 16/170 was assigned a content of 50 µg/ampoule for use in therapeutic drug monitoring. No unitage was assigned to ADCC or CDC activities.

The Committee noted that this international standard was intended to support assay calibration by defining international units of bioactivity. It is not intended to form the basis of any revised product labelling or dosing requirements as any decisions regarding the use of the IU for specific activity specifications are solely the responsibility of the relevant competent regulatory authorities. The international standard should also be very clearly distinguished from the reference product mAb to be used in the comparability studies of SBPs.

6. International reference materials – blood products and related substances

All reference materials established at the meeting are listed in Annex 6.

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

6.1.1 First WHO Reference Reagent for activated blood coagulation factor X (human)

Activated blood coagulation factor X (FXa) is a trypsin-like serine protease that plays a crucial role in the coagulation cascade. FXa is regarded as an inherent impurity of factor eight inhibitor bypassing activity (FEIBA), an activated prothrombin complex concentrate used in the treatment of haemophilia in patients with inhibitory antibodies against FVIII. In the absence of an international reference standard for FXa, direct measurements of FXa activity in FEIBA have been made relative to working standards calibrated against the non-WHO NIBSC Reference Material for blood coagulation factor Xa (NIBSC code 75/595). This reference material had been sourced from bovine plasma, prepared by an external group and arbitrarily assigned a potency of 1 U/ampoule. With no information on the uniformity of the fill or on the stability of the material, its continued use had been questioned and the development of a replacement material proposed.

Although the measurement of FXa levels in FEIBA is the primary regulatory use for an FXa reference standard, the current standard is also used routinely to calibrate local standards. Potential future uses for a replacement FXa reference material also include the standardization of measurement of FXa contamination in non-activated products such as prothrombin complex concentrates used in the reversal of anticoagulant therapy, and for a recently licensed FX concentrate product used in the treatment of congenital FX deficiency. Standardizing the biological activity of direct FXa inhibitors (as used in anticoagulation therapies) is another significant potential use which would require a human FXa standard due to key differences between bovine and human FXa. The development of a WHO international standard for human FXa would require a large-scale study at a time when the full range of its intended uses were not yet known. It had therefore been proposed that a WHO reference reagent be established as an interim measure.

Donated source material had been formulated and lyophilized, and the resulting candidate material (NIBSC code 15/102) calibrated against 75/595 using direct chromogenic assays. Based on the results of a total of 12 independent assays conducted by two laboratories, a potency estimate for 15/102 was assigned. Intra-laboratory variability was low (geometric coefficient

of variation (GCV) < 4%) with inter-laboratory geometric mean estimates of potency also in close agreement (6.5 and 6.8 U/ampoule). The overall geometric mean estimate of potency of 6.7 U/ampoule was therefore assigned to candidate material 15/102. Long-term stability was assessed in accelerated-degradation studies, with data indicating that the candidate material 15/102 remained stable after 10 months, with no measurable degradation. Predicted activity loss per year at normal storage temperature (–20 °C) was 0.008 % by activated partial thromboplastin time (APTT) assay and 0.012 % by prothrombin time (PT) assay. On-bench stability (after reconstitution) was assessed over 6 hours using PT and APTT assays. No measurable loss of potency was detected within 4 hours of reconstitution.

The Committee considered the report of the study (WHO/BS/2017.2324) and recommended that the candidate material 15/102 be established as the First WHO Reference Reagent for activated blood coagulation factor X (human), with an assigned potency of 6.7 U/ampoule. During discussion, the Committee raised a number of issues in relation to the original selection of bovine material and its continued use. It was further noted that since the change from bovine to human material may require the additional calibration of clotting assays, a statement to this effect should be included in the IFU.

6.1.2 Second WHO International Standard for activated blood coagulation factor IX (human)

Activated blood coagulation factor IX (FIXa) is a highly thrombogenic process-related impurity found in therapeutic prothrombin complex concentrates and monocomponent plasma-derived and recombinant FIX concentrates. As new-generation modified FIX products are now licensed for replacement therapy, there has been a recent increase in demand for the current WHO international standard. As stocks of this international standard were now close to exhaustion, a replacement standard was urgently required.

Bulk starting material prepared by the activation of recombinant human FIX followed by size exclusion chromatographic purification had been donated by Pfizer. The purity of the material was assessed and confirmed by PAGE with silver staining. The estimated specific activity of the bulk was 612 IU/mg. Following formulation at NIBSC, 18 000 ampoules were produced of the candidate material (NIBSC code 14/316). During an international collaborative study, 19 laboratories participated in the value assignment of the proposed Second WHO International Standard for activated blood coagulation factor IX (human) relative to the first international standard. Data sets were generated using purified reagent chromogenic/fluorogenic based assays, one-stage clotting assays based on APTT, one-stage clotting assays based on NAPTT and a thrombin generation test (TGT).

With the exception of one laboratory, similar potencies were obtained for the coded duplicates provided. The overall geometric mean potency determined by purified reagent assays was 10.48 IU/ampoule, with values of 11.67 and 12.10 IU/ampoule produced by assays based on APTT and NAPTT respectively. The results obtained using TGT were similar to those obtained using purified reagent assays. Given some degree of uncertainty concerning the influence of other components involved in clot- and plasma-based assays on the measurement of FIXa it was proposed by NIBSC that the value assigned to the replacement standard should be based on the results of purified reagent assays only – as had been the case with the current WHO international standard.

The Committee considered the report of the study (WHO/BS/2017.2325) and recommended that the candidate material 14/316 be established as the Second WHO International Standard for activated blood coagulation factor IX (human) with an assigned potency of 10.5 IU/ampoule.

6.1.3 First WHO International Standard for blood coagulation factor XII (plasma, human) via assignment of additional analytes to the current Second WHO International Standard for blood coagulation factor XI (plasma, human)

The role of blood coagulation factor XII (FXII) in haemostasis was not previously considered important because its deficiency is not associated with bleeding. However there is now emerging interest in FXII. The finding that it is activated by agents such as mast cells, platelet polyphosphates and clinically used materials such as stents and mechanical valves suggests that it may play a significant role in thrombogenesis, especially in patients with prothrombotic conditions. FXII inhibition may therefore present an attractive option for antithrombotic therapy and various antibodies and inhibitors are in development. There is therefore now a need for supporting assays for FXII, for which there is currently no international standard. A plasma international standard for FXII functional activity (FXII:C) and antigen (FXII:Ag) would support the development of such assay methods and the clinical monitoring of patients.

The 2015 collaborative study required to replace the First WHO International Standard for blood coagulation factor XI (plasma, human) had presented an opportunity to assess the feasibility of establishing an international standard for FXII. Since similar handling and processing conditions (such as avoidance of cold activation and contact with negatively charged surfaces) are needed for both contact factors, the same candidate material could therefore be assigned both FXI and FXII values. The feasibility of this was assessed based on the number and type of assays performed by participating laboratories and on the precision of the data returned. The results indicated that there were a sufficient number of laboratories capable of performing functional and antigenic assays

with reasonable precision, and that this data set could be used for assignment of both FXII:C and FXII:Ag values to the candidate material.

Twenty laboratories took part in a collaborative study to assign FXII:C and FXII:Ag values to the Second WHO International Standard for blood coagulation factor XI (plasma, human) (NIBSC code 15/180). Value assignment was against local normal pooled plasmas which were assumed to have 1 U/ml of functional activity or antigen content. For FXII:C, 28 sets of results from one-stage clotting assays using 13 different APTT reagents against local plasma pools (total number of donors = 566) were returned. Intra-laboratory GCVs were in the range 1–20%, with the majority being < 10%. The overall geometric mean was 0.86 IU/ampoule, with an inter-laboratory GCV of 10%. For FXII:Ag, nine sets of results obtained using three different commercial kits/paired antibody sets and one in-house reagent were analysed against local plasma pools (total number of donors = 216). Intra-laboratory GCVs were in the range 4–12%, with the majority being < 10%. The overall geometric mean was 0.80 IU/ampoule, with an inter-laboratory GCV of 11%.

The Committee considered the report of the study (WHO/BS/2017.2326) and recommended that the First WHO International Standard for blood coagulation factor XII (human, plasma) be established via assignment of an FXII:C unitage of 0.86 IU/ampoule and an FXII:Ag unitage of 0.80 IU/ampoule to the current Second WHO International Standard for blood coagulation factor XI (plasma, human).

7. International reference materials – in vitro diagnostics

All reference materials established at the meeting are listed in Annex 6.

7.1 WHO International Standards and Reference Reagents – in vitro diagnostics

7.1.1 First WHO Reference Reagent for lupus anti-dsDNA serum

Systemic lupus erythematosus (SLE) is a severe autoimmune connective tissue disease in which autoantibodies are generated to a range of antigens. Among these autoantibodies, antibody to double-stranded DNA (anti-dsDNA) is highly specific to SLE, occurring in 70% of cases of SLE against a non-SLE background of < 5%. The level of anti-dsDNA can also be used to monitor disease activity. As a result, the measurement of anti-dsDNA is a widely used diagnostic test for SLE and a range of kits and diagnostic tests are available.

In 1985, a freeze-dried preparation of plasma obtained from a patient with definite SLE had been established as the First WHO International Standard for anti-double-stranded DNA serum and had been used to assign units to diagnostic tests. The Committee was informed that this material was now exhausted and that calls had been made for its replacement with a suitable preparation.

Oligo-specific SLE plasma from a single donor was therefore obtained and following appropriate processing was prepared as a lyophilized candidate standard for lupus anti-dsDNA serum. The candidate material (NIBSC code 15/174) was evaluated in an international collaborative study involving 36 laboratories in 17 countries. Comparisons of the candidate material were made against both local standards (some of which were previously calibrated against the First WHO International Standard for anti-double-stranded DNA serum) and three individual plasma donations from patients with SLE in order to support the evaluation of commutability of the candidate material.

In all laboratories and for all test methods used, the candidate material exhibited anti-dsDNA reactivity. In approximately half of the laboratories, the material behaved in an apparently similar way to local standards and, by inference, to the previous international standard. However in a similar number of laboratories there was observable non-parallelism and no quantitative traceability to the unitage of the previous international standard could be established. Moreover, across the entire study, it was not possible to establish commutability, as a consistent ranking order for the three patient samples was not obtained.

Given the apparent lack of qualitative comparability of this candidate material with the previous international standard, it was considered unwise to establish it as a replacement international standard with a defined unitage in IU based on the previous international standard. It was proposed that candidate material 15/174 be established de novo as the First WHO International Standard

for lupus anti-dsDNA serum with a nominal potency of 100 IU/ampoule, noting the name change from the previous standard for anti-dsDNA serum. Moreover, it was proposed that information be provided to users emphasizing that caution would be needed in transferring the new unitage to existing assay methods.

The Committee questioned why plasma from only one patient had been used for candidate material generation. Despite this also being the case for the establishment of the previous international standard it was suggested that as each patient has different autoantibodies, the use of a pooled sample would be better for the preparation of an international standard for anti-dsDNA serum. The Committee also concluded that it would not be appropriate to establish a first WHO international standard with a similar reagent for the same analyte based on a name change alone and that the previous international standard could not be replaced by any preparation due to the inability to maintain continuity of unitage. The Committee considered the report of the study (WHO/BS/2017.2306) and recommended instead that the candidate material 15/174 be established as the First WHO Reference Reagent for lupus anti-dsDNA serum with a nominal value of 100 U/ampoule. The Committee further indicated that labelling should inform users of the lack of continuity to the First WHO International Standard for anti-double-stranded DNA serum, and that parallelism and commutability had not been established.

7.1.2 Third WHO International Standard for hepatitis A virus RNA for NAT-based assays

Hepatitis A virus (HAV) nucleic acid amplification technique (NAT)-based assays are primarily used in the safety testing of plasma intended for use in medicinal products. It is the recommended method as directed by the European Pharmacopeia monograph, with assays required to detect 100 IU/ml HAV RNA. The WHO international standard is used by blood product manufacturers, clinical laboratories, control authorities and IVD manufacturers to calibrate the secondary standards used in such assays.

The First WHO International Standard for hepatitis A virus RNA for NAT-based assays (NIBSC code 00/560) was established by the Committee in 2003. Although a second batch (NIBSC code 00/562) had also been made from the same material (but lyophilized at a different time point) its stability was brought into question at the time of replacement of 00/560. In 2013, a proposed replacement material was made (NIBSC code 12/234). However, the stability of this material had also been found to be questionable and in 2014 the Committee recommended that the previous candidate material 00/562 be established for an interim period as the Second WHO International Standard for hepatitis A virus RNA for NAT-based assays, and shipped on dry ice to allow continuity of supply, but that an investigation into a stable replacement should begin immediately.

Following a successful pilot study, two candidate materials were taken forward for evaluation in an international collaborative study. One of the candidate materials consisted of human plasma spiked with HAV (NIBSC code 15/276) and the other of human plasma spiked with HAV with the addition of hepes buffer and trehelose (NIBSC code 15/278). Eleven laboratories in 10 countries assessed a total of eight panel members which also included the current international standard. Variability in reported laboratory mean estimates for a number of samples was higher for qualitative assays compared to quantitative assays. Stability data at a 12-month time point suggested that both candidate materials were stable and would therefore be shipped at ambient temperature. It was proposed that the candidate material 15/276 (consisting only of human plasma spiked with HAV) should considered for establishment as the replacement standard, with a potency of 4.49 $\log_{10}$ IU/ml based on the qualitative assay results.

There was some discussion regarding the reasons for the lack of stability observed in previous preparations, which remains unexplained. It was noted that lyophilization of the previous international standard had taken place off site from NIBSC and that the specifics of the process used were not completely known. The candidate materials used for this study had been lyophilized at NIBSC. It was also clarified that the rationale for using only qualitative data sets was based on these being the predominant assay type in the field. However, it was agreed that given the observed high level of consistency of the comprehensive data set that the value assigned to the international standard would be 4.42 $\log_{10}$ IU/ml.

The Committee considered the report of the study (WHO/BS/2017.2308) and recommended that the candidate material 15/276 be established as the Third WHO International Standard for hepatitis A virus RNA for NAT-based assays with an assigned potency of 4.42 $\log_{10}$ IU/ml.

7.1.3 Fourth WHO International Standard for HIV-1 RNA for NAT-based assays

The advent of NAT-based assays in the 1990s allowed for the direct detection of HIV, thus providing a positive indication of infection weeks in advance of the traditionally used serological tests. However, inter-assay sensitivity varied greatly and the need for harmonization across this new technology was recognized, including in the field of blood transfusion safety where concerns had been raised regarding the risk of transfusion transmitted infections occurring through false-negative screening results.

In 2015, the Committee had been informed that stocks of the current international standard were diminishing and the proposal to develop a replacement standard was endorsed. A candidate material (NIBSC code 16/194) was therefore developed using an HIV-1 primary isolate (subtype B) which derived from the same viral stocks used for the production of the two most

recent international standards. As with the current international standard the viral stocks were heat-inactivated using established procedures. Inactivation was then confirmed by cell culture and p24 antigen expression. More than 9000 vials of candidate material were produced and the material assessed in a collaborative study which also included the current international standard.

Twenty one laboratories from 11 countries participated in the study and returned a total of 23 data sets (17 from quantitative assays and 6 from qualitative assays). Analysis of the data for both the candidate material and current international standard revealed two distinct data groups – one consisting of qualitative data and the other of quantitative data. When expressing data as a relative potency to the current international standard this disparity was not repeated. It was also noted that four laboratories that had used the same quantitative assay (Siemens) reported higher results more consistent with the qualitative assays. Stability of the candidate material up to a 12-month time point was satisfactory, with no loss of titre to suggest degradation.

There was some discussion as to the possible reasons for the observed discrepancies. It was highlighted that the only difference between the materials evaluated in this study and previous materials was that the former had been heat-inactivated. However, insufficient earlier data was available to investigate this further. It was also reiterated that limited conclusions regarding commutability could be drawn from the inclusion of only one patient sample.

The Committee considered the report of the study (WHO/BS2017.2314) and recommended that the candidate material 16/194 be established as the Fourth WHO International Standard for HIV-1 RNA for NAT-based assays with an assigned potency of 5.10 $\log_{10}$ IU/ml.

7.1.4 First WHO International Standard for Ebola virus antibodies (plasma, human); and First WHO Reference Panel for Ebola virus antibodies (plasma, human)

The Committee was reminded that in response to the 2014–2016 Ebola epidemic, a First WHO Reference Reagent for Ebola virus antibodies had been developed and established in 2015 as an interim standard with an assigned potency of 1 U/ml. The standard comprised a preparation of convalescent plasma obtained from a patient recovering from Ebola virus disease (EVD) and had been chosen as it could quickly be formulated into an urgently needed standardization material.

Further work had since been undertaken to fully evaluate and develop EVD convalescent plasma samples for evaluation in an international collaborative study with the aim of establishing materials to serve as the First WHO International Standard and First WHO Reference Panel for Ebola virus antibodies (plasma, human). The study assessed six different anti-Ebola samples comprising convalescent plasma pools from Africa, Italy, Norway, the United

Kingdom and the USA, along with a negative human plasma sample, for inclusion in the panel. A total of 17 laboratories in four countries (predominantly in the United Kingdom and the USA) returned 26 data sets from three categories of assay – neutralization of live Ebola virus, neutralization of Ebola pseudotypes and enzyme immunoassays (EIAs). It was highlighted that a number of laboratories using neutralization methods reported the negative plasma sample as positive, particularly laboratories using a lentivirus-vector system. In addition, as potency values derived for convalescent plasma samples from Norway, Italy and the United Kingdom were near the detection limit for some assays, some values were reported as negative. EIA data were analyzed separately and presented as relative potency expressed against the candidate international standard material (NIBSC code 15/262) and the current reference reagent (NIBSC code 15/220). GCVs were lower in all samples when expressed against the candidate material and were in the range 41–92%. The stability of the candidate material 15/262 was demonstrated to be suitable for its use as an international standard and for its shipment at ambient temperatures. Due to the paucity of materials no stability data could be generated for the proposed reference panel members (NIBSC code 16/344).

During discussion, the Committee expressed concern that the stability of the proposed reference panel members had not been assessed, and suggested that their real-time stability should be assessed annually to ensure fitness for purpose. Concern was also expressed regarding a specific proposal made to assign an IU value to a single panel member as an international standard would also be available, and as stability data were lacking.

The Committee considered the report of the study (WHO/BS/2017.2316) and recommended that the candidate material 15/262 (a freeze-dried pool of convalescent plasma from Sierra Leone) be established as the First WHO International Standard for Ebola virus antibodies (plasma, human) with an assigned potency of 1.5 IU/ml. The Committee further recommended that the candidate material 16/344) be established as the First WHO Reference Panel for Ebola virus antibodies (plasma, human) without assigned units. The Committee highlighted that the labelling of the reference panel should clearly indicate the potential for a false-positive result for the negative panel member in some assay types. The Committee also agreed that the current First WHO Reference Reagent for Ebola virus antibodies should remain available for use with its originally assigned potency of 1 U/ml.

7.1.5 First WHO Reference Panel for genomic KRAS codons 12 and 13 mutations

Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations are present in approximately 20% of all cancers and are especially prevalent in colorectal, lung and pancreatic cancers, with 90% of such mutations being located in codons

12 and 13. Patients with KRAS mutations do not respond to anti-Epidermal Growth Factor Receptor antibody therapies, and accurate and reproducible KRAS mutation testing is essential in ensuring that suitable and effective treatment is administered.

Following endorsement of the project by the Committee in 2015, an international collaborative study had been conducted to assess the suitability of a panel of genomic DNA materials as a proposed WHO reference panel for genomic KRAS codons 12 and 13 mutations (NIBSC code 16/250) for use in diagnostics standardization. The panel comprised eight freeze-dried purified genomic DNAs representing the seven most-common KRAS mutations associated with colorectal cancer plus wild-type KRAS codons 12 and 13. The materials were produced from cancer cell lines and from a wild-type lymphoblastoid cell line, respectively.

A total of 56 laboratories from 34 countries participated in the collaborative study with each sent triplicate coded samples of the eight panel members. Laboratories were requested to test these materials using their routine diagnostic assays. Of the 68 data sets returned 36 were derived from quantitative methods, with a wide variety of different methodologies used. Details were provided on the calculation performed to calculate KRAS copy number for each panel member. It was proposed that each panel member should be established with a value relating to genotype, consensus mutation percentage, consensus mutant KRAS copy number and consensus total KRAS copy number. These would be clearly stated in a table in the IFU.

During discussion, it was noted that the replacement of formalin fixation would not happen in the near future and that many laboratories use blood to study mutations. However, for fixed tissue and blood samples the levels of genomic fragmentation would be high, whereas the genomic DNA proposed in this study would be of higher quality than is routinely used in these methods. It was indicated that the IFU should highlight the potential for poor harmonization between assays other than NGS and digital PCR. It was also noted that the genomic DNA panel would provide an improved means of standardizing methods and that going forward it may be possible to produce secondary standards in a different format (such as fragmented DNA) back-calibrated to this high-quality primary reagent. It was further noted that cell line stability may be a problem for replacement panels in the future. With only 2000 panels available, it may also be necessary to consider restricting their distribution to laboratories producing kits, assays or secondary standards to be made available to others. It was also noted that due to the requirement for extended KRAS analysis in all patients presenting with colorectal cancer, the development of panel members representing codons 61, 117 and 146 was also planned.

The Committee considered the report of the study (WHO/BS/2017.2317) and recommended that the candidate material 16/250 be established as the First

WHO Reference Panel for genomic KRAS codons 12 and 13 mutations. The value details of each panel member, relating to genotype, consensus mutation percentage, consensus mutant KRAS copy number and consensus total KRAS copy number were to be provided in the IFU.

7.1.6 First WHO International Standard for human herpes virus 6B DNA for NAT-based assays

Human herpes virus 6A (HHV-6A) and HHV-6B are members of the β-subfamily of herpes viruses and share ~90% sequence homology. First isolated in the 1980s, HHV-6A and B are now recognized as separate viruses. Seroprevalance in adults is high (> 90%) with primary infection occurring in the first 2 years of childhood. HHV-6B predominates in Europe, Japan and the USA while HHV-6A is common in sub-Saharan Africa. Latency is established in monocytes with transplant recipients at increased risk from HHV-6B reactivation linked to organ dysfunction, myelosuppression, encephalitis and graft-versus-host disease. A number of previous studies have highlighted considerable variability in the detection of HHV-6, highlighting the need for a measurement standard.

Two candidate standards were prepared (> 5000 vials each for HHV-6A and HHV-6B) and evaluated for their suitability in an international collaborative study alongside seven other materials comprising the corresponding liquid bulk materials and various clinical samples in different matrices, including two chromosomally integrated samples. Twenty six laboratories from 12 countries returned 36 data sets, the majority of which (34) were from quantitative assays. Raw data estimates for both candidate materials were very similar showing an approximate 2.6 $\log_{10}$ variation across the different quantitative assays, which increased by a further one $\log_{10}$ with the inclusion of qualitative assays in the data set. Agreement between laboratories was improved by using the liquid preparation of the candidate (HHV-6B) material (NIBSC code 15/266) to derive a relative potency assessment. This effect was also mirrored in the HHV-6A candidate material. Evaluation of the stability of candidate material 15/266 up to a 6-month time point indicated no loss in potency. Stability data indicated that this material was stable at −20 °C and at higher ambient temperatures that reflect global shipment. Although study data showed that both candidate materials performed equally well, HHV-6B has the greater clinical diagnostic significance. It was therefore proposed that the candidate material 15/266 be established as the international standard with a value assignment of 7.75 $\log_{10}$ IU/ml. It was pointed out that this value had been derived only from the quantitative estimates.

The Committee considered the report of the study (WHO/BS/2017.2321) and recommended that the candidate material 15/266 be established as the First WHO International Standard for human herpes virus 6B DNA for NAT-based assays with an assigned potency of 7.75 $\log_{10}$ IU/ml.

7.1.7 First WHO International Standard for *Plasmodium falciparum* antigens

Malaria is a mosquito-borne disease endemic to 91 countries and territories. In 2015, there were an estimated 212 million cases of malaria worldwide resulting in 429 000 deaths, mainly among children under 5 years of age in Africa. Malaria is caused by protozoan *Plasmodium* parasites, with the majority of morbidity and mortality due to the species *P. falciparum*. The Committee was informed of the growing market for rapid detection tests (RDTs) for malaria antigens, which are now routinely used for both the diagnosis and management of malaria. The main targets of such products are histidine-rich protein 2 (HRP2), plasmodial lactate dehydrogenase (pLDH) and aldolase.

Although numerous malaria RDTs are currently available, their validation and quality control are limited as there is currently no international standard for the above antigens. At present, clinical isolates or culture-derived materials from the United States Centers for Disease Control and Prevention and the WHO Malaria Specimen Bank are typically used in the evaluation of new assays and technologies. However, demand for these materials is heavy and recent RDTs are characterized by improved sensitivity requiring appropriate reference materials. A suitable reference material produced from a single source could be used worldwide for the quality control and standardization of malaria RDTs.

Following provision of source material by the United States Centers for Disease Control and Prevention, an international collaborative study was conducted to assess the suitability of an in vitro-derived *P. falciparum* candidate material (NIBSC code 16/376) for use as a WHO international standard. The performance of 19 different RDTs from a range of manufacturers was assessed by 13 laboratories in 11 countries using 14 clinical isolates. Data sets obtained from laboratories which had additionally used ELISAs showed variable results with wide-ranging GCVs for both HRP2 and pLDH. However, when these values were evaluated as relative potencies to the proposed candidate material a significant reduction in variability was observed for both analytes. Separate analysis of the results obtained using RDTs showed that all participating laboratories detected HRP2 and pLDH end-point titres of the candidate material using all of the RDTs tested, while HRP2 and pLDH end-point titres of clinical isolates were detected in the majority of RDT assays carried out, indicating that HRP2/pLDH could be more reliably detected in the candidate material than in the clinical isolates tested. Stability evaluation of the candidate material 16/376 indicated no detectable degradation of HRP2 with the pLDH antigen predicted to be stable at −70 °C storage. However, some degradation was predicted at higher storage temperatures and accelerated and real-time stability studies were ongoing. Based on these findings it was recommended that ampoules should be stored at −70 °C and shipped at −20 °C or lower.

The Committee considered the report of the study (WHO/BS/2017.2329) and recommended that the candidate material 16/376 be established as the First WHO International Standard for *Plasmodium falciparum* antigens with the individually assigned unitages of 1000 IU/ampoule for HRP2 and 1000 IU/ampoule for pLDH.

7.1.8 First WHO International Standard for anti-cytomegalovirus immunoglobulin G

Cytomegalovirus (CMV) poses a major health threat to immunocompromised people, and to transplant recipients and other patients undergoing immunosuppressive therapy. Moreover, CMV is the leading viral cause of birth defects. Measurement of anti-CMV immunoglobulin G (IgG) is used for screening, determining serological status, assessing immunity and evaluating the risk of CMV disease. Despite there being no known correlation with protection, the quantitative determination of anti-CMV IgG is considered useful in identifying reactivation.

Due to the lack of an international standard, anti-CMV immunoglobulin tests differ widely in a number of key aspects and the values obtained using different tests are not comparable. The reliability of serological diagnosis therefore depends heavily on the assay used. In light of increasing recognition of the need for an anti-CMV IgG reference material for diagnostic purposes, a proposal to evaluate such a material in an international collaborative study had been endorsed by the Committee in 2013.

A freeze-dried anti-CMV IgG candidate material (PEI code A1) was produced from a pool of three human disease state plasmas purchased from Biomex GmbH – a commercial supplier in Germany. During pre-testing, the candidate material displayed, among other characteristics, both a high IgG level and high IgG avidity, and was shown to be anti-CMV IgM negative and CMV DNA negative. The candidate material was lyophilized to produce 1900 ampoules.

Sixteen laboratories from nine countries participated in the collaborative study, including reference, regulatory and research laboratories, IVD manufacturers and blood banks. A total of 10 study samples were used, with all samples diluted until the test cut-off to determine the endpoint titre. Calibration of the tests using the candidate material A1 was effective in harmonizing the results.

During discussions, it was noted that the test kits used in the study did not appear to correlate very well. It was clarified that whilst the raw data output from each assay did show differences this was not the case when Spearman Rank analysis was carried out. It was further clarified that IgG-only assays are designed for the quantitative interpretation of infectious stage and for following titre development. Such kits may be used for staging in neonatal infections and in post-transplantation situations but for acute infection the measurement of IgM (rather than IgG) or DNA was used.

The Committee considered the report of the study (WHO/BS/2017.2322) and recommended that the candidate material A1 be established as the First WHO International Standard for anti-cytomegalovirus immunoglobulin G with an assigned unitage of 46.4 IU/vial. The Committee also recommended that a number of statements should be made in the IFU regarding issues of commutability, the lack of analytical sensitivity in a minority of tests when diluting low-avidity anti-CMV IgG samples and the unsuitability of the standard for harmonizing anti-CMV IgG/IgM assays.

7.1.9 First WHO International Standard for chikungunya virus RNA for NAT-based assays

Chikungunya virus (CHIKV) is a member of the Alphavirus genus in the Togaviridae family and was first identified as a human pathogen in the early 1950s. The virus is present not only in Africa but also in Asia and the Indian subcontinent and, since 2013, has spread to the Americas, particularly central and southern areas. Small outbreaks have also occurred in Europe. For the laboratory diagnosis of acute CHIKV infections and blood screening, NAT-based assays are considered the most sensitive detection method. There is however currently no standardization of NAT-based assays for the detection of CHIKV RNA. Following endorsement by the Committee in 2010 of the proposal to establish an international standard for CHIKV RNA, an international collaborative study was conducted.

The candidate material chosen was a CHIKV isolate that had been imported into the USA in 2006 and which was characterized as the East-Central-South-African genotype. The virus was propagated in Vero cells and then heat-inactivated. A total of 3524 vials containing 0.5 ml of the candidate material (PEI code 11785/16) were produced in 2016.

Twenty five laboratories from 14 countries participated in the international collaborative study, including IVD manufacturers, regulatory authorities, and clinical, reference and research laboratories. The methods used involved automated or manual extraction followed by testing in a range of qualitative and quantitative in-house and commercial NAT-based assays. In total, 31 data sets (20 qualitative and 11 quantitative) were received from 24 laboratories. The majority of the methods used were real-time RT-PCR assays which targeted several different regions of the CHIKV genome. The lyophilized candidate material 11785/16 was detected by all assays evaluated in the study and was estimated to have a potency of 6.39 $\log_{10}$ U/ml. No significant titre loss was detected at 20 °C, 4 °C, or 20 °C for up to 6 months. However, under accelerated conditions at 37 °C, a log reduction of 0.5 was observed after 6 months. Both real-time and accelerated stability studies were currently ongoing.

The Committee considered the report of the study (WHO/BS/2017.2330) and recommended that the candidate material 11785/16 be established as the

First WHO International Standard for chikungunya virus RNA for NAT-based assays with an assigned unitage of 2.5 x 10^6 IU/ml. It was noted that although shipping on dry ice was proposed, the data suggested that shipping at ambient temperature might be acceptable. It was suggested that the stability of the material at ambient temperature should be further monitored in the follow-up stability studies.

7.1.10 First WHO International Standard for Zika virus antibodies (immunoglobulin G and immunoglobulin M) (human)

The accurate diagnosis of Zika virus (ZIKV) infection, particularly in pregnant women, is a crucial step in making appropriate health-care decisions. Following a negative PCR result, both serum immunoglobulin G and immunoglobulin M are measured to determine prior exposure, where immunoglobulin M would indicate a recent infection. The standardization of serological tests is now required to improve both their sensitivity and specificity. Following its endorsement of a proposal to develop an international standard for use in the calibration and control of ZIKV antibody assays in 2016 the Committee was provided with an update of the progress made.

A candidate material for establishment as an international standard had been formulated by NIBSC and sent out as part of a panel of samples for evaluation in an international collaborative study. Participating laboratories had indicated that they would be using a range of different serological assay types to assess the panel of anti-Zika-positive samples and negative specimens, along with samples containing antibodies to other flaviviruses. Samples were dispatched to laboratories in Central America, Europe, the Far East and the USA. It had been hoped that sufficient data would have been received to permit establishment of the standard during the current meeting of the Committee. However, data sets from a significant number of laboratories were still to be received and no study report was available.

The Committee discussed the possibility of establishing the candidate material as an interim standard but agreement was reached that this would be ill advised prior to the collaborative study data becoming available for analysis. The Committee then considered the possibility of establishing the international standard prior to its next annual meeting. It was however recognized that the Committee currently lacked any mechanism for recommending the establishment of reference preparations between its meetings. In view of these constraints, the Committee recommended that establishment of the international standard be deferred. An understanding was however reached that NIBSC would continue to make the candidate material available with an arbitrary interim unit defined by the institute.

7.2 Proposed new projects and updates – in vitro diagnostics

7.2.1 Proposed First WHO Reference Panel for cancer mutation detection

Cancer is the second leading cause of death globally and new cases are expected to increase by 70% in the next 20 years. There is therefore a strong need to improve the quality of cancer diagnostics and treatment worldwide. Drug therapies are advancing, with multiple globally licensed anti-cancer drugs now targeting specific tumour biomarkers. Despite the progress made, there remains a gap in the translational research between the identification of new biomarkers and the development and commercialization of genetic diagnostic tests. Standards for assay validation and verification are needed to ensure the consistency of these diagnostics.

To support standardization efforts in this rapidly advancing field, the development of a First WHO Reference Panel for cancer mutation detection is proposed. This would be the first panel of its kind and would support the standardization of multiple mutation detection in tumour DNA samples by next-generation sequencing (NGS). The application of NGS to the personalized diagnosis, treatment and monitoring of cancer is becoming more widespread, and is based on the parallel analysis of multiple genomic markers. However, it is recognized that NGS is a complex technology and variability can be introduced at many stages. The proposed reference panel is intended to serve as a benchmark across different platforms.

It is proposed that the panel would comprise approximately four genomic DNAs of varying mixtures derived from approximately six cancer cell lines. This will provide coverage for up to 300 mutations in around 60 key oncogenes. Approximately 2500 ampoules will be produced and the panel assessed in a variety of NGS platforms representing a range of input variables. It was emphasized that the development of such a panel will not negate the need to develop other panels covering additional genes and mutation types and that standards will continue to be proposed for certain single cancer mutations. It was envisaged that the final panel would be presented to the Committee for establishment in 2019.

Discussion topics included the amount of DNA that should be present in each ampoule. Previous materials contain 5 µg which would be overly sufficient. In view of this and the anticipated popularity of the panel it was suggested that consideration be given to increasing the anticipated production of 2500 ampoules by reducing the DNA content in each, while remaining mindful of potential stability issues. The possibility of increasing DNA volume through multiple cell passages was also discussed and it was concluded that this would not be a suitable approach to expanding production due to practical limitations. Instead, consideration could be given to limiting the number of ampoules that

laboratories could receive and ensuring that the material was only used in the calibration of kits, assays or secondary reference materials which would be made available to others. It was suggested that such an approach would also promote alignment to the primary standard.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO Reference Panel for cancer mutation detection.

7.2.2 Proposed Third WHO International Standard for prekallikrein activator

The international standard for prekallikrein activator (PKA) is used to determine the level of this impurity in albumin and is thus highly important in promoting patient safety. Albumin solutions contaminated with PKA can result in vasodilation thus complicating the use of albumin to manage hypovolemia.

The Committee was informed that there is frequent and worldwide demand for the current international standard, stocks of which were now running low. Therefore, a replacement standard was needed to ensure continuity of supply and ongoing comparability of test results across laboratories. The proposed candidate material was an albumin solution with a high level of PKA that had been donated by a manufacturer and filled into ampoules. The replacement preparation will be calibrated against the current international standard by end users in a joint NIBSC/EDQM international collaborative study scheduled for November 2017. During discussion, it was noted that both antithrombin activity and temperature need to be well controlled when performing PKA assays.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a Third WHO International Standard for prekallikrein activator.

7.2.3 Proposed First WHO Reference Reagent for anti-human platelet antigen 15b

The detection of alloantibodies against human platelet antigens (HPAs) in patient serum is important in the diagnosis and treatment of thrombocytopenias, including fetal and neonatal alloimmune thrombocytopenia, platelet refractoriness and post-transfusion purpura. Thrombocytopenias relating to alloantibodies to HPA-15b have been reported in both Caucasian and Japanese populations. As with HPA-1a, -3a and -5b (for which WHO potency references exist) HPA-15b is therefore also of clinical importance, with studies indicating that it is as immunogenic as HPA-5. Currently, the methods used to detect anti-HPA-15b antibodies can be unreliable and vary in their sensitivity.

Following requests made to NIBSC for a reference material for anti-HPA-15b alloantibodies, the Committee was asked to endorse a proposal to develop a First WHO Reference Reagent for anti-human platelet antigen 15b. The proposed reference reagent would be used to validate the minimum sensitivity

of anti-HPA-15b antibody test methods used by clinical laboratories, test kit manufacturers, research institutions and proficiency testing organizations. This will allow users to determine the sensitivity of their antibody detection assays, thus providing confidence that a negative result is not simply due to poor assay sensitivity. The proposed reference reagent would also improve the comparability of results obtained using different tests.

The intended source material is a serum containing anti-HPA-15b antibody obtained from one or more donors. Currently approximately 2 litres of serum from one donor is available containing low titres of anti-HPA-15b. The material requires further evaluation and the anti-HPA-15b antibodies may need concentrating. However, as anti-HPA-15b antibody is relatively rare, additional material could be difficult to source. Testing for infection markers will also be required. The Committee was informed that submission of the proposed reference reagent for establishment is scheduled for 2019.

During discussion it was clarified that although the current serum also contained other anti-HPA antibodies, the high specificity of testing methods meant that this would not affect its suitability as a reference reagent. In addition, although material from only one patient would not represent patient variability in the real world, this would have to be accepted due to the rarity of the antibody. It was suggested that contacting experts in the field could be very helpful in determining whether the IgG subclasses of the proposed source material required characterization and whether or not further source materials could be obtained.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO Reference Reagent for anti-human platelet antigen 15b.

7.2.4 Proposed First WHO International Standard for anti-cyclic citrullinated peptide antibodies

Anti-cyclic citrullinated peptide (anti-CCP) is an autoantibody found in patient sera that is widely used as a diagnostic and prognostic marker, along with rheumatoid factor (RF), for rheumatoid arthritis, a common systemic autoimmune disease. Unlike RF determination, anti-CCP measurement can provide a differential diagnosis of rheumatoid arthritis from other diseases such as SLE. Furthermore, anti-CCP is now recommended by The European League Against Rheumatism as a marker for the identification of rheumatoid arthritis patients with erosive disease, thus aiding in its therapeutic management.

A number of qualitative and quantitative first-, second- and third-generation test kits are commercially available and use different combinations of cyclic citrullinated peptides for capturing anti-CCP antibodies. However, the controls and calibrators provided with the kits only have an arbitrarily assigned unitage (U/ml). As a result, quantitative values obtained using different test kits are not comparable and cut-off values for a positive result vary significantly between kits. The calibration of test kits using an international standard for

anti-CCP antibodies would allow for meaningful comparisons to be made, facilitate the use of anti-CCP antibodies as prognostic and diagnostic markers of rheumatoid arthritis, and improve disease monitoring. Several organizations have now highlighted the need for such an international standard and a proposal was made to establish a First WHO International Standard for anti-cyclic citrullinated peptide antibodies. The international standard would be used by diagnostic test kit manufacturers, research institutions, proficiency testing organizations and clinical laboratories. It was further proposed that the same preparation used for this international standard could also serve as the Second WHO International Standard for rheumatoid factor (see section 7.2.5 below).

If successfully sourced, the candidate material will comprise pooled serum from rheumatoid arthritis patients free from infection markers and with a sufficient concentration of anti-CCP and RF. International units would be assigned based on results generated by end users in an international collaborative study, with submission of the study report to the Committee in 2019. The Committee was informed that a reference serum had already been prepared by the Autoantibody Standardisation Committee (ASC) of the International Union of Immunological Societies. Consideration had been given to possible approaches to making the unitages of the two materials consistent. During discussion it was pointed out that the ASC standard was not a WHO standard. However, this standard should be included in the collaborative study to ensure consistency of unitage.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO International Standard for anti-cyclic citrullinated peptide antibodies.

7.2.5 Proposed Second WHO International Standard for rheumatoid factor

Rheumatoid factor (RF) is an autoantibody found in the serum of patients with rheumatoid arthritis, a common, systemic autoimmune disease which affects at least 1.3 million adults in the USA and approximately 1–2% of adults worldwide. RF binds to IgG Fc resulting in immune-complex formation, inflammation and joint damage and is a diagnostic marker of rheumatoid arthritis.

The current First WHO International Reference Preparation for rheumatoid arthritis serum (lyophilized) has been distributed worldwide and is used to calibrate assays and diagnostic test kits which measure RF levels in patient serum. It is used by clinical laboratories, test kit manufacturers and research institutes to calibrate their working standards. Stocks of this material are now running low and a proposal was made to develop a replacement standard to ensure the continuity of test result comparability across laboratories. The replacement preparation will be calibrated against the current reference preparation by end users in a collaborative study using methods which are not RF-isotype specific. In addition, nominal values for specific RF isotypes (IgM,

IgA and IgG) may be assigned based on the results obtained using isotype-specific detection methods. It was pointed out that different antibody subclasses have different prevalences in different geographical areas of the world.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a Second WHO International Standard for rheumatoid factor.

7.2.6 Proposed Second WHO reference reagents for dengue virus subtypes 1–4

The Committee was informed that the previous reference materials had proved very useful during the 2015–2016 ZIKV epidemic in understanding flavivirus serological cross-reactivity with dengue viruses. As these materials were now fully depleted a replacement was urgently required, not only for continuity but also to support the initiation of a new dengue vaccination programme.

It was intended that the replacement materials would represent the same four serotypes (dengue serotypes 1–4) as the previous reference reagents. However, due to insufficient stocks of the material used to produce the reference materials a direct replacement cannot be produced and new material is required. Sourcing suitable replacement material may prove to be problematic. In addition, previous issues encountered during efforts to standardize neutralization assays had not been resolved and may arise again during the replacement study. It is acknowledged that there is some urgency concerning the need to replace the previously used materials. For example, it is expected that the forthcoming ZIKV vaccine roll-out may initiate increased anti-dengue testing, while the possibility has been raised that prior exposure to dengue viruses increases the likelihood of haemorrhagic fever following ZIKV vaccination. Therefore every effort was being made to present the finalized study report to the Committee in 2018–2019.

During discussion it was pointed out that the National Institutes of Health had supported studies of both dengue and Zika in Brazil and may have access to positive samples. Other possibilities were also highlighted along with the importance of ensuring that any candidate source material would need to be free of antibodies to ZIKV and yellow fever virus.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop replacements for the First WHO reference reagents for dengue virus serotypes 1–4.

7.2.7 Proposed First WHO International Standard for cutaneous leishmaniasis; and First WHO Reference Panel for cutaneous leishmaniasis

Cutaneous leishmaniasis is a protozoan parasitic disease transmitted via the infectious bite of sand flies belonging to the genus *Phlebotomus* in Europe, North Africa, the Middle East and Asia and to the genus *Lutzomyia* in the southern USA and Central and South America. The epidemiology of the disease is complex

with different protozoan species present in the eastern and western hemispheres. It is estimated that there are over 1 million new cases each year with varying levels of clinical manifestation. Early detection and diagnosis improves treatment efficacy and it was proposed that the development of both an international standard and a species-specific reference panel would facilitate this.

It is intended that preparations will be formulated from cultured pathogens and a range of species types assessed in an international collaborative study. The candidate material demonstrating the largest degree of assay harmonization would be used as the proposed WHO international standard, while the other species types would be used in a WHO reference panel. Depending on the availability of source materials, it is anticipated that the study report will be presented to the Committee in either 2019 or 2020. During discussion the issue of using a common matrix for testing was discussed. It was clarified that as laboratories often use tissue preparations instead of whole blood the suitability of the reference materials should be evaluated in both matrix types.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO International Standard for cutaneous leishmaniasis and a First WHO Reference Panel for cutaneous leishmaniasis.

7.2.8 Proposed First WHO International Standard for *Plasmodium vivax* antigens; and First WHO Reference Reagent for anti-malaria (*Plasmodium vivax*) serum

The need to standardize RDTs for malaria detection is well recognized. While there are a range of reference preparations, both molecular and serological, to support *Plasmodium falciparum* assay standardization, significant differences between *P. falciparum* and *P. vivax* necessitate the development of separate materials. The Committee was provided with an outline of the challenges inherent in *P. vivax* diagnosis. These include the lower parasite densities characteristic of the clinical stage and the inability of diagnostic tests to detect the hypnozoite stage of the life-cycle. The availability of standards that would allow for the development of assays with improved sensitivity to *P. vivax* LDH and the inclusion of serological markers for hypnozoites would significantly improve the diagnostic field.

A proposal was made to develop a First WHO International Standard for *Plasmodium vivax* antigens and a First WHO Reference Reagent for anti-malaria (*Plasmodium vivax*) serum for evaluation in two separate collaborative studies. Each of the projects would be undertaken in conjunction with the Foundation for Innovative New Diagnostics (FIND). It was pointed out that obtaining *P. vivax* other than from clinical isolates was problematic and such isolates may be difficult to source in the quantities required. In addition, the standards developed would need to appropriately address the need for assay

standardization in the areas of diagnosis, seroepidemiology and immunogenicity of potential vaccines. A realistic timeframe was being allocated to the sourcing of materials and it was anticipated that the completed study report would be presented to the Committee in 2020.

During discussion the possibility of developing antigen standards for *P. vivax* using recombinant proteins was raised given the difficulty of acquiring high-titre clinical source material. A further suggestion was made to acquire blood specimens during the acute, convalescent and recrudescent phases of *P. vivax* infection.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO International Standard for *Plasmodium vivax* antigens, and a First WHO Reference Reagent for anti-Malaria (*Plasmodium vivax*) serum.

7.2.9 Proposed First WHO International Standard for anti-MERS-CoV serum

Despite the rising global incidence of infection with the Middle East respiratory syndrome coronavirus (MERS-CoV) there is currently no international standard for use in diagnostics and research. Following a pilot study carried out at NIBSC to assess a small number of materials for their potential suitability as a candidate international standard it was proposed that an international collaborative study now be conducted. This collaborative study would involve a range of different laboratories and methods and would focus on the establishment of an international standard for use in harmonizing diagnostic assays for MERS-CoV infection. It was envisaged that the biggest issue would be the sourcing of suitable volumes and titres of material. The ideal source material would be serum obtained from convalescent patients that had confirmed MERS-CoV infection.

It was pointed out that in the pilot study a transchromosomal bovine IgG sample had been included even though this type of material had never been used previously for such a purpose. Were it to be selected as the candidate reference material its suitability and commutability would need to be rigorously assessed.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO International Standard for anti-MERS-CoV serum.

7.2.10 Proposed First WHO International Standard for MERS-CoV RNA for NAT-based assays

The rapid diagnosis and quarantining of individuals infected with MERS-CoV is extremely important in preventing the spread of infection to health-care workers and the wider population. As the use of molecular methods to detect MERS-CoV infection increases there is a growing need for standardization to ensure their accuracy and sensitivity. The Committee was provided with an overview of the findings of an external quality assessment study of MERS-CoV

RNA assays. The study had highlighted wide variability in reporting based on different MERS-CoV assays. In addition, there was a high possibility of under-reporting of MERS incidence owing to the patient sampling sites utilized. In some infected individuals MERS-CoV was not always detected in upper respiratory tract samples. Furthermore, upper and lower respiratory tract swabs, potentially containing only low concentrations of MERS-CoV RNA, are the most commonly used diagnostic samples.

A proposal was made to develop a first WHO international standard for MERS-CoV RNA. Following the earlier identification of a potential source of suitable stock material the intention was to provide this to NIBSC as a heat-inactivated high-titre tissue culture that could further be diluted into a suitable matrix. This material would then be evaluated in an international collaborative study. Although sourcing suitable clinical material for the study may be problematic, the group responsible for conducting the external quality assessment study outlined above had indicated that they may be able to assist with this. It is anticipated that the results of the collaborative study would be presented to the Committee in 2019.

During discussion it was noted that MERS-CoV has a rapidly changing genome and attention would need to be given to ensuring the relevance of the chosen sample to circulating strains. The sequence of the candidate material would have to be determined and a broad range of variants included in the study to better understand the efficiency of variant detection.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a First WHO International Standard for MERS-CoV RNA for NAT-based assays.

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

The WHO international standard for HCV RNA is used to calibrate secondary reference materials for use in NAT-based assays. In addition to its use in the safety testing of blood donations, and in testing for HCV in cells, tissues and organs, NAT-based assays are also widely used in the management of HCV infection, particularly in the diagnosis of disease and the initiation and monitoring of antiviral therapies.

Despite efforts to ensure its exclusive use in the calibration of secondary reference materials, demand for this international standard remains high. As HCV cannot be propagated in tissue culture, standard development relies upon sourcing HCV-positive plasma of suitable volume and titre. Historically, this has proved problematic and has led to the production of small batches (< 2000 vials). The proposal to replace the current international standard was being brought to the Committee with sufficient lead time to allow for an attempt to be made to source higher volumes of starting material. At the current rate of dispatch, there

is approximately a 3-year supply of the current international standard, which should allow sufficient time to source suitable replacement materials with a view to presenting the outcome of their evaluation to the Committee in either 2019 or 2020.

During discussion it was pointed out that the improvement of HCV treatment protocols could lead to an upsurge in demand for HCV testing. This should be borne in mind when deciding upon the volume of material to be produced. It was also recommended that the genotype 1a should continue to be used for the international standard. It was agreed that material does not have to be from a single source and that pooling could be used to ensure sufficient volumes. The suggestion was made that suitable source material might be obtained from commercial plasma-collection establishments.

The Committee endorsed the proposal (WHO/BS/2017.2320) to develop a Sixth WHO International Standard for hepatitis C virus RNA for NAT-based assays.

8. International reference materials – vaccines and related substances

All reference materials established at the meeting are listed in Annex 6.

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

8.1.1 First WHO international standards for oral poliomyelitis vaccines

As the WHO Global Polio Eradication Initiative approaches completion a number of changes in vaccination policy have been necessary. Following the certified global eradication of wild-type poliovirus serotype 2 in 2015, the highly successful trivalent oral poliomyelitis vaccine (tOPV) used for routine immunization was replaced with a bivalent OPV (bOPV) containing only poliovirus serotypes 1 and 3. Monovalent versions of the three poliovirus serotypes (mOPV1, mOPV2 and mOPV3) have also been produced and maintained as stockpiles to respond to polio outbreaks now and in the post-eradication era. The Committee was informed that all of these products will be required for the foreseeable future.

Accordingly, the manufacture and control of these vaccines must be maintained to the highest level in support of polio eradication. Relevant standard preparations must therefore be available to ensure that testing meets appropriate worldwide regulatory requirements. The Second WHO International Standard for the potency testing of trivalent OPV was established in 2004 and has been used for the calibration of regional working references, manufacturer in-house references and NCL references worldwide. However, due to the raised containment requirements for poliovirus serotype 2 following its eradication, this standard is no longer suitable for testing bOPVs at lower containment levels.

The Committee was informed that candidate WHO international standards for bOPV, mOPV1, mOPV2 and mOPV3 had been produced from three commercially produced monovalent bulks. A collaborative study had then been conducted to establish the first WHO international standards for bOPVs and mOPVs. The NIBSC codes 16/164 (bOPV1+3), 16/196 (mOPV1), 15/296 (mOPV2) and 16/202 (mOPV3) were assigned and the candidate materials tested in an international collaborative study involving 14 laboratories. The bOPV 1+3, mOPV1 and mOPV3 candidate materials were tested in duplicate by all laboratories, while the mOPV2 candidate material was tested by the four laboratories that were able to meet the raised containment requirements for poliovirus serotype 2. Overall levels of intra-assay and intra-laboratory variation in the results obtained were in line with the previous study to establish the Second WHO International Standard for the potency testing of trivalent

OPV, thus demonstrating a high level of consistency within laboratories. This was supported by the range of values obtained between laboratories for all the candidates which were within 0.5 $\log_{10}$ $TCID_{50}$ of the mean, indicating that the methods are well standardized. All of the candidate materials exhibited good stability at −70 °C indicating their suitability for use as international standards. It was expected that under long-term storage they would behave in a very similar way to the current second international standard. Data also indicated that the materials are stable for 1 month at 4 °C. Annual monitoring of the potency of these materials will be carried out. The recommended shipping and storage requirements will be in dry ice and −70 °C respectively and will be reflected in the IFU.

The Committee considered the report of the study (WHO/BS/2017.2313) and recommended that: (a) candidate material 16/164 be established as the First WHO International Standard for the potency testing of bivalent OPV, with potencies of 7.19, 6.36 and 7.32 $\log_{10}$ $TCID_{50}$/ml for serotypes 1, 3 and total virus content respectively; (b) candidate material 16/196 be established as the First WHO International Standard for the potency testing of monovalent serotype 1 OPV, with a potency of 7.32 $\log_{10}$ $TCID_{50}$/ml; (c) candidate material 15/296 be established as the First WHO International Standard for the potency testing of monovalent serotype 2 OPV, with a potency of 6.74 $\log_{10}$ $TCID_{50}$/ml; and (d) candidate material 16/202 be established as the First WHO International Standard for the potency testing of monovalent serotype 3 OPV, with a potency of 6.66 $\log_{10}$ $TCID_{50}$/ml. The Committee noted that NIBSC should address storage and distribution needs in relation to mOPV2 in view of its increased containment level and should reflect such needs in the IFU.

8.1.2 Second WHO International Standard for pertussis toxin

Reference preparations of pertussis toxin (PTx) are required for the quality control and assessment of pertussis vaccines. Acellular pertussis vaccines contain pertussis toxin in its detoxified form and regulatory safety tests are required to ensure that residual levels of pertussis toxin activity and reversion to toxicity do not exceed levels shown to be safe in clinical lots. This is usually evaluated using the murine histamine sensitization test (HIST) for final formulated lots and/or CHO cell clustering assay for purified pertussis toxoids. Biochemical assays comprising fetuin-binding ELISA and enzymatic reaction coupled to HPLC have also been developed to measure PTx activity in vitro. However, most manufacturers and control laboratories routinely use only the HIST and CHO cell tests to monitor residual PTx activity in acellular pertussis vaccines and whole cell vaccines. As all of these assays require active PTx as a control, a WHO international standard is required and the First WHO International Standard for pertussis toxin was established in 2003. Stocks of this international standard were

now low, with the development of a replacement standard having been endorsed by the Committee in 2014.

The Committee was informed that a frozen preparation of PTx manufactured by the Serum Institute of India had been donated to NIBSC where it had been formulated and freeze-dried in sealed glass ampoules. An international collaborative study had then been conducted to determine the suitability of the candidate material (NIBSC code 15/126) to replace the current international standard. A total of 14 laboratories from 12 countries took part in the study with 11 performing HIST, 14 performing CHO cell clustering assay and three performing biochemical assays to measure the enzymatic and carbohydrate-binding activities of the toxin.

Study data confirmed that the candidate material 15/126 exhibited biological activity both in HIST and CHO cell assays. However, unlike the current international standard, the levels of activity in HIST and CHO cell assays did not accord and the use of an individual unitage for each assay type was proposed. The candidate material also exhibited activity in the biochemical assays used. Accelerated degradation studies indicated that the material would be stable at recommended storage conditions with further stability studies showing that the material is less stable once reconstituted – a characteristic of other PTx materials.

The Committee considered the report of the study (WHO/BS/2017.2315) and after discussion recommended that the candidate material 15/126 be established as the Second WHO International Standard for pertussis toxin with an assigned unitage of 1881 IU/ampoule for HIST and 680 IU/ampoule for the CHO cell clustering assay.

8.1.3 First WHO international standards for *Citrobacter freundii* and *Salmonella* Typhi Vi polysaccharides

Typhoid fever is caused by an infection with *Salmonella enterica* subspecies *enterica* serovar Typhi (*Salmonella* Typhi). *Salmonella* Typhi expresses a capsular polysaccharide – Vi polysaccharide (Vi PS) – which is a virulence factor and considered the main protective antigen. A Vi PS capsule which has similar physicochemical and immunological characteristics to that of *Salmonella* Typhi is expressed by the soil bacterium *Citrobacter freundii*. Vaccination is the most cost-effective preventative strategy to control typhoid, especially in areas where multidrug resistant strains are endemic. Since plain Vi PS vaccines are unable to provide immunoprotection for young children or infants, conjugate Vi PS vaccines have been developed. The Committee was informed that two Vi PS-tetanus toxoid conjugate vaccines had been licensed in India and that several more Vi PS conjugate vaccines (using a variety of carrier proteins) were in development. Vi PS-based typhoid vaccine production relies on physicochemical and serological methods for estimating polysaccharide content as a measure of potency and to ensure that batches are consistently manufactured. However,

the methods used are currently not well standardized between individual laboratories and different reference materials are in use. The intended use of WHO international standards for Vi PS was to provide globally standardized Vi PS quantification with the aim of harmonizing measurement of the Vi PS content of typhoid vaccines.

The standards would be used to facilitate calibration of the various assays and in-house reference materials, and were likely to be in considerable demand, particularly by manufacturers and NCLs in low- and middle-income countries. The Committee was reminded that at its meeting in 2015 it endorsed a project to develop WHO international standards for *Salmonella* Typhi Vi PS and for *C. freundii* Vi PS, since both have been used to produce conjugate vaccines.

Source materials had been donated by the Novartis Vaccines Institute for Global Health (now the GSK Vaccines Institute for Global Health) and by GlaxoSmithKline Biologicals for the development of *C. freundii* and *Salmonella* Typhi Vi PS international standards respectively. These materials had been filled into ampoules at NIBSC and assigned the NIBSC codes 12/244 (*C. freundii* Vi PS) and 16/126 (*Salmonella* Typhi Vi PS). An international collaborative study had then been conducted involving 20 laboratories from 12 countries. During the study, two separate evaluations were made – one to assign unitage using qNMR to quantitate Vi PS/ampoule using the N-acetyl and O-acetyl resonances and the second to assess the suitability of the candidate materials in determining the Vi PS and O-acetyl content of vaccine samples using commonly used physicochemical assays and immunoassays and in comparison with in-house standards. Stability studies performed over 6 months indicated that both candidate materials were stable at temperatures used for storage (−20 °C) and during laboratory manipulation (4 °C). Accelerated degradation studies showed no observable size reduction for either material following storage at up to 56 °C for 6 months. The amount of polysaccharide per ampoule remained constant under all conditions. Further real-time and accelerated stability studies were ongoing.

The Committee considered the report of the study (WHO/BS/2017.2310) and after further discussion and clarification of the need for the two international standards recommended that: (a) the candidate material 12/244 be established as the First WHO International Standard for *Citrobacter freundii* Vi polysaccharide with a content of 1.94 ± 0.12 mg/ampoule; and (b) the candidate material 16/126 be established as the First WHO International Standard for the *Salmonella* Typhi Vi polysaccharide with a content of 2.03 ± 0.10 mg/ampoule. The Committee further noted that the intended use of both WHO international standards was for the quantification of the Vi PS component of vaccines containing Vi PS using the HPAEC-PAD, ELISA, rate nephelometry or rocket immuno-electrophoresis assays, with the observation that the latter may not be suitable for all bulk Vi PS conjugates.

8.1.4 First WHO International Standard for anti-typhoid capsular Vi polysaccharide immunoglobulin G (human)

The Committee was provided with a brief overview of the history of efforts to establish a WHO international standard for anti-typhoid capsular Vi polysaccharide IgG. These efforts had led to the conducting of an international collaborative study to evaluate the performance of a number of candidate reference materials using the commercial assay VaccZyme ELISA as well as in-house ELISAs. The study found considerable variation in the results obtained using in-house assays, and the Committee at its 2014 meeting recommended against the establishment of the proposed candidate material (NIBSC code 10/126) as an international standard at that time. Possible causes of the variation included differences in ELISA procedures, in the quality of Vi PS used or differences in the anti-Vi IgG.

The Committee was informed that a new IgG preparation had been obtained and a collaborative study of its performance completed. The new candidate material (NIBSC code 16/138) had been obtained from healthy volunteers immunized with licensed typhoid vaccines, and been donated by the Oxford Vaccine Group with full ethical approval. The candidate material was filled into ampoules, freeze-dried and evaluated for stability. The collaborative study involved seven laboratories (including vaccine manufacturers, NCLs and research laboratories) in six countries. The suitability of candidate material 16/138 for use as a reference material for anti-Vi IgG serum (human) was evaluated alongside a previously used reference reagent (Vi-$IgG_{R1, 2011}$), the previous candidate material 10/126, three post-vaccination sera and one pre-vaccination serum in several Vi ELISA formats.

In the majority of cases, valid estimates were obtained for the potency of coded samples relative to both the candidate material 16/138 and Vi-$IgG_{R1, 2011}$ and for the relative potency of the two standards to each other. Despite giving valid estimates, the NIBSC ELISA (using biotinylated Vi) showed no concordance with the VaccZyme ELISA, suggesting that the modification of Vi makes these types of ELISAs unsuitable as an alternative to the VaccZyme ELISA. Study data indicated that the candidate material 16/138 would be suitable for use as a reference standard for anti-Vi IgG serum (human) in the VaccZyme ELISA and in in-house ELISAs where the commutability of 16/138 with the coded samples and Vi-$IgG_{R1, 2011}$ was evident. Stability evaluation of candidate material 16/138 indicated adequate stability when stored at −20 °C.

The Committee considered the report of the study (WHO/BS/2017.2307) and, following considerable discussion and clarification of a number of points, recommended that the candidate material 16/138 be established as the First WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) with an assigned unitage of 100 IU/ampoule. Relative unitages of 54 IU/

ampoule and 163 IU/vial were also assigned to candidate material 10/126 and Vi-$IgG_{R1, 2011}$ respectively. The Committee agreed that a follow-up collaborative study might be helpful in clarifying whether in-house ELISAs based on poly-L-Lysine and native Vi could be suitable alternatives to the commercial VaccZyme ELISA.

8.1.5 First WHO International Standard for antiserum to respiratory syncytial virus

The development of a respiratory syncytial virus (RSV) vaccine is a widely recognized global health priority. The Committee was informed that activity in this area had increased significantly in recent years, with numerous RSV vaccine candidates in development – 14 of which were now in human clinical trials. RSV-neutralizing activity in serum has been reported to correlate with protection against acute lower respiratory infection with RSV in both rodent models and human infants. Accurately quantifying this neutralizing activity will be vital in the development of RSV vaccines.

There are multiple formats of RSV-neutralization assays, and accurately comparing the neutralization titres in sera from multiple clinical trials using different neutralization assay formats is challenging. A reference antiserum is therefore urgently needed to standardize clinical trial data and outcomes. Sixty serum samples obtained from healthy adults were provided by PATH for use as source material for a proposed WHO international standard. Samples with high and medium RSV antibody titres were selected and two candidate pools were prepared, processed, filled, freeze-dried and assigned the NIBSC codes 16/284 and 16/322. A collaborative study was then conducted to characterize the performance of the two candidate materials in a range of diverse RSV-neutralization assays and to assess their suitability for use as international standards for anti-serum to RSV. The study involved 21 laboratories from 9 countries, including university laboratories, manufacturers/developers of RSV vaccines and public health laboratories. All participants used their own in-house virus-neutralization assay and their own virus stocks. Study samples comprised the two candidate materials 16/284 and 16/322, naturally infected adult sera, age-stratified naturally infected paediatric sera, sera from RSV vaccine clinical trials in maternal and elderly subjects, a mAb to RSV (palivizumab), two cotton rat serum samples, and samples from the BEI Resources Panel of Human Antiserum and Immune Globulin to Respiratory Syncytial Virus.

Study results indicated that inter-laboratory variability in neutralization titres was significantly reduced when values were expressed relative to those of either of the two candidate materials. The collaborative study also indicated that the standards were useful for multiple sample types across a wide variety of assay formats. However, analysis suggested that the cotton rat serum samples and mAb

sample behaved differently from the human serum samples and that a more suitable standard should be considered for these sample types. This would not be an issue for the establishment of a WHO international standard as its main role would be to look at neutralizing antibody activity in human serum mostly produced during RSV vaccine clinical trials.

Stability data for 16/284 indicated a low predicted loss in activity per year (< 0.01%) when stored at −20 °C, suggesting that it is sufficiently stable to serve as a WHO international standard. A long-term stability-monitoring programme will be needed to show that candidate material 16/284 remains stable over its lifetime. Stability data for 16/322 were not currently available but it too will be monitored for stability over its lifetime. Furthermore, stability analysis indicated that the candidate materials were also stable after reconstitution. Both candidate materials showed loss of activity at 37 °C after 2 weeks, with 16/322 showing a greater loss than 16/284.

The Committee considered the report of the study (WHO/BS/2017.2318) and recommended that the candidate material 16/284 be established as the First WHO International Standard for antiserum to respiratory syncytial virus with an assigned unitage of 1000 IU/vial. The Committee also assigned a unitage of 960 IU/vial to candidate material 16/322 as a potential future replacement standard.

8.2 Proposed new projects and updates – vaccines and related substances

8.2.1 Proposed First WHO Reference Panel for *Vibrio cholera* O1 and O139 lipopolysaccharides

Vibrio cholera O1 and O139 are leading causes of bacterial diarrhoea and bacteraemia in Africa, South-East Asia and the Caribbean. Cholera outbreaks occur frequently in refugee camps and following natural disasters. Young children and infants are particularly vulnerable. Oral cholera vaccine (OCV), inactivated, is the most cost-effective measure to contain and prevent the disease in such settings. Three such vaccines have now been prequalified by WHO and are part of a WHO-maintained stockpile. To be effective, cholera vaccines require appropriate regulation at the national level to ensure their efficacy. As standardization is a prerequisite for achieving appropriate quality control of these vaccines the availability of WHO standards for the lipopolysaccharides (LPS) of O1 Inaba, O1 Ogawa and O139 *V. cholera* would be expected to support the assay development needed for the technology transfer and quality control of inactivated OCV.

Purified LPS O1 Inaba, O1 Ogawa and O139 will be obtained from the International Vaccine Institute, Republic of Korea, and the material filled and

freeze-dried at the NIBSC. Approximately 1000 ampoules of each serotype LPS would be produced. A collaborative study would then be required to assess the suitability of the three candidate materials for use in a WHO reference panel for the inhibition ELISA assay used to determine the potency of OCVs. The study would also compare the reactivity of the candidate materials and in-house standards using in-house inhibition ELISAs. Collaborative study participants are expected to include vaccine developers/manufactures and NCLs, with the total number likely to be quite small. However, three or four new manufacturers from low- and middle-income countries were expected to enter the field. It was anticipated that the reference panel would be used by NCLs and vaccine manufacturers to calibrate the immunoassays used to determine OCV potency, with a predicted demand of approximately 10 vials per year.

Following discussion of timelines, funding and details of the proposed assay, as well as issues related to the donation of reference material, the Committee endorsed the proposal (WHO/BS/2017.2319) to develop a First WHO Reference Panel for *Vibrio cholera* O1 and O139 lipopolysaccharides and requested that feedback be provided on progress.

8.2.2 Proposed First WHO Reference Panel for anti-*Vibrio cholera* O1 and O139 lipopolysaccharide serums (rabbit)

The Committee was informed that this proposal was part of a joint effort with the International Vaccine Institute, and the Bill & Melinda Gates Foundation to produce three sets of antisera in rabbits – anti-O1 Inaba serum, anti-O1 Ogawa serum and anti-O139 serum. The materials would be filled and freeze-dried at NIBSC to produce approximately 500 ampoules, each containing 1 ml of freeze-dried antiserum. An international collaborative study would then be undertaken to assess the suitability of the candidate materials for use in a WHO reference panel for anti-O1 Inaba, anti-O1 Ogawa and anti-O139 for the inhibition ELISA used to determine the potency of OCVs. The collaborative study would also compare the reactivity of the candidate materials and in-house standards using in-house inhibition ELISAs.

It was envisaged that the resulting rabbit antisera panel would be used by NCLs and vaccine manufacturers, primarily in low- and middle-income countries, to calibrate the immunoassays used to determine the potency of OCV batches. The panel was also expected to be used to compare the potency and stability of vaccines with respect to a proposed new standard OCV (see section 8.2.3 below). The predicted level of demand was approximately 10 vials per year.

The Committee endorsed the proposal (WHO/BS/2017.2319) to develop a First WHO Reference Panel for anti-*Vibrio cholera* O1 and O139 lipopolysaccharide serums (rabbit).

8.2.3 Proposed First WHO International Standard for *Vibrio cholera* vaccine (oral, inactivated)

The Committee was informed that the availability of a mixture of killed *Vibrio cholera* O1 Inaba, O1 Ogawa and O139 whole cells in a composition similar to that of OCVs would enhance and support the further development of assays required to ascertain vaccine quality and facilitate technology transfer for cholera vaccine manufacturing. A candidate material will be evaluated in a collaborative study to assess its suitability for use as an international standard vaccine for use in in-house inhibition ELISAs to determine the OCV potency. Approximately 1000 ampoules containing 1 ml of freeze-dried cells were expected to be made available. This proposal is part of a joint effort with the International Vaccine Institute and the Bill & Melinda Gates Foundation to ensure sufficient supplies of inactivated OCV of appropriate quality, safety and efficacy.

The Committee endorsed the proposal (WHO/BS/2017.2319) to develop a First WHO International Standard for *Vibrio cholera* vaccine (oral, inactivated).

8.2.4 Proposed First WHO International Standard for antibody to the influenza virus haemagglutinin stem domain

The need for improved, longer lasting and more broadly protective influenza vaccines has long been recognized. Such vaccines would have a public health impact worldwide, in both low- and high-income countries, and would potentially improve the public health response to seasonal and pandemic influenza. The Committee was informed that many antibodies binding to the stem domain of the haemagglutinin (HA) of influenza A viruses have been found to be cross-reactive between drifted viruses of the same subtype, and in some cases between viruses of different subtypes. As a result, attempts were now under way to develop broadly reactive and protective influenza vaccines that elicit HA stem-binding antibodies, with clinical trials expected soon. In addition, a number of laboratories are developing therapeutic mAbs against the HA stem domain, and such efforts might also benefit from the development of a WHO international standard.

Various assays are used to measure HA stem-binding antibodies, including virus neutralization assays and binding assays. As vaccine candidates progress through pre-clinical and clinical testing, the harmonization of serological read-outs would be beneficial. A WHO international standard would help achieve such harmonization thus improving comparability both between laboratories and between studies. The optimal format such a standard was not yet clear and it is not known whether a single mAb, an oligoclonal mixture of mAbs or a polyclonal antiserum (of human or animal origin) would perform best. Obtaining mAbs from commercial entities may involve lengthy MTA negotiations.

The Committee was informed that the proposed project would therefore be conducted in two phases and would take more time than a more typical standardization project. In the first phase of the project, candidate materials will be sent to a small number of laboratories and tested for HA stem-binding antibodies using their in-house assays. The materials will also be evaluated in terms of their ability to harmonize assay results. Based on the outcome of this first study, a second larger study would then be undertaken which would include the best performing standard types identified in the first study, as well as samples from human clinical trials.

The Committee considered this to be an exciting and forward-looking project and expressed its support in principle. However, it also noted the exploratory nature of the first phase of the project and therefore endorsed the first exploratory phase of the proposal (WHO/BS/2017.2319) to develop a First WHO International Standard for antibody to the influenza virus haemagglutinin stem domain. The Committee asked that NIBSC report back at its next meeting on the progress made, as well as on the state of the influenza vaccine field, to allow for further review and consideration of the proposal by the Committee.

8.2.5 Proposed First WHO international standards for influenza virus pathogenicity for safety testing

The safety testing of an influenza candidate vaccine virus (CVV) is based upon comparison against the parental wild-type virus in ferret studies. Although the CVV must be attenuated relative to the corresponding wild-type virus there are currently no accepted criteria for attenuation. The Committee was informed that the WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines – which specifies safety-testing procedures for CVVs – was currently under review. It had been proposed that standards of pathogenicity could be used instead of reliance upon wild-type viruses specific to each CVV during the development of CVVs for influenza vaccines for pandemic preparedness purposes. Such CVVs are generated on a continual basis and need to be assessed for attenuation. It was proposed that guidance on the use of the standard viruses would be included in the revised version of the WHO guidelines.

CVVs are crucial in the production of influenza vaccines and in the case of a pandemic, must be generated and tested very rapidly. Having the proposed standard viruses available before a pandemic would enable laboratories to harmonize their safety testing. In the event of a pandemic – when access to a newly emerging wild-type virus may be difficult – tests could be conducted faster and CVVs released sooner without compromising safety. Ultimately with fewer animals being required for testing, a greater range of CVVs could be tested in a pandemic situation if required.

Animal tests are also subject to variability and the ferret safety test is no exception. Having standards that allow for the bench-marking of the test will lead to better understanding of test results and greater confidence in them. Moreover, this proposal is in line with moves towards reducing the use of animals in the research and development of biological medicines. Instead of comparing every new CVV with its respective wild-type parental virus, the occasional testing of the standard viruses would be conducted, thus reducing the overall number of ferrets required. The use of standard viruses in this way – in combination with defined cut-off values/ranges – will not only reduce the number of animals used but will also provide a means of assessing CVVs derived from parental wild-type viruses which are non-pathogenic in ferrets, which cannot currently be demonstrated to be attenuated as per the existing WHO guidance.

Following discussion and clarification of how the proposed standards would be used the Committee endorsed the proposal (WHO/BS2017.2319) to develop the First WHO international standards for influenza virus pathogenicity for safety testing. However, in view of ongoing discussions on the revision of the existing WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines, the Committee requested that it be updated on the progress made in this project and on all other relevant developments at its next meeting.

8.2.6 Proposed Third WHO International Standard for anti-rabies immunoglobulin (human)

Rabies is a neglected zoonosis with substantial public health and economic impacts worldwide. Rabies antibody assays are used to evaluate the immunogenicity of human rabies vaccines and the potency of immunoglobulins used in post-exposure prophylaxis. The standardization of assays used for the detection and quantification of rabies antibodies is crucial. The Committee was informed that stocks of the current international standard are nearing depletion and are now under restricted sales. A replacement standard was now urgently needed as many national regulatory requirements for rabies IgG potency assays state that a reference preparation calibrated in IU must be used.

The development of a new international standard would require the donation of human immunoglobulin preparations from manufacturers. As the ability of manufacturers to donate such preparations is currently uncertain there is concern that the current international standard may become depleted before its replacement is established. The calibration of a candidate replacement standard would require a collaborative study in the usual way. In this case, the aim of the collaborative study would be to calibrate the candidate material in IUs against the current international standard in assays such as the rapid fluorescent focus inhibition test, the mouse neutralization test and the plaque reduction assay.

A rabies challenge virus standard 11 (G protein) pseudotyped lentiviral particle neutralization assay was also available and was currently undergoing a feasibility study for adoption into the European Pharmacopoeia.

The collaborative study would involve up to 12 laboratories worldwide, performing a range of rabies vaccine assays, and representing manufacturers of rabies vaccines and NCLs. Study samples would include the candidate replacement standard, the current international standard and, if possible, EDQM reference material.

The Committee noted that the availability of IgG to serve as the proposed third international standard was uncertain but was assured that sufficient quantities of the current international standard would be retained for calibrating its replacement. The Committee therefore endorsed the proposal (WHO/BS/2017.2319) to develop a Third WHO International Standard for anti-rabies immunoglobulin (human).

Annex 1

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

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

Recommendations concerned with biological substances used in medicine are formulated by international groups of experts and are published in the WHO Technical Report Series[1] as listed below. A historical list of Requirements and other sets of Recommendations is available on request from the World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

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

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

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

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

[1] 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 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)
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)

Recommendations, Guidelines and other documents	Reference
Diphtheria vaccines (adsorbed)	Revised 2012, TRS 980 (2014)
DNA vaccines: assuring quality and nonclinical safety	Revised 2005, TRS 941 (2007)
Ebola vaccines	Adopted 2017, TRS 1011 (2018)
Haemophilus influenzae 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 1987, TRS 771 (1988)
Hepatitis B vaccines made by recombinant DNA techniques	Revised 2010, TRS 978 (2013)
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, biosafety risk assessment and safe production and control for (human) pandemic vaccines	Adopted 2005, TRS 941 (2007)
Influenza vaccines (inactivated)	Revised 2003, TRS 927 (2005)
Influenza vaccines (inactivated): labelling information for use in pregnant women	Addendum to TRS 927; TRS 1004 (2017)
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)
In vitro diagnostic medical devices, establishing stability of, Technical Guidance Series for WHO Prequalification – Diagnostic Assessment	Adopted 2017, TRS 1011 (2018)

Recommendations, Guidelines and other documents	Reference
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 848 (1994); Note 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)
Pneumococcal conjugate vaccines	Revised 2009, TRS 977 (2013)
Poliomyelitis vaccines (inactivated)	Revised 2014, TRS 993 (2015)
Poliomyelitis vaccines (inactivated): guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses	Adopted 2003, TRS 926 (2004)
Poliomyelitis vaccines (oral)	Revised 2012, TRS 980 (2014)
Quality assurance for biological products, guidelines for national authorities	Adopted 1991, TRS 822 (1992)

Recommendations, Guidelines and other documents	Reference
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)
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 removal	Adopted 2003, TRS 926 (2004)
Thromboplastins and plasma used to control oral anticoagulant therapy	Revised 2011, TRS 979 (2013)
Tick-borne encephalitis vaccines (inactivated)	Adopted 1997, TRS 889 (1999)
Transmissible spongiform encephalopathies in relation to biological and pharmaceutical products[2]	Revised 2005, WHO (2006)
Tuberculins	Revised 1985, TRS 745 (1987)
Typhoid vaccines, conjugated	Adopted 2013, TRS 987 (2014)
Typhoid vaccines (live, attenuated, Ty21a, oral)	Adopted 1983, TRS 700 (1984)
Typhoid vaccines, Vi polysaccharide	Adopted 1992, TRS 840 (1994)

[2] Available online at: http://www.who.int/biologicals/publications/en/whotse2003.pdf

Recommendations, Guidelines and other documents	Reference
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, clinical evaluation: use of human challenge trials	Adopted 2016, TRS 1004 (2017)
Vaccines, lot release	Adopted 2010, TRS 978 (2013)
Vaccines, nonclinical evaluation	Adopted 2003, TRS 926 (2004)
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	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)

Annex 2

Guidelines on the quality, safety and efficacy of Ebola vaccines

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

Abbreviations

Ad	human adenovirus
AESI	adverse event of special interest
AR	attack rate
ARU	attack rate in unvaccinated individuals
ARV	attack rate in vaccinated individuals
BCG	bacillus Calmette–Guérin
BDBV	Bundibugyo ebolavirus
BSL	biosafety level
CBER	Center for Biologics Evaluation and Research
CEF	chick embryo fibroblast
ChAd3	chimpanzee adenovirus type 3
DCVMN	Developing Countries Vaccine Manufacturers Network
DNA	deoxyribonucleic acid
EBOV	Ebola virus
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
ERA	environmental risk assessment
EUAL	WHO emergency use assessment and listing (procedure)
EVD	Ebola virus disease
GLP	good laboratory practice(s)
GMO	genetically modified organism
GMP	good manufacturing practice(s)
GP	glycoprotein
HIV	human immunodeficiency virus
HVAC	heating, ventilation and air conditioning
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

ICP	immune correlate of protection
ICS	intracellular cytokine staining
Ig	immunoglobulin
LAL	Limulus amoebocyte lysate
LVV	lentiviral vector
MARV	Marburg virus
MCB	master cell bank
MVA	modified vaccinia Ankara
NAT	nucleic acid amplification technique
NRA	national regulatory authority
PCR	polymerase chain reaction
PDL	population doubling level
qPCR	quantitative polymerase chain reaction
RDT	rapid diagnostic test
RESTV	Reston ebolavirus
RNA	ribonucleic acid
RR	relative risk
RT	reverse transcriptase
rVSV	recombinant vesicular stomatitis virus
SAE	serious adverse event
SAGE	WHO Strategic Advisory Group of Experts
SPF	specific pathogen-free
SUDV	Sudan ebolavirus
SWRCT	stepped wedge randomized cluster trial
TAFV	Tai Forest ebolavirus
TSE	transmissible spongiform encephalopathy
VLP	virus-like particle
VSV	vesicular stomatitis virus
WCB	working cell bank
ZEBOV	Zaire ebolavirus

Introduction

The unprecedented scale and severity of the Ebola virus disease (EVD) epidemic in West Africa in 2014–2016 led to calls for the urgent development and licensing of an Ebola vaccine (*1*, *2*). A considerable amount of work was subsequently undertaken over a short period of time and a series of international consultations held on related public health issues and on Ebola vaccine development, evaluation and licensing (*2–4*). The development of Ebola vaccines and implications for future immunization policy recommendations are being monitored by the WHO Strategic Advisory Group of Experts (SAGE) on Immunization (*5*). In addition, as part of ongoing WHO measures to support the development of Ebola vaccines, guidance was prepared on the scientific and regulatory considerations relating to their quality, safety and efficacy. In March 2015, WHO convened an informal consultation in Geneva attended by scientific experts, regulatory professionals and other stakeholders involved in Ebola vaccine development, production, evaluation and licensure. The purpose of this consultation was to review initial draft guidelines prepared by a drafting group, and to seek consensus on key technical and regulatory issues (*6*). The draft guidelines were revised in the light of comments made, and then underwent public consultation which resulted in a large number of further comments and suggestions. The draft guidelines, together with the comments, were discussed by the WHO Expert Committee on Biological Standardization at its meeting in October 2015. During 2016, further revisions were made following public consultations and working group discussions. One major challenge during the development of these Ebola vaccine guidelines was that they were initially prepared during the rapidly evolving epidemic situation when the need for a vaccine was most urgent. With the end of the large-scale EVD outbreak in Africa, declared by WHO in June 2016, EVD returned to its previous sporadic pattern – an epidemiological situation which made the evaluation of Ebola vaccine efficacy, and thus licensing, more challenging. Interest also shifted from the development of monovalent Ebola virus (EBOV) Zaire vaccines to multivalent preparations directed against more than one EBOV strain, as well as against the Marburg virus (MARV).

The WHO Expert Committee on Biological Standardization reviewed the draft document again in October 2016 and after extensive discussion agreed that the guidance should be extended to include multivalent Ebola vaccines and the clinical evaluation of candidate vaccines using innovative clinical trial designs. There was also a recognized need to provide guidance on how to evaluate and license Ebola vaccines subsequent to the potential licensure of one of the advanced vectored vaccines. These WHO Guidelines are the result of these discussions.

This document provides information and guidance on the development, production, quality control and evaluation of candidate Ebola vaccines in the form of WHO Guidelines rather than WHO Recommendations. This allows

for greater flexibility with respect to the expected future of Ebola vaccine development, production, quality control and evaluation. Given that this is a very dynamic field both in terms of technologies and clinical trial designs, these WHO Guidelines should be read in conjunction with other relevant recent guidelines.

A model protocol for the manufacturing and control of viral-vectored Ebola vaccines is provided in Appendix 1 of these WHO Guidelines. This protocol outlines the information that should be provided as a minimum by a manufacturer to the NRA in support of a request for the release of a vaccine for use. The protocol is not intended to apply to material intended for clinical trials. A Lot Release Certificate signed by the appropriate NRA official should be provided if requested by a manufacturer, and should certify whether or not the lot of vaccine in question meets all national requirements and/or Part A of these WHO Guidelines. The purpose of this is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 2.

Purpose and scope

These WHO Guidelines provide scientific and regulatory guidance for national regulatory authorities (NRAs) and vaccine manufacturers on the quality, nonclinical and clinical aspects of Ebola vaccines relevant to marketing authorizations. In particular, the document deals with Ebola vaccines based on viral vectors, which are currently at the most advanced stage of development and for which no specific WHO guidance is available. The document also discusses opportunities to accelerate vaccine development and product availability during a public health emergency.

The document does not address access programmes or regulatory pathways for making investigational Ebola vaccines available for situations where their use is not primarily intended to obtain safety and efficacy or effectiveness information.

Although recombinant viral-vectored Ebola vaccines are the main category of vaccine considered in this document, some aspects of the guidance provided are relevant to other approaches. General guidance on other technologies relevant to Ebola vaccine development has been published elsewhere by WHO, including guidance on:

- inactivated vaccines (*7*–*9*)
- protein antigens produced by recombinant technology (*10*–*13*)
- DNA vaccines (*14*, *15*).

In the past 10 years, WHO has convened two consultations to consider the development, production and evaluation of viral-vectored vaccines in general,

and the reports of those meetings provide useful discussion and opinions on the quality, safety and efficacy aspects of such vaccines (*16*, *17*). A regional guideline is also available for live recombinant viral-vectored vaccines (*18*).

Although recombinant viral-vectored Ebola vaccines are by far the most advanced candidates, other approaches to the development of Ebola vaccines are also being investigated. These include different production platforms, such as recombinant DNA vaccines expressing an EBOV antigen produced in *Escherichia coli* (*19*), Ebola virus-like particles (VLPs) expressed from recombinant baculovirus in insect cells, and other forms of subunit vaccines. Most developmental approaches to Ebola vaccines involve recombinant DNA technology.

Part A of this document focuses on the development, manufacturing and quality control issues relevant to viral-vectored vaccines against EBOV. Although the key principles related to nonclinical development (Part B) and clinical development (Part C) may apply to vaccine approaches other than those based on viral vectors, special considerations and guidance would be required for such products – and they are therefore not elaborated upon in this document. Any mention of specific vaccines is for information only and should not be considered as an endorsement of a particular candidate.

Parts A, B and C provide guidance in general terms on the full quality, nonclinical and clinical requirements for a license submission for viral-vectored Ebola vaccines. The document also considers the principles which may be applied to product development, manufacturing and control – and to nonclinical and clinical evaluation – during a public health emergency to allow for the rapid introduction of an Ebola vaccine. Wherever appropriate, discussions on the minimum dataset required are highlighted and aspects of vaccine development which may be accelerated during a public health emergency are indicated. These context-specific discussions and indications are shown as indented smaller text in Parts A, B and C. In addition, special considerations regarding the quality requirements at different stages of clinical development are discussed in sections A.2.4, A.3.6, A.3.7 and A.3.8.

These WHO Guidelines should be read in conjunction with other relevant WHO guidelines such as those on nonclinical (*20*, *21*) and clinical (*22*) evaluation of vaccines, as well as relevant documents that describe the minimum requirements for an effective National Pharmacovigilance System (*23*). Other WHO guidance, such as that on the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*), should also be consulted as appropriate.

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

Terminology

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

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

Adverse event of special interest (AESI): an adverse event (serious or non-serious) that is of scientific and medical concern specific to the sponsor's product or programme, and for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to be characterized and understood. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (for example, regulators) might also be warranted.

Attenuated virus: a strain of virus in which pathogenicity has been reduced so that the virus strain will initiate an immune response without producing the disease.

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

Candidate vaccine: an investigational vaccine which is in the research and clinical development stages and has not been granted marketing authorization or licensure by a regulatory agency.

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

Cell substrate: cells used to manufacture a biological product.

Expression construct: an expression vector containing the genetic coding sequence of the recombinant protein.

Expression system: the host cell containing the expression construct and the cell culture process that is capable of expressing protein encoded by the expression construct.

Final bulk: a formulated vaccine preparation from which the final containers are filled. If applicable, the final bulk may be prepared from one or more monovalent antigen bulks and, in this case, mixing should result in a uniform preparation to ensure that final containers are homogenous.

Final lot: a collection of sealed final containers of formulated 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 single vessel of final bulk or prepared in one working session.

Heterologous gene: a transgene from the disease-causing organism that is integrated into the genomic sequence of the viral vector.

Immune correlate of protection (ICP): an immunological response that correlates with vaccine-induced protection from disease and is considered predictive of clinical efficacy. The ICP may be mechanistic (that is, causative for protection) or it may be non-mechanistic (that is, an immune response that is present in persons protected by vaccination but that is not the cause of protection).

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

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

Master cell bank (MCB): a quantity of well-characterized cells of animal or other 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. In some cases, such as genetically engineered cells, the MCB may be prepared from a selected cell clone established under defined conditions. Frequently, however, the MCB is not clonal. It is considered best practice for the MCB to be used to derive working cell banks.

Monovalent vaccine: a vaccine containing immunizing antigen, or a gene encoding an immunizing agent, against a single strain or type of disease agent.

Platform technology: a production technology in which different viral-vectored vaccines are produced by incorporating heterologous genes for different proteins into an identical viral vector backbone.

Multivalent vaccine: a vaccine containing a mixture of more than one immunizing antigen or genes encoding several immunizing agents active against more than one strain or type of disease agent.

Pooled virus harvest: a homogeneous pool of two or more single virus harvests.

Public health emergency: an extraordinary event that is determined, as provided in the International Health Regulations (*25*), to: (a) constitute a public health risk to other States through the international spread of disease; and (b) potentially require a coordinated international response.

Seed lot: a system according to which successive batches of viral-vectored vaccine are derived from the same virus master seed lot of viral vector at a given

passage level. For routine production, a virus working seed lot is prepared from the virus master seed lot. The final product is derived from the virus working seed lot and has not undergone more passages from the virus master seed lot than the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Single virus harvest: a quantity of virus suspension of one virus strain harvested from cell cultures derived from the same working cell bank and prepared from a single production run.

Vaccine efficacy: measures direct protection (that is, protection induced by vaccination in the vaccinated population sample). Vaccine efficacy is most commonly a measure of the proportionate reduction in disease attack rate (AR) between the control group that did not receive vaccination against the infectious disease under study (ARU) and the vaccinated group (ARV). Vaccine efficacy (expressed as a percentage) can be calculated from the relative risk (RR = ARV/ARU) of disease when comparing the vaccinated group to the unvaccinated control group as [(ARU-ARV)/ARU] x 100 – that is, as (1-RR) x 100. This estimate may be referred to as absolute vaccine efficacy. Alternatively, vaccine efficacy may be defined as a measure of the proportionate reduction in disease AR in a group vaccinated with the candidate vaccine relative to a control group vaccinated with a licensed vaccine against the infectious disease under study. This estimate may be referred to as relative vaccine efficacy (*22*).

Vaccine effectiveness: an estimate of the protection conferred by vaccination. It is usually obtained by monitoring the disease to be prevented by the vaccine during routine use in a specific population. Vaccine effectiveness measures both direct and indirect protection (for example, the estimate may in part reflect protection of unvaccinated persons secondary to the effect of use of the vaccine in the vaccinated population) (*22*). Evidence for vaccine effectiveness may also be derived from challenge-protection studies conducted in animal models or from a vaccine-induced immune response (for example, pre-specified antibody threshold induced by the vaccine in vaccinated persons).

Virus master seed: a collection of appropriate containers whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of virus vector particles of defined passage from which the virus working seed is derived.

Virus pre-master seed: a single pool of virus vector particles of defined passage from which the virus master seed is derived.

Virus working seed: a collection of appropriate containers whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of virus vector particles of defined passage derived directly from the virus master seed lot and which is the starting material for individual manufacturing batches of viral-vectored vaccine product.

Working cell bank (WCB): a quantity of cells of uniform composition derived from one or more ampoules of the MCB at a finite passage level, stored frozen at −70 °C or below in aliquots, one or more of which is used for vaccine production. All containers are treated identically and once removed from storage are not returned to the stock.

General considerations

Ebola viruses, Ebola virus disease and epidemiology

Ebola viruses belong to the *Filoviridae* family of filamentous, negative-stranded RNA, enveloped viruses consisting of three genera: Ebola virus, Marburg virus and Cueva virus – the latter being a pathogen of bats in Spain (*26*). There are five distinct species of EBOV: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SUDV), Tai Forest ebolavirus (TAFV), Reston ebolavirus (RESTV) and Bundibugyo ebolavirus (BDBV) (*26*, *27*). Marburg virus (MARV) appears to be antigenically stable and at present there is only a single species. The first recognized MARV outbreak in humans was in 1967 and was linked to infected monkeys imported from Uganda that infected laboratory workers in Marburg and Belgrade (*28*). Bats are believed to be the natural reservoir of all filoviruses. EBOV and MARV cause severe haemorrhagic fever in humans and non-human primates alike, with high morbidity and mortality rates (*29*, *30*). Outbreaks of infection with Ebola filoviruses have been noted since 1976, mainly in Central Africa, and recur at intervals. Prior to the 2014–2016 EVD epidemic in West Africa there had not been such a large-scale outbreak and the disease had not been recorded in West Africa, apart from a single infection with TAFV.

The incubation period following infection with EBOV and prior to the onset of symptoms is believed to be approximately 2–21 days, with initial symptoms being similar to diseases such as influenza or malaria (*31*, *32*). Patients then progress rapidly to a life-threatening disease (*33*). From a practical perspective, infected individuals rarely if ever become infective before symptoms appear, but those who survive remain infective until the virus is cleared from their blood and other bodily fluids. It has been reported that viable EBOV can persist in ocular fluid for at least 9 weeks following clearance of viraemia (*34*). EBOV has also been detected in semen for months following recovery from EVD, which is consistent with the possible persistence of the virus within immune-privileged tissue sites in the body (*35*, *36*). Presumptive sexual transmission of EBOV from recovered individuals has also been reported (*37*, *38*). Individuals suffering from EVD have been treated aggressively with oral and intravenous fluids, including electrolyte replacements, to combat severe diarrhoea and dehydration, with some surviving the infection (*33*).

Filoviruses are high-risk agents classified as biosafety level 4 (BSL-4) pathogens. They consist of a non-segmented RNA genome of approximately 19 kb containing 7 genes encoding viral proteins VP24, VP30, VP35, VP40, a nucleoprotein, a glycoprotein (GP) and a polymerase (39). The GP is a type-1 transmembrane GP that is cleaved into disulphide-linked GP1 and GP2 subunits. The mature GP forms homotrimers that are presented as spikes on the surface of infected cells and virions, and is responsible for receptor binding, viral entry and, most likely, immunity (*40*, *41*). Most of the vaccines currently under development are based on the EBOV GP and have been shown to confer protection from lethal EBOV challenge in animal models – including, importantly, in non-human primates (*42*, *43*).

Natural immune responses to Ebola viruses

Filovirus infection in humans elicits innate, cellular and humoral responses. Immunoglobulin M (IgM) and IgG antibodies have been reported to develop early in infected patients who survive, whereas fatal cases are associated with immune dysregulation and high viraemia (*44*). Some cross-reactive immune responses across the five EBOV species have been reported (*45*). Cellular responses can also be detected. The generation of neutralizing antibodies during filovirus infection and the passive transfer of neutralizing monoclonal antibodies or monkey convalescent immunoglobulin preparations have been shown to sometimes protect non-human primates against lethal filovirus challenge – though overall the data are somewhat conflicting (*44*, *46*). Data suggest that antibodies play a significant role in protection against filovirus infection but correlates of protection have not been established and the importance of cellular immunity is uncertain (*47*, *48*).

Ebola vaccines development

A large number of candidate Ebola vaccines are under development. Some of these vaccines had already been in preclinical development prior to the 2014–2016 EVD epidemic and are significantly more advanced than the others. To date, several candidate vaccines (including monovalent, bivalent and multivalent candidate vaccines) have undergone or are undergoing clinical development at different trial phases. The Phase III trial for a recombinant vesicular stomatitis virus (rVSV)-vectored candidate vaccine (rVSVΔG-ZEBOV-GP), undertaken in Guinea, is the only study that has reported clinical efficacy and effectiveness for any candidate Ebola vaccine. This candidate vaccine was granted access to the Priority Medicine (PRIME) scheme by the European Medicines Agency, and Breakthrough Therapy designation by the United States Food and Drug Administration (*5*). Examples of Ebola vaccines

currently under clinical development are provided in a WHO Working Group background paper (*49*).

The most advanced Ebola vaccines are based on live recombinant virus vector platforms. Such vaccines have been developed in Canada, China, Europe, Russia and the USA. Five of the most advanced platforms used to engineer these vaccines are rVSV (*50*, *51*), chimpanzee adenovirus type 3 (ChAd3) (*52*), human adenovirus type 26 (Ad26) (*53*), human adenovirus type 5 (Ad5) (*51*, *54*) and the modified vaccinia Ankara (MVA) strain (*55*). To date, the virus vectors have been produced in a wide variety of cell lines including PER.C6 (Ad26.ZEBOV), chick embryo fibroblasts (MVA-BN-Filo), Procell-92.S (ChAd3-EBOZ), Vero (rVSV-ZEBOV) and HEK 293 (Ad5-EBOV). Monovalent candidate vaccines have been constructed to express the EBOV GP of one EBOV strain, such as the Zaire strain responsible for the epidemic in West Africa. Others have been developed as multivalent vaccines expressing the GP of more than one EBOV strain and/or MARV and/or the TAFV nucleoprotein. Multivalent vaccines have also been produced by blending monovalent bulks expressing glycoproteins from different EBOV and/or MARV strains. These candidates are currently under study in non-human primates and in humans, either as single vaccines or for use in heterologous prime-boost vaccine schedules where priming is done with one vaccine and boosting with another – as for example, Ad26.ZEBOV/MVA-BN-Filo (*56*, *57*) and rVSV/Ad5 (*51*).

The viral-vectored vaccines under development include those that are replication-incompetent in the human host or in human cells as well as those that are replication-competent but likely to be highly attenuated because of their recombinant gene inserts and cell culture passage. Replication-incompetent vectors include adenoviral vectors derived both from human adenoviruses (such as Ad26 and Ad5) and from non-human primate adenoviruses (such as ChAd3), as well as MVA. MVA is a highly attenuated vaccinia strain, derived by more than 500 passages in hens' eggs. The non-recombinant MVA was used as a human smallpox vaccine in Germany in the 1970s and a derivative has now been licensed for use in a future smallpox emergency in Canada and Europe. Vectors that are replication-competent but attenuated include rVSV (a negative-stranded RNA virus animal pathogen) in which attenuation is due to the insertion of a recombinant heterologous gene such as the EBOV GP in place of the VSV GP. These viral-vector platforms have been used to produce other investigational products – including gene therapy products, and both prophylactic and therapeutic vaccines – and data from their quality, nonclinical and clinical evaluations provide supporting safety data for their use in Ebola vaccine production (*50*, *58*, *59*).

The need for careful clinical studies using candidate vaccines in the target population will be of paramount importance. WHO has developed

a document – *Ebola virus disease (EVD) vaccine target product profile* (*60*) – which provides guidance on WHO preferred options in relation to two categories of Ebola vaccine (reactive use and prophylactic use). Encouraging results on the immunogenicity and safety of these candidate options, as well as on their clinical efficacy based on disease end-points, have already been generated and their evaluation in larger Phase II and Phase III trials is ongoing. This includes novel trial design clinical studies (ring vaccination) using the rVSV-ZEBOV vaccine (*32*, *54*, *61–64*). A prime-boost approach, using a two-dose schedule with different vector vaccines, is also being explored. Boosting of Ad26.ZEBOV responses by MVA-BN-Filo resulted in sustained elevation of specific immunity with no vaccine-related serious adverse responses reported (*56*, *57*). Administration of rVSV-ZEBOV vaccine resulted in low-level viraemia detectable by polymerase chain reaction (PCR) during the first and sometimes second week after vaccination (*63*). The vaccine virus was also detected by PCR in the urine and saliva of a minority of the recipients. An unexpected safety signal was detected in one study when mild-to-moderate and generally short-lived arthritis developed during the second week following immunization in a minority of recipients and at one site in particular (*63*). In subsequent studies in healthy North American and European adults which carefully assessed joint-related adverse events, transient post-vaccination arthritis was noted in approximately 5% of vaccine recipients (*65*, *66*). However, the epidemiological situation has now changed significantly. Using strict infection control and public health measures, the EBOV epidemic has been ended – though there will still be a risk of new Ebola cases or clusters occurring through, for example, sexual transmission or new introduction of the virus into the human population. WHO declared Sierra Leone free of EBOV transmission in March 2016 and Guinea and Liberia free of EBOV transmission in June 2016, bringing to an end the large-scale Ebola outbreak in the three African countries mainly affected (*67*). In the absence of ongoing disease transmission, the assessment of Ebola vaccine efficacy will now be more challenging. Nevertheless, it is expected that current clinical trials of candidate vaccines will provide key data on safety, reactogenicity and immunogenicity to inform licensure.

Accelerated availability of vaccines during a public health emergency – general principles

The quality of a vaccine must always be taken into account during the process of evaluating whether the benefit derived from its administration is greater than any risks which might be associated with its use. This is a principle by which all pharmaceuticals, whether they are chemical or biological, medicine or vaccine, are evaluated to decide whether they should be made available for use or not. The principle applies equally to a product intended for use in a clinical trial or

as a licensed product, or to be made available through emergency procedures. In addition, there is an obligation to provide full assurance that the vaccine will not cause harm to the recipient due to a failure of manufacture and control that results in contamination of the product with unwanted components such as microorganisms or toxic materials. This requirement is absolute, regardless of the stage of development of the product or the urgency of the need for its availability.

Beyond this, the process and product characterization requirements will depend on the prevailing clinical situation and the urgency of need for the product. However, it is generally accepted that in order to gain marketing authorization for a vaccine the usual standards for quality development, manufacture and control will apply. During the assessment of a marketing authorization application, the balance of benefits and risks of the vaccine to the intended population is taken into consideration and must be found to be positive if the product is to be granted marketing approval. The specific findings related to the assessment of product quality are taken into account in this benefit–risk assessment.

It is not possible to provide a "road map" of the minimum process and product characterization and control requirements for a viral-vectored vaccine against EVD, or against any other disease with the potential to cause a public health emergency, since the requirements will be partially dependent on the ongoing epidemic situation in the affected countries.

In the case of viral-vectored vaccines, many of the opportunities to accelerate development and product availability during a public health emergency are likely to involve exploiting the knowledge gained from similar products manufactured with the same vector backbone (that is, platform technology). If a new vaccine is based on a well-characterized platform technology, then key aspects of manufacture and control (but not stability) can be based on the specific platform with only confirmatory information required for the new vaccine. This principle is especially applicable during the phase of clinical trial development. For licensure, product-specific data will be required but supportive platform-derived data may decrease the requirement for some product data if it can be shown that the benefit–risk assessment remains positive. Scientific advice should be sought from relevant regulatory authorities.

During product development, it might be possible to defer certain tests and development procedures provided it can be justified that their deferral does not affect product safety – and if it can also be argued that performing the tests or development procedures would hinder the availability of the product (for example, where performing the tests are on the critical path for product availability, or where large quantities of scarce material required for clinical purposes would need to be used). Such deferrals should be identified on a case-by-case basis and discussed with the NRA.

In some cases, even if the nature of a public health emergency affects the benefit–risk balance in such a way as to justify the accelerated development and approval of a vaccine for use in a public health emergency, the manufacturer would still be responsible for completing the full development work to the same standard required for a new vaccine under non-emergency conditions should it be decided to subsequently submit the product for full licensure. The required supplementary data and timelines for submission should be agreed between the applicant and the NRA.

Similar considerations apply to the nonclinical evaluation of candidate Ebola vaccines. For nonclinical evaluation during a public health emergency, it is paramount to determine a minimum nonclinical package (see section B.4) that can reasonably support initiation of early Phase I clinical trials. This should take into account the characteristics and novelty of candidate vaccines and the supportive information derived from the platform technology on which the vaccine is based. For example, the presence of nonclinical data and/or clinical experience gained with the same vector may support the omission of a specific safety test or toxicity testing programme. For a candidate vaccine derived from a novel platform, a certain amount of toxicity data (see section B.4) should at a minimum be obtained, and should focus on unexpected direct and indirect consequences that might result from vaccination.

In general, the use of a minimum safety package during nonclinical evaluation should be backed up by the continuous assessment of additional data collected during clinical development. At the time of the licensing application, the complete nonclinical programme data appropriate for a particular vaccine should be submitted, or the application should be otherwise adequately justified.

Clinical development of an Ebola vaccine in the setting of an outbreak is complex, and close collaboration between public health authorities, NRAs, the community, clinical investigators and the vaccine developer is essential to ensure that studies will meet authorization requirements, including requirements for ethical study conduct.

A WHO emergency use assessment and listing (EUAL) procedure (*68*) has been developed to expedite the availability of unlicensed vaccines needed during a public health emergency of (usually) international concern.

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

At the time of writing this document, no WHO guidance on viral-vectored vaccines was available. Consequently, this section focuses on issues relevant to the development, manufacturing and quality control steps leading to the licensing of such vaccines developed to protect against EVD.

The lead viral-vectored vaccines and their replication abilities are summarized in a WHO document (*49*). The relevance of aspects of the guidance provided in this document should be considered with respect to the replication status of the products. For example, tests for reversion to competency apply to replication-incompetent viral vectors where genes required for replication are not present in the vector. On the other hand, for replication-competent viral-vectored vaccines, the level of attenuation of the parent and recombinant viral vectors should be considered.

A.1 General manufacturing guidelines

The WHO Target Product Profile (*60*) prioritizes the development of multivalent vaccines from 2016 onwards and seeks at a minimum coverage for MARV and for both Zaire and Sudan species of EBOV.

The general manufacturing requirements contained in the WHO good manufacturing practices for pharmaceutical products: main principles (*69*) and WHO good manufacturing practices for biological products (*70*) should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for recombinant Ebola vaccines.

Quality control during the manufacturing process relies on the implementation of quality systems, such as good manufacturing practice (GMP), to ensure the production of consistent vaccine lots with characteristics similar to those of lots shown to be safe and effective in clinical trials. Throughout the process, a number of in-process control tests should be established (with acceptable limits) to allow quality to be monitored for each lot from the beginning to the end of production. It is important to note that most release specifications are product specific and should be agreed with the NRA as part of the clinical trial or marketing authorization.

Manufacturers should present a risk assessment regarding the biosafety level of their manufacturing facility and of the vaccine product. The principles presented in the WHO *Laboratory biosafety manual* (*71*) should be followed to justify the classification. Approval for the classification should be sought from the relevant authority in the country/region in which the manufacturing facility is located.

A.1.1 International reference materials

The highly pathogenic nature of EBOV raises particular concerns for the preparation of international reference materials as they must be both safe for use and representative of clinical samples to be analysed. Generally, plasma reference preparations are used for the standardization of assays for evaluating immune response, and artificial RNA viruses containing part of the EBOV genome are used for the standardization of nucleic acid assays for assessing viraemia.

Plasma from a recovered repatriated patient who contracted Ebola in West Africa one month before the plasma was collected was established by the 2015 WHO Expert Committee on Biological Standardization as the First WHO Reference Reagent for Ebola virus antibodies, with an assigned unitage of 1 U/ml (*72*). As the reference material resulted from a natural infection it is likely to have relevant antibody specificities. It is considered to be of acceptable safety for three reasons: (a) the patient was fully recovered clinically; (b) the plasma was negative for EBOV nucleic acid in PCR assays performed in various laboratories; and (c) the plasma was treated with solvent/detergent (an established method used in the blood products industry for decades for the inactivation of enveloped viruses).

Following evaluation and characterization of candidate materials (*73*, *74*), the First WHO International Standard for Ebola virus antibodies (assigned unitage = 1.5 IU/ml) and the First WHO Reference Panel for Ebola virus antibodies were established by the 2017 WHO Expert Committee on Biological Standardization. The First WHO International Standard for Ebola virus antibodies is intended for standardizing assays used in the detection and quantitation of EBOV antibodies. It is not intended to be used to set a protective threshold, which is currently unknown (see section C). The First WHO Reference Reagent for Ebola virus antibodies and the First WHO Reference Panel for Ebola virus antibodies can be used in the assessment of factors that affect assay variability (*75*).

Following evaluation and characterization of candidate materials (76, 77), two EBOV RNA preparations were also established as reference reagents by the 2015 WHO Expert Committee on Biological Standardization for use in the standardization of nucleic acid amplification technique (NAT)-based assays. One of these materials (Ebola NP-VP35-GP-LVV) consists of the RNA encoding the nucleoprotein VP35 and GP genes and is intended for use in standardizing assays directed at these genes only. The second (Ebola VP40-L-LVV) consists of the RNA encoding the VP40 and L genes and again is intended to standardize assays directed only at these genes. Both preparations are packaged in non-replicating lentiviral vectors (LVVs) with the EBOV genes incorporating mutations that make them inactive. Collectively the two materials were established as the First WHO reference reagents for Ebola virus RNA for NAT-based assays with assigned unitages of 7.5 $\log_{10}$ U/ml and 7.7 $\log_{10}$ U/ml respectively.

The First WHO Reference Panel for Ebola virus VP40 antigen was established by the 2016 WHO Expert Committee on Biological Standardization (*78*). The panel consists of different recombinant VP40 antigens and may be suitable for the evaluation and quality control of Ebola antigen assays based on VP40 detection.

All the reference materials listed above are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom.

For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations (*79*) should be consulted.

A.2 Control of source materials

A.2.1 Viral vector

A.2.1.1 Virus master and working seeds

The use of any viral vector should be based on a master and working seed lot system, analogous to the cell banking system used for production cells described below in section A.2.2.

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

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

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

The nucleotide sequence of the gene insert and of adjacent segments of the vector should be provided, along with restriction-enzyme mapping of the vector containing the gene insert. The genetic stability of the vector with the recombinant construct should be demonstrated. The stability of a recombinant vector should be assessed by comparing the sequence of the vector at the level of a virus pre-master seed or virus master seed to its sequence at, or preferably beyond, the anticipated maximum passage level. The comparison should demonstrate that no changes occur in regions involved in attenuation (where known) or replication deficiency. Any modifications to the sequence of the heterologous insert should be investigated and demonstrated to have no impact

on the resulting amino acid sequence (that is, it should be a conservative change) or on the antigenic characteristics of the vaccine.

A.2.1.2 Tests on virus master seed and virus working seed

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

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

Genetic characterization will involve nucleotide sequence analysis of the vaccine vector. Restriction mapping, southern blotting, PCR analysis or DNA fingerprinting will also be useful adjuncts. Individual elements involved in the expression of the heterologous gene(s) (including relevant junction regions) should be described and delineated.

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

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

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

Information should be given on the testing carried out for adventitious agents.

> During a public health emergency it is anticipated that the majority of the above information should be available and submitted in full for evaluation since it is essential to demonstrate the suitability and safety of the product.

It may be justified to initiate clinical trials using a product which is manufactured prior to establishment of the seed banking system. In such a case, the suitability and safety of the product must be established prior to its use – especially with regard to adventitious agents (*24*), replication competence, attenuation and other phenotypic characteristics, stability and suitable genetic sequence.

A.2.2 Cell substrates

The cell substrate for the manufacture of Ebola vaccine should be based on a cell banking system or on controlled primary cells.

A.2.2.1 Cell banks and primary cells

A.2.2.1.1 *Master and working cell banks (MCBs and WCBs)*

The cell banks should conform to 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 (*24*).

An appropriate history of the cell bank should be provided. This should include information on its origin, identification, development manipulations and characteristics for the purposes of the vaccine. Full details of the construction of packaging cell lines should be given, including the nature and identity of the helper viral nucleic acid and its encoded proteins/functions. If available, information on the chromosomal location of the helper viral nucleic acid should also be provided.

Genetic stability of the cell lines should be demonstrated. The stability of a production cell line should be assessed by comparing the critical regions of the cell line (and flanking regions) at the level of a pre-cell or master cell to its sequence at or beyond the anticipated maximum passage level. Stability studies should also be performed to confirm cell viability after retrieval from storage, maintenance of the expression system, and so on. These studies may be performed as part of routine use in production or may include samples taken specifically for this purpose.

With regard to cell cultures, the maximum number of passages (or population doublings) allowable from the MCB through to the WCB, and through production in cells should be defined on the basis of the stability data generated above, and should be approved by the NRA.

A.2.2.1.2 *Primary cells*

Primary cells are used within the first passage after establishment from the original tissue, and so it is not possible to carry out extensive characterization of the cells prior to their use. Therefore additional emphasis is placed on the origin of the tissues from which the cell line is derived. Tissues should be derived

from healthy animals/embryonated eggs subjected to veterinary and laboratory monitoring to certify the absence of pathogenic agents. Whenever possible, donor animals/embryonated eggs should be obtained from closed, specific-pathogen-free colonies or flocks. Animals used as tissue donors should not have been used previously for experimental studies. Birds and other animals should be adequately quarantined for an appropriate period of time prior to use for the preparation of cells.

Information on the materials and components used for the preparation of primary cell substrates should be provided, including the identity and source of all reagents of human or animal origin. A description of the testing performed on components of animal origin to certify the absence of detectable contaminants and adventitious agents should be included.

The methods used for the isolation of cells from tissue, establishment of primary cell cultures and maintenance of cultures should be described.

A.2.2.2 Testing of cell banks and primary cells

A.2.2.2.1 *Tests on MCBs and WCBs*

MCBs and WCBs should be tested for the absence of bacterial, fungal, mycoplasmal and viral contamination by appropriate tests, as specified 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 (*24*), or by a method approved by the NRA, to demonstrate that they are not contaminated with adventitious agents.

Rapid sterility methods to demonstrate the absence of bacteria and fungi, as well as NAT-based assays alone or in combination with cell culture, may be used as an alternative to one or both of the compendial mycoplasmal detection methods after suitable validation and agreement from the NRA (*24*).

The cell bank should be tested for tumorigenicity if it is of mammalian origin, as described in Part B 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 (*24*). The tumorigenic potential of the cell bank(s) should be described and strategies to mitigate risks that might be associated with this biological property should be described and justified.

> During a public health emergency, it is anticipated that the majority of the above information should be available and submitted for evaluation since it is essential to demonstrate the suitability and safety of the product. However, it may be justified to initiate clinical trials using a product which is manufactured prior to establishment of the cell banking system. In such a case, the suitability and safety of the product must be established prior to its use, especially with regard to adventitious agents (*24*).

A.2.2.2.2 *Tests on primary cells*

The nature of primary cells precludes extensive testing and characterization before use. Testing to demonstrate the absence of adventitious agents (bacteria, fungi, mycoplasmas and viruses) is therefore conducted concurrently, and should include, where relevant, the observation of control (uninfected) cultures during parallel fermentations to the production runs. The inoculation of culture fluid from production cultures and (where available) control cultures into various susceptible indicator cell cultures capable of detecting a wide range of relevant viruses (followed by examination for cytopathic changes and testing for the presence of haemadsorbing viruses) should also be performed routinely for batch release. In addition, pharmacopoeial testing for bacteria, fungi and mycoplasmas in the production cultures and (if relevant) control cultures should be conducted. Mycoplasmas and specific viruses of notable concern may also be tested for by additional methods such as PCR.

In the specific case of chick embryo fibroblasts (CEFs), the tissue should be sourced from specific-pathogen-free eggs. After preparation, the CEF cells should be tested for: (a) bacterial, fungal and mycoplasmal contamination; (b) viral adventitious agents by in vitro assay using three cell lines, including avian and human cells (such as CEF, MRC-5 and Vero); (c) viral adventitious agents by in vivo assay using mice and embryonated eggs; (d) avian leukosis virus contamination; and (e) the presence of retroviruses by measuring reverse transcriptase (RT) activity. Testing should take into consideration that CEF cells are expected to be positive for RT activity due to the presence of endogenous avian retroviral elements not associated with infectious retroviruses. It may be necessary to use an amplification strategy (for example, co-culturing of RT-positive fluids on an RT-negative, retrovirus-sensitive cell line) to determine whether a positive RT result can be attributed to the presence of an infectious retroviral agent.

A.2.3 Source materials used for cell culture and virus propagation

If serum is used for the propagation of cells it should be tested to demonstrate the absence of bacteria, fungi and mycoplasmas, as specified in the requirements given in Part A – section 5.2 (*80*) and section 5.3 (*81*) – of the WHO General requirements for the sterility of biological substances. Testing should also be conducted to demonstrate freedom from adventitious viruses.

Detailed guidance on detecting bovine viruses in serum used to establish MCBs and WCBs is provided 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 (*24*) and should be applied as appropriate. This same guidance may also be applicable to production cell cultures. As an additional monitor of quality, sera may be

examined for endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation. Whichever viral inactivation process is used, a validation study must be conducted to determine its consistency and effectiveness while still maintaining serum performance. The use of non-inactivated serum should be justified and is not advised without strong justification. Any non-inactivated serum must meet the same criteria as inactivated serum when tested for sterility and absence of mycoplasmal and viral contaminants.

The source(s) of animal components used in culture medium should be approved by the NRA. These components should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*82*).

Bovine or porcine trypsin used to prepare cell cultures should be tested and found free of bacteria, fungi, mycoplasmas and adventitious viruses, as appropriate. The methods used to ensure this should be approved by the NRA. The source(s) of trypsin of bovine origin (if used) should be approved by the NRA and should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*82*).

In some countries, irradiation is used to inactivate potential contaminant viruses in trypsin. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough for the biological properties of the reagents to be retained while being high enough to reduce virological risk. Consequently, irradiation cannot be considered a sterilizing process (*24*). The irradiation method should be validated and approved by the NRA.

Recombinant trypsin is available and should be considered – however, it should not be assumed to be free of risk of contamination and should be subject to the usual considerations for any reagent of biological origin (*24*).

Human serum should not be used.

If human serum albumin derived from human plasma is used at any stage of product manufacture, the NRA should be consulted regarding the requirements for this, 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 (*83*). In addition, human albumin and materials of animal origin should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*82*). Recombinant human serum albumin is available and should be considered as a substitute for the plasma-derived product.

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 at any stage of manufacture, provided that the quantity present in the final product is acceptable to the NRA.

Non-toxic pH indicators may be added (for example, phenol red at a concentration of 0.002%). Only substances that have been approved by the NRA may be added.

A.2.4 Special considerations for the development and testing of the viral vector and production cell lines

Early-phase nonclinical and clinical studies are generally supplied with product for which the level of knowledge of manufacture and control is expected to be quite rudimentary since few batches will have been manufactured and analytical methods will be in the early stages of development. The provision of material is required for early safety and proof-of-concept studies, as well as to initiate the dose-finding evaluation. Product will be tested initially in animals and then in a small number of human subjects in a well-controlled environment. This is the normal situation when there is no public health emergency and, in these circumstances, guidance on the quality requirements for investigational medicinal products in clinical trials is available (*84*).

Most data to be provided to the NRA before human studies can begin will concern the derivation and safety of the viral vector and the production cell line. The data will aim to show that the product and production system are well designed, the function of each genetic element is known and its inclusion in the product or cell line is justified. It should be confirmed that the expected elements are present in the product and cell line and that the final structure of the product is as predicted. A full description of the origin and construction of the genetic components of the viral vector and cell line should be provided, along with data on genetic stability up to (or preferably beyond) the anticipated maximum passage level in manufacture. Ideally, a virus master seed/virus working seed for the viral vector and MCB/WCB for the production cell line should be prepared early in the development of the product – though it is acknowledged that this may not be practical in the initial stages. Testing of the seed lots and cell banks at the time of their establishment should confirm comparability to the parental material. Any starting material (viral seeds and production cell lines) used to manufacture product for clinical use must be fully tested to ensure the absence of bacteria, fungi, mycoplasmas and adventitious viruses (*24*, *80*). Where applicable, freedom from TSEs must also be addressed (*82*). The potential for tumorigenicity of the cell line should also be tested and should meet current regulatory standards if it is of mammalian origin. All reagents used in the manufacture of the virus seed or cell lines (including cell culture solutions) should be tested and characterized as being of adequate quality, particularly regarding freedom from adventitious agents.

A.3 Control of Ebola vaccine production

A.3.1 Manufacture and purification

The manufacture of monovalent vaccine vectors starts with the amplification of the vaccine vector seed stock in a suitable cell line. The number of passages between the virus working seed lot and viral-vectored vaccine product should be kept to a minimum and should not exceed the number used for production of the vaccine shown in clinical studies to be satisfactory, unless otherwise justified and authorized.

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

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

Multivalent vaccines are generally prepared by combining batches of purified monovalent bulk that contain more than one EBOV strain and/or MARV strain. However, if the vaccine consists of a single vector containing genes encoding multiple antigens, then the recommendations for monovalent bulk manufacturing should be followed, but testing should take into account the multivalent identity and potency of the product.

By the time a marketing authorization application is submitted the manufacturing process should be adequately validated by demonstrating that a sufficient number of commercial-scale batches can be manufactured routinely under a state of control by meeting predetermined in-process controls, critical process parameters and lot release specifications. Any materials added during the purification process should be documented and their removal should be adequately validated or residual amounts tested for, as appropriate. Validation should also demonstrate that the manufacturing facility and equipment have been qualified, cleaning of product contact surfaces is adequate, and critical process steps (such as sterile filtrations and aseptic operations) have been validated.

The purified viral vector bulk and intermediates should be maintained under conditions shown by the manufacturer to ensure the retaining of the desired biological activity. Hold times should be defined.

During early clinical trials it is unlikely that there will be data from sufficient batches to validate/qualify product manufacture. However, as development progresses, data should be obtained from subsequent manufacture

and should be used in support of an eventual application for commercial supply of the product.

> During a public health emergency, on a case-by-case basis, some requirements of process validation may be abbreviated provided it can be demonstrated that the product will remain safe and well controlled. For example, if platform-specific data have demonstrated that scale-up for a vector is independent of the specific heterologous insert, this information may be used to justify fewer full-scale batches with the EBOV gene insert and a greater reliance on pre-validation and pilot-plant-scale batches. Validation data from the manufacture of platform-related products may provide useful supportive information, particularly in the identification of critical parameters.
>
> Since it is likely that there will initially be insufficient time to generate full validation data during an emergency situation, as much information as possible on the control of each batch should be presented to the NRA as supporting evidence that batch manufacture is sufficiently controlled. However, manufacturers should agree on the strategy with the NRA before relying on platform-specific validation data.

In addition to control during manufacture, the products should be adequately characterized by the stage of development. These attributes facilitate understanding of the biology of the candidate vaccine and assessment of the impact of any changes in manufacturing that are introduced as development advances or following licensure. Assessing the immunogenicity of the product, when relevant, should also be included in the characterization programme (for example, as part of the nonclinical pharmacodynamic evaluation).

A.3.1.1 Tests on control cell cultures (if applicable)

When control cells are included in the manufacturing process due to limitations on the testing of primary cells or viral harvests, or when their inclusion is required by the NRA, the following procedures should be followed. From the cells used to prepare cultures for vaccine production, a fraction equivalent to at least 5% of the total or 500 ml of cell suspension or 100 million cells should be used to prepare uninfected control cell cultures.

These control cultures should be observed microscopically for cytopathic and morphological changes attributable to the presence of adventitious agents for at least 14 days (at a temperature of 35–37 °C) after the day of inoculation of the production cultures, or until the time of final virus harvest, whichever comes last. At the end of the observation period, supernatant fluids collected from the control culture should be tested for the presence of adventitious agents, as described below. Samples that are not tested immediately should be stored at −60 °C or lower until such tests can be conducted.

If testing the control cultures for adventitious agents yields a positive result, the harvest of virus from the parallel vaccine-virus-infected cultures should not be used for production.

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

A.3.1.1.1 *Tests for haemadsorbing viruses*

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

In some countries the NRA requires that additional tests for haemadsorbing viruses are performed using other red blood cells, including human (blood group O), monkey and/or chicken (or other avian species). All haemadsorption tests should be read after incubation for 30 minutes at 0–4 °C, and again after further incubation for 30 minutes at 20–25 °C. Tests using monkey red blood cells should be read once more after additional incubation for 30 minutes at 34–37 °C.

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.

A.3.1.1.2 *Tests for other adventitious agents*

At the end of the observation period, a sample of the pooled fluid and/or cell lysate from each group of control cell cultures should be tested for adventitious agents. For this purpose, an aliquot of each pool should be tested in cells of the same species used for the production of virus, but not cultures derived directly from the production cell expansion series for the batch which is subject to the test. If primary cells are used for production then a different batch of that primary cell type should be used for the test than was used for production. Samples of each pool should also be tested in human cells and in a simian kidney cell line. At least one culture vessel of each kind of cell culture should remain uninoculated as a control.

The inoculated cultures should be incubated at the appropriate growth temperature and should be observed for cytopathic effects 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 7 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.

A.3.2 Single virus harvest

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

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

A.3.2.1 Control tests on single virus harvest

Tests for adventitious agents should be performed on each single virus harvest according to the relevant parts of section B.11 of the WHO Recommendations for the evaluation of animal cells as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*). Additional testing for adventitious viruses may be performed using validated NAT-based assays.

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

Single or pooled virus harvests should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, as specified in the requirements given in Part A – section 5.2 (*80*) and section 5.3 (*81*) – of the WHO General requirements for the sterility of biological substances.

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

Provided that the cell banks and viral seed stocks have been comprehensively tested and released, demonstrating that they are free of adventitious agents, the possibility of delaying in vitro testing for adventitious

agents (viral pathogens and mycoplasmas) in the cell harvest or bulk substance, or replacing it with validated PCR tests, could be evaluated subject to the agreement of the NRA. The method of production should be taken into account when deciding upon the nature of any specified viruses being sought.

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

A.3.3 Pooled monovalent virus harvests

Single virus harvests may be pooled to form virus pools from which the final bulk vaccine will be prepared. The strategy for pooling single virus harvests should be described. All processing of the virus pool should be described in detail.

A.3.3.1 Control tests on pooled virus harvests

Virus pools should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, as specified in the requirements given in Part A – section 5.2 (*80*) and section 5.3 (*81*) – of the WHO General requirements for the sterility of biological substances. Alternatively, if single virus harvests have been tested to demonstrate freedom from bacteria, fungi and mycoplasmas then these tests may be omitted on the pooled virus harvests.

A.3.4 Monovalent bulk vaccine

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

A.3.4.1 Control tests on monovalent bulk

The monovalent bulk vaccine should be tested and consideration given to using the tests listed below for the individual products as appropriate. Alternatively, if the monovalent bulk will be held for only a short period of time, some of the tests listed below could – if appropriate – be performed instead on the final bulk or final lot. If sufficiently justified, some of the tests may be performed on an earlier intermediate instead of on the monovalent bulk. All quality-control release tests for monovalent bulk should be validated and shown to be suitable for the intended purpose. Assay validation or qualification should be appropriate

for the stage of the development life-cycle. Additional tests on intermediates during the purification process may be used to monitor consistency and safety.

> During an emergency situation it is anticipated that critical assays would be fully validated. Specifications should also be given for each critical parameter. Qualification or validation, as well as specifications for some assays, may be based on related products (for example, products with the same vector backbone but differing in heterologous gene from the Ebola GP gene) where it can be justified that the specific heterologous gene used is unlikely to have an impact on the result. An example of this would be particle quantification by qPCR where the probe is demonstrated to be a non-EBOV sequence in the vector.
>
> With appropriate justification, validation for non-critical assays could be completed after product approval, provided that assay verification adequately demonstrates that the assay is fit for purpose and under control.
>
> Similarly, if adequately justified, not all of the proposed assays may need to be completed for clinical trial batch release. If it can be justified that product safety and potency are not compromised, that completion of the test(s) would delay product availability for use in clinical trials, and/or that the test(s) would use up an unacceptably large volume of the product urgently required for clinical trials, it may be possible to omit or delay the test, or replace it with one that is more acceptable in terms of the overall aims of the clinical trials in an emergency situation.
>
> However, all of the approaches discussed above should be agreed with the NRA on a case-by-case basis.

A.3.4.1.1 ***Purity***

The degree of purity of each monovalent bulk vaccine should be assessed using suitable methods. This should include testing for the presence of fragments, aggregates or empty particles of the product, as well as for contamination by residual cellular proteins. Residual cellular DNA levels should also be assessed when non-primary cell substrates are used for production. The content and size of 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 (*24*).

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

> In a public health emergency, theoretical calculations to determine residual levels of process contaminants (except DNA and proteins) may be acceptable at the time of licensure – data should however be submitted as soon as possible post-licensure.

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

A.3.4.1.2 *Potency*

Each monovalent bulk vaccine should be tested for potency using a combination of the following methods.

Particle number

For relevant vectors (for example, adenovirus vectors) the total number of virus particles per millilitre, quantitated by techniques such as qPCR or high-performance liquid chromatography, should be determined for each batch of monovalent bulk.

Infectivity

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

Expression of the heterologous antigen in vitro

The ability of the viral particles to express the heterologous gene should be demonstrated (for example, by the generation of immunoblots using antigen-specific antibodies) following amplification of the vector in a suitable cell line.

A.3.4.1.3 *Identity*

Tests used for assessing relevant properties of the viral vector – such as antigen expression, restriction analysis, PCR with a specific probe or sequencing – will generally be suitable for assessing the identity of the product.

A.3.4.1.4 *Sterility or bioburden tests for bacteria and fungi*

Each monovalent bulk should be tested for bacterial and fungal bioburden or sterility. Bioburden testing should be justified in terms of product safety. Sterility testing should be as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (*80*), or by methods approved by the NRA.

A.3.4.1.5 *Bacterial endotoxins*

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

A.3.4.1.6 *Reversion to replication competency or loss of attenuation*

The viral-vectored Ebola vaccines under development are either replication-incompetent in human cells or adequately attenuated to prevent disease symptoms related to the viral vector backbone. Although manufacturers generally provide theoretical justifications for why reversion to competency or virulence is unlikely to occur, low levels of viral particles may emerge that have gained the complementing gene from the production cell line by an unknown or poorly characterized mechanism. These viral particles are considered to be an impurity – it is not known whether they represent a safety concern. It should also be taken into account that many individuals within the Ebola target population could be immunocompromised. Consequently, it should be shown that the product is still replication-incompetent or fully attenuated (whichever is relevant) in initial batches of the product. After demonstrating this, it may be possible to omit such tests in future batches provided a sufficient justification is made. Such justification should include the demonstration of replication incompetence/attenuation, and discussion of why reversion to competency or loss of attenuation will not occur in future batches.

A.3.4.1.7 *Preservative content (if applicable)*

The monovalent bulk may be tested for the presence of preservative, if added. The method used and the permitted concentration should be approved by the NRA.

A.3.5 Final bulk vaccine

To manufacture the final bulk vaccine, appropriate quantities of different monovalent bulk vaccines should be pooled, mixed and formulated (if required) to form an homogeneous solution. The final bulk can be made up of one or more batches of a single monovalent vaccine, to give a monovalent vaccine product or alternatively, batches of several different monovalent bulks may be mixed to yield a multivalent vaccine.

For multi-dose preparations, the need for effective antimicrobial preservation should be evaluated, taking into account possible contamination during use and the maximum recommended period of use after opening the container or after reconstitution of the vaccine. If an antimicrobial preservative

is used, it should not impair the safety or potency of the vaccine; the intended concentration of the preservative should be justified and its effectiveness should be validated (*85*).

A.3.5.1 Control tests on final bulk vaccine

The following tests should be performed on the final bulk vaccine, unless otherwise justified and agreed with the NRA.

A.3.5.1.1 *Identity*

See section A.3.4.1.3.

A.3.5.1.2 *Antimicrobial preservative*

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

A.3.5.1.3 *Sterility tests for bacteria and fungi*

See section A.3.4.1.4.

A.3.6 Special considerations for manufacture and validation

It is acknowledged that the fermentation and downstream processes might undergo considerable optimization after the initial clinical batches are produced. Where control cells are grown in parallel to production cells, their raw materials and fermentation should be aligned with production cell manufacturing procedures. Process and product characterization should ensure the comparability of product throughout development. Some changes in product characteristics can be anticipated (for example, intended improvements due to optimization studies, or unintended changes due to a process change). All such changes should be identified and presented in clinical trial submissions or during an application for a product licence and the implications of the change should be discussed. It is not expected that process consistency will be demonstrated during early clinical development, partly because insufficient batches will have been produced to allow for adequate process validation and also because the process is likely to be undergoing optimization. However, all available batch data (including qualitative and quantitative data) should be presented. The product must be demonstrated to be free from contaminants and sufficiently characterized to allow bridging to later clinical material and commercial product. Process validation should address safety issues such as aseptic operations, sterile filtrations, cleaning validations, environmental control of facilities and validation of process utilities – such as heating, ventilation and air conditioning (HVAC) systems, and water for injection systems.

It is expected that during an emergency situation these validation criteria would be adequately addressed.

During early development, validation of pooling of single viral harvests may not have been completed and so the number of harvests pooled should be defined based on other criteria such as production requirements.

During later clinical stages and at licensing submission, the manufacturing process is normally firmly established and process-specific validation completed by demonstrating that several consecutive full-scale commercial batches can be made that conform to predetermined criteria.

Although the "Quality-by-Design" approach is not considered in these WHO Guidelines, such an approach is not excluded provided that the principles discussed throughout this document are adequately addressed.

A.3.7 Special considerations for Good Manufacturing Practice

The principles of GMP should be adhered to during the manufacture of product for clinical studies – even during a public health emergency. This may be particularly important if some normal elements of development or control have been omitted because of the urgent need for product. For example, if certain testing is to be omitted on the basis that the test is also conducted on an upstream intermediate, it is essential that the process is operated under full control. Validation and specifications are likely to be provisional during the manufacture of product for clinical trials, and additionally the process is not likely to be well understood since only a limited number of batches will have been produced. Therefore, it becomes essential that the principles of GMP, as laid down for the manufacture of investigational medicinal products, are followed (*69*, *70*, *86*).

A.3.8 Special considerations for analytical procedures and specifications

Testing of critical intermediates and of the final product, as well as in-process control testing, should primarily confirm product safety for early clinical trial batches. In this regard, tests for bioburden/sterility, endotoxin and freedom from adventitious agents should be fully developed and validated and should be applied to each batch (although some flexibility towards adventitious virus testing is also discussed in these WHO Guidelines). Other tests may not be fully validated. However, even from an early clinical phase, assay verification should have been performed. This is likely to fall short of the full validation requirements detailed in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline Q2(R1) (*87*), but should nevertheless give an indication that each method is fit for purpose.

Tests for safety, quantity, potency, identity and purity are mandatory. Upper limits should be set for quantity of impurities, taking safety considerations

into account. For relevant virus vectors, reversion to competency should be tested for. A justification should be provided for the quality attributes included in the specification and for the acceptance criteria for purity, impurities, quantity, potency and any other quality attributes which may be relevant to vaccine performance. The justification should be based on relevant development data, the batches used in nonclinical and/or clinical studies, and data from stability studies. It is acknowledged that during early clinical development, the acceptance criteria may be wider than the final specification for product intended for Phase III studies and for commercial product. During the manufacture of products for initial clinical trials, not all attributes tested may have established specification ranges since insufficient batches may have been made to know what an acceptable range is. Nor at this time is a clinically meaningful range always known. However, as the clinical programme continues – and certainly by the time of initiation of Phase III trials – specification ranges should be set for each attribute.

Product characteristics that are not completely defined in the early stages of development, or for which the available data are too limited to establish relevant acceptance criteria, should also be recorded. As a consequence, such product characteristics could be included in the specification without predefined acceptance limits. At the initial stages of development, testing may not be required to determine residual levels of process contaminants (except DNA and proteins) if sufficient justification can be provided by theoretical calculation. However, data to confirm the calculations should be provided prior to the licensing application.

For later-stage clinical trials, it is expected that all analytical procedures would be validated according to the principles set out in ICH Q2(R1) (*87*). Specifications for each parameter should be justified by process capability as well as by clinical suitability. If justified, following the manufacture of additional batches of product, the sponsor should commit to revise the specifications as data on process capability are accumulated.

> During a public health emergency, data on clinical suitability are likely to be limited and should be taken into account to the extent that they are available.

A.4 Filling and containers

The general requirements concerning filling and containers given in WHO good manufacturing practices for biological products (*70*) should apply to vaccine filled in the final form.

Care should be taken to ensure that the materials of which the containers and closures (and, if applicable, the transference devices) are made do not adversely affect the quality of the vaccine. To this end, a container closure integrity test and assessment of extractables and/or leachables for the

final container closure system are generally required for the qualification of containers, and may be needed as part of stability assessments.

If multi-dose vaccine vials are used and these vaccines do not contain preservative then their use should be time-restricted, as is the case for reconstituted vaccines such as bacillus Calmette–Guérin (BCG) and measles-containing vaccines (*85*). In addition, the multi-dose container should prevent microbial contamination of the contents after opening. The extractable volume of multi-dose vials should be validated.

The manufacturers should provide the NRA with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.5 Control tests on final lot

Samples should be taken from each final vaccine lot – which may be monovalent or multivalent. These samples must fulfil the requirements of this section. All tests and specifications should be approved by the NRA. The specifications should be defined on the basis of the results of tests on lots that have been shown to have acceptable performance in clinical studies.

A.5.1 Inspection of containers

Every container in each final lot should be inspected visually or mechanically. Those showing abnormalities should be discarded and each relevant abnormality should be recorded. A limit should be established for the maximum number of containers which can be discarded before investigation of the cause; potentially resulting in batch failure.

A.5.2 Appearance

The appearance of the vaccine should be described with respect to its form and colour.

A.5.3 Identity

See section A.3.4.1.3. For multivalent vaccine each antigen component should be identified.

A.5.4 Sterility tests for bacteria and fungi

See section A.3.4.1.4.

A.5.5 General safety test (innocuity)

The need to test the final lots of the Ebola vaccine for unexpected toxicity (also known as abnormal toxicity) should be discussed and agreed with the NRA.

Some countries no longer require this test (*88*, *89*).

A.5.6 Purity

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

A.5.7 pH and osmolality

The pH and osmolality values of each final lot of containers should be tested. Lyophilized products should be reconstituted with the appropriate diluent prior to testing.

A.5.8 Test for pyrogenic substances

Each final lot should be tested for pyrogenic substances through intravenous injection into rabbits. A Limulus amoebocyte lysate (LAL) test may be used in lieu of the rabbit pyrogen test if it has been validated and the presence of non-endotoxin pyrogens has been ruled out. A suitably validated monocyte-activation test may also be considered as an alternative to the rabbit pyrogen test. The endotoxin content or pyrogenic activity should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and should be approved by the NRA.

A.5.9 Potency, particle number and infectivity

See section A.3.4.1.2.

The potency specifications for live viral-vectored vaccines should be set based on the minimum dose used to demonstrate efficacy or effectiveness in human clinical trials and/or challenge studies with a suitable non-human preclinical model plus human immunogenicity data. An upper limit should also be defined based on available human safety data. For multivalent vaccines it may be necessary to perform this test on the monovalent bulks instead if analytical methods cannot distinguish between the different monovalent vaccines in the final lot.

A.5.10 Extractable volume

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

A.5.11 Aggregates/particle size

Since virus particles are susceptible to aggregation, each final lot should be examined for particle size/aggregate content at lot release and at end of shelf-life unless it can be shown that the test is not necessary.

A.5.12 Preservatives (if applicable)

Each final lot should be tested for the presence of preservative, if added.

A.5.13 Residual moisture (if applicable)

For freeze-dried final product, the residual moisture should be shown to be within acceptable limits.

A.5.14 Reconstitution time (if applicable)

For freeze-dried final product, the reconstitution time of the product should conform to specification.

A.6 Records

The requirements given in section 17 of WHO good manufacturing practices for biological products (*70*) should apply.

A.7 Retained samples

The requirements given in section 16 of WHO good manufacturing practices for biological products (70) should apply.

A.8 Labelling

The requirements given in section 14 of WHO good manufacturing practices for biological products (*70*) should apply.

The label on the carton, the container or the leaflet accompanying the container should state:

- the name of the vaccine;
- the lot number;
- the nature of the cells used to grow the viral vector;
- the volume of one recommended human dose, the immunization schedule and the recommended routes of administration;
- the amount of active substance(s) contained in one recommended human dose;

- the number of doses, if the product is issued in a multi-dose container;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the name and concentration of any preservative added;
- the temperature recommended during storage and transport;
- the expiry/retest date;
- any special dosing schedules; and
- contraindications, warnings and precautions, concomitant vaccine use advice, and potential adverse reactions.

Labelling should conform to the national requirements of the region in which the vaccine will be used.

A.9 Distribution and transport

Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (*90*).

Efforts should be made to ensure that shipping conditions are such as to maintain the vaccine in an appropriate environment. Temperature indicators should be packaged with each vaccine shipment to monitor fluctuations in temperature during transportation.

A.10 Stability testing, storage and expiry date

A.10.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (*91*). Stability testing should be performed at different stages of production, namely: on single harvests or single harvest pools (if the process is held up for a period of time, which may affect product attributes at these points); final monovalent bulk; final bulk; whenever materials are stored for a period of time before further processing (which may affect product attributes); and final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. A shelf-life should be established and assigned to all in-process materials during vaccine production, and particularly to the vaccine intermediates.

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

For vaccine licensure, the stability and expiry date of the vaccine in its final container, when maintained at the recommended storage temperature, should be demonstrated to the satisfaction of the NRA using final containers from at least three final lots made from different vaccine bulks. During clinical trials, fewer data are likely to be available. However, the stability of the vaccine under the proposed storage conditions should be demonstrated for at least the expected duration of the clinical trial.

Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (*91*). Data should be provided to the NRA according to local regulatory requirements.

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

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

> In an emergency situation and during early clinical trials, limited stability data on the monovalent or final bulk vaccine and finished product may be acceptable to preserve scarce stocks of product for use in clinical trials, or if there is insufficient time to generate real-time stability data. Data from one batch of bulk and final product may be sufficient initially but this should be supplemented with data from at least two more batches of bulk and final product as material that is surplus to clinical trial requirements becomes available.
>
> Even if limited stability data are available, it is preferable to provide an expiry or retest date on the immediate product label since this provides important information to the user. If this goes beyond the available real-time data, accelerated stability data should be available to help support the proposed extrapolation to the shelf-life, and the clinical trial sites should be able to demonstrate a robust system for recalling the product if real-time data do not support the extrapolated shelf-life. In exceptional circumstances, the rationale for omitting this information from the label may be discussed with NRAs.

A.10.2 Storage conditions

Storage conditions should be fully validated. The vaccine should have been shown to maintain its potency for a period equal to that from the date of release to the expiry date. During clinical trials, this period should ideally be at least equal to the expected duration of the clinical trial.

A.10.3 Expiry date

The expiry date should be based on the shelf-life supported by stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of final bulk, date of filling or the date of the first valid potency test on the final lot.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals are inoculated.

Part B. Nonclinical evaluation of Ebola vaccines

B.1 General remarks

The design, conduct and analysis of nonclinical studies should be based on the WHO Guidelines on nonclinical evaluation of vaccines (*20*). Further guidance can be found in WHO and national and regional documents on DNA vaccines (*14*, *15*) and live recombinant viral-vectored vaccines (*16–18*).

The nonclinical safety evaluation, whenever necessary, should yield sufficient information to demonstrate that the candidate vaccine is reasonably safe for use in humans.

The following sections describe the types of nonclinical information that should be submitted to support the licensing of a new Ebola vaccine. Wherever appropriate, recommendations are also made on the minimum dataset required.

B.2 Product characterization and process development

It is vitally important that vaccine production processes are standardized and appropriately controlled to ensure consistency in manufacturing. The extent of process validation may vary with the stage of product development. The vaccine lots produced for nonclinical good laboratory practice (GLP) safety studies should be manufactured with production process, formulation and release specifications similar to those of the lots intended for clinical use. Supporting stability data generated under conditions of use should be provided.

For a live viral-vectored vaccine, the degree of attenuation and the stability of the phenotype should be evaluated. The critical genetic and phenotypic markers of stability of the vector genome should as far as is practical be defined. Phenotypic markers are useful for the detection of reversion events and may include, though are not restricted to, vector replication efficiency, induction of viraemia and level of virulence, and neurovirulence. The need for neurovirulence testing is discussed below in section B.4.

B.3 Pharmacodynamic studies

B.3.1 Challenge-protection studies

In the past, rodents (mouse, guinea-pig) and non-human primates (cynomolgus or rhesus macaques) have been used to study the pathogenesis of EBOV infection and the mechanism of immune protection. Rodent models are frequently used to provide initial evidence for the immunogenicity or efficacy of candidate vaccines. However, non-human primates display natural susceptibility to EBOV infection and similarity in genetics, morphology and immunology with humans, and more closely mimic EVD observed in humans. As a consequence, the non-human primate models are particularly useful for proof-of-concept challenge studies and characterization of the mechanism of protection. It is expected that proof-of-concept data be collected for each virus strain included in the candidate vaccines.

It should be noted that conducting proof-of-concept challenge studies with wild-type EBOV requires a BSL-4 containment facility. The same requirement may apply to running virus-neutralization assays when wild-type EBOV is used to evaluate vaccine immunogenicity and to evaluate serology samples obtained from animals after EBOV challenge. A BSL-2 facility is sufficient to contain animals until the time of challenge, to run other immunological assays – such as enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISpot) and intracellular cytokine staining (ICS) – without involvement of a wild-type EBOV, or to manufacture a genetically modified organism (GMO).

Due to limited availability of BSL-4 laboratories, the proof-of-concept challenge studies will generally be small. Nonetheless, these studies are of higher predictive value than immunogenicity studies for forecasting vaccine performance in humans. The parallel assessment of vaccine immunogenicity and efficacy (protection from EVD) in proof-of-concept challenge studies may permit the establishment of an immune correlate of protection (ICP) and an understanding of the underlying protective mechanism.

> Either during a public health emergency or in a normal situation, the challenge studies are not required prior to initiating Phase I clinical trials. However, it is nevertheless desirable for proof-of-concept challenge studies to be conducted early during product development since these studies, in combination with immunogenicity assessment, could provide important information regarding an ICP and protective mechanism, which would assist in the selection of immunological end-points in subsequent clinical trials.

The design of challenge-protection studies should take into account the planned posology for a specific route of administration and valency of candidate vaccines. For a multivalent candidate vaccine intended to induce durable protective immunity, a heterologous prime-boost regimen may need to

be considered. The protective activity of the vaccine with respect to each of the Ebola strains targeted should be assessed.

As in any challenge-protection animal study, the end-points used to define protection should normally correlate with the desired effect in humans – typically a survival benefit or attenuation of severe disease indicators such as viral shedding, body weight changes and other relevant clinical signs. Other key characteristics of the experimental design include the use of appropriate challenge virus strains, dose(s) and route of challenge. The challenge dose should be sufficiently high to produce an appropriate degree of lethality in the control group of animals so that the vaccine protective effect can be shown with adequate statistical power. For example, doses of 100–1000 plaque-forming units (PFU) have been used (92).

The collection of challenge-protection data should take account of the proposed indication for use – that is, pre-exposure versus post-exposure prophylaxis against EVD. Appropriate timing of the challenge is another important consideration. For pre-exposure prophylaxis, animals are usually challenged at the time when the peak level of vaccine response (for example, peak antibody titres) has developed post-vaccination. Where feasible, it would also be informative for various public health vaccine strategies to challenge animals at other times (for example, before the peak response or after the immune responses have waned). For post-exposure prophylaxis, challenge at various time points should be considered.

B.3.1.1 Use of a challenge-protection animal study to support licensure

In some circumstances in which demonstrating vaccine efficacy in clinical trials is not feasible – due to low rates of EVD or absence of an EVD outbreak, or when a human ICP has not been established for a vaccine – manufacturers may propose an alternative approach to estimating vaccine effectiveness to support licensing (for example, by inferring animal challenge results to humans). If this course is pursued – and agreed to by the relevant NRA – the study should be adequately designed to generate reliable data for inferring effectiveness in humans (see section C.2.5).

Beyond the key design elements discussed above, further considerations may include the use of non-human primates, vaccinating animals with an appropriate range of doses of the vaccine so that the level of immune response developed in animals (for example, range of relevant antibody titres) can match that in humans. Compliance with GLP also brings significant advantages and is encouraged. However, it is acknowledged that compliance with GLP may not be possible in BSL-4 laboratories. Consequently, well-controlled and well-documented non-GLP studies are also acceptable. The use of good documentation practices to ensure data integrity is required.

The standardization of non-human primate challenge models is important for generating reproducible and relevant data for the purpose of supporting licensure, especially when different candidate Ebola vaccines are compared. Relevant aspects here include species and age of animals, challenge material (including virus strain/variant and passage number), challenge route, challenge dose, criteria for animal euthanasia, and standardized data collection and reporting. Further current thinking on this issue can be found elsewhere (*4*).

B.3.2 Immunogenicity studies

Immunogenicity studies in animal models can generate important information on the immunological properties of the candidate vaccine. These studies should evaluate immune responses both quantitatively and qualitatively as per intended posology. The immune responses to each of the Ebola strains in a multivalent vaccine should be assessed, including any potential immunological interference between strains. Data on cross-neutralizing antibodies and cross-reactivity should be obtained for monovalent and multivalent vaccines through the use of heterologous viruses.

Such studies can provide evidence for the appropriateness of the vaccine dose, the number of doses, dosing interval and dose–response relationship.

> Either during a public health emergency or in a normal situation, immunogenicity data derived from a relevant species responsive to the vaccine antigen in terms of desired immune responses are an expected minimum requirement prior to starting Phase I clinical trials. Alternatively, strong supportive data generated from the same platform technology (for example, the same vector and manufacturing process, but expressing different vaccine antigens) may be considered sufficient for Phase I trial initiation.

Immunogenicity should be measured as humoral, cellular or functional immune responses, as appropriate to each of the intended protective antigens and to the antigens of the vector used. For several leading candidate vaccines using Ebola GP as a sole protective antigen, antigen-specific ELISA (which measures the quantity of serum GP-specific IgG antibodies) has been routinely used to characterize the humoral response. Evaluation of cellular responses should include the phenotypic and functional characterization of CD8+ and CD4+ T cell responses using sensitive and highly specific assays such as ELISpot and ICS by multiparameter flow cytometry. The functional activity of immune responses may be measured in vitro in neutralization assays using either wild-type virus or pseudovirion virus. More extensive analyses may include examination of Th1 and Th2 responses, the kinetics and duration of CD8+ and CD4+ T cells and antibody responses, as well as assessment of the quality or fine specificity of the antibody response.

As discussed in section B.3.1, the assessment of immunogenicity parameters in proof-of-concept challenge studies may allow for the establishment of a correlation between an antibody or other immune response (such as cellular immunity or cytokine response) and the level of protection from disease or death, or for understanding the underlying protective mechanisms. These key data may be expected to be generated during the development of the product.

Assessment of immunogenicity against multiple EBOV types should be performed for multivalent vaccines and should also be considered for monovalent vaccines.

B.4 Nonclinical safety studies (toxicity testing)

A safety assessment, including repeat-dose toxicity and local-tolerance studies, is generally required for all new candidate vaccines, unless otherwise adequately justified (*20*). In general, these studies will have been completed and analysed prior to the initiation of Phase I clinical trials. Additional safety testing may be necessary depending on the properties of the candidate vaccines. For a replicating recombinant vaccine vector with neurovirulent potential, neurovirulence testing in an animal species acceptable to the relevant NRA is an important consideration and should be conducted before proceeding to trials in humans.

> During a public health emergency, interim data from ongoing toxicity studies (including on the immediate effect on survival and vital physiological functions) and the submission of draft unaudited toxicity study reports may be sufficient to support proceeding to Phase I clinical trials with a novel platform/candidate vaccine.
>
> As in a normal non-emergency situation, the omission of toxicity studies may be possible if there are adequate platform toxicology data and clinical safety experience. For example, for the viral-vectored vaccines that this document focuses on, toxicity studies were not required during the 2014–2016 EVD epidemic.
>
> Such a limited dataset should be of good quality – that is, it should be generated from a relevant animal species and should follow GLP principles.
>
> Since the use of a reduced toxicity dataset during a public health emergency provides less certainty about the safety of the product, additional data should be submitted once they become available, including data on any delayed effect observed at later time points in repeat-dose toxicity studies, histopathological data and the final signed audited reports. Early discussion with NRAs in the countries where the Phase I clinical trials are to be conducted is encouraged.

Since Ebola vaccines are also beneficial for women of childbearing potential, a reproductive-toxicity study will need to be conducted at an appropriate point during product development. Serious consideration should be given to vaccine administration that results in the exposure of pregnant animals to a vaccine response during the early phase of implantation/organogenesis. For a replicating recombinant vaccine vector that may have a direct effect on the embryo/fetus, the dosing regimen should ensure a sufficient level of vaccine vector in the blood of exposed pregnant animals.

> The requirement for a developmental toxicity study is an important issue for consideration, depending on the level of threat or control of the disease. During the 2014–2016 EVD epidemic, large-scale Phase III efficacy trials were approved in endemic countries without intentionally enrolling pregnant women. With decreasing numbers of cases as the 2014–2016 epidemic was brought under control, the local NRAs required that developmental toxicity data be made available to support the enrolment of pregnant women.

B.5 Pharmacokinetic (biodistribution) studies

Classic pharmacokinetic studies with live viral-vectored vaccines are normally not required. However, a biodistribution study in a relevant species should generally be considered if the recombinant viral vector has any of the following characteristics: (a) it is a novel viral vector or a known vector with a novel envelope and there are no existing biodistribution data for the platform; (b) there is a likelihood of altered infectivity and tissue tropism due to recombination; or (c) a novel route of administration and formulation is to be used.

B.6 Environmental risk

The use of Ebola vaccines based on recombinant viral vectors could result in the release of recombinant microorganisms into the environment. Some countries have legislation covering environmental and other concerns related to the use of live vaccines derived by recombinant DNA technology since they may be considered as GMOs, and an environmental risk assessment (ERA) must be submitted with any application to market these products. The specifics of the ERA assessment within each country/region vary. Manufacturers are encouraged to start a dialogue with the responsible authorities, including regulatory authorities in countries where clinical trials are planned, early in the development of this class of product.

The WHO Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) (*93*) provide advice in this respect that may also be useful in the case of Ebola vaccines.

The primary environmental risk of a replicating recombinant vaccine vector relates to vaccine vector shedding and shedding-based transmission to third parties – that is, to unvaccinated humans or domestic animals following human administration. In the case of a replication-incompetent recombinant viral vector, no shedding experiment is required. For future candidate novel live recombinant vaccines based on a GMO, an ERA of the possible shedding of the vaccine organisms following administration is required as part of the preclinical evaluation.

Part C. Clinical evaluation of Ebola vaccines

C.1 General considerations

Clinical development programmes for Ebola vaccines must take into account the epidemiology of the disease, the infrastructure for conducting clinical trials in affected areas and the regulatory frameworks of particular NRAs. However, key points that should be common to all such programmes are: (a) the standards for demonstrating Ebola vaccine safety and effectiveness are the same as for other vaccines; and (b) clinical studies are to be conducted in accordance with the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (*94*) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*22*).

As for all vaccines, close monitoring of studies by an independent data monitoring committee (if warranted), the ethics committee(s) and the sponsor should help to ensure study integrity. Meetings between sponsors and the relevant NRA at critical time points during clinical development should be encouraged, as well as meetings to discuss scientific and medical questions that may arise at any time during an investigation.

C.1.1 Study population

Study population characteristics (for example, demographics, location, underlying medical conditions and Ebola immune status) may vary by phase of clinical development, as further discussed in section C.1.2. Specific considerations for the evaluation of Ebola vaccines in the paediatric population are discussed in section C.7.2.

Inclusion and exclusion criteria for participants should be defined for each study planned. Exclusion criteria may include previous receipt of an Ebola vaccine and possible previous contact with a person with EVD. Consideration should be given to excluding subjects at risk of loss to follow-up (for example, individuals not planning to live in the area for the duration of safety follow-up), as well as immunodeficient or immunosuppressed subjects, particularly in the

case of live vaccines based on replication-competent viral vectors. Additional exclusion criteria should be based on clinical experience with the particular vaccine, with the aim of excluding individuals who may have an increased risk of significant adverse reactions, and individuals whose underlying conditions may make it difficult to interpret safety data. For example, an investigational recombinant VSV-vectored Ebola vaccine has been associated with arthritis in one study. Consideration should be given to excluding individuals with arthritis or related conditions (active or in past medical history) from participating in initial studies of this vaccine, taking into account their risk of contracting Ebola, and pending subsequent determination of the frequency, duration and severity of this adverse event. Thus, considerations for exclusion would likely differ for studies of healthy volunteers with a low risk of exposure to EBOV and for studies conducted in the setting of an active outbreak.

> The phase of clinical development and circumstances of the study should also be considered when developing inclusion and exclusion criteria. For example, a later-phase study being conducted in an emergency situation in a population at high risk of EVD would probably have fewer exclusion criteria than a Phase I study of healthy volunteers not at risk of EVD. The phases of clinical development are described below in section C.1.2.

Pre-vaccination sera should be collected, at least in early-phase trials, to assess pre-existing antibodies to EBOV and vaccine vector viruses, as well as to assess aspects of baseline health status. The laboratory values expected for the study population and any exclusion criteria should be specified in the study protocol. Stored pre-vaccination serum may also be useful in the assessment of certain post-vaccination adverse events that may occur. Assessment of possible causal associations between vaccination and adverse events can also be facilitated by knowledge of the background rates of events in the relevant general population.

C.1.2 Phases of clinical development

The phases of vaccine clinical development are typically a continuum from Phase I, which often includes the first-in-human clinical trials carried out primarily to assess safety and preliminary immunogenicity, to Phase II to further describe safety and dose relationship to immunogenicity, and then to Phase III pivotal studies to demonstrate the safety and effectiveness of a product in support of licensure.

> As for all vaccines, Phase I and Phase II studies of investigational Ebola vaccines are expected to provide initial safety and immunogenicity data, and to assess the optimal dose. The epidemiology of the disease is likely to have a major impact on the timing and design of Phase III studies. In the face of an outbreak, without available preventive vaccines, vaccine

evaluation should adhere to the principles of this phased approach but intervals between phases of evaluation may be compressed and overlapping. For example, compressed timelines for clinical development may be achieved by initiating Phase III studies based on interim safety and immunogenicity data from earlier-phase studies rather than on data from final study reports. Clinical development of an Ebola vaccine in the setting of an outbreak is complex. Close collaboration between public health authorities, NRAs, the community, clinical investigators and the vaccine developer is essential to ensure that studies will meet licensure requirements, including requirements for ethical conduct. Phase II and Phase III clinical trials may be designed with prospectively planned adaptive features that allow for changes in design or analyses based on examination of the accumulated data at pre-specified interim points in the trial. Such adaptive features may make trials more efficient. For detailed considerations regarding approaches and the designing of studies to demonstrate vaccine effectiveness see section C.2.

C.1.2.1 Phase I studies

The primary purpose of Phase I vaccine studies is to obtain preliminary safety and immunogenicity data. For Ebola vaccines, these studies would generally be first conducted in a small number (for example, < 100) of healthy adult volunteers previously unexposed to EBOV and at low risk of EVD.

However, in the face of an outbreak, NRAs may consider larger Phase I clinical studies (for example, by enrolling more sites) to increase the early safety and immunogenicity database, as well as the use of study populations similar to the eventual target population, thus facilitating timely initiation of Phase II clinical studies.

The design of Phase I studies can be uncontrolled and open label or may include a placebo control. When possible, the concomitant use of other vaccines should be avoided to optimize the safety evaluation. The study design may include sequential dose-escalation whereby subjects enrolled in lower-dose cohorts are closely monitored for safety for a defined period (for example, 1–2 weeks or as appropriate for the characteristics of the vaccine) and the resulting data are reviewed before subsequent enrolment of additional subjects in successively higher-dose cohorts. All study participants should be actively and closely monitored for safety.

C.1.2.2 Phase II studies

Phase II studies are initiated once satisfactory safety and immunogenicity data from Phase I studies are available. In the absence of safety concerns from short-term post-vaccination follow-up in Phase I studies (for example, 7 days or as

appropriate for the specific vaccine), development may in some cases proceed to Phase II studies in parallel with the continued collection of longer-term safety data from Phase I studies. Phase II studies provide further information on safety and immunogenicity to determine the optimal dose and dosing regimen, and to support initiation of Phase III studies. Phase II studies typically involve up to several hundred subjects and are frequently randomized, double-blind and controlled. The comparator is usually an inert placebo or a control vaccine that provides protection against disease unrelated to EVD. Phase II trials should be of sufficient size to test hypotheses on dose and dosing regimen. Phase II studies should be conducted in the proposed target population or in a population similar to the target population in terms of demographic and ethnic factors, and other factors that might impact on vaccine effectiveness or safety (for example, concomitant infections). Detailed safety and immunogenicity data should be obtained in Phase II studies.

C.1.2.3 Phase III studies

Large-scale Phase III clinical studies involve more-extensive testing to provide a rigorous assessment of vaccine effectiveness that may include direct evaluation of efficacy in protecting against clinical disease, expanded safety evaluation and opportunities to potentially identify an ICP. Definitions of vaccine effectiveness and vaccine efficacy are provided in section C.2.1. Phase III clinical trials may also permit clinical evaluations of lot-to-lot manufacturing consistency. The target population for Phase III clinical trials with candidate Ebola vaccines should consist of individuals at high risk for the disease (that is, populations residing in EVD outbreak areas, relevant health-care providers, laboratory personnel or first responders). The design of Phase III effectiveness studies must be of adequate scientific rigour to support effectiveness claims, while adhering to ethical standards. Ideally, effectiveness is evaluated in randomized, double-blind, well-controlled trials with a parallel control group receiving an inert placebo such as saline injection or a vaccine that provides protection against another disease. In some settings, the balance between scientific rigour and ethical standards may preclude the use of a placebo group – for example, if there is an existing efficacious Ebola vaccine that those in the trial might be eligible to receive. Ethical considerations for the use of placebos in vaccine research, including in circumstances in which an efficacious vaccine is already available, are discussed in the WHO meeting report *Expert consultation on the use of placebos in vaccine trials* (95). As discussed in section C.2 below, other study designs for obtaining effectiveness data for candidate Ebola vaccines may be considered if a placebo-controlled trial is not considered ethical or is not feasible.

To demonstrate vaccine effectiveness, Phase III trials may be based on a disease end-point or, as described in section C.2, they may be based on

the attainment of a level of an immune marker predictive of protection. The incidence of EVD and ethical considerations will be primary determinants of the approach used to evaluate vaccine effectiveness and the design of clinical end-point efficacy studies, as also discussed in more detail in section C.2. For many disease end-point clinical efficacy study designs, large sample sizes may be needed, particularly if the incidence of the disease in the study population is expected to be low or to decline during the study period. Adequate statistical justification of the size and duration of the trial should be provided, and trial end-points and criteria for trial success specified prior to initiation of the study. Plans should be included to monitor the conduct of the trial, taking into consideration the potential for changes in disease incidence which may necessitate trial design modification. It is important that some attempt should be made to define an ICP as part of efficacy studies. For such an evaluation to be clinically meaningful, validated standardized assays are essential.

Clear and definite evidence that the vaccine is safe and effective is required for regulatory decision-making. Discussions should be held with relevant NRAs on the study design and on plans for conducting the study and analysing its results at the early conceptual stage of the Phase III study, and agreement reached with the NRAs prior to trial initiation. Close consultation with local community leaders, health policy-makers and ethics committee(s) in EVD outbreak regions where efficacy studies are planned is also crucial.

C.2 Demonstration of effectiveness of candidate Ebola vaccines

C.2.1 Definitions of effectiveness and efficacy

It is important to distinguish vaccine effectiveness from vaccine efficacy. Vaccine efficacy is an estimate of the reduction in the incidence of clinical disease observed in a vaccinated group relative to the incidence of disease in a group not vaccinated against the disease to be prevented. Vaccine efficacy measures direct protection (that is, protection induced by vaccination in the vaccinated population sample). The best estimates of vaccine efficacy come from randomized controlled clinical trials.

Vaccine effectiveness is an estimate of the protection conferred by vaccination. It is usually obtained by monitoring the disease to be prevented by the vaccine during routine use in a specific population. It may measure both direct and indirect protection (for example, the estimate may reflect in part the protection of non-vaccinated people secondary to the effect of the vaccine in the vaccinated population). Thus, the term vaccine effectiveness may be used broadly to encompass vaccine efficacy (direct protection) as well as indirect protection. Evidence for vaccine effectiveness may be derived from challenge-protection studies conducted in animal models or from a vaccine-induced

immune response (for example, pre-specified antibody threshold induced by the vaccine in vaccinated people).

For any preventive vaccine, the most direct approach for demonstrating effectiveness is based on clinical end-point efficacy trials showing protection against disease, or alternatively, based on clinical trials evaluating a scientifically well-established ICP (for example, antibody response).

C.2.2 Immunological evaluation of Ebola vaccines

Clinical disease end-point efficacy trials provide an opportunity to identify an ICP. The derivation of an ICP is facilitated by the availability of post-vaccination serum samples from a relatively large number of protected trial participants as well as from vaccinated participants who develop disease. Thus, for all Ebola vaccine clinical disease end-point efficacy trials, post-vaccination serum samples (and preferably also pre-vaccination serum samples) would ideally be collected from all subjects, with post-vaccination sampling at regular predefined intervals throughout the study period. If this is not feasible, pre- and post-vaccination serum samples should be collected from as many subjects as possible. Ebola prevalence studies in various African countries have revealed unexpectedly high rates of baseline Ebola seropositivity in some regions, as measured by serum IgG antibodies, underscoring the importance of collecting baseline serum samples in studies conducted in these countries (*96–101*). Consideration should also be given to the collection of blood samples for the evaluation of cell-mediated immunity which may play a role in protection for some vaccines.

Even if it is not possible to identify an ICP from a clinical end-point efficacy trial, immunogenicity data from Phase II and Phase III studies are crucially important for the use of alternative approaches to assess vaccine effectiveness based on surrogate immune response end-points likely to predict protection and/or for challenge-protection studies conducted in animal models (see sections C.2.4 and C.2.5 respectively).

Potentially important immunogenicity end-points include EBOV IgG ELISA antibody titre and presence/levels of EBOV neutralizing antibody. End-points evaluating T cell mediated responses following vaccination may also be considered. Specific considerations regarding immunological assays are discussed below in section C.6.

In evaluating antibody response to vaccination, it is important to stratify analyses by baseline serostatus and to pre-specify the definition of seroresponse, and seroconversion. Seroresponse is typically based on an x-fold rise in antibody level from pre-vaccination to post-vaccination in initially seropositive individuals. Seroconversion is typically based on achieving a measurable antibody level post-vaccination in individuals who were initially

seronegative. A detailed justification for the definition of each term should be provided. The definition of seroresponse may differ for different Ebola vaccines and assays. Serological end-points and evaluation criteria should be determined following input from, and agreement by, the NRA before study un-blinding and serological analysis.

As an ICP (including potential antibody thresholds associated with protection) or a surrogate immune marker may differ for different vaccines, it is important to obtain vaccine-specific human serological data. Ideally, vaccine-specific human cellular immune response data would also be obtained (*102*). Applicability of an ICP or a surrogate immune marker will depend on specific vaccine characteristics such as antigen structure, mode of delivery, antigen processing in the vaccinee and virus serotype. For example, an ICP established for an adenovirus-vectored Ebola vaccine cannot be presumed to be applicable to a VSV-vectored Ebola vaccine given that the two vaccines present antigen differently and engender different types of protective immune responses. Similarly, Ebola vaccines that are, for example, based on VSV and adenovirus vectors and administered using a prime-boost regimen may induce different protective immune responses than Ebola vaccines based on different platforms or technologies and administered using a different regimen. As another example, an ICP or a surrogate immune marker identified for a vaccine containing a particular EBOV (for example, ZEBOV) cannot be assumed to be applicable to another vaccine containing a different EBOV (for example, SUDV).

C.2.3 Clinical disease end-point studies

C.2.3.1 General principles of clinical disease end-point studies

In general, the crucially important aspects of clinical disease end-point efficacy studies include: (a) an appropriate control group; (b) appropriate methods for randomization, as applicable; (c) masking procedures, as applicable; (d) a pre-specified primary end-point (for example, EVD confirmed by PCR); (e) pre-specified important secondary end-points (for example, EVD not laboratory confirmed); (f) pre-specified, detailed clinical case definitions for the primary end-point; (g) validated diagnostic assays to support the pivotal efficacy analyses; (h) unbiased case-ascertainment methods; and (i) adherence to relevant statistical principles. Measures to reduce potential bias are important in all trials, but particularly so for designs other than randomized, double-blind, controlled trials with a parallel control group. Specific considerations regarding the design of clinical end-point efficacy studies and diagnostic tests for EVD are discussed below in sections C.2.3.2 and C.6.1, respectively. Consideration should be given to the establishment of an independent data-monitoring committee for clinical end-point efficacy studies of Ebola vaccines in order to advise the sponsor on the continuing validity and scientific merit of the study.

C.2.3.2 Design of clinical disease end-point studies

C.2.3.2.1 *Randomized controlled trials*

The prospective randomized, double-blind, placebo-controlled trial with an EVD end-point is the gold standard for demonstrating the efficacy of any investigational Ebola vaccine(s) when no licensed efficacious vaccine is available. This design avoids potential bias in the assessment of end-points and maximizes the chance that a difference in disease incidence observed between the vaccinated and unvaccinated groups is due to a true effect of the vaccine being evaluated. The unit of randomization is usually the individual subject enrolled in the trial, although other units of randomization may be considered. While direct assessment of vaccine efficacy in randomized controlled trials provides the most definitive evidence of effectiveness, it requires a sufficiently high disease incidence and a correspondingly adequate sample size.

C.2.3.2.2 *Ring vaccination design*

In settings with relatively low disease incidence, vaccine efficacy clinical trial designs – such as ring vaccination in which people at highest risk of infection are recruited – may be considered in order to maximize statistical power (*64*, *103*).

> A novel cluster randomized controlled trial design to evaluate vaccine efficacy and effectiveness during outbreaks, the ring vaccination trial design was developed with special reference to Ebola (*101*). The approach taken to increase statistical power is to recruit those at highest risk of infection (for example, individuals who are socially or geographically connected to an index case). An important consequence of this increase in power is that this trial design has the potential to yield an estimate of vaccine efficacy within a shorter period of time and possibly with a smaller sample size, compared to more-common trial designs.
>
> A ring is a socio-geographical population group made up of the contacts and contacts of contacts of the index case. Rings are randomly assigned to immediate or delayed vaccination, with the delayed vaccination rings serving as controls. Vaccine efficacy is calculated on the basis of the relative rates of disease in the immediate and the delayed vaccination rings. An efficacy trial using ring vaccination with an investigational Ebola vaccine was conducted in Guinea in 2015 (*64*, *103*).

C.2.3.2.3 *Stepped wedge randomized cluster trial*

After licensure of an Ebola vaccine – and in some settings even before licensure – the high case-fatality rate of EVD may raise ethical concerns about non-vaccination in a parallel control group. To mitigate these concerns, a stepped wedge randomized cluster trial (SWRCT) design in which clusters of participants

are sequentially vaccinated over a number of time periods, may be considered. In this design, all participants start in the control group and, at predefined time points, a cluster of participants is vaccinated in a random order (known as "steps"). Vaccine efficacy is calculated on the basis of the relative rate of disease in the vaccinated population compared to the unvaccinated population. This design, in which all participants are vaccinated by the end of the study, may be ethically acceptable in settings where the candidate vaccine is not available simultaneously for all participants and where the use of a placebo group is considered unacceptable.

Disadvantages of the SWRCT design include difficulty in blinding, attrition in the later vaccinated clusters and the more complex analysis required. In addition, an underlying requirement for validity of an SWRCT design is that disease incidence rates must remain fairly stable throughout the trial. If disease incidence rates are not expected to remain reasonably constant during the course of the trial, data analyses may be performed separately within narrow windows of time (for example, by day or week) within which it can be assumed that disease incidence rates are stable. This time stratification will necessitate more careful recording of disease incidence rate with time. The impact of misclassification of disease incidence rate with time will need to be considered. Another issue is that SWRCT designs randomize the timing of vaccination of the clusters, which unlike most designs disallows the flexibility to move vaccination to high-risk areas that evolve while the trial is ongoing, and which could also potentially cause the SWRCT to take longer to complete compared to other trial designs (*104*).

C.2.3.2.4 *Test-negative case control design*

Once a vaccine has been deployed in a population, it may be possible to estimate vaccine effectiveness using a test-negative case control design (*105–107*). In the test-negative case control design, patients seeking health care for symptoms compatible with EVD are recruited into the study and tested for the disease. Vaccine effectiveness is estimated by comparing the odds of vaccination in subjects testing positive for Ebola (cases) to the odds of vaccination in subjects testing negative (controls).

Test-negative case control studies are relatively low cost and easy to conduct. However, controlling for potential bias in this non-randomized design is particularly challenging because vaccinated and unvaccinated individuals may have different risk factors for disease.

Test-negative case control studies are also subject to the same sources of bias and measurement error as other non-randomized studies – some of which may not be recognized or adequately adjusted for in the statistical analyses. Furthermore, it may be difficult to assure comparable disease severity across participants at study entry or to achieve complete ascertainment of vaccination

status. Potential sources of bias and limitations inherent in this design need to be carefully considered in planning study procedures and statistical analysis, as well as in interpreting the results.

C.2.4 Surrogate end-points for demonstration of effectiveness

For diseases like EVD, for which there is no well-established ICP, if disease incidence is too low to feasibly conduct clinical end-point efficacy studies then effectiveness may be based on controlled clinical studies which establish an effect on a surrogate end-point (for example, immune response) considered likely to predict clinical benefit. The surrogate end-point used to evaluate effectiveness could be derived from human studies – for example, immune responses in vaccinated individuals from Phase II and Phase III studies and/or from a comparison of antibody responses post-vaccination in protected vaccinees with those of vaccinees who contract EVD. In this scenario, immune responses such as antibody titres achieved in vaccinated non-human primates that correlate with protection from challenge may also help in determining an immunogenicity end-point likely to predict protection in humans. Some NRAs may have provisions that would allow for the licensing of an Ebola vaccine based on such an approach for demonstrating effectiveness. Specific regulatory requirements associated with such provisions (for example, post-licensure studies to verify clinical benefit, and requirements for pre-licensure clinical safety studies in humans) must be adhered to.

As discussed above in section C.2.2, a surrogate immune marker identified for a particular vaccine may not be applicable to another vaccine.

C.2.5 Animal efficacy data for demonstration of effectiveness

If clinical end-point efficacy studies in humans are not ethical or feasible and there is no well-established ICP or surrogate immune marker likely to predict protection then evidence for effectiveness may be based on controlled challenge-protection studies conducted in an appropriate animal model (see section B.3.1) and clinical immunogenicity data. A central principle of approaches based on animal efficacy data is that the results of the animal studies establish that the vaccine is likely to produce clinical benefit in humans. Some NRAs may have provisions that would allow for the licensing of an Ebola vaccine based on such an approach for demonstrating effectiveness. Specific regulatory requirements associated with such provisions (for example, meeting certain criteria for the animal model(s), accrual of information in animals and humans to allow for selection of an effective dose in humans, pre-licensure safety studies in humans, and post-licensure studies to verify clinical benefit when such studies are feasible and ethical) must be adhered to.

C.2.6 Special considerations

C.2.6.1 Evaluation of effectiveness of candidate vaccines after initial licensure of an Ebola vaccine

Licensure of an Ebola vaccine may facilitate the evaluation of effectiveness of a new candidate Ebola vaccine if an ICP is established or a surrogate immune marker likely to predict clinical benefit is identified during development of the licensed vaccine and is considered to be applicable to new candidate vaccines. In such cases, an adequately conducted, randomized, controlled clinical trial(s) comparing the immune response, as measured by the relevant immunological parameter(s), in recipients of the candidate vaccine to that of recipients of the already licensed vaccine, using pre-specified statistical criteria, appropriate statistical methods and validated assays, could provide sufficient evidence of effectiveness to support licensure. As previously described, if the estimate of effectiveness is based on a surrogate marker likely to predict clinical benefit, approval may be subject to post-marketing requirements to verify the clinical benefit of the vaccine.

Alternatively, an Ebola vaccine may be licensed without an ICP or surrogate immune marker likely to predict protection considered to be applicable to other candidate Ebola vaccines. It may therefore be necessary to demonstrate vaccine effectiveness using other approaches (for example, animal challenge-protection studies combined with clinical immunogenicity studies). For this purpose, the animal challenge-protection studies should be adequately designed to provide reliable data, as discussed in B.3.1.

Licensure of an Ebola vaccine may make it infeasible and unethical to conduct pre-licensure clinical end-point efficacy trials with new candidate Ebola vaccines. Even conducting a comparative efficacy trial to demonstrate non-inferiority of a new candidate vaccine to the licensed vaccine would be challenging.

C.2.6.2 Evaluation of effectiveness of multivalent vaccines

For multivalent vaccines (for example, containing more than one EBOV strain, or an EBOV strain(s) and MARV) effectiveness (that is, based on clinical end-point efficacy studies, animal efficacy data and/or human immune response data) will need to be demonstrated for each strain contained in the vaccine.

C.2.6.3 Duration of immune response and protection, and need for booster vaccinations

The long duration of the 2014–2016 EVD epidemic and the potential for future exposures highlight the need to consider the durability of vaccine-induced protection and the potential need for booster doses in the evaluation of Ebola vaccines. This evaluation could be facilitated by the identification of an ICP.

Importantly, Phase II and Phase III clinical trials should attempt to identify ICPs and should evaluate the kinetics of the immune response and induction of immunological memory.

C.3 Safety evaluation of candidate Ebola vaccines

C.3.1 General considerations

Sponsors must comply with the adverse event reporting requirements of the relevant NRA and the independent ethics committee(s). Templates of the forms used to monitor and document adverse events should be provided with each protocol. Sponsors are encouraged to initiate early dialogue with the appropriate NRAs to reach agreement on the size of the safety database needed to support licensure of a particular vaccine. As with all vaccines, the size of the safety database depends in part on the characteristics of the candidate vaccine as well as on available preclinical and clinical safety data. Safety data from previous preclinical and clinical experience with related vaccines using the same platform may also be considered when determining the size of the safety database.

Safety-monitoring methods should be tailored to the specific study population (for example, children, adults, pregnant women or people living in areas where EVD is endemic), with consideration given to adverse events known to be associated with a particular vaccine – for example, in some Ebola vaccine studies, fever, arthralgia and arthritis have been observed. Study protocols should specify methods for monitoring and documenting adverse events, including: (a) use of standardized subject diaries and case report forms; (b) procedures for inquiring about adverse events at study visits; (c) severity grading scales; (d) definitions for adverse event categories – for example, serious, new-onset chronic medical condition, and adverse event of special interest (AESI); and (e) requirements for prompt reporting of serious adverse events (SAEs) to the sponsor.

In early-phase clinical studies (and at later phases if warranted), consideration may be given to pre- and post-vaccination assessment of safety laboratory parameters, including haematological and clinical chemistry evaluations. If such parameters are monitored, grading scales appropriate for the study population should be utilized.

It is also important to establish stopping rules for subsequent doses for individual study participants who experience an SAE, as well as study pausing/stopping rules for SAEs overall. Consideration should also be given to the establishment of an independent data-monitoring committee to advise the sponsor with regard to the continuing safety of trial participants and those to be recruited into the trial, particularly for any trials involving children and any large-scale later-phase trials.

Other aspects of safety that should be addressed in the study protocol include assessment of virus shedding and the potential for secondary transmission of replicating or potentially replication-competent live vaccine virus vectors, at least in early-phase studies, as well as procedures to minimize the risk of EBOV transmission to study personnel involved in clinical end-point efficacy studies.

C.3.2 Monitoring for common, solicited adverse reactions

In Phase I and Phase II studies, all participants should be monitored for pre-specified, solicited local and systemic adverse reactions at specified time points, for a specified period following vaccination (for example, daily for at least 7 days, or longer if warranted based on vaccine characteristics and available preclinical and clinical data). In Phase III studies, it may be acceptable to actively monitor only a subset of participants (for example, several hundred per group) for common, non-serious local and systemic adverse reactions. Data-collection methods may include the use of memory aids in literate populations and telephone interviews.

C.3.3 Monitoring for unsolicited adverse events

All study participants should also be monitored for unsolicited adverse events, including new-onset chronic medical conditions and exacerbation of medical conditions that may not necessarily meet the NRA's definition of serious. Whereas monitoring for all unsolicited adverse events may be conducted for relatively short periods post-vaccination (for example, 21 days, or 42 days for replicating live viral vaccines), monitoring for new-onset chronic medical conditions for a longer period (for example, 6–12 months) may be useful in detecting unexpected safety signals.

C.3.4 Monitoring for serious adverse events

While the exact definition of an SAE can vary across different NRAs, the ICH Guideline E2A defines an SAE as any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect (*108*). WHO considers an adverse event following immunization as "serious" if it meets any of the above criteria or if it requires intervention to prevent permanent impairment or damage (*109*).

All participants in pre-licensure clinical trials of Ebola vaccines should be closely and actively monitored (for example, with diary cards or follow-up visits) for SAEs for at least 21 days (or 42 days for replicating live viral vaccines) after each vaccination. A method to further query for SAEs over a minimum of 6 months following the last vaccination should also be incorporated into

the study protocol. A longer-term safety follow-up period for the assessment of SAEs (for example, through the 12 months following the last vaccination) may be warranted for some vaccines (for example, vaccines containing novel adjuvants). Long-term safety follow-up (that is, for 6–12 months post-vaccination) may be accomplished by telephone follow-up or other methods appropriate for the setting.

C.3.5 Monitoring for adverse events of special interest

All study participants should be monitored for any AESIs for a particular vaccine for a specified period post-vaccination (for example, 6–12 months). The period of follow-up may vary for different AESIs, depending on the anticipated window of risk.

C.4 Ethical considerations

Compliance with good clinical practice standards (*22*, *94*) provides assurance that the rights, safety and well-being of study participants are protected and study integrity is preserved. For any clinical study, a review by an independent ethics committee is mandatory and the approval of this committee must be obtained prior to study initiation. Informed consent must be given freely by every study participant and should be documented. For children participating in clinical studies, consent must be given by their parent or legal guardian. The informed consent process may need to be more specifically tailored to take into account local cultural views or practices. Child participants should be informed about the study to the extent compatible with their understanding and, if capable, should provide their assent. Participants in vaccine studies should not be exposed to unreasonable or serious risks of illness or injury. A study should be initiated and continued only if the anticipated benefits justify the risks. Low-resource communities, which are often those at greatest risk of EVD, should not be exploited in conducting research (for example, where there will be no long-term benefit to the community because the developer does not intend to seek licensure in the country where the vaccine is studied).

See section C.7.2 for considerations regarding initiation of clinical studies in the paediatric population.

C.5 Statistical considerations

C.5.1 General statistical principles

General statistical principles for clinical trials should be based on the relevant WHO document (*21*), where available, and other guidelines such as ICH E9 (*110*). Phase I studies are generally exploratory and may lack statistical power for hypothesis testing.

Phase II studies are for selecting the final optimal dose and dosing regimen and should be rigorously designed and analysed. The potential role of immunogenicity data should be taken into consideration to ensure the adequacy of data to support licensure if necessary.

Phase III studies are designed to provide robust data on vaccine effectiveness and more-extensive data on safety. The study protocols should clearly describe the procedures for randomization and blinding, primary and secondary objectives, end-points to be analysed, null and alternative hypotheses to be tested, level of type I error, sample size calculations, statistical methods for assessing each end-point, and analysis populations (per-protocol and intent-to-treat). If interim analyses for efficacy are planned, detailed information should be included in the protocol regarding the timing of interim analyses, type I error allocated to each analysis, and stopping rules. The study reports should include detailed information on subject disposition. Statistical estimates should be presented along with confidence intervals.

C.5.2 Statistical considerations for evaluating vaccine effectiveness

The effectiveness of a new Ebola vaccine is most convincingly demonstrated in a randomized, double-blind, placebo-controlled study based on an EVD end-point – though circumstances may dictate that alternative trial designs be considered. Vaccine efficacy and the corresponding confidence interval (usually 95%) should be estimated. Sample size for these trials depends on disease incidence rates in the study population, the level of vaccine efficacy considered to be clinically relevant and the chosen trial design.

Rapidly changing and/or declining incidence rates during an outbreak may need to be considered when choosing a study design. In some circumstances, designs such as cluster randomization may need to be used. For cluster-randomized trials, data should be analysed using statistical methods appropriate for the study design and study objectives. If inference will be at the usual individual level rather than the cluster level, sample size calculations and statistical analysis methods should appropriately address the within-cluster correlation, as feasible. Randomization should be carefully planned to avoid imbalance in disease risk or incidence rate between clusters randomized to be vaccinated or to serve as controls. As mentioned in section C.2.3.2.2, seeking to confine a trial to individuals at relatively high risk of EVD (as with the ring vaccination trial design) may have higher statistical power to detect vaccine efficacy than a trial in a population at lower risk of disease and, as a consequence, can potentially require a smaller sample size and achieve faster completion time compared to other study designs.

When ICPs established in animal challenge studies are being used to define immune response end-points for effectiveness evaluation or to infer

clinical benefit under other alternative licensure pathways, these studies (for example, in non-human primates) should be conducted using an appropriate dose range and an adequate number of animals such that the relationship between immune response and protection, and the protective threshold, can be estimated with satisfactory precision (see Part B).

C.5.3 Statistical considerations for evaluating vaccine safety

Safety evaluation is inherently exploratory and typically uses descriptive statistics. The calculation of p-values is sometimes useful as a flagging device applied to a large number of outcomes to detect differences that may need further evaluation. Multiplicity adjustment is not performed in order to increase the ability to detect potential signals. However, the potential for false-positive signals resulting from multiple tests must be considered prior to drawing firm conclusions.

If detection of several pre-specified SAEs is the primary focus of a large pre-licensure safety trial then multiplicity adjustment for testing a small number of hypotheses can be considered. When specific safety issues are identified during preclinical studies or early clinical trials (for example, cases of post-vaccination arthritis in clinical studies with certain viral-vectored vaccines) then prospective monitoring for related events as well as formal statistical testing should be considered.

C.6 Serological and diagnostic assays

The incubation period for EVD is 2–21 days. While patients are infectious by the time symptoms are evident, levels of virus in saliva or blood may not reach detectable levels until two or three days later. At this point in the course of infection, viral antigen can be detected by immunoassay and viral nucleic acid by a NAT-based assay. For both antigen and nucleic-acid-based tests the use of blood is preferred due to lower sensitivity of these assays with saliva. While serum IgM may also be detectable at this time, there is a risk of obtaining false-negative results so early in the course of infection. Serological testing should therefore be reserved for confirming prior infection or for evaluating vaccine responses. Isolation of EBOV in tissue culture must be performed in a high-containment laboratory, of which there are few, and this is therefore not routinely performed.

C.6.1 Diagnostic tests

All currently available EBOV NAT-based assays are based on the same principle – detection of an EBOV nucleic acid target sequence after extraction of viral nucleic acids from clinical samples, reverse transcription of RNA and in vitro amplification. The primers used in different NAT-based assays target different viral genome regions, which should be considered, particularly when used

in vaccine trials, so that infection can be distinguished from vaccination. For example, if the EBOV gene targeted by the NAT-based assay is also expressed by the vaccine, a positive result on a blood sample could mean that the subject may have EVD or it could mean that the subject is shedding vaccine virus.

Although many EBOV diagnostic kits have received approval for emergency use, this should not be taken to mean that they have been validated for non-emergency purposes, such as establishing vaccine efficacy in field trials. Assay performance parameters investigated as part of emergency-use approval often do not include more rigorous assessments, such as repeatability over the operating range, inter-assay precision or performance in the field. Appropriate RNA process controls and international reference standards became available for these assays in 2015 (see section A.1.1), which should now enable assessment of assay performance and comparison of results across different assay platforms.

Rapid diagnostic tests (RDTs) designed for EBOV antigen detection provide results more rapidly (sometimes within minutes), are easier to perform compared to NAT-based assays and do not require complex equipment (or electricity). However such tests are less sensitive than NAT-based assays and results should be confirmed by NAT-based assay where possible. As with NAT-based assays, care should be exercised when interpreting the results of RDTs using samples obtained from vaccinees, given that the antigen targeted by the kit may share homology with vaccine antigen.

C.6.2 Immunological tests

Although an ICP against EVD has not been established, myriad immunological tests have been developed. Of these, the EBOV IgG ELISA has gained the greatest acceptance based mostly on studies of experimentally vaccinated non-human primates in which high IgG levels have been linked to protection against subsequent challenge. Whether protection was via antibody detected by ELISA, or whether the presence of high levels of ELISA antibody is a marker of some other more meaningful form of immune response, is not known. In the absence of available data from humans defining an ICP (for example, data from a successful vaccine efficacy trial), an ICP may have to be established in an animal model. On the basis of data available to date, non-human primates appear to be an acceptable animal model for such an exercise, with inadequate information to support the use of other animal species.

Few EBOV immunoassays are commercially available – most reside in research laboratories where they were developed for use in preclinical or clinical trials of investigational vaccines. For this reason, most ELISAs are designed to detect antibodies against the EBOV GP – that is, the protein expressed by most investigational vaccines. There are numerous concerns about these tests and care should be taken in interpreting the data they produce. ELISA plates coated with lysates of cells expressing non-EBOV antigen that is also contained

in, or expressed by, the vaccine may be prone to yielding false-positive results. Other issues for consideration are the source of virus antigen used in the ELISA (reduced cross-recognition between virus strains), conformational changes of the antigen upon binding to the plate and antigen stability over time.

Since ELISAs are not necessarily informative of functional immunity, assays that measure virus neutralization and cell-mediated responses have been developed. The neutralization assays generally employ pseudovirions (such as VSV in which the GP gene has been replaced with that of EBOV) or lentivirus packaging systems. Consideration should be given as to whether virus-neutralizing activity detected in these in vitro assays is predictive of EBOV-neutralizing activity in vivo. It is also important to consider false positivity through the use of ELISA plates coated with non-EBOV vaccine components. For example, non-EBOV antibodies generated in response to receipt of a VSV-vectored Ebola vaccine may have an impact on the performance of VSV-based neutralization assays. This is less of a problem for neutralization assays using wild-type EBOV as the target virus but it highlights the need for careful evaluation of assay specificity as part of assay validation.

Although not well established, there is evidence supporting the importance of T cell-mediated responses in preventing EVD. In a study of an Ad5-vectored Ebola vaccine in non-human primates, depletion of CD8+ T cells in vaccinated animals before challenge abrogated protection (*111*). Several different types of tests for cell-mediated immunity have been developed, including the ELISpot and ICS tests. These tests present additional challenges, including determination of the appropriate peptide pools to be used and logistical and safety issues concerning the collection and storage of peripheral blood mononuclear cells, as well as assay validation issues.

In general, there are few published data on the performance of assays to detect immunological responses to EBOV infection or to Ebola vaccines. Where available, international standards or reference reagents (see section A.1.1) should be used to standardize assay performance, and improve comparison of results across vaccines, across studies and across different assay platforms.

C.7 Special populations

Ideally, developers of candidate Ebola vaccines will perform studies to gather data in at least some, if not all, of the relevant populations discussed below.

C.7.1 Pregnant women

Evidence from the 2014–2016 EVD epidemic suggests that EVD is associated with high rates of maternal and neonatal mortality (112). The use of Ebola vaccine in pregnant women may have potential benefits in: (a) preventing EVD in the mother and reducing maternal morbidity; (b) preventing EVD in the

early neonatal period; and (c) limiting the spread of EVD from pregnant women during labour and delivery to health-care workers in an outbreak setting (*113*).

The following concepts should be considered when planning clinical trials in pregnant women. Details regarding such trials should be discussed with the respective NRA(s) and can also be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*22*). Prior to enrolling pregnant women in clinical trials, developmental toxicity studies in animal models are needed to address the potential reproductive risk of the product (see section B.4). In addition, supportive safety data from completed Phase I and Phase II clinical trials in healthy men and non-pregnant women should be available. The consent form should include information on what is known and unknown regarding the potential risks and benefits of the investigational product to both mother and infant, and should reflect available data from non-pregnant adults and nonclinical studies. A reasonable effort should be made to accurately calculate gestational age for pregnant participants prior to enrolment, taking into consideration the standard of care in the region where the clinical trial is being conducted. For studies of preventive vaccines in general (including Ebola vaccines), consideration should be given, as part of a cautious approach, to excluding women in the first trimester of pregnancy.

Safety data specific to both the pregnant mother and her fetus should be collected. Information on pregnancy-related outcomes (such as spontaneous abortion or intrauterine growth restriction) and on pregnancy-related complications (such as new-onset gestational diabetes or placenta previa) should be collected. In addition, severity scales used for the grading of adverse outcomes should be based on pregnancy-specific physiological and laboratory values, if available. Efforts should be made to monitor infants for developmental abnormalities.

C.7.2 Paediatric populations

A paediatric clinical development plan for a vaccine to protect against EVD should be considered early (prior to Phase III) and should take into account the incidence and prevalence of EVD, as well as existing therapies, in the paediatric population, including neonates. In general, enrolment of children in Ebola vaccine studies should be considered when there is sufficient evidence to support the safety of studies in the paediatric population and there is a reasonable demonstration of a sufficient prospect of direct benefit from animal and/or human adult studies to justify the risks. Scientific and ethical considerations regarding the initiation of paediatric studies of Ebola vaccines should be discussed with the relevant NRA early in clinical development. Available preclinical data and clinical data in older age groups should support the paediatric dose and regimen to be evaluated, and should guide decisions on

the potential need for incremental evaluation in older paediatric groups first, followed by younger children and possibly infants. Safety considerations will be critical when deciding upon the potential study of Ebola vaccines based on live, replication-competent viral vectors in infants younger than 1 year of age.

Whether evidence of effectiveness can be extrapolated from adults to specific paediatric age groups or from older to younger paediatric age groups will depend on the similarities between the relevant age groups with respect to factors such as the course of the disease and the immune response to vaccination. Consideration may also be given to bridging effectiveness from older to younger populations on the basis of a comparison of immune responses, as measured by a validated assay using an immune marker that is thought to predict clinical benefit. In some cases, immunological markers that are thought to contribute to protection may be used to bridge across age groups even if they are not scientifically well-established correlates of protection.

If the adult formulation of a vaccine is not suitable for certain paediatric age groups (for example, due to the large dose volume), sponsors should plan for the development of an age-appropriate paediatric formulation.

In paediatric studies, grading scales for adverse events and normal ranges for laboratory tests should be specifically tailored to the age group studied.

C.7.3 Immunocompromised individuals and individuals with underlying disease

Countries that have experienced prior Ebola outbreaks frequently have a relatively high prevalence of concomitant illnesses or conditions such as HIV/AIDS, tuberculosis, malaria and malnutrition. This prompts a number of unique considerations with respect to clinical development programmes for Ebola vaccines. Information on underlying medical conditions that may have an impact on the safety and effectiveness evaluations of a vaccine should be collected for participants in clinical trials.

The safety evaluation of investigational vaccines in immunocompromised individuals should include assessment of exacerbation of the underlying disease post-vaccination. For example, plasma HIV viral load has been shown in some studies, but not in others, to transiently increase following vaccination with influenza and pneumococcal vaccines – though without established clinical consequence. Product-specific considerations may preclude the use of some vaccines in certain populations due to unacceptable risks (for example, risk of disseminated disease following immunization of HIV-infected individuals with BCG vaccine).

The effectiveness of an Ebola vaccine may differ in countries according to the prevalence of certain underlying medical conditions. Thus, effectiveness data should be obtained in the region where the vaccine is most likely to be used.

C.8 Post-marketing surveillance

As part of preparing for marketing approval of any new Ebola vaccine, pharmacovigilance plans specific to each vaccine should be developed. Depending on the situation, these plans could be prepared/implemented by vaccine manufacturers and public health authorities in the countries where the vaccine will be used, or through cooperative efforts that could also include participation by regulators, WHO and other institutions.

According to the ICH, a pharmacovigilance plan should be prepared for any new vaccine (*114*). A first step towards the preparation of such a plan is the "safety specification" which summarizes: (a) the important identified and potential risks of the vaccine; and (b) the important missing information. The safety specification should also describe the populations that are potentially at risk for EVD (that is, the populations in which the vaccine will most likely be used) and any outstanding safety questions which warrant further investigation. The safety specification is intended to help industry, regulators and other institutions involved in the process to identify any need for specific data collection and to facilitate preparation of the pharmacovigilance plan (*114*). The safety specification is usually prepared by the sponsor (the institution submitting the vaccine for marketing authorization, which is usually, but not always, the manufacturer) during the pre-marketing phase. For products of international public health importance, such as Ebola vaccines, pharmacovigilance planning would benefit from dialogue not only with regulators but also with public health authorities, WHO and other institutions involved in the process.

In the case of vaccines for which no specific concerns have arisen, routine pharmacovigilance should be sufficient for post-approval safety monitoring. Nevertheless, for products with important identified risks, important potential risks or important missing information (which may be the case with new Ebola vaccines) the pharmacovigilance plan should consider appropriate risk-management and risk-minimization activities to address these concerns (*114*).

The strategies proposed for the identification and investigation of vaccine safety signals should be specified in the pharmacovigilance plan. These may depend, in part, on decisions made regarding the use of the vaccine(s) during epidemic and inter-epidemic periods. Specifically, pharmacovigilance activities may need to be adapted to situations in which the vaccine is recommended for: (a) well-defined and relatively small groups (for example, first responders, health-care workers and/or specific groups at high risk such as the close contacts of suspected cases); (b) large demographic groups (for example, all individuals in a certain age range or the inhabitants of a specific geographical region); or (c) the overall population of a country or region.

Ideally, the pharmacovigilance plan should permit the detection of new safety signals (a role performed mainly by spontaneous or passive reporting

systems) and confirmation of the association between the suspected event(s) and the vaccine being investigated (*115*, *116*). Currently, no effective post-marketing surveillance systems with clear protocols, tools and a mandate exist in countries affected by the 2014–2016 EVD epidemic. Thus, enhanced capacity for vaccine pharmacovigilance may be needed, in accordance with the WHO *Global vaccine safety blueprint* (*23*). This blueprint defines the need for enhanced capacity as follows:

> *Enhanced vaccine pharmacovigilance, at a minimum level, includes improved data collection, in passive surveillance, towards higher data quality and more complete data sets, but also improved collation, verification, analysis and communication by building capacity for stimulated and active surveillance. It also includes the ability to perform population-based studies and appropriate epidemiologic studies testing hypotheses by assessing relative and absolute risk ratios, when appropriate.*

The document goes on to state that:

> *Spontaneous reporting systems are insufficient to enable rapid assessment and adequate public health response to vaccine safety signals. Rapid response to vaccine safety signals is required to identify those rare instances where real adverse reactions occur, so that their impact can be minimized as they emerge. Countries where an increased level of vaccine safety activity is judged to be necessary are those where newly developed vaccines are being introduced and in countries that manufacture and use prequalified vaccines (23).*

The WHO Global Advisory Committee on Vaccine Safety (GACVS) has reviewed safety data from Phase I studies of two investigational Ebola vaccines (*117*). The adverse event profiles from these studies provide useful information for planning safety evaluations in further studies of these vaccines. Pharmacovigilance plans for the introduction of Ebola vaccines should take into account the observed safety profiles from clinical studies and should be aligned with WHO guidance.

In summary, the implementation of an adequate pharmacovigilance plan for the post-marketing evaluation of adverse events following the introduction of Ebola vaccines requires a functioning spontaneous reporting system, active surveillance systems and the ability to perform appropriate epidemiological studies to further investigate any possible association between suspected event(s) and the vaccine. Given existing limitations in countries that were affected by the 2014–2016 EVD epidemic, an enhanced capacity for pharmacovigilance may be needed in some countries, and more than one active surveillance approach may need to be implemented to achieve effective pharmacovigilance.

Part D. Guidelines for NRAs

D.1 General

The general recommendations for control laboratories given in the WHO Guidelines for national authorities on quality assurance for biological products (*118*) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (*119*) should apply after the vaccine product has been granted a marketing authorization. These recommendations specify that no new biological substance should be released until consistency of batch manufacturing and quality has been established and demonstrated. The recommendations do not apply to material for clinical trials.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of viral-vectored vaccines, should be discussed with and approved by the NRA.

The NRA may obtain the product-specific working reference from the manufacturer to be used for lot release until the international or national standard preparation is established.

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

D.2 Release and certification

A vaccine lot should be released to the market only if it fulfils all national requirements and/or satisfies Part A of these WHO Guidelines (*119*). A protocol for the manufacturing and control of Ebola vaccines, based on the model protocol provided in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A Lot Release Certificate signed by the appropriate NRA official should then be provided if requested by a manufacturing establishment, and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these WHO Guidelines. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided below in Appendix 2.

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Appendix 1

Model protocol for the manufacturing and control of viral-vectored Ebola vaccines

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

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

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

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

International name:
Commercial name:
Product licence (marketing authorization) number:
Country:
Name and address of manufacturer:
Name and address of product licence-holder if different:
Viral vector(s):
Ebola virus strain(s):
Batch number(s):
Type of container:
Number of filled containers in this final lot:
Number of doses per container:
Composition (viral vector concentration)/volume of single human dose:

Target group:
Expiry date:
Storage conditions:

2. Control of source material

2.1 Virus seeds (repeat for each monovalent vaccine component)

2.1.1 Seed banking system

- Name and identification of viral vector:
- Origin of all genetic components:
- Construction of viral vector:
- Nucleotide sequence of the transgene and flanking regions:
- Antigenic analysis, infectivity titre, in vitro yield:
- Comparison of genetic and phenotypic properties with parental vector:
- Seed bank genealogy with dates of preparation, passage number and date of coming into operation:
- Tests performed for detection of adventitious agents at all stages of development:
- Freedom from TSE agents:
- Details of animal or human components of any reagents used in the manufacture of seed banks, including culture medium:
- Genetic stability at the level of a virus pre-master seed or virus master seed to its sequence at, or preferably beyond, the anticipated maximum passage level:
- Confirmation of approval for use by manufacturer, and the basis for that approval:

2.2 Cell cultures (if applicable) (repeat for each monovalent vaccine component)

2.2.1 Cell banking system

- Name and identification of cell substrate:
- Origin and history of cell substrate:
- Details of any manipulations (including genetic manipulations) performed on the parental cell line in the preparation of the production cell line:
- Cell bank genealogy with dates of preparation, passage number and date of coming into operation:

- Confirmation of approval for use by manufacturer, and the basis for that approval:
- Tests performed for detection of adventitious agents at all stages of development:
- Test for tumorigenic potential (if of mammalian origin):
- Details of animal or human components of any reagents used in manufacture of cell banks, including culture medium:
- Freedom from TSE agents:
- Genetic stability (if genetically manipulated):

2.2.2 Primary cells (if generated)

- Source of animals and veterinary control (for example, specify if animals or eggs are sourced from closed, pathogen-free colonies):
- Name, species and identification of primary cell batches:
- Details of animal or human components of any reagents used in manufacture of cells:
- Methods of isolation of the cells:
- Tests performed for detection of adventitious agents during manufacture (may be performed on control cells if necessary):
- Freedom from TSE agents:

3. Control of vaccine production (repeat for each monovalent vaccine component)

3.1 Control of production cell cultures/control cells

3.1.1 Information on preparation

- Lot number of master cell bank:
- Lot number of working cell bank:
- Date of thawing ampoule of working cell bank:
- Passage number of production cells:
- Date of preparation of control cell cultures:
- Result of microscopic examination:

3.1.2 Tests on cell cultures or control cells

- Adventitious agents:
- Sterility (bacteria, fungi, mycoplasmas):

3.2 Viral vector harvests or pooled viral vector harvests

3.2.1 Information on manufacture

- Batch number(s):
- Date of inoculation:
- Date of harvesting:
- Lot number of virus master seed lot:
- Lot number of virus working seed lot:
- Passage level from virus working seed lot:
- Methods, date of purification if relevant:
- Volume(s), storage temperature, storage time and approved storage period:

3.2.2 Tests

- Adventitious virus tests:
- Bacteria/fungi/mycoplasmas:
- Virus titre:

3.3 Monovalent viral vector bulk

3.3.1 Information on manufacture

- Batch number(s):
- Date of formulation:
- Total volume of monovalent bulk formulated:
- Virus pools used for formulation:
- Lot number/volume added:
- Virus concentration:
- Name and concentration of added substances (for example, diluent, stabilizer if relevant):
- Volume(s), storage temperature, storage time and approved storage period:

3.3.2 Tests

- Identity:
- Purity:
- Residual HCP:
- Residual HC DNA (if non-primary cell lines):

- Potency:
  - Particle number (for adenovirus):
  - Infectious virus titre:
  - Particle-to-infectivity ratio (for adenovirus):
  - Expression of heterologous antigen in vitro:
- Replication competence (for adenovirus):
- pH:
- Preservative content (if applicable):
- Endotoxin:
- Sterility or bioburden:

3.4 Final viral vector bulk

3.4.1 Information on manufacture

- Batch number(s):
- Date of formulation:
- Total volume of final bulk formulated:
- Monovalent virus pools used for formulation:
- Lot number/volume added:
- Virus concentration:
- Name and concentration of added substances (for example, diluent, stabilizer if relevant):
- Volume(s), storage temperature, storage time and approved storage period:

3.4.2 Tests

- Identity:
- Sterility or bioburden:
- Concentration of antimicrobial agent, if relevant:

4. Filling and containers

Lot number:
Date of filling:
Type of container:
Volume of final bulk filled:
Filling volume per container:
Number of containers filled (gross):

Number of containers rejected during inspection:
Number of containers sampled:
Total number of containers (net):
Maximum period of storage approved:
Storage temperature and period:

5. Control tests on final vaccine lot

Inspection of containers (that is, inspection container integrity):
Appearance (that is, appearance of container content):
Identity:
pH and osmolality:
Potency (if feasible to measure in a multivalent system):

- Particle number (adenovirus):
- Infectious virus titre:
- Particle-to-infectivity ratio (for adenovirus):
- Expression of heterologous antigen in vitro:

General safety tests (initial batches only):
Endotoxin:
Sterility:
Extractable volume:
Aggregate/particle size:
Presence of preservative (if relevant):
Residual moisture content (for freeze-dried product):
Reconstitution time (for freeze-dried product):

6. Certification by the manufacturer

Name of Head of Production (typed) _____

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

I certify that lot no. _____ of Ebola vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A[1] of the WHO Guidelines on the quality, safety and efficacy of Ebola vaccines[2] (if applicable)

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

[2] WHO Technical Report Series, No. 1011, Annex 4.

Name (typed) _____

Signature _____

Date _____

7. Certification by the NRA

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

Appendix 2

Model NRA Lot Release Certificate for viral-vectored Ebola vaccines

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

Certificate no. _____

The following lot(s) of Ebola vaccine produced by _____[1] in _____[2] whose lot numbers appear on the labels of the final containers, complies with the relevant specification in the marketing authorization and provisions for the release of biological products[3] and Part A[4] of the WHO Guidelines on the quality, safety and efficacy of Ebola vaccines[5] and comply with WHO good manufacturing practices for pharmaceutical products: main principles,[6] WHO good manufacturing practices for biological products,[7] and Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on _____[9]

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);

[1] Name of manufacturer.

[2] Country of origin.

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

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

[5] WHO Technical Report Series, No. 1011, Annex 2.

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

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

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

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

- type of container used;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other authority as appropriate):

Name (typed) _____
Signature _____
Date _____

Annex 3

Guidelines on procedures and data requirements for changes to approved biotherapeutic products

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

Abbreviations

ALIFAR	Asociación Latinoamericana de Industrias Farmacéuticas
BSE	bovine spongiform encephalopathy
DNA	deoxyribonucleic acid
GCP	good clinical practice
GLP	good laboratory practice(s)
GMP	good manufacturing practice(s)
HPLC	high-performance liquid chromatography
HSA	Health Sciences Authority
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations
IGBA	International Generic and Biosimilar Medicines Association
IQ	installation qualification
MCB	master cell bank
NRA	national regulatory authority
OQ	operational qualification
PAS	prior approval supplement
PDA	Parenteral Drug Association
PK/PD	pharmacokinetic/pharmacodynamic
PPTA	Plasma Protein Therapeutics Association
PQ	performance qualification
SBP	similar biotherapeutic product
TSE	transmissible spongiform encephalopathy
WCB	working cell bank

1. Introduction

Biotherapeutic products are an increasingly important component of global health care. Several WHO guidelines on the evaluation of biotherapeutic products have been produced (*1–3*) that provide a set of principles on the regulatory evaluation of such products. During international consultations on the development of these guidelines, and their subsequent implementation, it became clear that there was a need for WHO guidance on making post-approval changes to biotherapeutic products in order to help address the complexity and other challenges associated with the global life-cycle management of such products. In May 2014, the Sixty-seventh World Health Assembly adopted two relevant resolutions: one on promoting access to biotherapeutic products and ensuring their quality, safety and efficacy (*4*) and the other on regulatory systems strengthening (*5*). In support of these resolutions, WHO was requested to provide guidance on how to deal with increasingly complex biotherapeutic products, including similar biotherapeutic products (SBPs). In addition, the 16th International Conference of Drug Regulatory Authorities recommended that WHO assist Member States in ensuring regulatory oversight throughout the life-cycle of biotherapeutic products (*6*).

This document is intended to provide guidance to national regulatory authorities (NRAs) and manufacturers on regulating changes to already licensed biotherapeutic products in order to assure their continued quality, safety and efficacy, as well as continuity in supply and access. The term "biotherapeutic products" as used in this document collectively includes the originator products and SBPs (also called "biosimilars").

Changes are essential for the continual improvement of the manufacturing process and for maintaining state-of-the-art control of biotherapeutic products, and often need to be implemented after the product has been approved (that is, when it has been licensed or when marketing authorization has been received). Changes may be made for a variety of reasons, including: (a) to maintain routine production (for example, replenishment of reference standards, or change of raw materials); (b) to improve product quality, or the efficiency and consistency of manufacture (for example, changes in the manufacturing process, equipment or facility, or adding a new manufacturing site); (c) to make safety or efficacy changes (for example, adding a new indication, changing the dosage regimen, or adding information on co-administration with other medicines); (d) to update product labelling information (for example, improvement of the management of risk by addition of a warning statement for a particular target population, or limiting the target population); or (e) to address administrative changes (for example, change in the proper/nonproprietary or trade name of a biotherapeutic product).

NRAs and marketing authorization holders should recognize that:

- any change to a biotherapeutic product has a potential impact on the quality, safety and/or efficacy of that product;
- any change to the information associated with the product (that is, product labelling information) may have an impact on its safe and effective use.

The regulation of changes to approved biotherapeutic products is key to ensuring that products of consistent quality, safety and efficacy are marketed after they receive authorization or licensure. Many NRAs of Member States have requested guidance on the data needed to support changes to approved biotherapeutic products in order to ensure comparability of the pre-change and post-change products with respect to quality, safety and efficacy. Although it is difficult to provide a set of guidelines that apply to all national situations, an attempt has been made to cover a range of possible changes in manufacture, quality control, safety, efficacy and product labelling information.

This document is intended to serve as a guide for establishing national requirements for the regulation of post-approval changes to biotherapeutic products. The categories of changes and reporting procedures are provided in the main body of the document and the data requirements to support the proposed changes are provided in the appendices. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements. It is possible that modifications to this document may be justified due to risk–benefit and legal considerations specific to each NRA. In such cases, it is recommended that any modifications should not depart from the principles outlined in this document. NRAs are encouraged to apply the concepts of reliance or work-sharing or to use collaborative approaches when reviewing post-approval changes, as indicated in section 8 below.

2. Purpose and scope

These WHO Guidelines provide guidance for NRAs and marketing authorization holders on the regulation of changes to the original marketing authorization dossier or product licence for an approved biotherapeutic product in terms of: (a) the procedures and criteria for the appropriate categorization and reporting of changes; and (b) the data required to enable NRAs to evaluate the potential impact of the change on the quality, safety and efficacy of the product. Additionally, the purpose of these WHO Guidelines is to assist NRAs in establishing regulatory procedures for post-approval changes to such products.

The guidance applies in principle to all biologically active protein products used in the treatment of human diseases (for example, plasma-fractionated products) and those intentionally modified by, for example, fusion

proteins, PEGylation, conjugation with a cytotoxic drug or modification of rDNA sequences. The guidance also applies to protein products used for in vivo diagnosis (for example, monoclonal antibody products used for imaging).

While these WHO Guidelines apply to products that have received a licence or a marketing authorization, the principles described herein may also apply to quality changes that occur during development of a product and where comparability needs to be demonstrated. However, the amount and type of data submitted for such products will be limited and will vary according to the nature of each product and its stage of development. In addition, the legal status of investigational products varies from country to country and should therefore be discussed with the NRA.

Prophylactic vaccines against infectious diseases, and gene and cell therapy products, are not covered by these WHO Guidelines. Detailed and specific guidance for prophylactic vaccines are available in a separate WHO Guidelines document (*7*). However, the principles set out in this document may apply to low molecular weight heparins. Other WHO guidelines with relevance to this area include those covering good manufacturing practices (GMP) for biological and pharmaceutical products (*8*, *9*).

3. Terminology

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

Acceptance criteria: criteria, expressed by numerical limits, ranges or other suitable measures, which should be met to release the drug substance or drug product or materials at different stages of their manufacture.

Biotherapeutic product: a biological medicinal product with the indication of treating human disease. For the purpose of these WHO Guidelines, biotherapeutic products include all biologically active protein products (including plasma-fractionated products) which are used in the treatment of human diseases, and those intentionally modified by, for example, fusion proteins, PEGylation, conjugation with a cytotoxic drug or modification of rDNA sequences. They also include protein products used for in vivo diagnosis (for example, monoclonal antibody products used for imaging).

Change: refers to a change that includes, but is not limited to, the product composition, manufacturing process, quality controls, analytical methods, equipment, facilities or product labelling information made to an approved marketing authorization or licence by the marketing authorization holder. Also referred to as "variations" or "post-notice of compliance changes" in other documents (*10–14*).

Comparability exercise: the activities – including study design, conducting of studies and evaluation of data – that are designed to investigate whether a pre-change product and a post-change product are highly similar (*1*).

Comparability protocol: a well-defined plan for future implementation of quality change(s) (for example, manufacturing-related changes, change of analytical method or site transfer). Also referred to as "post-approval change management protocol" in other documents (*15*). A comparability protocol establishes the tests to be performed and acceptable limits to be achieved to demonstrate the comparability of pre-change and post-change products following specific quality change(s).

Container closure system: refers to the following components:

- A primary container closure system is a packaging component that is in, or may come into, direct contact with the drug product dosage form (for example, vial or pre-filled syringe) or components that contribute to the container/closure integrity of the primary packaging material for a sterile product.
- A secondary container closure system is a packaging component that is not, and will not be, in direct contact with the dosage form (for example, carton or tray).
- A functional secondary container closure system is a packaging material that is not in direct contact with the product and that provides additional protection or serves to deliver the product.

Control strategy: a planned set of controls derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control (*16*).

Critical quality attribute: a physical, chemical, biological or microbiological property or characteristic that is selected for its ability to indicate the consistent quality of the product within an appropriate limit, range or distribution to ensure the desired product quality (*1*).

Design space: the multidimensional combination and interaction of input variables (for example, material attributes) and process parameters that have been demonstrated to provide assurance of quality (*16*).

Dosage form: the physical form in which a pharmaceutical product is presented by the manufacturer (form of presentation) and the form in which it is administered (form of administration). Also referred to as "pharmaceutical form" in other documents.

Drug product: a pharmaceutical product type in a defined container closure system that contains a drug substance, generally in association with excipients.

Drug substance: the active pharmaceutical ingredient and associated molecules that may be subsequently formulated to produce the drug product.

Excipient: any component of the drug product, other than the active component/drug substance and the packaging material, generally added during formulation. Also referred to as "inactive ingredient" in other documents.

Final batch: a collection of sealed final containers that is homogeneous with respect to the composition of the product. A final batch must have been filled in one continuous working session.

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

In-process control: checks performed during manufacture to monitor or to adjust the process in order to ensure that the intermediate or final product conforms to its specifications. The control of the production environment or equipment may also be regarded as part of in-process control.

Intermediate: a material produced during steps in the manufacture of a biotherapeutic product that undergoes further processing before it becomes the drug product. See also the definition for Drug substance.

Manufacturer: any person or legal entity engaged in the manufacture of a product subject to marketing authorization or licensure. In other documents, "manufacturer" may also refer to any person or legal entity that is an applicant or holder of a marketing authorization or product licence where the applicant assumes responsibility for compliance with the applicable product and establishment standards. See also the definition for Marketing authorization holder.

Marketing authorization: a formal authorization for a medicine to be marketed. Once an NRA approves a marketing authorization application for a new medicine, the medicine may be marketed and may be available to be prescribed by physicians. Also referred to as "product licence" or "licence" in this and other documents.

Marketing authorization application: a formal application to the NRA for approval to market a new medicine. The purpose of the marketing authorization application is to determine whether the medicine meets the statutory standards for safety, efficacy, product labelling information and manufacturing. Also referred to as "product licence application" or "licence application" in this and other documents.

Marketing authorization holder: any person or legal entity that has received a marketing authorization or licence to manufacture and/or distribute

a medicine. It also refers to a person or legal entity allowed to apply for a change to the marketing authorization or licence.

Master cell bank (MCB): an aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions.

Primary packaging site: site involved in the activity of putting a drug in its primary container which is, or may be, in direct contact with the dosage form.

Process validation: documented evidence which provides a high degree of assurance that a specific process will consistently result in a product that meets its predetermined specifications and quality characteristics.

Product labelling information: refers to printed materials that accompany a prescription medicine and all labelling items, namely:

- prescribing information (an instruction circular that provides product information on indication, dosage and administration, safety and efficacy, contraindications, warnings and a description of the product for health-care providers (also referred to as "summary of product characteristics" or "package insert" in various countries);
- patient labelling or consumer information;
- inner label or container label;
- outer label or carton.

Quality attribute: a physical, chemical, biological or microbiological property or characteristic.

Quality change: a change in the manufacturing process, product composition, quality control testing, equipment or facility. Also referred to as "chemistry manufacturing and control (CMC) change" in other documents.

Raw materials: a general term used to denote the culture media components, reagents or solvents intended for use in the production of starting material, drug substance, intermediates or drug products.

Real-time release testing: testing that provides the ability to evaluate and ensure the quality of in-process and/or final product based on process data, which typically include a valid combination of measured material attributes and process controls (*16*, *17*).

Reference standards/materials: well-characterized materials used as references against which batches of biological products are assessed. These materials remain fundamental to ensuring the quality of biological products as well as the consistency of production, and are essential for the establishment of appropriate clinical dosing.

Safety and efficacy change: a change that has an impact on the clinical use of the biotherapeutic product in relation to safety, efficacy, dosage and

administration, and that requires data from clinical or post-marketing studies, and in some instances clinically relevant nonclinical studies, to support the change.

Secondary packaging facility: site involved in packaging activities using a packaging component that is not, and will not be, in direct contact with the dosage form (for example, putting the primary container in the outer container or affixing labels).

Shelf-life: the period of time during which a drug substance or drug product, if stored under the conditions defined on the container label, is expected to comply with the specification, as determined by stability studies on a number of batches of the product. The expiry date is assigned to each batch by adding the shelf-life period to the date of manufacture.

Similar biotherapeutic product (SBP): a biotherapeutic product that is similar in terms of quality, safety and efficacy to an already licensed reference biotherapeutic product, and which was developed and approved on the basis of the principles outlined in relevant WHO guidelines (*2*, *3*).

Source material/starting material: material from a biological source that marks the beginning of the manufacturing process of a drug as described in a marketing authorization or licence application and from which the active ingredient is derived either directly (for example, plasma derivatives, ascitic fluid or bovine lung) or indirectly (for example, cell substrates, host/vector production cells, eggs or viral strains).

Specification: a list of tests, references to analytical procedures and appropriate acceptance criteria which are numerical limits, ranges or other criteria for the tests described. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by the regulatory authorities.

Supplement: a written request submitted to the NRA to approve a change in the original application for the marketing authorization (or product licence) or any other notification to add to (that is, to supplement) the information in the original marketing authorization or product licence file. A prior approval supplement (PAS) is a supplement requiring approval from the NRA prior to implementation of the change. Also referred to as "change application dossier" in other documents.

Validation: the demonstration, with documentary evidence, that any procedure, process, equipment, material, activity or system will consistently produce a result meeting predetermined acceptance criteria.

Working cell bank (WCB): the working cell bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the master cell bank under defined culture conditions.

4. General considerations

Changes to approved biotherapeutic products or SBPs are categorized on the basis of a risk analysis which takes into consideration the complexity of the production process and product, the patient population and the proposed changes. When a change affects the manufacturing or the control strategy, the assessment should include evaluation of the impact of the change on quality (that is, identity, strength, purity and potency) as it may relate to the safety and/or efficacy of the product. When a change affects the clinical use of a product or of product labelling information, this assessment should include evaluation of the effect of the change on the safety and efficacy of the product.

Prior to implementing a change with a potential impact on quality, the marketing authorization holder should demonstrate through appropriate studies (analytical testing, functional assays and, if needed, clinical and/or nonclinical studies) that the pre-change and post-change products are comparable in terms of quality, safety and efficacy.

For each change, the marketing authorization holder should decide if the information in the original marketing authorization or product licence needs to be supplemented (that is, requires an official submission of a supplement to the NRA) based on the recommendations provided in these WHO Guidelines. Supplements requiring approval by the NRA prior to the implementation of a change – that is, for changes that potentially have a major or moderate impact – are referred to as prior approval supplements (PASs) and must be submitted in advance to the NRA. For supplements that do not require approval prior to implementation – that is, for changes that potentially have a minor impact on product quality – the NRA should be notified following implementation of the change.

For each change, the supplement should contain information developed by the marketing authorization holder to allow the NRA to assess the effects of the change. All changes, regardless of their impact on quality, safety and efficacy, should be recorded and retained by the manufacturer or marketing authorization holder in accordance with the applicable regulatory requirements for document retention (*8*, *9*).

For manufacturing changes not specifically described in these WHO Guidelines, the marketing authorization holder is encouraged to use scientific judgement, leverage competent regulatory authority guidance or to contact the NRA to determine the potential impact of the change on quality, safety and efficacy in order to discuss the appropriate reporting category.

Assessment of the extent to which a quality change (also referred to as a manufacturing change) affects the quality attributes of the product is generally

accomplished by comparing manufacturing steps and test results from in-process, release, and characterization testing of the pre-change product (for example, using historical data) with those of the post-change product. It can then be determined if the test results are comparable – that is, if the drug substance, intermediate or drug product made after the change is comparable to, and/or meets the predefined acceptance criteria of, the drug substance or drug product made before the change. Where minor differences in quality are identified, these may be considered acceptable provided that they are shown not to have an adverse impact on the quality, safety or efficacy of the product (see sections 5.1 and 5.2). In some cases, additional supporting data may be required, as noted in Appendices 2, 3 and 4 below.

A marketing authorization holder or manufacturer making a change to an approved biotherapeutic product should also conform to other applicable laws and regulations, including good manufacturing practices (GMP), good laboratory practices (GLP) and good clinical practices (GCPs). Marketing authorization holders and drug substance/product manufacturers should also comply with relevant GMP validation and record-keeping requirements and should ensure that relevant records are readily available for examination by authorized NRA personnel during inspections. For example, changes in equipment used in the manufacturing process generally require installation qualifications (IQs), operational qualifications (OQs) and performance qualifications (PQs). This information does not need to be included in a PAS for equipment changes but is part of GMP requirements and should be available during inspections. Inspections (on-site or paper-based) may occur routinely or may be required during submission review of a PAS for a major manufacturing change such as a move to a new facility.

Certain major changes, such as changes to the molecule (for example, changing amino acid sequence or conjugating to PEG moieties) will lead to a new molecular entity and are not considered as post-approval changes. For these changes, submission of a product licence application for a new marketing authorization may be required. In some countries, a change in the quantity of drug substance per dose of biotherapeutic product also requires a product licence application for a new marketing authorization.

The implementation of new regulations for post-approval changes should take product supply into consideration. Any negative impact on access to approved products should be minimized. Therefore, NRAs are strongly encouraged to establish requirements that are commensurate with their own regulatory capacity, experience and resources. NRAs of countries procuring products are encouraged to consider establishing procedures for the expedited approval of changes based on previous expert review and approval of the same changes by the NRAs of the countries where these products are licensed, or based on the decision of a recognized regional regulatory authority. If a change has been

approved by another competent NRA, the NRA receiving the submission may choose to recognize this approval decision or may make an independent decision based on its own assessment. Foreign approval documentation may accompany the required information and may be used as supporting evidence for the post-approval change, as outlined in this document. The responsibility for the final regulatory decision on the approval of the change still lies with the receiving NRA (see section 8 and Appendix 1).

To ensure product supply and encourage adequate reporting of changes by manufacturers, NRAs should consider establishing procedures for the concurrent (that is, parallel) review of changes to the product. The manufacturing of biotherapeutic products requires, for example, the replenishment of biological starting materials such as WCBs and secondary/working reference standards which are considered as routine changes. Consequently, these changes often need to be reviewed concurrently with other manufacturing or safety and efficacy changes. Conversely, clinical safety and efficacy changes, such as the addition of a new indication or new age group for the use of a biotherapeutic product, require considerable supporting data including clinical studies; thus, review time should not impact the review of unrelated manufacturing changes or the immediate implementation of urgent changes to product labelling information. However, multiple related changes, or those supported by the same information, may be submitted in the same supplement (see "Multiple changes" in section 8).

In these WHO Guidelines, descriptions of the reporting categories for quality changes are provided in section 6, and the reporting categories for information changes on safety, efficacy and product labelling are provided in section 7. Proposed regulatory procedures for the reporting of changes to NRAs are described in section 8. Examples of suggested review timelines for changes in the various categories are given in Appendix 1. A comprehensive list of quality changes and the type of information that should be included in a supplement application are provided in Appendix 2 (for the drug substance and intermediates) and in Appendix 3 (for the drug product). Examples of changes that affect clinical use of a product and product labelling information (on safety, efficacy, dosage, administration and product components) are provided in Appendix 4.

5. Special considerations

5.1 Comparability exercise

The need for – and extent of – a comparability exercise depends upon the potential impact of the change(s) on the quality, safety and efficacy of the product. Comparability exercises can range from analytical testing alone (for example, where process changes have no impact on any quality attribute) to a comprehensive exercise requiring nonclinical and clinical bridging studies. For example, a change in the culture conditions or in the purification process may

cause the alteration of the glycosylation profile of the product, including site-directed glycosylation. Alteration of glycosylation profiles may cause a change in the pharmacokinetic/pharmacodynamic (PK/PD) profile of the product (see also section 5.2 on "Bridging studies"). If comparability can be demonstrated through analytical studies alone, nonclinical or clinical studies with the post-change product are not necessary. However, where the relationship between specific quality attributes and safety and efficacy has not been established, and/or differences are observed between some critical quality attributes of the pre-change and post-change product, it may be necessary to include a combination of quality, nonclinical and/or clinical studies in the comparability exercise (*1*, *11*).

5.2 Bridging studies

Nonclinical and clinical bridging studies are studies in which a parameter of interest (such as a manufacturing process or formulation) is directly compared with a changed version of that parameter with respect to the effect of the change on the product's clinical performance. If the physicochemical properties, biological activity, purity and/or level of impurities of the pre-change and post-change product are comparable, the safety and efficacy of the biotherapeutic product can be inferred. However, nonclinical and/or clinical bridging studies may be required when analytical data alone either do not establish comparability or are insufficient to do so. The comparison of efficacy responses and safety outcomes (for example, PK/PD profile, or rates of common adverse events and serious adverse events) is often the primary objective. For ethical reasons, it is desirable to apply the 3R principles (Replacement, Reduction, Refinement) to the use of animals where scientifically appropriate. The following are examples of changes that are likely to require nonclinical and/or clinical bridging studies: (a) generation of a new MCB derived from a different host cell line; (b) a new dosage form; (c) a new formulation (for example, a new excipient); (d) a new presentation (for example, addition of pre-filled pens to vials); (e) a new route of administration; and (f) a new dosing schedule. For these and comparable changes, any proposed use of alternative approaches to a bridging study must be justified and discussed with the NRA.

5.3 Similar biotherapeutic products

Following approval, an SBP is considered to be independent from the reference product and has its own life-cycle (*3*). The manufacturer is not required to re-establish similarity to the reference product when comparability exercises are conducted.

A major change in clinical use for an SBP that relies on the previously demonstrated similarity provided in the original approval of the SBP may be considered by the NRA on a case-by-case basis. For example, a new indication

given to the reference product after approval of an SBP should not automatically be given to the SBP. However, when new safety information on the reference product is added after the original approval of the SBP, the labelling information changes of the SBP should follow the changes made for the reference product unless it can be demonstrated that the new information on the reference product is not relevant to the SBP.

6. Reporting categories for quality changes

On the basis of the potential effect of the quality change (for example, manufacturing change) on the quality attributes (that is, identity, strength, purity and potency) of the biotherapeutic product, and on the potential impacts of this on the safety or efficacy of the product, a change should be categorized as:

- a major quality change
- a moderate quality change
- a minor quality change, or
- a quality change with no impact.

The implementation of changes in the major or moderate categories must be reported to the NRA in order to supplement the information in the original marketing authorization or product licence. Major and moderate quality changes should be reviewed and approved by the NRA prior to implementation of the change (that is, prior to distribution of the post-change product).

Quality changes that are expected to have minimal potential to have an impact, or to have no impact on the quality, safety or efficacy of the biotherapeutic product, do not require submission of a PAS. The changes included in these categories may be implemented by the marketing authorization holder without prior review and approval by the NRA. However, quality changes with minimal potential to have an impact should be notified to the NRA within established timelines following implementation.

For each approved product, the marketing authorization holder or manufacturer should maintain a comprehensive chronological list of all quality changes, including minor quality changes. Additionally, this list should include a description of the quality changes, including the manufacturing site(s) or area(s) involved, the date each change was made, and references to relevant validations and standard operating procedures. All data supporting minor quality changes, as listed in Appendices 2 and 3 below, should be available on request to the NRA or during inspections in accordance with local regulations.

Further information on each category of change is given below in sections 6.1–6.4, with Appendices 2 and 3 providing a comprehensive list of major, moderate and minor quality changes, and the information required to

support each change. The quality changes listed in Appendices 2 and 3 should be reported or recorded in the appropriate categories, as recommended in this section and in the appendices. If a quality change may potentially have an impact on the quality, safety and efficacy of the biotherapeutic product, but is not included in Appendix 2 or 3, the NRA may be consulted for the correct classification. When procedures and timelines for such consultations are not in place, manufacturers should determine the classification of the change on the basis of a change-specific risk assessment using the principles and examples provided in these WHO Guidelines. The NRA should consider establishing a mechanism that allows for its guidelines to be updated to address technological changes requiring regulatory category classifications.

6.1 Major quality changes

Major quality changes are changes to the product composition, manufacturing process, quality controls, facilities or equipment that have significant potential to have an impact on the quality, safety or efficacy of the biotherapeutic product or SBP. The marketing authorization holder should submit a PAS and receive a notification of approval from the NRA before implementing the change. NRAs should consider establishing a mechanism that allows for clear review timelines and a consistent means of ensuring that those timelines are met (see section 8 and Appendix 1).

For a change in this category, the PAS should specify the products concerned and should include a detailed description of the proposed change. Additional supporting information is needed for the drug substance (as noted in Appendix 2) and for the drug product (as noted in Appendix 3) and could include: (a) information on the methods used and studies performed to evaluate the effect of the change on the product's quality attributes; (b) the data derived from those studies; (c) relevant validation protocols and results; and (d) updated product labelling information. In some cases, major quality changes may also require nonclinical and/or clinical data. Relevant considerations on the data required can be found in the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*1*).

6.2 Moderate quality changes

Moderate quality changes are changes to the product composition, manufacturing process, quality controls, facilities or equipment that have a moderate potential to have an impact on the quality, safety or efficacy of the biotherapeutic product or SBP. The marketing authorization holder should submit a PAS and receive a notification of approval from the NRA before implementing the change. The

requirements for the PAS for moderate quality changes are the same as those for major quality changes (see section 6.1); however, the amount of supporting data required will generally be less than that required for major changes and the review timeline should be shorter.

6.3 Minor quality changes

Minor quality changes are changes to the product composition, manufacturing process, quality controls, facilities or equipment that have a minimal potential to have an impact on the quality, safety or efficacy of the biotherapeutic product or SBP. Changes in this category may be implemented by the marketing authorization holder without prior review by the NRA. However, the NRA should be notified of the changes within a specified timeline (see Appendix 1). The justification and supporting documentation for minor quality changes are not needed for such notification but should be made available by the marketing authorization holder upon request from the NRA.

When a minor quality change affects the lot release specifications (for example, narrowing of a specification, or compliance with pharmacopoeial changes) and affects the quality control testing as summarized in the lot release protocol, the marketing authorization holder should inform the institution responsible for reviewing the release of lots (see introductory sections in Appendices 2 and 3).

Minor quality changes that are related to a major or moderate change should be described in the supplement for the major or moderate quality change (see section 8.2 for additional details).

6.4 Quality changes with no impact

Quality changes that have no impact on product quality, safety or efficacy may be implemented by the marketing authorization holder without prior review by the NRA. Information on such changes must be retained as part of the manufacturer's GMP records or marketing authorization holder's product records, as applicable. These changes must comply with the applicable GMP requirements and must be available for review during GMP inspections. Examples of such changes include, but are not limited to:

- non-critical changes to the licensed application, including spelling corrections and editorial clarifications made to documents (such as validation summaries and/or reports, analytical procedures, standard operating procedures or production documentation summaries) that have no impact on the quality, safety and efficacy of the product;
- replacement of equipment with identical equipment;

- change in specifications for a compendial raw material, a compendial excipient or a compendial container closure component to comply with an updated pharmacopoeial standard/monograph;
- transfer of quality control testing activities to a different facility within a GMP-compliant site;
- with the exception of a potency assay or a bioassay, transfer of the quality control testing activities for a pharmacopoeial assay to a different facility within the same company;
- change in the in-process controls performed at non-critical manufacturing steps;
- addition of a new GMP-compliant storage warehouse for raw materials, master and working cell banks, and drug substance;
- installation of non-process-related equipment or rooms to improve the facility, such as warehousing refrigerators or freezers;
- addition of time point(s) into the post-approval stability protocol;
- deletion of time point(s) from the post-approval stability protocol beyond the approved shelf-life.

7. Reporting categories for safety, efficacy and/or product labelling information changes

After assessing the effect of a change related to the clinical use of a product or to product labelling information on the safe and effective use of a biotherapeutic product, marketing authorization holders should classify this change as one of the following reporting categories:

- safety and efficacy change;
- product labelling information change;
- urgent product labelling information change; or
- administrative product labelling information change (in cases where prior approval before implementation is needed).

The product labelling information includes prescribing information (or package insert) for health-care providers or patients, outer label (that is, carton) and inner label (that is, container label). After approval, the marketing authorization holder should promptly revise all promotional and advertising items relating to the biotherapeutic product to make them consistent with implementation of the product labelling information change.

Further information on each category is provided below in sections 7.1–7.4. In addition, examples of efficacy, safety and product labelling

information changes considered to be appropriate for each category are provided in Appendix 4.

7.1 Safety and efficacy changes

Safety and efficacy changes are changes that have an impact on the clinical use of the biotherapeutic product in relation to safety, efficacy, dosage and administration. To support such changes, data are required from clinical studies and, in some cases, from clinically relevant nonclinical studies. Safety and efficacy changes also require supplement submission and approval prior to implementation of the change.

In general, safety and efficacy changes affect the product labelling information and have the potential to increase or decrease the exposure levels of the biotherapeutic product either by expanding the population that is exposed or by changing dosage or dosing. These changes may be related to clinical use of the biotherapeutic product, and can include:

- addition or expansion of a safety claim or efficacy claim, including expansion of the population that is exposed;
- change in the strength or route of administration;[1]
- change in the recommended dose and/or dosing schedule;
- co-administration with other biotherapeutic products or medicines;
- deletion or reduction of existing risk-management measures (for example, contraindications, adverse events, warnings or cautionary text/statements in the product labelling information).

The type and scope of the required nonclinical and/or clinical safety and efficacy data are determined case by case on the basis of risk–benefit considerations related to the impact of the changes, the biotherapeutic product attributes and the disease that the biotherapeutic product is designed to prevent. Other considerations include:

- the nature of the disease treated (that is, morbidity and mortality, acute or chronic disease, current availability of disease therapy, and size and nature of patient population);
- safety considerations (for example, adverse drug reactions observed, adverse events in specific patient populations, management of adverse reactions and change in rates of adverse reactions);
- the availability of animal models.

[1] Some NRAs consider that changes in the route of administration or strength may require a new marketing authorization.

Marketing authorization holders are encouraged to consult with the NRA on the adequacy of the clinical and/or nonclinical data needed to support a safety and efficacy change, if deemed necessary. Additionally, some changes such as dosage form, content of excipients or residual components, or delivery device may require clinical data as well as revision of the product labelling information. The NRA should be consulted on the data required to support such changes.

For nonclinical and clinical studies, the recommendations given in the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*1*) should apply. Guidance on approaches to the nonclinical and clinical comparability exercise can also be found in WHO guidelines on the evaluation of SBPs (*2*, *3*).

For a change under this category, the marketing authorization holder should submit a supplement to the NRA that includes the following where applicable:

- a detailed description of – and rationale for – the proposed change;
- a summary of the methods used and studies performed to evaluate the effect of the change on the safety or efficacy of the biotherapeutic product;
- amended product labelling information;
- information on clinical studies (protocol, statistical analysis plan and clinical study report);
- information on clinical assay methods (standard operating procedures) and validations; and
- the pharmacovigilance plan.

7.2 Product labelling information changes

Product labelling information changes are changes to the labelling items that have the potential to improve the management of risk to the population for which use of the biotherapeutic product is currently approved through:

- the identification or characterization of any adverse event resulting in the addition or strengthening of risk-management measures for an adverse event considered to be consistent with a causal association with the biotherapeutic product concerned;
- the identification of subgroups for which the benefit-to-risk profile of the biotherapeutic product has the potential to be less favourable; and
- the addition or strengthening of risk-management measures, including instructions on dosing or any other conditions of use.

Product labelling information changes require the filing of a PAS and a notification of approval from the NRA prior to distribution of the product. Supplements for product labelling information changes related to the clinical use of a product often require data from pharmacovigilance reports (that is, periodic safety update reports). Changes supported by large clinical or nonclinical studies are usually not considered as product labelling information changes but as safety and efficacy changes.

For a change under this category, the marketing authorization holder should submit to the NRA a PAS that includes the following where applicable:

- a detailed description of – and rationale for – the proposed change;
- pharmacovigilance reports and statistical analysis of results; and
- amended product labelling information.

7.3 Urgent product labelling information changes

Urgent product labelling information changes are changes to the labelling items that need to be implemented in an expedited manner in order to mitigate a potential risk to the population in which the biotherapeutic product is currently approved for use. Marketing authorization holders should consult with the NRA and agree on the required supporting documentation and time frames for the labelling changes or the need for a Dear Health-Care Professional Letter (that is, a formal letter from a manufacturer to health-care professionals) to convey the information prior to the submission of the supplement(s).

7.4 Administrative product labelling information changes

Administrative product labelling information changes are changes that are not expected to affect the safe and efficacious use of the biotherapeutic product. In some cases these changes may require reporting to the NRA and receipt of approval prior to implementation, while in other cases reporting may not be required.

- Examples of product labelling information changes that require approval by the NRA prior to implementation are changes in the proper/nonproprietary name or trade name of the biotherapeutic product. Changes in this category are considered important for reasons of liability and monitoring.
- Examples of product labelling information changes that do not require approval by the NRA prior to implementation are administrative changes such as those related to labelling (for example, minor changes in format without any negative effect on

readability). These changes should be reported to the NRA as part of a subsequent PAS for safety and efficacy changes or product labelling information changes when updated product labelling information is included.

Manufacturers are encouraged to consult with the NRA regarding the appropriate reporting category for labelling changes to approved products.

8. Procedures

The establishment of procedures and criteria for the adequate oversight of changes to approved biotherapeutic products is the responsibility of the regulator. Therefore, NRAs should establish written instructions regarding submission procedures and timelines (with action dates) for consultation by marketing authorization holders as they prepare to submit a supplement for a change. These instructions should cover: (a) the identification of emergency use; (b) expanded access; and (c) expedited and/or priority review, timelines and procedures for life-saving medications to address an unmet need. As supplements for a major quality change or an efficacy and safety change require extensive documentation and data, the review times should be longer than those for supplements for moderate quality changes or product labelling information changes. Furthermore, NRAs may establish different timelines for the review of major quality changes that do not require clinical data as compared with safety and efficacy changes that do require clinical data. Appendix 1 provides examples of different regulatory categories and their suggested review timelines.

If a change is not included in Appendices 2, 3 or 4, marketing authorization holders are encouraged to use scientific judgement, leverage competent regulatory authority guidance or to contact the NRA to determine the appropriate category of a supplement prior to submission of the information in support of a change. Similarly, marketing authorization holders should consult NRAs for major changes that require the inclusion of a GMP certificate and which may trigger a pre-submission inspection, or that may require clinical and/or nonclinical data to support a change in safety and efficacy or in product labelling information. Marketing authorization holders are encouraged to contact the NRA regarding plans for future changes and proposed filing dates for changes to existing products in order to assist NRAs in planning the allocation of review resources. NRAs should establish procedures with appropriate timelines for the conducting and recording of communications between themselves and marketing authorization holders.

To assist in the acceptance of submissions for review, the covering letter or the Module 1 documentation of the Common Technical Document

accompanying a supplement for a quality change should clearly specify the selected category by labelling the submission as either a major quality change or a moderate quality change.

The covering letter accompanying a supplement for a safety, efficacy or product labelling information change should specify that the change is being reported in the selected category by labelling the submission as:

- a safety and efficacy change;
- a product labelling information change;
- an urgent product labelling information change; or
- an administrative product labelling information change (in cases where prior approval is needed before implementation).

Major quality change supplements that contain both quality data and revised product labelling information but no clinical and/or nonclinical data should be labelled "Major quality change and Product labelling information change" and the covering letter should specify that the submission includes both quality changes and revised product labelling information items.

Major quality change supplements that contain quality, safety and efficacy data (from clinical studies and/or clinically relevant nonclinical studies) and revised product labelling information, should be labelled "Major quality change and Safety and efficacy change" and the covering letter should specify that the submission includes quality changes, results from clinical and/or nonclinical studies, and revised product labelling information items.

Each supplement should include a list of all the changes contained in the submission. The list should describe each change in sufficient detail to allow the NRA to determine quickly whether the appropriate reporting category has been used. If the submission has been inappropriately classified, the marketing authorization holder should be notified. Minor quality changes that are related/consequential to moderate or major quality changes should be described in the PAS. In addition, any minor changes that have been implemented should be annotated in the affected documents (for example, Common Technical Document sections) and reported in any future filing to the NRA. For example, a minor change such as narrowing of a specification should be included in a supplement for a moderate or major change which includes updated quality control release information.

The regulation of post-approval changes is part of the entire regulatory framework which includes marketing authorization, GMP inspection and post-marketing surveillance. These activities are often performed by different units of the NRA. It is essential that these different units – especially the marketing authorization (or regulatory affairs) and GMP inspection units – interact and

exchange information effectively, and that the roles and responsibilities of each unit are clearly defined, particularly when they operate as separate entities. When multiple units are involved in the evaluation of a supplement, a formal decision-making process should be in place to discuss, for example, whether a change may require a GMP inspection or may be reviewed during the next routine inspection. Procedures should also be established so that the outcomes of inspections are verified or taken into account prior to the approval of supplements. Good coordination and communication between different units of the NRA are pivotal in ensuring continuity of supply and access to products of assured quality, safety and efficacy. Some regulatory authorities may be willing to cooperate more closely and to share information on GMP inspections under a mutual agreement (for example, the Pharmaceutical Inspection Cooperation Scheme – PIC/S).

Expedited review procedures

NRAs of product-procuring countries that decide to recognize or rely on the decisions of other NRAs should establish alternative regulatory procedures for the expedited approval of changes based on previous expert review and approval by the NRA of the country where the biotherapeutic products are licensed (see Appendix 1). Accordingly, the product-procuring NRAs should also create a list of the NRA approvals they will recognize. On the basis of regulatory and regional considerations, procedures for recognition of the decisions of other NRAs on the approval of changes could include the following pathways:

- The NRA recognizes the decision of other regulatory authorities and does not perform a review of supporting data, but is notified of the change. The submission consists of a covering letter from the marketing authorization holder informing the procuring NRA about the change and including as an attachment a copy of the approval letter from the NRA of the licensing country stating the relevant changes.
- The NRA performs an assessment of the decision of the NRA of the licensing country to determine whether recognition of that NRA's decision is appropriate. The submission consists of:
  - the covering letter from the marketing authorization holder informing the procuring NRA of the change;
  - a copy of the approval letter issued by the NRA of the licensing country;
  - assessment reports and relevant correspondence from the NRA of the licensing country (if made available by the NRA);
  - a detailed description of the change; and

  - supporting data submitted as necessary if assessment reports are not available.
- The NRA performs a partial review and evaluation of a complete package of supporting data, as originally submitted in the product-licensing country.

Similarly, recognition of inspection activities conducted by the authorities that license the product may be considered as part of the expedited review process and may be included in the regulatory pathways listed above.

Additionally, for previously approved changes addressing urgent safety issues in the product labelling information, procedures should be in place to allow for the expedited implementation of such changes (see section 8.3 and Appendix 1).

In special or urgent circumstances, a marketing authorization holder may ask the NRA to expedite the review of a supplement for public health reasons (for example, a product shortage or safety update) or if a delay in making the change would impose extraordinary hardship on the marketing authorization holder or manufacturer.

Multiple changes

Multiple related changes, involving various combinations of individual changes, may be submitted in the same supplement. For example, a manufacturing site change may also involve changes to the equipment and manufacturing process. For submissions that include multiple changes, the marketing authorization holder should clearly specify which data support each change.

Multiple major or moderate quality changes for the same product may be filed in a single submission provided that the changes are related and/or supported by the same information. Minor quality changes that were implemented previously and that are related and/or consequential to a moderate or major quality change should be described in the PAS for the moderate or major quality change. If the proposed changes are related, the marketing authorization holder should indicate the association between them. The marketing authorization holder should also clearly specify which supporting data support which change. Such changes could affect both the drug substance and the drug product. If too many changes are filed within the same submission, or if major issues are identified with a change and extensive time would be required to review them, the NRA may ask the marketing authorization holder to divide the changes into separate submissions and to resubmit the file. If the recommended reporting categories for the individual changes differ, the submission should be in accordance with the most restrictive of the categories recommended for the individual changes. In the case of numerous changes of the same category, the NRA may reclassify

the submission to the next higher level on the basis of the potential impact of the totality of the changes on the quality, safety and efficacy of the biotherapeutic product or SBP. This reclassification should be communicated to the marketing authorization holder at the start of the assessment.

8.1 Procedures for prior approval supplements

The procedures in this section apply to all changes requiring approval prior to implementation: namely, major and moderate quality changes, safety and efficacy changes, product labelling information changes, urgent product labelling information changes and selected administrative product labelling information changes.

The following items should be included, where applicable, in the supplement submission for post-approval changes:

- a covering letter that includes:
  - the type of submission (for example, major quality change, moderate quality change or safety and efficacy change),
  - a list of the change(s) and a rationale for the change(s) with sufficient detail (including a justification for the selected reporting category) to allow for processing and reviewer assignments by NRAs,
  - an indication of the general type of supporting data, and
  - cross-referenced information (including product name, marketing authorization holder's name, submission type and date of submission/approval);
- completed documents or forms based on NRA requirements, such as a medicine submission application form, signed and dated;
- the anticipated date for implementation of the change (recognizing that in some cases the implementation of the change may be delayed after approval to allow for depletion of the previously approved biotherapeutic or to allow for global staggered approval depending on supply/demand);
- GMP information (for example, inspection history and/or evidence of GMP compliance rating by experienced NRAs), as applicable;
- when relevant, a side-by-side comparison showing the differences between the approved manufacturing process (including quality control tests) and the proposed one(s) (see section 5);
- when relevant, clinical and/or nonclinical study reports, pharmacovigilance reports, and annotated and clean drafts of product labelling information (see section 7).

In addition to the above general information, the specific information required to support the various quality changes is outlined in Appendices 2 and 3. It should be noted that the general information is not repeated under each of the various changes outlined in the appendices. All data recommended to support a change should be provided with the submission, in addition to the general information as appropriate. If recommended supporting data are not submitted, a detailed rationale should be provided to explain why.

If the same change is applicable to multiple products, a separate submission is generally required for each product – though the data may be cross-referenced. NRAs may in some cases allow a common change to be bundled into one submission for multiple products. When cross-references are made to information that has been submitted previously, details of the cross-referenced information should be provided in the covering letter.

Submissions filed in electronic or paper format should be based on the requirements of the NRA. The data submitted should be well organized and should be provided in the format defined by the NRA.

After the NRA completes the review of the supporting data in a supplement, the following outcomes are possible:

- If the NRA determines that the information submitted in a supplement supports the quality, safety and efficacy of the product manufactured with the change, the NRA will issue a written notification of approval stating that the change can be implemented and the product manufactured with the change can be distributed.
- If the NRA determines that the information submitted in a supplement fails to support the quality, safety or efficacy of the product manufactured with the change, the NRA will issue a written request notification for additional documentation, information and clarification to be submitted by the marketing authorization holder. If the identified deficiencies are minor, they may be addressed without stopping the review process. If the deficiencies are major or are not resolved during the allotted review period following rounds of questions and requests for more information, the NRA may decide to issue a written notification of noncompliance, as a result of which the review process is stopped, the change may not be implemented and the product manufactured with the change may not be distributed. In the case of a notification of noncompliance being issued, the following outcomes are possible:
  - If the marketing authorization holder's response document to the notification of noncompliance is adequate and all identified deficiencies are resolved in a satisfactory manner, the NRA will

issue a written notification of approval stating that the change can be implemented and the product manufactured with the change can be distributed.

– If the information in the marketing authorization holder's response document to the notification of noncompliance is not adequate and not all identified deficiencies are resolved in a satisfactory manner, the NRA will issue a written notification of rejection stating that the change cannot be implemented and the product manufactured with the change cannot be distributed.

The NRA should establish procedures and timelines for the review of marketing authorization holders' responses to the notification of noncompliance in cases where the review has been stopped. Documentation subsequent to the original supplement submission (in response to information requests or notifications of noncompliance) should be submitted and filed as amendments to the original supplement, and all communications with sponsors should be properly recorded.

Appeal procedures should be established for resolving disagreements and disputes between the NRA and the marketing authorization holder. Such procedures should allow the marketing authorization holder to request a re-evaluation of the submitted application in case the application is initially rejected by the NRA.

NRAs may consider the use of a "comparability protocol" when a marketing authorization holder submits changes:

Comparability protocol

A comparability protocol (also referred to as "post-approval change management protocol" in other documents) establishes a framework for a well-defined plan for future implementation of a quality change. This will include the tests to be done and acceptable limits to be achieved when assessing the effect of specific changes on the quality, safety or efficacy of a biotherapeutic product or SBP. For some changes, the routine quality tests performed to release the drug substance or drug product are not considered sufficient for assessing the impact of the change, and additional in-process tests and characterization tests may be needed. Comparability protocols are often used for the routine replenishment of WCBs and reference standards used in quality control tests when the remaining aliquots of reference standards expire or diminish.

The purpose of a comparability protocol is to provide transparency in the data requirements for changes and increase the predictability of the effects of changes. This allows for the more expedient distribution of a product by

permitting the marketing authorization holder to submit a protocol for a change which, if approved, may justify a reduced reporting category for the change when the comparability data are obtained and the change is implemented. It is for the NRA to decide whether or not to include the review and approval of comparability protocols in its approach to regulating changes to approved biotherapeutic products or SBPs; however, the concept of using comparability protocols is encouraged. For NRAs currently taking this approach, a comparability protocol can be provided in the original submission. Otherwise, a new comparability protocol, or a change to an existing one, requires submission of a supplement and approval prior to implementation because it may result in a lower reporting category for the changes covered in the comparability protocol once the actual comparability data are submitted. The change in reporting category for a change covered by a comparability protocol and the supporting data to be generated should be established by the NRA at the time the comparability protocol is approved. For a minor quality change that results from the execution of a comparability protocol, the change should be notified to the NRA immediately after implementation. For some marketing authorization holders with multiple related products and facilities, an expanded change protocol can be proposed. The scope of an expanded change protocol may cover multiple related products or manufacturing changes (for example, facility changes) (*15*).

Production documents

Production documents (that is, executed batch records) are not generally required to support changes to the marketing authorization dossier or product licence. However, such documents may be requested during review and should be made available to the NRA on request. These documents should be retained in accordance with GMP and should be available in their local official language during inspections. If English translations are required, NRAs are encouraged to establish a mechanism to make this requirement known to marketing authorization holders accordingly.

8.2 Procedures for minor quality changes and quality changes with no impact

Implementation of minor quality changes does not require prior approval from the NRA but should be notified to the NRA. Each NRA is responsible for determining the timelines for reporting the notification (for example, annually). Supporting data should not be provided with the notification unless it may help in justifying the reporting category. However, as recommended in Appendices 2 and 3 below, the minor quality changes should be recorded or compiled with related supporting data generated by the manufacturer in a document or file

dedicated to minor changes. The documents or files for all minor quality changes should be available to the NRA on request or during inspection.

NRAs may audit minor quality changes by requesting and reviewing the supporting data, as deemed appropriate during an inspection or review of related changes. If the classification of a change or the supporting data are not considered to be acceptable then the marketing authorization holder may be requested to file a supplement for a major or moderate quality change.

Minor quality changes that have previously been implemented and are related and/or consequential to a major or moderate quality change should be described in the relevant parts of the documentation when submitting a PAS for the major or moderate change. As for all minor quality changes, the supporting data for these changes do not need to be included in the supplement but should be retained by the manufacturer.

Changes that have no impact on the quality, safety and efficacy of the product are not reported, but if the NRA determines (during an inspection or a review of related changes) that the information for the change fails to demonstrate the continued safety or efficacy of the product manufactured using the changes, the NRA may work to resolve the problem with the marketing authorization holder. If the NRA finds that the product in distribution poses a danger to public health, or if it determines that there are unresolved issues, it may require the marketing authorization holder to cease distribution of the product manufactured using the changes or to remove the product from distribution pending resolution of the issues related to the changes.

8.3 Procedures for urgent product labelling information changes

For urgent changes to product labelling information which address safety updates and have the potential to have an impact on public health (for example, addition of a contraindication or a warning), NRAs should establish a specific mechanism to allow for the immediate or expedited approval and implementation of such changes on a case-by-case basis after previous agreement between the NRAs and marketing authorization holders.

Since product labelling safety updates invariably need to be implemented and are generally approved, NRAs in procuring countries should establish a mechanism by which urgent product labelling changes that have been approved in the country where the biotherapeutic products in question are produced and/or licensed may be implemented immediately upon receipt of the supplement from marketing authorization holders or manufacturers. Such accelerated procedures would contribute to the dissemination of the most current information to health-care providers and would help to mitigate discrepancies between the labels used in the various countries and posted on websites.

8.4 Procedures for administrative product labelling information changes

Depending on the scope of the change, administrative product labelling information changes may require approval prior to implementation. For example, changes in the proper/nonproprietary name or trade name of the biotherapeutic product require approval before implementation, while minor formatting changes do not (see section 7.4 for further details).

For an administrative product labelling information change that requires approval prior to implementation the marketing authorization holder should submit a supplement containing background information on the change and annotated and clean drafts of the product labelling information.

Administrative product labelling information changes that do not need prior approval and that have been implemented since the last approved product labelling information should be included when submitting a subsequent PAS for safety and efficacy changes or for product labelling information changes. In these cases, the product labelling information should be annotated when filing the next PAS to indicate the new changes and those administrative changes that have been implemented since the last approval.

9. Authors and acknowledgements

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Appendix 1

Reporting categories and suggested review timelines

It is recommended that NRAs establish review timelines to allow marketing authorization holders or applicants to plan the implementation of changes. The review timelines are established taking into consideration the country or regional situation, the capability of the NRA, the impact of the change and the amount of data required to support the change. Consequently, the review time frames for major changes should be longer than those for moderate changes. The suggested review times in the table below are shown as examples; they are based on the experience of several NRAs and apply to situations where the NRA performs a full review or assessment of the supplement. The review time would start when the supplement has been accepted for review and found to be complete, and would end at the time when the initial assessment is shared with the marketing authorization holder by the issuance of either a notification of approval or a notification of noncompliance with a list of comments and deficiencies. In case of the latter, the marketing authorization holder may seek approval for the change by submitting an amendment to the supplement with responses to all the comments in the notification of noncompliance. The NRA should also establish timelines for the secondary review cycle following the receipt of responses from the marketing authorization holder. If minor deficiencies are identified during the initial review cycle, the NRA may communicate these to the marketing authorization holder without stopping the review clock in order to try to finalize the assessment within the established timeline (see section 8.1).

Expedited implementation procedures should be in place for dealing with product labelling information changes which address urgent safety issues (see section 8.3).

Reporting categories for post-approval changes and suggested review timelines

Quality changes		
Reporting category	**Procedure**	**Suggested review timeline**
Major quality changes	PAS	3–6 months
Moderate quality changes	PAS	1–3 months
Minor quality changes	Require notification to the NRA[a, b]	N/A
Quality changes with no impact	Do not require notification to the NRA	N/A

Table *continued*

Safety, efficacy and product labelling information changes		
Reporting category	**Procedure**	**Suggested review timeline**
Safety and efficacy changes	PAS	10 months
Product labelling information changes	PAS	5 months
Urgent product labelling information changes[c]	PAS for urgent safety restrictions	Immediate implementation on receipt of supplement by the NRA
Administrative product labelling information changes	PAS	30 days
	Do not require approval prior to implementation[d]	N/A

N/A: not applicable.

[a] Each NRA is responsible for determining the timeline for reporting the notification (for example, annually). However, NRAs should establish a mechanism to ensure that notifications are received no later than one year post-implementation. In a case where a minor quality change results from the use of a comparability protocol, the change should be notified to the NRA immediately after implementation.

[b] Minor quality changes impacting the registered details may be bundled with moderate or major quality changes, if needed.

[c] Urgent product labelling information changes are applicable only to label changes which address urgent safety updates or have the potential to have an impact on public health, with immediate implementation allowed after prior agreement between NRAs and marketing authorization holders.

[d] Administrative product labelling information changes that do not require approval prior to implementation and that have been implemented since the last approved product labelling information change should be reported by including all changes in subsequent PAS for safety and efficacy changes or product labelling information changes when updated product labelling information is included.

NRAs that procure biotherapeutic products from countries other than their own are encouraged to establish alternative accelerated timelines for changes that have previously been approved by the other NRAs. Accordingly, those NRAs should create a list of the NRA approvals they will recognize. On the basis of the regulatory pathway options provided in section 8, the following examples of accelerated timelines could be established:

- The NRA recognizes the decision of other regulatory authorities and does not perform a review of supporting data but is informed of the change. Using this approach, NRAs could allow changes to be implemented immediately after receipt of the change notification.

- The NRA performs an assessment of the decision of the NRA of the licensing country to determine if recognition of the latter NRA's decision is appropriate. Using this approach, NRAs could establish abbreviated review timelines – such as 2 months for major quality changes, 4 months for safety and efficacy changes, and immediate implementation on receipt of the change notification for moderate quality changes and product labelling information changes.
- The NRA performs a partial review and evaluation of a complete supporting data package, as originally submitted to the licensing country. Using this approach, timelines would be expected to be shorter than the timelines described in the above table.

Appendix 2

Changes to the drug substance

The examples presented in this appendix are intended to assist with the classification of changes made to the quality information for the drug substance. The information summarized in the table below provides guidance on:

- the **conditions to be fulfilled** for a given change to be classified as major, moderate or minor (if any of the conditions outlined for a given change are not fulfilled, the change is automatically considered to be at the next higher reporting category – for example, if any conditions recommended for a moderate quality change are not fulfilled, the change is considered to be a major quality change);
- the **supporting data** for a given change, either to be submitted to the NRA or maintained by the marketing authorization holder (if any of the supporting data outlined for a given change are not provided, are different or are not considered applicable, adequate scientific justification should be provided); and
- the **reporting category** (major, moderate or minor quality change).

Marketing authorization holders should use scientific judgement, leverage competent regulatory authority guidance or contact the NRA if a change is not included in the table and has the potential to impact on product quality. Marketing authorization holders should also contact the NRA when a change is considered at the next higher reporting category because any of the conditions outlined are not fulfilled and where the supporting data are not described. NRAs should establish procedures, with appropriate timelines, on the conducting and recording of communications between themselves and marketing authorization holders.

Supporting data should be provided according to the submission format accepted by the NRA – see for example (*1*, *2*).

Additional information on data requirements to support quality changes can be found in WHO good manufacturing practices for biological products (*3*), WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*4*) and in relevant International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (*5*, *6*).

Quality changes to comply with updated compendia and/or pharmacopoeias

NRAs should make a list of the recognized compendia and/or pharmacopoeias. Manufacturers are expected to comply with the current versions of compendia/pharmacopoeias, as referenced in the approved marketing authorization. Changes linked to a change in the compendial/pharmacopoeial methods or specifications for a drug substance do not need to be submitted for review if reference is made to the current edition of the compendium or pharmacopoeia, but the changes should be notified to the NRA with information on them available for inspection.

In some cases, changes introduced to comply with recognized compendia/pharmacopoeias may require approval by the NRA prior to implementation regardless of the timing of the change in relation to the date when the compendium/pharmacopoeia was updated. For example, supplement submission and approval by the NRA may be required for some changes to quality control tests performed for product release (for example, to potency tests), for changes that have an impact on any product labelling information items, and for changes that may affect the quality, safety or efficacy of the product.

Quality changes affecting lot release

While WHO recognizes that independent lot release by NRAs or national control laboratories is required for vaccines, in some countries this lot release system also applies to other types of products such as plasma-fractionated products. Where post-approval changes to the drug substance affect the lot release protocol (for example, changes to test procedures, reference standards or laboratory sites) or sample testing requirements for lot release, the marketing authorization holder should inform the institution responsible for reviewing the release of product lots. These procedures apply to changes that have been authorized by the NRA in the case of major and moderate quality changes and to changes that have been implemented in the case of minor quality changes. For example, the qualification of a new lot of reference standard against the approved reference standard may be considered a minor quality change if the qualification of a new standard is performed in accordance with an approved protocol and specification. Nevertheless, these changes must be reported to the NRA or national control laboratory as appropriate.

Manufacture

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
1. Change to a drug substance manufacturing facility: *Note: For the purpose of this change, manufacturing refers to unit operations in the manufacturing process of the drug substance and is not intended to refer to quality control testing, storage or transportation.*			
a. Replacement or addition of a manufacturing facility for the bulk drug substance or any intermediate	None	1–4, 6–8	Major
	1–3	1–8	Moderate
b. Conversion of a drug substance manufacturing facility from single-product to multi-product	4	9, 10	Moderate
c. Deletion of a manufacturing facility or manufacturer of an intermediate drug substance, or bulk	5, 6	None	Minor

Conditions

1. The proposed facility is an approved drug substance facility for biotherapeutics (for the same company/marketing authorization holder).
2. Any changes to the manufacturing process and/or controls are considered either moderate or minor (for example, duplication of product line).
3. The new facility/suite is under the same quality assurance/quality control oversight.
4. The proposed change does not involve additional containment requirements.
5. There should remain at least one site/manufacturer, as previously authorized, performing the same function as the one(s) to be deleted.
6. The deletion should not be due to critical deficiencies in manufacturing (for example, recurrent out-of-specification events, environmental monitoring failures, etc.).

Supporting data

1. Evidence of GMP compliance of the facility.
2. Name, address and responsibilities (for example, fermentation, purification) of the proposed facility.
3. Summary of the process validation studies and results.

Table *continued*

4. Comparability of the pre-change and post-change drug substance with respect to physicochemical properties, biological activity, purity, impurities and contaminants, as appropriate. Nonclinical and/or clinical bridging studies may be required if quality data alone are insufficient to establish comparability. The extent and nature of nonclinical and/or clinical studies should be determined on a case-by-case basis, taking into consideration the quality comparability findings, the nature and level of the knowledge of the product, existing relevant nonclinical and clinical data, and aspects of their use.
5. Justification for the classification of any manufacturing process and/or control changes as moderate or minor.
6. Description of the batches and summary of in-process control and release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Matrixing, bracketing, use of smaller-scale batches, use of fewer than three batches and/or leveraging data from scientifically justified representative batches, or batches not necessarily manufactured consecutively, may be acceptable where justified and agreed by the NRA.
7. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug substance batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug substance under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, use of smaller-scale batches and/or use of fewer than three batches of drug substance for stability testing may be acceptable where justified (*6*).
8. Updated post-approval stability protocol.
9. Information describing the change-over procedures for shared product-contact equipment and the segregation procedures, as applicable. If no revisions, the manufacturer should state that no changes were made to the change-over procedures.
10. Cleaning procedures (including data in a summary validation report and the cleaning protocol for the introduction of new products, as applicable) demonstrating lack of carry-over or cross-contamination.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
2. Change to the cell banks: *Note: New cell substrates that are unrelated to the licensed master cell bank (MCB) or pre-MCB material may require a new application for marketing authorization or licence application.*			
a. Adaptation of an MCB into a new culture medium	None	1, 2, 5–8, 10	Major
b. Generation of a new MCB	1	1, 2, 5–8	Moderate
c. Generation of a new working cell bank (WCB)	2–4	1, 2	Minor
3. Change in the cell bank manufacturing site	None	1, 2, 9	Moderate
4. Change in the cell bank testing/storage site	5, 7	9	Minor
5. Change in the cell bank qualification protocol	None	3, 4	Moderate
	6	4	Minor

Conditions

1. The new MCB is generated from the original clone or from a pre-approved MCB and is grown in the same culture medium.
2. The new cell bank is generated from a pre-approved MCB.
3. The new cell bank is at the pre-approved passage level.
4. The new cell bank is released according to a pre-approved protocol/process or as described in the original licence.
5. No changes have been made to the tests/acceptance criteria used for the release of the cell bank.
6. The protocol is considered more stringent (that is, addition of new tests or narrowing of acceptance criteria).
7. No changes have been made to the storage conditions used for the cell bank, and the transport conditions of the cell bank have been validated.

Supporting data

1. Qualification of the cell bank according to guidelines considered acceptable by the NRA.
2. Information on the characterization and testing of the MCB/WCB, and cells from the end-of-production passage or post-production passage.
3. Justification of the change to the cell bank qualification protocol.
4. Updated cell bank qualification protocol.

Table *continued*

5. Comparability of the pre-change and post-change drug substance with respect to physicochemical properties, biological activity, purity, impurities and contaminants, as appropriate. Nonclinical and/or clinical bridging studies may occasionally be required when quality data are insufficient to establish comparability. The extent and nature of nonclinical and/or clinical studies should be determined on a case-by-case basis, taking into consideration the quality-comparability findings, the nature and level of knowledge of the product, existing relevant nonclinical and clinical data, and aspects of its use.
6. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the drug substance derived from the new cell bank. Matrixing, bracketing, use of smaller-scale batches, use of fewer than three batches and/or leveraging data from scientifically justified representative batches, or batches not necessarily manufactured consecutively, may be acceptable where justified.
7. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug substance batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug substance under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug substance for stability testing may be acceptable where justified (*6*).
8. Updated post-approval stability protocol.
9. Evidence that the new company/facility is GMP-compliant.
10. Supporting nonclinical and clinical data or a request for a waiver of in vivo studies with justification.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
6. Change to the fermentation or cell culture process:			
a. A critical change (a change with high potential to have an impact on the quality of the drug substance or drug product; for example, incorporation of disposable bioreactor technology)	None	1–7, 9, 11	Major
b. A change with moderate potential to have an impact on the quality of the drug substance or drug product (for example, extension of the in vitro cell age beyond validated parameters)	1, 3	1–6, 8, 10	Moderate
c. A noncritical change with minimal potential to have an impact on the quality of the drug substance or drug product, such as: • a change in harvesting and/or pooling procedures which does not affect the method of manufacture, recovery, intermediate storage conditions, sensitivity of detection of adventitious agents or production scale; • duplication of a fermentation train; or • addition of similar/comparable bioreactors	1–5, 7–10	1, 2, 4, 8	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
7. Change to the purification process, involving the following:			
a. A critical change (a change with high potential to have an impact on the quality of the drug substance or drug product, for example, a change that could potentially have an impact on the viral clearance capacity of the process or the impurity profile of the drug substance)	None	1, 2, 5–7, 9, 11, 12	Major
b. A change with moderate potential to have an impact on the quality of the drug substance or drug product (for example, a change in the chemical separation method, such as ion-exchange HPLC[1] to reversed-phase HPLC)	1, 3	1, 2, 5–7, 10–12	Moderate
c. A noncritical change with minimal potential to have an impact on the quality of the drug substance or drug product (for example, addition of an in-line filtration step equivalent to the approved filtration step)	1–4	1, 2	Minor
8. Change in scale of the manufacturing process:			
a. At the cell culture stage	3, 9–11	2, 3, 5–7, 9, 11	Moderate
b. At the purification stage	1, 2, 4, 6	2, 5–7, 9, 11	Moderate
9. Introduction of reprocessing steps	12, 13	8, 10, 11, 13	Minor
10. Addition of a new holding step or change in the parameters of an approved holding step	None	5, 14	Moderate

[1] HPLC = high-performance liquid chromatography.

Table *continued*

Conditions

1. The change does not have an impact on the viral clearance data or the chemical nature of an inactivating agent.
2. There is no change in the drug substance specification outside the approved limits.
3. There is no change in the drug substance impurity profile outside the approved limits.
4. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
5. The change does not affect the purification process.
6. The change in scale is linear with respect to the proportionality of production parameters and materials.
7. The new fermentation train is identical to the approved fermentation train(s).
8. There is no change in the approved in vitro cell age.
9. The change is not expected to have an impact on the quality, safety or efficacy of the final product.
10. There is no change in the proportionality of the raw materials (that is, the change in scale is linear).
11. The change in scale involves the use of the same bioreactor (that is, it does not involve the use of a larger bioreactor).
12. The need for reprocessing is not due to recurrent deviations from the validated process, and the root cause triggering reprocessing is identified.
13. The proposed reprocessing steps have been shown to have no impact on product quality.

Supporting data

1. Justification for the classification of the change(s) as critical, moderate or noncritical in terms of its impact on the quality of the drug substance.
2. Flow diagram (including process and in-process controls) of the proposed manufacturing process(es) and a brief narrative description of the proposed manufacturing process(es).
3. If the change results in an increase in the number of population doublings or subcultivations, information on the characterization and testing of the post-production cell bank for recombinant product or of the drug substance for non-recombinant product.
4. For drug substance obtained from, or manufactured with, reagents obtained from sources that are at risk of transmitting bovine spongiform encephalopathy/transmissible spongiform encephalopathy (BSE/TSE) agents (for example, ruminant origin), information and evidence that the material does not pose a potential BSE/TSE risk (for example, name of manufacturer, species and tissues from which the material is a derivative, country of origin of the source animals, use and previous acceptance of the material) (*7*).
5. Process validation results.

Table *continued*

6. Comparability of the pre-change and post-change drug substance with respect to physicochemical properties, biological activity, purity, impurities and contaminants, as appropriate. Nonclinical and/or clinical bridging studies may occasionally be required when quality data are insufficient to establish comparability. The extent and nature of nonclinical and/or clinical studies should be determined on a case-by-case basis, taking into consideration the quality–comparability findings, the nature and level of knowledge of the product, existing relevant nonclinical and clinical data, and aspects of its use.
7. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Matrixing, bracketing, the use of smaller-scale batches, the use of fewer than three batches and/or leveraging data from scientifically justified representative batches, or batches not necessarily manufactured consecutively, may be acceptable where justified.
8. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for one commercial-scale batch of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Batch data on the next two full-production batches should be made available on request and should be reported by the marketing authorization holder if outside the specification (with proposed action). The use of a smaller-scale batch may be acceptable where justified and.
9. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug substance batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months and one batch of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug substance under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug substance for stability testing may be acceptable where justified (*6*).

Table *continued*

10. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes with at least one commercial-scale drug substance batch produced with the proposed changes under real-time/real-temperature testing conditions. Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug substance under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified.
11. Updated post-approval stability protocol and stability commitment to place the first commercial-scale batch of the drug product manufactured using the post-change drug substance into the stability programme.
12. Information assessing the risk with respect to potential contamination with adventitious agents (for example, impact on viral clearance studies and BSE/TSE risk) (*7*).
13. Data describing the root cause triggering the reprocessing, as well as validation data (for example, extended hold-times, resistance to additional mechanical stress) to help prevent the reprocessing from having an impact on the drug substance.
14. Demonstration that the new or revised holding step has no negative impact on the quality of the drug substance (data from one commercial-scale or scientifically justified representative drug substance batch should be provided).

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
11. Change in equipment used in the drug substance manufacturing process, involving the following:			
Note: New bioreactor technology (for example, a change from stainless steel bioreactor to disposable bioreactor) is excluded from this table and should be filed according to ***change 6a****.*			
a. Introduction of new equipment with different operating principles and different product contact material	None	1–5	Moderate
	3, 4	1, 2, 5	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
b, Introduction of new equipment with the same operating principles but different product contact material	None	1, 3–5	Moderate
	3, 4	1, 4, 5	Minor
c. Introduction of new equipment with different operating principles but the same product contact material	None	1–3, 5	Moderate
	4	1, 2, 5	Minor
d. Replacement of product-contact equipment with equivalent equipment	None	3	Minor
e. Change of product-contact equipment from dedicated to shared	1, 2	1, 6	Minor
f. Relocation of major equipment to another room in the same facility/suite/ premises	2, 4, 5	None	Minor

Conditions

1. The site is approved as a multi-product facility.
2. The change has no impact on the risk of cross-contamination and is supported by validated cleaning procedures.
3. The manufacturing process is not impacted by the change in product-contact equipment.
4. The change has no impact on product quality.
5. Re-qualification of the equipment follows the original qualification protocol.

Supporting data

1. Information on the in-process control testing.
2. Process validation study reports.
3. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for one commercial-scale batch of the drug substance produced with the approved and proposed product contact equipment/material. Batch data on the next two full-production batches should be made available on request and reported by the marketing authorization holder if outside specification (with proposed action).

Table *continued*

4. Information on leachables and extractables.
5. Information on the new equipment and comparison of similarities and differences regarding operating principles and specifications between the new and the replaced equipment.
6. Information describing the change-over procedures for the shared product-contact equipment.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
12. Change in specification for the materials, involving the following:			
a. Narrowing of the approved specification limits for starting materials/ intermediates	1–4	1–3, 5	Minor
b. Widening of the approved specification limits for starting materials/ intermediates	None	1–3, 5, 7	Moderate
	3–7	3–6	Minor
13. Change in supplier of raw materials of biological origin (for example, fetal calf serum, insulin, trypsin)	None	4, 6, 9, 10	Moderate
	8	4, 6	Minor
14. Change in source of raw materials of biological origin (for example, bovine trypsin to porcine trypsin)	None	4, 7, 9, 10	Moderate
	8	4, 7	Minor

Conditions

1. The change in specification for the materials is within the approved limits.
2. The grade of the materials is the same or is of higher quality, where appropriate.
3. There is no change in the drug substance specification outside the approved limits.
4. There is no change in the impurity profile of the drug substance outside the approved limits.
5. The change has no significant effect on the overall quality of the drug substance and/or drug product and there are no changes to the cell banks.
6. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.

Table *continued*

7. The test does not concern a critical attribute (for example, content, impurity, any critical physical characteristics or microbial purity). 8. The change is for compendial raw materials of biological origin (excluding human plasma-derived materials).	
Supporting data	
1. Revised information on the quality and controls of the materials (for example, raw materials, starting materials, solvents, reagents and catalysts) used in the manufacture of the post-change drug substance. 2. Updated drug substance specification, if changed. 3. Copies or summaries of analytical procedures if new analytical procedures are used. 4. For drug substance obtained from, or manufactured with, reagents obtained from sources that are at risk of transmitting bovine spongiform encephalopathy/ transmissible spongiform encephalopathy (BSE/TSE) agents (for example, ruminant origin), information and evidence that the material does not pose a potential BSE/TSE risk (for example, name of manufacturer, species and tissues from which the material is a derivative, country of origin of the source animals, use and previous acceptance of the material) (*7*). 5. Comparative table or description, where applicable, of pre-change and post-change in-process tests/limits. 6. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for one commercial-scale batch of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Batch data on the next two full-production batches should be made available on request and reported by the marketing authorization holder if outside specification (with proposed action). The use of a smaller-scale batch may be acceptable where justified. 7. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for three consecutive commercial-scale batches of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Matrixing, bracketing, the use of smaller-scale batches, the use of fewer than three batches and/or leveraging data from scientifically justified representative batches, or batches not necessarily manufactured consecutively, may be acceptable where justified. 8. Justification/risk assessment showing that the attribute is non-significant. 9. Information assessing the risk with respect to potential contamination with adventitious agents (for example, impact on viral clearance studies and BSE/TSE risk) (*7*). 10. Information demonstrating suitability of the auxiliary materials/reagents of both sources through the comparability of the drug substance.	

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
15. Change to in-process tests and/or acceptance criteria applied during manufacture of the drug substance, involving the following:			
a. Narrowing of approved in-process limits	1, 3, 6, 7	1, 4	Minor
b. Addition of new in-process test and limits	2, 3, 6	1–5, 8	Minor
c. Deletion of a non-significant in-process test	1–4, 6	1, 4, 7	Minor
d. Widening of the approved in-process limits	None	1–4, 6, 8	Moderate
	1–4	1, 4, 5, 8	Minor
e. Deletion of an in-process test which may have a significant effect on the overall quality of the drug substance	None	1, 4, 6, 8	Moderate
f. Addition or replacement of an in-process test as a result of a safety or quality issue	None	1–4, 6, 8	Moderate
16. Change in the in-process controls testing site *Note: Transfer of in-process control testing to a different facility within a GMP-compliant site is not considered to be a reportable change but is treated as a minor GMP change and is reviewed during inspections.*	1–3, 5, 6	9	Minor

Conditions

1. No change in the drug substance specification outside the approved limits.
2. No change in the impurity profile of the drug substance outside the approved limits.
3. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
4. The test does not concern a critical attribute (for example, content, impurity, any critical physical characteristics or microbial purity).
5. The replaced analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity, if applicable.
6. No change in the approved in-process controls outside the approved limits.
7. The test procedure remains the same, or changes in the test procedure are minor.

Table *continued*

Supporting data

1. Revised information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed drug substance.
2. Updated drug substance specification, if changed.
3. Copies or summaries of analytical procedures if new analytical procedures are used.
4. Comparative table or description, where applicable, of pre-change and post-change in-process tests/limits.
5. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for one commercial-scale batch of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Batch data on the next two full-production batches should be made available on request and reported by the marketing authorization holder if outside specification (with proposed action). The use of a smaller-scale batch may be acceptable where justified.
6. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for three consecutive commercial-scale batches of the pre-change and post-change drug substance. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Matrixing, bracketing, the use of smaller-scale batches, the use of fewer than three batches and/or leveraging data from scientifically justified representative batches, or batches not necessarily manufactured consecutively, may be acceptable where justified.
7. Justification/risk assessment showing that the attribute is non-significant.
8. Justification for the new in-process test and limits.
9. Evidence that the new company/facility is GMP-compliant.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
17. Change in the approved design space, involving the following:			
a. Establishment of a new design space	None	1	Major
b. Expansion of the approved design space	None	1	Major
c. Reduction in the approved design space (any change that reduces or limits the range of parameters used to define the design space)	1	1	Minor

Table *continued*

Conditions

1. The reduction in design space is not necessitated by recurring problems arising during manufacture.

Supporting data

1. Manufacturing development data to support the establishment of, or changes to, the design space.

Control of the drug substance

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
18. Change affecting the quality control (release and stability) testing of the drug substance, involving the following:			
Note: Transfer of testing to a different facility within a GMP-compliant site is not considered to be a reportable change but is treated as a minor GMP change and is reviewed during inspections.			
a. Transfer of the quality control testing activities for a non-pharmacopoeial assay to a new company not approved in the current marketing authorization or licence, or to a different site within the same company	None	1, 2	Moderate
	1–3	1, 2	Minor
b. Transfer of the quality control testing activities for a pharmacopoeial assay to a new company not approved in the current marketing authorization or licence	None	1, 2	Moderate
	1	1, 2	Minor

Conditions

1. The transferred quality control test is not a potency assay or bioassay.
2. No changes are made to the test method.
3. The transfer is within a facility approved in the current marketing authorization for the performance of other tests.

Supporting data

1. Information demonstrating technology transfer qualification for the non-pharmacopoeial assay or verification for the pharmacopoeial assay.
2. Evidence that the new company/facility is GMP-compliant.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
19. Change in the standard/monograph (that is, specifications) claimed for the drug substance, involving the following:			
a. A change from a pharmacopoeial standard/ monograph to an in-house standard	None	1–5	Moderate
b. A change from an in-house standard to a pharmacopoeial standard/monograph or from one pharmacopoeial standard/ monograph to a different pharmacopoeial standard/monograph	1–4	1–3	Minor
20. Change in the specifications for the drug substance in order to comply with an updated pharmacopoeial standard/monograph	1, 2	1, 2	Minor

Conditions

1. The change is made exclusively in order to comply with a pharmacopoeial monograph.
2. There is no change in drug substance specifications outside the approved ranges.
3. There is no deletion of tests or relaxation of acceptance criteria of the approved specifications, except to comply with a pharmacopoeial standard/monograph.
4. There are no deletions or changes to any analytical procedures, except to comply with a pharmacopoeial standard/monograph.

Supporting data

1. Revised drug product labelling information, as applicable.
2. Updated copy of the proposed drug substance specifications.
3. Where an in-house analytical procedure is used and a pharmacopoeial standard/ monograph is claimed, results of an equivalency study between the in-house and pharmacopoeial methods.
4. Copies or summaries of validation reports if new analytical procedures are used.
5. Justification of specifications with data.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
21. Changes in the control strategy of the drug substance, involving the following:			
a. Change from end-product testing to upstream controls for some test(s) (for example, real-time release testing, process analytical technology)	None	1–3, 5	Major
b. Addition of a new critical quality attribute in the control strategy	None	1–5	Moderate
c. Deletion of a critical quality attribute from the control strategy	None	1, 5	Moderate
Conditions None			
Supporting data 1. Information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed drug substance. 2. Updated copy of the proposed drug substance specifications. 3. Copies or summaries of analytical procedures if new analytical procedures are used. 4. Copies or summaries of validation reports if new analytical procedures are used to monitor the new CQA at release. 5. Justification and supporting data for each proposed change to the control strategy.			

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
22. Change in the specification/analytical procedure used to release the drug substance, involving the following:			
a. Deletion of a test	None	1, 5, 6	Moderate
b. Addition of a test	1–3	1–3, 5	Minor
c. Replacement of an analytical procedure	None	1–5	Moderate
	5, 6, 8	1, 4, 5	Minor
d. Changes to an approved analytical procedure	None	1–5	Moderate
	2, 4–6	1, 4, 5	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
e. Change from an in-house analytical procedure to a recognized compendial/ pharmacopoeial analytical procedure	None	1–5	Moderate
	2, 6	1–3	Minor
f. Widening of an approved acceptance criterion	None	1, 5, 6	Moderate
g. Narrowing of an approved acceptance criterion	1, 4, 7	1	Minor

Conditions

1. The change does not result from unexpected events arising during manufacture (for example, new unqualified impurity, change in total impurity limits).
2. There is no change in the limits/acceptance criteria outside the approved limits for the approved assays used at release/ stability.
3. The addition of the test is not intended to monitor new impurity species.
4. The method of analysis is the same and is based on the same analytical technique or principle (for example, change in column length or temperature, but not a different type of column or method) and no new impurities are detected.
5. The modified analytical procedure maintains or improves performance parameters of the method.
6. The change does not concern potency-testing.
7. Acceptance criteria for residual solvent are within recognized or approved acceptance limits (for example, within ICH limits for a Class 3 residual solvent, or pharmacopoeial requirements).
8. The change is from one pharmacopoeial assay to another pharmacopoeial assay or the marketing application holder has demonstrated an increased understanding of the relationship between method parameters and method performance defined by a systematic development approach including robustness studies.

Supporting data

1. Updated drug substance specifications.
2. Copies or summaries of analytical procedures if new analytical procedures are used.
3. Validation/qualification results if new analytical procedures are used.
4. Comparative results demonstrating that the approved and proposed analytical procedures are equivalent.
5. Justification for the proposed drug substance specification (for example, tests, acceptance criteria or analytical procedures).
6. Documented evidence that consistency of quality is maintained.

Reference standards or materials

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
23. Replacement of a primary reference standard	None	1, 2	Moderate
24. Change of the reference standard from pharmacopoeial or international standard to in-house (no relationship with international standard)	None	1, 2	Moderate
25. Change of the reference standard from in-house (no relationship with international standard) to pharmacopoeial or international standard	3	1, 2	Minor
26. Qualification of a new batch of reference standard against the approved reference standard (including qualification of a new batch of a secondary reference standard against the approved primary standard)	1	1, 2	Minor
27. Change to reference standard qualification protocol	None	3, 4	Moderate
28. Extension of the reference standard shelf-life or re-test period	2	5	Minor

Conditions

1. Qualification of the new reference standard is in accordance with an approved protocol.
2. The extension of the shelf-life of the reference standard is in accordance with an approved protocol.
3. The reference standard is used for a physicochemical test.

Table *continued*

Supporting data

1. Justification for the change in reference standard.
2. Information demonstrating qualification of the proposed reference standards or materials (for example, source, characterization, certificate of analysis, comparability data).
3. Justification of the change to the reference standard qualification protocol.
4. Updated reference standard qualification protocol.
5. Summary of stability testing and results to support the extension of reference standard shelf-life.

Drug substance container closure system

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
29. Change in the primary container closure system(s) for the storage and shipment of the drug substance	None	1, 2, 4, 5	Moderate
	1	1, 3, 5	Minor

Conditions

1. The proposed container closure system is at least equivalent to the approved container closure system with respect to its relevant properties (including results of transportation or compatibility studies, if appropriate).

Supporting data

1. Updated dossier sections describing information on the proposed container closure system (for example, description, composition, materials of construction of primary packaging components, specifications).
2. Data demonstrating the suitability of the container closure system (for example, extractable/leachable testing) and compliance with pharmacopoeial standards, if applicable.
3. Results demonstrating that the proposed container closure system is at least equivalent to the approved container closure system with respect to its relevant properties (for example, results of transportation or compatibility studies, and extractable/leachable studies).

Table *continued*

4. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating parameters with commercial-scale drug substance material using several container batches (for example, three different batches) produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug substance under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three container batches for stability testing may be acceptable where justified (*6*).
5. Comparative table of pre-change and post-change specifications of the container closure system.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
30. Change in the supplier for a primary container closure, involving the following:			
a. Replacement or addition of a supplier	None	1–3	Moderate
	1, 2	None	Minor
b. Deletion of a supplier	None	None	Minor

Conditions

1. There is no change in the type of container closure, the materials of construction or the sterilization process for a sterile container closure component.
2. There is no change in the specifications of the container closure component outside the approved ranges.

Supporting data

1. Data demonstrating the suitability of the container closure system (for example, extractable/leachable testing).
2. Information on the proposed container closure system (for example, description, materials of construction of primary packaging components, specifications).

Table *continued*

3. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug substance under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug substance for stability testing may be acceptable where justified (*6*).

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
31. Change in the specification/analytical procedure of the primary container closure system for the drug substance, involving the following:			
a. Deletion of a test	1, 2	1, 2	Minor
b. Addition of a test	3	1–3	Minor
c. Replacement of an analytical procedure	6, 7	1–3	Minor
d. Minor changes to an analytical procedure	4–7	1–3	Minor
e. Widening of an acceptance criterion	None	1, 2	Moderate
f. Narrowing of an acceptance criterion	8	1	Minor

Conditions

1. The deleted test has been demonstrated to be redundant compared to the remaining tests or is no longer a pharmacopoeial requirement.
2. The change to the specification does not affect the functional properties of the container closure component and does not result in a potential impact on the performance of the drug substance.
3. The change is not necessitated by unexpected recurring events arising during manufacture of the primary container closure system or because of stability concerns.
4. There is no change in the acceptance criteria outside the approved limits.
5. The new analytical procedure is of the same type.

Table *continued*

6. Results of method validation demonstrate that the new or modified analytical procedure is at least equivalent to the approved analytical procedure.
7. The new or modified analytical procedure maintains or tightens precision, accuracy, specificity or sensitivity.
8. The change is within the range of approved acceptance criteria.

Supporting data

1. Updated copy of the proposed specification for the primary container closure system.
2. Rationale for the change.
3. Description of the analytical procedure and, if applicable, validation data.

Stability

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
32. Change in the shelf-life of the drug substance or for a stored intermediate of the drug substance, involving the following:			
a. Extension	None	1–5	Moderate
	1–4	1, 2, 5	Minor
b. Reduction	None	1–5	Moderate
	5	2–4	Minor

Conditions

1. There are no changes to the container closure system in direct contact with the drug substance with the potential of impact on the drug substance, or to the recommended storage conditions of the drug substance.
2. Full long-term stability data are available covering the proposed shelf-life and are based on stability data generated on at least three commercial-scale batches.
3. Stability data were generated in accordance with the approved stability protocol.
4. Significant changes were not observed in the stability data.
5. The reduction in the shelf-life is not necessitated by recurring events arising during manufacture or because of stability concerns *(Note: Problems arising during manufacturing or stability concerns should be reported for evaluation)*.

Supporting data

1. Summary of stability testing and results (for example, studies conducted, protocols used, results obtained).
2. Proposed storage conditions and shelf-life, as appropriate.
3. Updated post-approval stability protocol and stability commitment.

Table *continued*

4. Justification for the change to the post-approval stability protocol or stability commitment.
5. Results of stability testing (that is, full real-time/real-temperature stability data covering the proposed shelf-life generated on stability testing of at least three commercial-scale batches unless otherwise justified). For intermediates, data to show that the extension of shelf-life has no negative impact on the quality of the drug substance. Under special circumstances, interim stability-testing results and a commitment to notify the NRA of any failures in the ongoing long-term stability studies may be provided. In such cases, the extrapolation of shelf-life should be made in accordance with ICH Q1E guidelines (*8*).

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
33. Change in the post-approval stability protocol of the drug substance, involving the following:			
a. Substantial change to the post-approval stability protocol or stability commitment, such as deletion of a test, replacement of an analytical procedure or change in storage temperature	None	1–5	Moderate
	1	1, 2, 4, 5	Minor
b. Addition of test(s) into the post-approval stability protocol	2	1, 2, 4, 5	Minor
c. Deletion of time point(s) from the post-approval stability protocol within the approved shelf-life	3	4, 5	Minor

Conditions

1. In the case of replacement of an analytical procedure, the new analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity.
2. The addition of test(s) is not due to stability concerns or to the identification of new impurities.
3. Deletion of time point(s) is made in accordance with relevant guidelines (for example, (*6*)).

Table *continued*

Supporting data

1. Copies or summaries of analytical procedures if new analytical procedures are used.
2. Validation results if new analytical procedures are used.
3. Proposed storage conditions and/or shelf-life, as appropriate.
4. Updated post-approval stability protocol including justification for the changes, and stability commitment.
5. If applicable, stability-testing results to support the change to the post-approval stability protocol or stability commitment (for example, data to show greater reliability of the alternative test).

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
34. Change in the storage conditions for the drug substance, involving the following:			
a. Addition or change to storage conditions for the drug substance (for example, widening or narrowing of a temperature criterion)	None	1–4	Moderate
	1, 2	1–3	Minor
b. Addition of a cautionary statement	None	1, 3, 4	Moderate
	1	1, 3, 4	Minor
c. Deletion of a cautionary statement	None	1, 3, 5	Minor

Conditions

1. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
2. The change consists in the narrowing of a temperature criterion within the approved ranges.

Supporting data

1. Proposed storage conditions and shelf-life.
2. Updated post-approval stability protocol and stability commitment.
3. Justification of the change in the storage conditions/cautionary statement.
4. Results of stability testing (that is, full real-time/real-temperature stability data covering the proposed shelf-life generated on one commercial-scale batch).
5. Results of stability testing (that is, full real time/real temperature stability data covering the proposed shelf-life generated on at least three commercial-scale batches).

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8. Evaluation for stability data. ICH Guideline Q1E. Geneva: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2003 (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1E/Step4/Q1E_Guideline.pdf, accessed 12 December 2017).

Appendix 3

Changes to the drug product

The examples presented in this appendix are intended to assist with the classification of changes made to the quality information of the drug product. The information summarized in the drug product table provides guidance on:

- the **conditions to be fulfilled** in order for a given change to be classified as major, moderate or minor (if any of the conditions outlined for a given change are not fulfilled, the change is automatically considered to be at the next higher reporting category – for example, if any of the conditions recommended for a moderate quality change are not fulfilled, the change is considered to be a major quality change);
- the **supporting data** for a given change, either to be submitted to the NRA and/or maintained by the marketing authorization holder (if any of the supporting data outlined for a given change are not provided, are different or are not considered applicable, adequate scientific justification should be provided); and
- the **reporting category** (major, moderate or minor quality change).

Marketing authorization holders should use scientific judgement, leverage competent regulatory authority guidance or contact the NRA if a change is not included in the table and has the potential to impact on product quality. Marketing authorization holders should also contact the NRA when a change is considered at the next higher reporting category because any of the conditions outlined are not fulfilled and where the supporting data are not described. NRAs should establish procedures, with appropriate timelines, on the conducting and recording of communications between themselves and marketing authorization holders.

Supporting data should be provided according to the submission format accepted by the NRA – see for example (*1*, *2*).

Additional information on data requirements to support quality changes can be found in WHO good manufacturing practices for biological products (*3*), WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (4) and in relevant ICH guidelines (*5*, *6*).

Quality changes to comply with updated compendia and/or pharmacopoeias

NRAs should make a list of the recognized compendia and/or pharmacopoeias. Manufacturers are expected to comply with the current version of compendia/pharmacopoeias as referenced in the approved marketing authorization. Changes in the compendial/pharmacopoeial methods or specifications for a drug product do not need to be submitted for review if reference is made to the current edition of the compendium or pharmacopoeia, but the changes should be notified to the NRA, with information on them available for inspection.

In some cases, changes made to comply with recognized compendia/pharmacopoeias may require approval by the NRA prior to implementation regardless of the timing of the change in relation to the date when the compendium/pharmacopoeia was updated. For example, supplement submission and approval by the NRA may be required for some changes to quality control tests performed for product release (for example, to potency tests), for changes that have an impact on any product labelling information item, and for changes that may affect the quality, safety or efficacy of the product.

Quality changes affecting lot release

While WHO recognizes that independent lot release by NRAs or national control laboratories is required for vaccines, in some countries this lot release system also applies to other types of products, such as plasma-fractionated products. Where post-approval changes to the final product affect the lot release protocol (for example, changes to test procedures, reference standards or laboratory sites) or sample testing requirements for lot release, the marketing authorization holder should inform the institution responsible for reviewing the release of product lots. These procedures apply to changes that have been authorized by the NRA in the case of major and moderate quality changes and to changes that have been implemented in the case of minor quality changes. For example, the qualification of a new lot of reference standard against the approved reference standard may be considered a minor quality change if the qualification of a new standard is performed in accordance with an approved protocol and specification. Nevertheless, these changes must be reported to the NRA or national control laboratory as appropriate.

Description and composition of the drug product

Note: Changes in dosage form and/or presentation may, in some cases, necessitate the filing of a new application for marketing authorization or licensure. Marketing authorization holders are encouraged to contact the NRA for further guidance.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
35. Change in the description or composition of the drug product, involving the following:			
a. Addition of a dosage form or change in the formulation (for example, lyophilized powder to liquid, change in the amount of excipient, new diluent for lyophilized product)	None	1–10	Major
b. Change in fill volume (same concentration, different volume)	None	1, 5, 7, 9, 10	Major
	1, 2	1, 5, 7, 9	Moderate
	1–3	5, 7, 9	Minor
c. Change in the concentration of the active ingredient (for example, 20 units/ml versus 10 units/ml)	None	1, 5, 7, 9, 10	Major
	2, 4, 5	1, 5, 7	Moderate
d. Addition of a new presentation (for example, addition of a new pre-filled syringe where the approved presentation is a vial for a biotherapeutic in a liquid dosage form)	None	1, 5, 7–10	Major

Conditions

1. No changes are classified as major in the manufacturing process to accommodate the new fill volume.
2. No change in the dose is recommended.
3. The change involves narrowing the fill volume while maintaining the lower limit of extractable volume.
4. The new concentration is bracketed by existing approved concentrations.
5. More than two concentrations are already approved (that is, linear PK/PD profile of the product from at least three different concentrations over the bracketed range has been demonstrated and the two extreme concentrations of the bracketed range have been shown to be bioequivalent or therapeutically equivalent).

Table *continued*

Supporting data

1. Revised drug product labelling information, as applicable.
2. Characterization data demonstrating comparability of the new dosage form and/or formulation.
3. Description and composition of the dosage form if there are changes to the composition or dose.
4. Discussion of the components of the drug product, as appropriate (for example, choice of excipients, compatibility of drug substance and excipients, leachates, compatibility with new container closure system).
5. Information on the batch formula, manufacturing process and process controls, controls of critical steps and intermediates, process validation results.
6. Control of excipients if new excipients are proposed (for example, specification).
7. Information on specification, analytical procedures (if new analytical methods are used), validation of analytical procedures (if new analytical methods are used), batch analyses (certificate of analysis for three consecutive commercial-scale batches should be provided). Bracketing for multiple-strength products, container sizes and/or fills may be acceptable if scientifically justified.
8. Information on the container closure system and leachables and extractables, if any of the components have changed (for example, description, materials of construction and summary of specification).
9. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug product batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug product under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug product for stability testing may be acceptable where justified (*6*).
10. Supporting clinical data or a justification for why such studies are not needed.

Description and composition of the drug product: change to a diluent

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
36. Change to the diluent, involving the following:			
a. Change in manufacturing process	None	1–5	Moderate
	1, 3	1–4	Minor
b. Replacement of or addition to the source of a diluent	None	1–6	Moderate
	1–3	1–3	Minor
c. Change in facility used to manufacture a diluent (same company)	1, 2	1, 3, 5	Minor
d. Addition of a diluent filling line	1, 2, 4	1, 3, 5	Minor
e. Deletion of a diluent	None	None	Minor

Conditions

1. The diluent is water for injection or a salt solution (including buffered salt solutions) – that is, it does not include an ingredient with a functional activity such as a preservative, and there is no change to its composition.
2. After reconstitution, there is no change in the drug product specification outside the approved limits.
3. The proposed diluent is commercially available in the country/jurisdiction of the NRA.
4. The addition of the diluent filling line is in an approved filling facility.

Supporting data

1. Flow diagram (including process and in-process controls) of the proposed manufacturing process(es) and a brief narrative description of the proposed manufacturing process(es).
2. Updated copy of the proposed specification for the diluent.
3. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the approved and proposed diluent. Comparative test results for the approved diluent do not need to be generated concurrently; relevant historical testing results are acceptable.
4. Updated stability data on the product reconstituted with the new diluent.
5. Evidence that the facility is GMP-compliant.
6. Revised drug product labelling information, as applicable.

Manufacture

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
37. Change in the approved design space, involving the following:			
a. Establishment of a new design space	None	1	Major
b. Expansion of the approved design space	None	1	Major
c. Reduction in the approved design space (any change that reduces or limits the range of parameters used to define the design space)	1	1	Minor
Conditions			
1. The reduction in design space is not necessitated by recurring problems that have arisen during manufacture.			
Supporting data			
1. Pharmaceutical development data to support the establishment or changes to the design space.			

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
38. Change involving a drug product manufacturer/ manufacturing facility, involving the following:			
a. Replacement or addition of a manufacturing facility for the drug product (including formulation/filling and primary packaging)	None	1–7	Major
	1–5	1–3, 5–8	Moderate
b. Conversion of a drug product manufacturing facility from single-product to multi-product facility	None	9, 10	Moderate
c. Replacement or addition of a secondary packaging facility, including secondary functional packaging (that is, assembly) facility	2, 3	1–3	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
d. Deletion of a drug product manufacturing facility or packaging site	6, 7	None	Minor

Conditions

1. The proposed facility is an approved formulation/filling facility (for the same company/marketing authorization holder).
2. There is no change in the composition, manufacturing process and drug product specification.
3. There is no change in the container/closure system and storage conditions.
4. The same validated manufacturing process at critical steps (that is, compounding and filling) is used.
5. The newly introduced product is in the same family of product(s), or in the same therapeutic classification, as the products already approved at the site, and also uses the same filling process/equipment.
6. There should remain at least one site/manufacturer, as previously authorized, performing the same function as the one(s) to be deleted.
7. The deletion should not be due to critical deficiencies in manufacturing (for example, recurrent out-of-specification events, environmental monitoring failures, etc.).

Supporting data

1. Name, address and responsibilities (for example, formulation, filling, primary/secondary packaging) of the proposed production facility involved in manufacturing and testing.
2. Evidence that the facility is GMP-compliant.
3. Confirmation that the description of the manufacturing process of the drug product has not changed (other than the change in facility), or submission of supporting data on the revised description of the manufacturing process if the process has changed.
4. Comparative description of the manufacturing process, if different from the approved process, and information on the controls performed at critical steps of the manufacturing process and on the intermediate of the proposed final product.
5. Summary of the process validation studies and results.
6. Description of the batches and summary of in-process control and release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the pre-change and post-change drug product. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Bracketing for multiple-strength products, container sizes and/or fills may be acceptable if scientifically justified.

Table *continued*

7. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug product batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug product under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug product for stability testing may be acceptable where justified (*6*).
8. Rationale for considering the proposed formulation/filling facility as equivalent.
9. Information describing the change-over procedures for shared product-contact equipment and the segregation procedures, as applicable. If there are no revisions, the manufacturer should state that no changes were made to the change-over procedures.
10. Cleaning procedures (including data in a summary validation report and the cleaning protocol for the introduction of new products, as applicable) demonstrating lack of carry-over or cross-contamination.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
39. Change in the drug product manufacturing process, involving the following:			
a. Scale-up of the manufacturing process at the formulation/filling stage	None	1–6	Major
	1–4	1–6	Moderate
b. Addition or replacement of equipment (for example, formulation tank, filter housing, filling line and head, lyophilizer)	None	1–7	Moderate
	5	2, 7, 8	Minor
c. Addition of a new scale bracketed by the approved scales or scale-down of the manufacturing process	None	1, 3–5	Moderate
	1–4, 8	1, 4	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
d. Addition of a new step (for example, filtration)	3	1–6	Moderate
e. Product-contact equipment change from dedicated to shared (for example, formulation tank, filter housing, filling line and head, lyophilizer)	6, 7	2, 9	Minor

Conditions

1. The proposed scale uses similar/comparable equipment to the approved equipment. Note: Change in equipment size is not considered as using similar/comparable equipment.
2. Any changes to the manufacturing process and/or to the in-process controls are only those necessitated by the change in batch size (for example, the same formulation, controls and standard operating procedures are utilized).
3. The change should not be a result of recurring events that have arisen during manufacture or because of stability concerns.
4. There is no change in the principle of the sterilization procedures of the drug product.
5. Replacement of equipment with equivalent equipment; the change is considered "like for like" (that is, in terms of product contact material, equipment size and operating principles).
6. The site is approved as a multi-product facility.
7. The change has no impact on the risk of cross-contamination and is supported by validated cleaning procedures.
8. The change does not affect the lyophilization step.

Supporting data

1. Description of the manufacturing process, if different from the approved process, and information on the controls performed at critical steps of the manufacturing process and on the intermediate of the proposed drug product.
2. Information on the in-process control testing, as applicable.
3. Process validation results (for example, media fills), as appropriate.
4. Description of the batches and summary of in-process control and release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the pre-change and post-change drug product. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Bracketing for multiple-strength products, container sizes and/or fills may be acceptable if scientifically justified.

Table *continued*

5. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug product batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug product under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug product for stability testing may be acceptable where justified (6).
6. Information on leachables and extractables, as applicable.
7. Information on the new equipment and comparison of similarities and differences regarding operating principles and specifications between the new and the replaced equipment.
8. The rationale for regarding the equipment as similar/comparable, as applicable.
9. Information describing the change-over procedures for the shared product-contact equipment.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
40. Change in the controls (in-process tests and/or acceptance criteria) applied during the manufacturing process or on intermediates, involving the following:			
a. Narrowing of approved in-process limits	2, 3, 7	1, 4	Minor
b. Addition of new in-process test and limits	2, 3, 6	1–5, 8	Minor
c. Deletion of a non-significant in-process test	2–4	1, 4, 7	Minor
d. Widening of the approved in-process limits	None	1–4, 6, 8	Moderate
	1–3	1, 4, 5, 8	Minor
e. Deletion of an in-process test which may have a significant effect on the overall quality of the drug product	None	1, 4, 6,8	Moderate

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
f. Addition or replacement of an in-process test as a result of a safety or quality issue	None	1–4, 6, 8	Moderate
41. Change in in-process controls testing site *Note: Transfer of in-process control testing to a different facility within a GMP-compliant site is not considered to be a reportable change but is treated as a minor GMP change and reviewed during inspections.*	1–3, 5, 6	9	Minor

Conditions

1. There is no change in drug product specification outside the approved limits.
2. There is no change in the impurity profile of the drug product outside the approved limits.
3. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
4. The test does not concern a critical attribute (for example, content, impurities, any critical physical characteristics or microbial purity).
5. The replaced analytical procedure maintains or improves precision, accuracy, specificity and sensitivity, if applicable.
6. There is no change in the in-process control limits outside the approved limits.
7. The test procedure remains the same, or changes in the test procedure are minor.

Supporting data

1. Revised information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed drug substance.
2. Updated drug product specification if changed.
3. Copies or summaries of analytical procedures if new analytical procedures are used.
4. Comparative table or description, where applicable, of current and proposed in-process tests.
5. Description of the batches and summary of in-process control and release testing results as quantitative data, in a comparative tabular format, for one commercial-scale batch of the pre-change and post-change drug product (certificates of analysis should be provided). Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Batch data on the next two full-production batches should be made available on request and reported by the marketing authorization holder if outside specification (with proposed action). The use of a smaller-scale batch may be acceptable where justified.

Table *continued*

6. Description of the batches and summary of in-process control and release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the pre-change and post-change drug product (certificates of analysis should be provided). Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable.
7. Justification/risk assessment showing that the attribute is non-significant.
8. Justification for the new in-process test and limits.
9. Evidence that the new company/facility is GMP-compliant.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
42. Change in the specification/analytical procedure used to release the excipient, involving the following:			
a. Deletion of a test	5, 8	1, 3	Minor
b. Addition of a test	4	1–3	Minor
c. Replacement of an analytical procedure	1–3	1, 2	Minor
d. Minor changes to an approved analytical procedure	None	1, 2	Minor
e. Change from an in-house analytical procedure to a recognized compendial analytical procedure	None	1, 2	Minor
f. Widening of an approved acceptance criterion	None	1, 3	Moderate
g. Narrowing of an approved acceptance criterion	3, 4, 6, 7	1	Minor

Conditions

1. Results of method validation demonstrate that the proposed analytical procedure is at least equivalent to the approved analytical procedure.
2. The replaced analytical procedure maintains or improves precision, accuracy, specificity and sensitivity.
3. The change is within the range of approved acceptance criteria or has been made to reflect the new pharmacopoeial monograph specification for the excipient.

Table *continued*

4. Acceptance criteria for residual solvents are within recognized or approved acceptance limits (for example, within ICH limits for a Class 3 residual solvent or pharmacopoeial requirements).
5. The deleted test has been demonstrated to be redundant compared to the remaining tests or is no longer a pharmacopoeial requirement.
6. The analytical procedure remains the same, or changes in the test procedure are minor.
7. The change does not result from unexpected events arising during manufacture (for example, new unqualified impurity, change in total impurity limits).
8. An alternative test analytical procedure is already authorized for the specification attribute/test and this procedure has not been added through a minor change submission.

Supporting data

1. Updated excipient specification.
2. Where an in-house analytical procedure is used and a recognized compendial standard is claimed, results of an equivalency study between the in-house and compendial methods.
3. Justification of the proposed excipient specification (for example, demonstration of the suitability of the monograph to control the excipient and potential impact on the performance of the drug product).

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
43. Change in the standard/ monograph (that is, specifications) claimed for the excipient	None	1–4	Moderate
	1–5	1–4	Minor

Conditions

1. The change is from a House standard to a pharmacopoeial standard/monograph.
2. The change is made exclusively to comply with a pharmacopoeial standard/ monograph.
3. There is no change to the specifications for the functional properties of the excipient outside the approved ranges, and no change that results in a potential impact on the performance of the drug product.
4. There is no deletion of tests or relaxation of acceptance criteria of the approved specifications, except to comply with a pharmacopoeial standard/monograph.
5. There is no deletion or change to any analytical procedures, except to comply with a pharmacopoeial standard/monograph.

Table *continued*

Supporting data

1. Updated excipient specifications.
2. Where a House analytical procedure is used and a pharmacopoeial/compendial standard/monograph is claimed, results of an equivalency study between the House and compendial methods.
3. Justification of the proposed excipient specifications (for example, demonstration of the suitability of the monograph to control the excipient and potential impact on the performance of the drug product).
4. A declaration that consistency of quality and of the production process of the excipient is maintained.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
44. Change in the source of an excipient from a vegetable or synthetic source to a human or animal source that may pose a TSE or viral risk	None	2–7	Major
45. Change in the source of an excipient from a TSE risk (for example, animal) source to a vegetable or synthetic source	None	1, 3, 5, 6	Moderate
46. Replacement in the source of an excipient from a TSE risk source to a different TSE risk source (for example, different animal source, different country of origin)	5, 6	2–7	Minor
47. Change in manufacture of a biological excipient	None	2–7	Major
	2	2–7	Moderate
	1, 2	2–7	Minor
48. Change in supplier for a plasma-derived excipient (for example, human serum albumin)	None	3–8	Major
	3, 4	5, 6, 9	Moderate

Table *continued*

49. Change in supplier for an excipient of non-biological origin or of biological origin (excluding plasma-derived excipient)	None 1, 5, 6	2, 3, 5–7 3	Moderate Minor
50. Change in excipient testing site *Note: Transfer of testing to a different facility within a GMP-compliant site is not considered to be a reportable change but is treated as a minor GMP change and is reviewed during inspections.*	1	10	Minor

Conditions

1. There is no change to the specification of the excipient or drug product outside the approved limits.
2. The change does not concern a human plasma-derived excipient.
3. The human plasma-derived excipient from the new supplier is an approved medicinal product and no manufacturing changes were made by the supplier of the new excipient since its last approval in the country/jurisdiction of the NRA.
4. The excipient does not influence the structure/conformation of the active ingredient.
5. The TSE risk source is covered by a TSE certificate of suitability and is of the same or lower TSE risk as the previously approved material (7).
6. Any new excipient does not require the assessment of viral safety data.

Supporting data

1. Declaration from the manufacturer of the excipient that the excipient is entirely of vegetable or synthetic origin.
2. Details of the source of the excipient (for example, animal species, country of origin) and the steps undertaken during processing to minimize the risk of TSE exposure (*7*).
3. Information demonstrating comparability in terms of physicochemical properties, and the impurity profile of the proposed excipient compared to the approved excipient.
4. Information on the manufacturing process and on the controls performed at critical steps of the manufacturing process, and on the intermediate of the proposed excipient.
5. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for at least three commercial-scale batches of the proposed excipient.

Table *continued*

6. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug product batches produced with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug product under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug product for stability testing may be acceptable where justified (*6*).
7. Information assessing the risk with respect to potential contamination with adventitious agents (for example, impact on the viral clearance studies, or BSE/TSE risk (*7*)), including viral safety documentation where necessary.
8. Complete manufacturing and clinical safety data to support the use of the proposed human plasma-derived excipient.
9. A letter from the supplier certifying that no changes were made to the plasma-derived excipient compared to the currently approved corresponding medicinal product.
10. Evidence that the new company/facility is GMP-compliant.

Control of the drug product

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
51. Change affecting the quality control testing of the drug product (release and stability), involving the following:			
Note: Transfer of testing to a different facility within a GMP-compliant site is not considered to be a reportable change but is treated as a minor GMP change and is reviewed during inspections.			
a. Transfer of the quality control testing activities for a non-pharmacopoeial assay (in-house) to a new company not approved in the current marketing authorization or licence or to a different site within the same company	None	1, 2	Moderate
	1–3	1, 2	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
b. Transfer of the quality control testing activities for a pharmacopoeial assay to a new company not approved in the current marketing authorization or licence	None	1, 2	Moderate
	1	1, 2	Minor

Conditions

1. The transferred quality control test is not a potency assay or bioassay.
2. There are no changes to the test method.
3. The transfer is within a facility approved in the current marketing authorization for the performance of other tests.

Supporting data

1. Information demonstrating technology transfer qualification for the non-pharmacopoeial assays or verification for the pharmacopoeial assays.
2. Evidence that the new company/facility is GMP-compliant.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
52. Change in the standard/monograph (that is, specifications) claimed for the drug product, involving the following:			
a. A change from a pharmacopoeial standard/ monograph to an in-house standard	None	1–5	Moderate
b. A change from an in-house standard to a pharmacopoeial standard/monograph or from one pharmacopoeial standard/ monograph to a different pharmacopoeial standard/monograph	1–4	1–3	Minor
53. Change in the specifications for the drug product to comply with an updated pharmacopoeial standard/ monograph	1, 2	1–3	Minor

Table *continued*

Conditions

1. The change is made exclusively to comply with a pharmacopoeial monograph.
2. There is no change in drug product specifications outside the approved ranges.
3. There is no deletion of tests or relaxation of acceptance criteria of the approved specifications, except to comply with a pharmacopoeial standard/monograph.
4. There is no deletion or change to any analytical procedures, except to comply with a pharmacopoeial standard/monograph.

Supporting data

1. Revised drug product labelling information, as applicable.
2. An updated copy of the proposed drug product specifications.
3. Where an in-house analytical procedure is used and a pharmacopoeial standard/monograph is claimed, results of an equivalency study between the in-house and pharmacopoeial methods.
4. Copies or summaries of validation reports if new analytical procedures are used.
5. Justification of specifications with data.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
54. Changes in the control strategy of the drug product, involving the following:			
a. Change from end-product testing to upstream controls for some test(s) (for example, real-time release testing, process analytical technology)	None	1–3, 5	Major
b. Addition of a new critical quality attribute to the control strategy	None	1–5	Moderate
c. Deletion of a critical quality attribute from the control strategy	None	1, 5	Moderate

Conditions

None

Supporting data

1. Information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed product.

Table *continued*

2. An updated copy of the proposed drug product specifications.
3. Copies or summaries of analytical procedures if new analytical procedures are used.
4. Copies or summaries of validation reports if new analytical procedures are used to monitor the new critical quality attribute at release.
5. Justification and supporting data for each proposed change to the control strategy.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
55. Change in the specification/analytical procedure used to release the drug product, involving the following:			
a. Deletion of a test analytical procedure and/or an acceptance criterion	None	1, 6, 7	Moderate
b. Addition of a test	1, 2, 7	1–3, 5	Minor
c. Replacement of an analytical procedure	None	1–5	Moderate
	4, 5, 8	1, 4, 5	Minor
d. Changes to an approved analytical procedure	None	1–5	Moderate
	1, 3–5	2, 4, 5	Minor
e. Change from an in-house analytical procedure to a recognized compendial analytical procedure	None	1–5	Moderate
	1, 5	1–3	Minor
f. Widening of an approved acceptance criterion	None	1, 5, 7	Moderate
g. Narrowing of an approved acceptance criterion	1, 3, 6, 7	1	Minor

Conditions

1. There is no change to the limits/acceptance criteria outside the approved limits for the approved assays used at release/ stability.
2. The additional test is not intended to monitor new impurity species.
3. The method of analysis is the same (for example, a change in column length or temperature, but not a different type of column or method) and no new impurities are detected.
4. The modified analytical procedure maintains or improves the performance parameters of the method.
5. The change does not concern potency-testing.

Table *continued*

6. Acceptance criteria for residual solvents are within recognized or approved acceptance limits (for example, within ICH limits for a Class 3 residual solvent, or pharmacopoeial requirements).
7. The change does not result from unexpected events arising during manufacture (for example, new unqualified impurity, or impurity content outside the approved limits).
8. The change is from a pharmacopoeial assay to another pharmacopoeial assay or the marketing application holder has demonstrated an increased understanding of the relationship between method parameters and method performance defined by a systematic development approach including robustness studies.

Supporting data

1. An updated copy of the proposed drug product specification.
2. Copies or summaries of analytical procedures if new analytical procedures are used.
3. Validation/qualification results if new analytical procedures are used.
4. Comparative results demonstrating that the approved and proposed analytical procedures are equivalent.
5. Justification for the change to the analytical procedure (for example, demonstration of the suitability of the analytical procedure in monitoring the drug product, including the degradation products) or for the change to the specification (for example, demonstration of the suitability of the revised acceptance criterion to control the drug product).
6. Justification for the deletion of the test (for example, demonstration of the suitability of the revised specification in controlling the final product).
7. Documented evidence that consistency of quality and of the production process is maintained.

Reference standards

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
56. Replacement of a primary reference standard	None	1, 2	Moderate
57. Change of the reference standards from a pharmacopoeial or international standard to in-house (no relationship with international standard)	None	1, 2	Moderate

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
58. Change of the reference standard from in-house (no relationship with international standard) to a pharmacopoeial or international standard	3	1, 2	Minor
59. Qualification of a new batch of reference standard against the approved reference standard (including qualification of a new batch of a secondary reference standard against the approved primary standard)	1	2	Minor
60. Change to the reference standard qualification protocol	None	3, 4	Moderate
61. Extension of the reference standard shelf-life or re-test period	2	5	Minor

Conditions

1. The qualification of a new standard is carried out in accordance with an approved protocol.
2. The extension of the shelf-life of the reference standard is carried out in accordance with an approved protocol.
3. The reference standard is used for a physicochemical test.

Supporting data

1. Revised product labelling to reflect the change in reference standard, as applicable.
2. Qualification data of the proposed reference standards or materials (for example, source, characterization, certificate of analysis).
3. Justification of the change to the reference standard qualification protocol.
4. Updated reference standard qualification protocol.
5. Summary of stability testing and results or retest data to support the extension of the reference standard shelf-life.

Drug product container closure system

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
62. Modification of a primary container closure system (for example, new coating, adhesive, stopper, type of glass) *Note: The addition of a new container closure system (for example, addition of a pre-filled syringe where the currently approved presentation is only a vial) is considered a change in presentation (see **change 35d**).*	None	1–7	Moderate
	4	3, 7	Minor
	1–3	3	Minor
63. Change from a reusable container to a disposable container with no changes in product contact material (for example, change from reusable pen to disposable pen)	None	1, 3, 6	Moderate
64. Deletion of a container closure system *Note: The NRA should be notified of the deletion of a container closure system, and product labelling information should be updated, as appropriate.*	None	1	Minor

Conditions

1. There is no change in the type of container closure or materials of construction.
2. There is no change in the shape or dimensions of the container closure.
3. The change is made only to improve the quality of the container and does not modify the product contact material (for example, increased thickness of the glass vial without changing interior dimensions).
4. The modified part is not in contact with the drug product.

Table *continued*

Supporting data

1. Revised product labelling information, as appropriate.
2. For sterilized products, process validation results, unless otherwise justified.
3. Update dossier containing information on the proposed container closure system, as appropriate (for example, description, materials of construction of primary packaging components).
4. Results demonstrating protection against leakage, no leaching of undesirable substance, compatibility with the product, and results from the toxicity and biological reactivity tests.
5. Summary of release testing results as quantitative data, in a comparative tabular format, for at least three consecutive commercial-scale batches of the pre-change and post-change drug product. Comparative pre-change test results do not need to be generated concurrently; relevant historical testing results are acceptable. Bracketing for multiple-strength products, container sizes and/or fills may be acceptable if scientifically justified.
6. Comparative pre-change and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three commercial-scale drug product batches produced (unless otherwise justified) with the proposed changes and stored under accelerated and/or stress conditions for a minimum of 3 months. Test results that cover a minimum of 6 months in real-time/real-temperature conditions should also be provided. A possibility of 3 months of real-time data could be acceptable if properly justified (for example, it can be proven that the relevant effect, if present, can already be observed within 3 months). Comparative pre-change test results do not need to be generated concurrently; relevant historical results for batches on the stability programme are acceptable. Additionally, the manufacturer should commit to undertake real-time stability studies to confirm the full shelf-life/hold-time of the drug product under its normal storage conditions and to report to the NRA any failures in these ongoing long-term stability studies. Matrixing, bracketing, the use of smaller-scale batches and/or the use of fewer than three batches of drug product for stability testing may be acceptable where justified (*6*).
7. Information demonstrating the suitability of the proposed container/closure system with respect to its relevant properties (for example, results from last media fills; results of interaction studies demonstrating preservation of protein integrity and maintenance of sterility for sterile products; maintenance of sterility in multidose containers; user testing).

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
65. Change in the supplier for a primary container closure component, involving the following:			
a. Replacement or addition of a supplier *Note: A change in container closure system involving new materials of construction, shape or dimensions would require supporting data, such as is shown for **change 62** on modification of a primary container closure system.*	1, 2	1, 2	Minor
b. Deletion of a supplier	None	None	Minor

Conditions

1. There is no change in the type of container closure, materials of construction, shape and dimensions, or in the sterilization process for a sterile container closure component.
2. There is no change in the specification of the container closure component outside the approved acceptance criteria.

Supporting data

1. Letter from the marketing authorization holder certifying that there are no changes to the container closure system.
2. Certificate of analysis, or equivalent, for the container provided by the new supplier and comparison with the certificate of analysis, or equivalent, for the approved container.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
66. Change in the specification used to release a primary container closure component or functional secondary container closure component, involving the following:			
a. Deletion of a test	1, 2	1, 2	Minor
b. Addition of a test	3	1, 2	Minor
c. Replacement of an analytical procedure	6, 7	1–3	Minor
d. Minor changes to an analytical procedure	4–7	1–3	Minor

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
e. Widening of an acceptance criterion	None	1, 2	Moderate
f. Narrowing of an acceptance criterion	8	1	Minor

Conditions

1. The deleted test has been demonstrated to be redundant compared to the remaining tests or is no longer a pharmacopoeial requirement.
2. The change to the specification does not affect the functional properties of the container closure component and does not have a potential impact on the performance of the drug product.
3. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
4. There is no change to the acceptance criteria outside the approved limits.
5. The new analytical procedure is of the same type.
6. Results of method validation demonstrate that the new or modified analytical procedure is at least equivalent to the approved analytical procedure.
7. The new or modified analytical procedure maintains or improves precision, accuracy, specificity and sensitivity.
8. The change is within the range of approved acceptance criteria.

Supporting data

1. An updated copy of the proposed specification for the primary or functional secondary container closure component.
2. Rationale for the change in specification for a primary container closure component.
3. Description of the analytical procedure and, if applicable, validation data.

Stability

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
67. Change in the shelf-life of the drug product, involving the following:			
a. Extension (includes extension of shelf-life of the drug product as packaged for sale, and hold-time after opening and after dilution or reconstitution)	None	1–5	Moderate

Table *continued*

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
b. Reduction (includes reduction as packaged for sale, after opening, and after dilution or reconstitution)	None	1–5	Moderate
Conditions None			
Supporting data 1. Updated product labelling information, as appropriate. 2. Proposed storage conditions and shelf-life, as appropriate. 3. Updated post-approval stability protocol. 4. Justification of the change to the post-approval stability protocol or stability commitment. 5. Results of stability testing under real-time/real-temperature conditions covering the proposed shelf-life generated on at least three commercial-scale batches unless otherwise justified.			

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
68. Change in the post-approval stability protocol of the drug product, involving the following:			
a. Substantial change to the post-approval stability protocol or stability commitment, such as deletion of a test, replacement of an analytical procedure, or change in storage temperature	None	1–5	Moderate
b. Addition of test(s) into the post-approval stability protocol	1	1, 2, 4, 5	Minor
c. Deletion of time point(s) from the post-approval stability protocol within the approved shelf-life	2	4, 5	Minor
d. Replacement of sterility testing by the container/ closure system integrity testing	None 3	1, 2, 4, 5 4, 5	Moderate Minor

Table *continued*

Conditions

1. The addition of the test(s) is not due to stability concerns or to the identification of new impurities.
2. Deletion of time point(s) is done according to relevant guidelines (for example, (*6*)).
3. The method used to demonstrate the integrity of the container/closure system has already been approved as part of a previous application related to the drug product.

Supporting data

1. Copies or summaries of analytical procedures if new analytical procedures are used.
2. Validation results if new analytical procedures are used.
3. Proposed storage conditions and or shelf-life, as appropriate.
4. Updated post-approval stability protocol, including justification for the change, and stability commitment.
5. Comparative results demonstrating that the approved and proposed analytical procedures are equivalent.

Description of change	Conditions to be fulfilled	Supporting data	Reporting category
69. Change in the labelled storage conditions for the drug product or the diluted or reconstituted biotherapeutic products, involving the following:			
a. Addition or change of storage condition(s) for the drug product, diluted or reconstituted drug product (for example, widening or narrowing of a temperature criterion, addition of or change to controlled temperature chain conditions)	None	1–4, 6	Moderate
b. Addition of a cautionary statement (for example, "Do not freeze")	None	1, 2, 4, 5	Moderate
c. Deletion of a cautionary statement (for example, "Do not freeze")	None	1, 2, 4, 6	Moderate
Conditions None			

Table *continued*

Supporting data
1. Revised product labelling information, as applicable.
2. Proposed storage conditions and shelf-life.
3. Updated post-approval stability protocol and stability commitment.
4. Justification of the change in the labelled storage conditions/cautionary statement.
5. Results of stability testing under appropriate stability conditions covering the proposed shelf-life, generated on one commercial-scale batch unless otherwise justified.
6. Results of stability testing under appropriate conditions covering the proposed shelf-life, generated on at least three commercial-scale batches unless otherwise justified.

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Appendix 4

Safety, efficacy and product labelling information changes

The examples of safety and efficacy changes, product labelling information changes and administrative product labelling information changes in this appendix are provided for clarification. However, such changes are not limited to those included in this appendix. They may also result in changes to the product labelling information for health-care providers and patients, and to inner and outer labels.

Because the amount of safety and efficacy data needed to support a change may vary according to the impact of the change, risk–benefit considerations and product-specific characteristics there is no "one size fits all" approach. This appendix therefore provides a list of examples of changes in the various categories rather than a detailed table linking each change with the required data needed to support that change (as is provided in Appendices 2 and 3 for quality changes). Marketing authorization holders or applicants are encouraged to contact the NRA for guidance on the data needed to support major changes if deemed necessary.

Safety and efficacy changes

Safety and efficacy change supplements require approval prior to implementation of the change and are generally submitted for changes related to clinical practice, safety and indication claims.

The following are examples of safety and efficacy changes requiring data from clinical studies and/or nonclinical studies, post-marketing observational studies or extensive post-marketing safety data:

- Change to the indication:
  - (a) addition of a new indication (for example, treatment of a previously unspecified disease);
  - (b) modification of an approved indication (for example, expansion of the age of use or restriction of an indication based on clinical studies demonstrating lack of efficacy).
- Change in the recommended dose and/or dosing schedule.
- Change to the use in specific at-risk groups (for example, addition of information on use in pregnant women or immunocompromised patients).

- Change to add information on co-administration with other medicines.
- Change to add a new route of administration.[1]
- Change to add a new dosage form[1] (for example, replacement of a suspension for injection with a lyophilized cake).
- Change to add a new strength.[1]
- Change to add a new delivery device[1] (for example, adding a pre-filled syringe or pen).
- Change in existing risk-management measures:
  (a) (deletion of an existing route of administration, dosage form and/or strength due to safety reasons;
  (b) (deletion of a contraindication (for example, use in pregnant women);
  (c) changing a contraindication to a precaution.

Product labelling information changes

Supplements on product labelling information changes should be submitted for changes which do not require clinical efficacy and/or safety data from clinical studies but normally require extensive pharmacovigilance (safety surveillance) data. Product labelling information changes require approval prior to implementation.

The following are examples of product labelling information changes that impact on the clinical use of a product:

- Addition of an adverse event that is identified as consistent with a causal association with administration of the biotherapeutic product concerned.
- Change in the frequency of occurrence of a given adverse reaction.
- Addition of a contraindication or warning (for example, identification of a specific subpopulation as being at greater risk, such as individuals with a concomitant condition or taking concomitant medicines, or a specific age group). These changes may include provision of recommended risk-management actions (for example, ensuring patient awareness of certain risks).

[1] Some NRAs consider that these changes may require a new application for a marketing authorization or licence.

- Strengthening, clarification or amendment of the text of the product labelling information relating to contraindications, warnings, precautions and adverse reactions.
- Revisions to the instructions for use, including dosage, administration and preparation for administration, to optimize the safe use of the biotherapeutic product.

In some cases, the safety-related changes listed above may be urgent and may require rapid implementation (for example, addition of a contraindication or warning). To allow for the speedy processing of such requests, the supplements for these changes should be labelled as "Urgent product labelling information changes" and should be submitted after prior agreement between the NRA and the marketing authorization holder (see section 8.3 and Appendix 1).

Administrative product labelling information changes

Administrative product labelling information changes are changes to any of the labelling items which are not expected to have an impact upon the safe and efficacious use of the biotherapeutic product. In some cases, these changes may need to be reported to the NRA and approval received prior to implementation, while in other cases reporting may not be required.

Examples of changes which **do** require reporting to the NRA and receipt of approval prior to implementation by the marketing authorization holder include:

- Change in the proper/nonproprietary name or trade name of the biotherapeutic product.

Examples of changes which **may not** require approval by the NRA prior to implementation include:

- Change in the name of the marketing authorization holder and/or manufacturer (for example, change of name due to a merger).
- Updated contact information for the marketing authorization holder (for example, customer service number or website address) or distributor's name.
- Minor changes to the layout of the product labelling information items or revision of typographical errors without changing the content of the label.
- Update of the existing information for referenced literature without adding or removing references.
- Changes made to comply with an official compendium (for example, change of the common name).

- Minor changes to the text to add clarity in relation to maintaining consistency with common label phrase standards (for example, change from "not recommended for children" to "not for use in children").

These administrative product labelling information changes (that is, changes not subject to prior approval that have been implemented since the last approved product labelling information) should be included when submitting subsequent PAS for safety and efficacy changes or for product labelling information changes (see section 8.4).

Annex 4

Technical Specifications Series (TSS) for WHO Prequalification – Diagnostic Assessment

Human immunodeficiency virus (HIV) rapid diagnostic tests for professional use and/or self-testing

TSS–1

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The draft technical specifications document was then posted on the WHO Prequalification website for public consultation on 15 September 2016. Various stakeholders, including manufacturers submitting to WHO prequalification of in vitro diagnostic medical devices (IVDs), IVD manufacturing industry associations, various national and international regulatory bodies, and IVD standards organizations, were informed of the consultation in order to solicit feedback. A two-month response period was provided.

Second-round public comments were then received from the following: Dr P Akolkar, Center for Biologics Evaluation and Research, United States Food and Drug Administration, MD, USA; Ms S Best, National Serology Reference Laboratory, Victoria, Australia; Dr J Duncan, London, the United Kingdom; Epicentre and Médecins sans Frontières International Office, Paris, France; Dr C Kosack, Dr A Page and Ms E Tran, Geneva, Switzerland; Dr R Galli, bioLytical™ Laboratories Inc., Vancouver, Canada; Ms D Lepine, Medical Devices Bureau, Health Canada, Ottawa, Canada; Dr M Nübling, WHO, Geneva, Switzerland; OraSure Technologies Inc., PA, the USA; Dr H Scheiblauer, Paul-Ehrlich-Institut, Langen, Germany; UNITAID/PSI HIV Self-Testing Africa (STAR) project

consortium members; and Dr E Cowan, Dr R Dacombe, Dr M Kumwenda, Dr M Neuman, Professor R Peeling, Dr M Taegtmeyer and Dr V Watson, Liverpool School of Tropical Medicine, Liverpool, the United Kingdom.

Following incorporation of the second-round public comments, a revised draft was published on the WHO Biologicals website for a final round of public consultation between 18 June and 18 September 2017. The comments received were incorporated to produce the document WHO/BS/2017.2305. The document was adopted by the WHO Expert Committee on Biological Standardization as a WHO written standard on 20 October 2017.

Abbreviations

Ag	antigen
CE	Conformité Européenne (European Conformity)
CRF	circulating recombinant form
HIV	human immunodeficiency virus
IVD	in vitro diagnostic medical device
RDT	rapid diagnostic test

A Introduction

The purpose of this document is to provide technical guidance to in vitro diagnostic medical device (IVD) manufacturers that intend to seek WHO prequalification of rapid diagnostic tests (RDTs) for the detection of human immunodeficiency virus (HIV).

The minimum performance requirements for WHO prequalification are summarized in this document, and apply equally to RDTs intended solely for HIV detection and to those tests where HIV detection is one component of a multi-detection assay (for example, an HIV/syphilis dual-detection RDT). This document applies to RDTs intended to be used as an aid to diagnosis of HIV infection. The current version of this document does not address IVDs that discriminate between the detection of HIV-1 and HIV-2 infection, IVDs intended as confirmatory tests, or the requirements for accompanying quality control materials.

For the purpose of this document, the use of certain verbal forms is as follows:

- "shall" indicates that the manufacturer is required to comply with the technical specifications;

- "should" indicates that the manufacturer is recommended to comply with the technical specifications but it is not a requirement; and
- "may" indicates that the technical specifications are a suggestion but not a requirement.

A documented justification and rationale shall be provided by the manufacturer when the WHO prequalification submission does not comply with the required technical specifications outlined in this document.

Minimum performance requirements for WHO prequalification are summarized in this document– and where possible, WHO performance requirements are aligned with published guidance, standards and/or regulatory documents. Although references to source documents are provided, it should be noted that WHO prequalification in some cases has additional requirements.

For WHO prequalification purposes, manufacturers shall provide evidence in support of the clinical performance of an IVD to demonstrate that reasonable steps have been taken to ensure that a properly manufactured IVD, when correctly operated by the intended user, will detect the target analyte and fulfil its indications for use.

The WHO prequalification requirements summarized in this document do not extend to the demonstration of clinical utility – that is, the effectiveness and/or benefits of an IVD, relative to and/or in combination with other measures, as a tool to inform clinical intervention in a given population or health-care setting. To demonstrate clinical utility, a separate set of studies is required. Clinical utility studies usually inform programmatic strategy and are thus the responsibility of programme managers, ministries of health and other related bodies in individual WHO Member States. Such studies do not fall under the scope of WHO prequalification.

B How to apply these specifications

For WHO prequalification purposes, an IVD intended for professional use only (by a laboratory professional, health-care worker or trained lay provider) shall be supported by studies outlined in Parts 1 and 2 of this document.

An IVD intended both for professional use and for self-testing shall be supported by the studies outlined in Parts 1 and 2 of this document. In addition, the claim for self-testing shall be supported by studies that qualify the usability of the IVD among a broad range of self-testing users, as outlined in Part 3.

An IVD intended for self-testing only shall be supported by studies outlined in Parts 1, 2 and 3.

For an IVD with an intended use that has been amended to include self-testing, and for which performance in professional use is already established,

and Parts 1 and 2 of this document have already been satisfied, the additional claim for self-testing shall be supported by studies outlined in Part 3.

These requirements are summarized below in Table 1.

Table 1
Summary of requirements for submission for WHO prequalification based on the intended use of the IVD

Intended use	Parts of the TSS to be fulfilled
Professional use	Parts 1 and 2
Self-testing	Parts 1, 2 and 3
Prequalified professional-use IVD with additional claim for self-testing	Part 3, with the provision that any adaptations made do not impact the established safety and performance

C Other guidance documents

This document should be read in conjunction with other relevant WHO guidance documentation, including:

- Technical Guidance Series for WHO Prequalification – Diagnostic Assessment
- Sample Product Dossiers for WHO Prequalification – Diagnostic Assessment
- Instructions for Compilation of a Product Dossier (WHO document PQDx_018).

These documents are available at: http://www.who.int/diagnostics_laboratory/evaluations/en/

D Performance principles for WHO prequalification

D.1 Intended use

An IVD intended for WHO prequalification shall be accompanied by a sufficiently detailed intended use statement. This should allow for an understanding to be gained of at least the following:

- the function of the IVD (for example, to detect antibodies to HIV-1, HIV-2 and/or HIV p24 antigen (Ag), etc.) and whether it is qualitative, semi-quantitative or quantitative;

- the testing population for which the functions are intended (for example, detection of susceptible individuals) and the intended operational setting (for example, for use in near-patient testing); and
- clinical indication (for example, aid to diagnosis of HIV infection).

D.2 Diversity of specimen types, users and testing environments and impact on required studies

For WHO prequalification submission, clinical performance studies should be conducted using the specimen types that are most likely to be used in resource-limited WHO Member States (for example, capillary whole blood and oral fluid) and claimed in the instructions for use. If this is not possible, substantial data shall be presented to show the equivalence between specimen types used in performance studies.

Prequalified RDTs in low- and middle-income countries are likely to be used by laboratory professionals[1] and at point-of-care by health-care workers, trained lay providers[2] or by individuals who self-test. Depending on the intended use of an RDT, performance studies shall be designed to take into account not only the diversity of knowledge and skills across the population of RDT users, but also the likely operational settings in which testing will occur. For example, studies that comprise the testing of left-over/repository specimens by research and development staff at a manufacturer's facility shall not, on their own, be considered sufficient to meet many of the performance requirements summarized in this document.

D.3 Applicability of supporting evidence to an IVD under review

Performance studies shall be undertaken using the specific locked-down version of the IVD intended to be submitted for WHO prequalification. Where this is not possible, a justification shall be provided and additional supporting evidence may also be required. This may occur in the case of minor variations in design where no negative impact on performance has been demonstrated.

Specific information is provided in Parts 1 and 2 of this document for the numbers of lots required for particular studies. Each lot should comprise different batches of critical components. It is a manufacturer's responsibility to ensure (via risk analysis of their IVD) that the minimum number of lots chosen

[1] Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certificate or tertiary education degree.

[2] Any person who performs functions related to health-care delivery and has been trained to deliver specific services but has received no formal professional or paraprofessional certification or tertiary education degree.

for estimating performance characteristics takes into account the variability in performance likely to arise from the diversity of key components and their formulation.

The true HIV status of a specimen shall be determined using a suitable reference method, for which justification shall be provided. Estimation (and reporting) of IVD performance shall include the rate of invalid test results. For certain analytical studies it may be acceptable to use contrived specimens (for example, normal human specimens that have been spiked with HIV antibodies). Although all reasonable attempts should be made to use natural specimens, justification should be provided where contrived specimens are used in the submitted studies. Clinical studies should be based on testing in natural specimens only.

For IVDs that include a claim for detection of multiple analytes, evidence of performance shall be provided for each claimed analyte. It should be noted that, depending on the design of an IVD, evidence generated in a similar, related product will usually not be considered sufficient by WHO to support performance claims in an IVD submitted for prequalification.

> *Example: an IVD designed to detect HIV antibodies only, and the same IVD designed for the dual detection of HIV and syphilis. It is unlikely that performance evidence presented for the HIV-only IVD would be acceptable for supporting performance claims for the dual-detection IVD.*

For an IVD with an intended use that has been expanded to include self-testing, changes are usually required to improve the usability of the IVD for this new testing population. Such changes may include the modification of:

- the instructions for use (for example, simplification of instructions to reflect new intended users);
- buffer vials;
- collection procedures;
- reading times, etc.

It is a manufacturer's responsibility to verify through testing (as summarized in Parts 1 and 2 of this document) that any changes made do not have an adverse impact on critical safety and performance characteristics of an IVD. Usability studies are undertaken to optimize the presentation of an IVD and the understanding of self-testing users. The minimum reporting requirements summarized in Part 3 of this document are not intended to be an exhaustive list or to indicate a particular order in which studies should be undertaken.

E Table of Requirements

Part 1	Establishing analytical performance characteristics
1.1	**Specimen type**
1.1.1	Demonstration of equivalence between specimen types
1.1.2	Demonstration of equivalence of claimed anticoagulants
1.2	**Specimen collection, storage and transport**
1.2.1	Specimen stability
1.3	**Precision of measurement**
1.3.1	Repeatability, reproducibility
1.4	**Performance panels**
1.4.1	Subtype panels
1.4.2	Mixed titre panels
1.5	**Validation of reading times**
1.5.1	Validation of reading times
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1.6.2	Limit of detection for HIV-1 p24 Ag, where appropriate
1.7	**Prozone/high-dose hook effect**
1.7.1	Prozone/high-dose hook effect
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1.9.1	Metrological traceability of control material values
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1.11.1	Flex studies

Table *continued*

Part 2	**Establishing clinical performance characteristics (professional use and/or self-testing)**
2.1	**Diagnostic sensitivity and specificity**
2.1.1	Diagnostic sensitivity
2.1.2	Diagnostic specificity
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3.1	**Qualification of usability (self-testing)**
3.1.1	Labelling comprehension study
3.1.2	Results interpretation study
3.1.3	Observed untrained user study

Part 1 Establishing analytical performance characteristics

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.1 Specimen type			
1.1.1 Demonstration of equivalence between specimen types	For each claimed specimen type, testing in at least: • 25 HIV-positive specimens • 25 HIV-negative specimens.	1. The relationship between IVD performance in claimed specimen types and reference materials used for analytical studies shall be established. The design of subsequent studies shall then take that relationship into account. 2. If there is no equivalence between claimed specimen types then the impact that this will have on each subsequent performance claim shall be fully understood and described. Where a significant difference in performance exists between specimen types, equivalence may need to be investigated as part of a larger clinical study (see Part 2 below). *Example: an IVD intended for testing whole blood for which seroconversion sensitivity is estimated using panels of serum/plasma specimens.* • *The relationship between seroconversion sensitivity in serum/plasma to that of the same characteristic in whole blood shall be understood.* • *This might be achieved by comparing titres between end-point dilution series of matched specimen types (whole blood versus serum/plasma) from a set of positive patients.*	Technical Guidance Series for WHO Prequalification – Diagnostic Assessment (*1*) European Commission (*2*)
1.1.2 Demonstration of equivalence of claimed anticoagulants	At least 25 HIV-positive and 25 HIV-negative specimens for each claimed anticoagulant. The equivalence of specimen types shall be determined for all claimed analytes (for example, HIV-1 antibody, HIV-2 antibody, p24 Ag, as appropriate) (see Note 3).		

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
		3. In some cases it may be acceptable to use diluted or spiked specimens. This approach is acceptable in early development work, but all reasonable attempts should be made to use natural specimens. Justification should be provided if diluted or spiked specimens are used in the submitted studies. 4. Positive specimens (undiluted) shall be chosen so that the majority of them are near the IVD cut-off. 5. Paired specimens should be used (for example, if claiming equivalence of four anticoagulants then each subject should provide four samples – one in each anticoagulant).	
1.2 Specimen collection, storage and transport			
1.2.1 Specimen stability	Real-time studies taking into account: • storage conditions (duration at different temperatures, temperature limits, freeze/thaw cycles); • transport conditions, where applicable; • intended use (see Note 1); • specimen collection and/or transfer devices intended to be used with the IVD.	1. Evidence shall be provided which validates the maximum allowable time between specimen collection and its addition to the IVD in the setting where testing takes place.	Technical Guidance Series for WHO Prequalification – Diagnostic Assessment (*3*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.3 Precision of measurement			
1.3.1 Repeatability, reproducibility	Both repeatability (within-condition – see Note 1) and reproducibility (between-condition – see Note 1) shall be estimated using panels of at least: • 1 negative specimen; • 1 low-reactivity positive specimen (near assay cut-off); • 1 medium-reactivity positive specimen. Each panel member shall be tested: • in 5 replicates; • using 3 different lots; • over 5 days (not necessarily consecutive) with one run per day (alternating morning/afternoon); • at each of 3 different testing sites.	1. For example, within- or between-run, -lot, -day, -site, etc. 2. Precision shall be determined for each pathogen and/or analyte for which detection is claimed (for example, HIV-1 antibody, HIV-2 antibody, HIV-1 p24 Ag, as appropriate). 3. The testing panel should be composed of natural (that is, undiluted) specimens. Where this is not feasible, the stock specimens that are to be diluted should represent a range of stages of infection (antibody maturation) in order to take into account the limitations of mimicking low IVD reactivity with a high-avidity specimen. 4. IVDs which include whole blood as a specimen type shall include evidence of precision in (at a minimum) spiked whole blood specimens (negative whole blood spiked with highly reactive plasma/serum specimens to produce an appropriate range of reactivities in the IVD). 5. Where possible, the testing panel should be the same for all operators, lots and sites. 6. Lots shall comprise different batches of critical components.	CLSI EP05-A3 (*4*) ISO 13612:2002 (*5*) CLSI EP12-A2 (*6*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
	The effect of operator-to-operator variation on IVD performance should be included as part of the precision studies (see also Note 8). Testing should be done: • by personnel representative of intended users; • unassisted; • using **only** those materials provided with the IVD (for example, instructions for use, labels and other instructional materials).	7. Results shall be statistically analyzed to identify and isolate the sources and extent of any variance. In addition, the percentage of correctly identified, incorrectly identified and invalid results shall be tabulated for each specimen and be separately stratified according to site, lot, etc. This type of analysis is especially important for rapid tests that may not have any numerical values. 8. The effect of operator-to-operator variation on IVD performance may also be considered as a human factor when designing robustness (flex) studies (see section 1.11.1 below) and may be addressed as part of clinical studies in representative populations (see Part 2). 9. Users should be selected based on a pre-determined and contextually appropriate level of education, literacy and auxiliary skills that will challenge the usability of the IVD and reflect the diversity of intended users and operational settings.	
1.4 Performance panels			
1.4.1 Subtype panels	Testing of WHO International Reference Preparations and/or commercial HIV subtype panels shall include:	1. Testing should be performed using more than 1 lot of the final design (locked-down). 2. All confirmed subtype-positive specimens shall be detected by the IVD.	Health Products and Food Branch, Health Canada (*7*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
	• all HIV-1 subtypes (for example, A, B, C, D, G, etc.), HIV-2, HIV-1 group O, and common circulating recombinant forms (CRFs); • at least 10 each of the most common subtypes (Subtype C, Subtype A, Subtype B, CRF02_AG, CRF01_AE, CRF07_BC and Subtype G); • at least 3 less-common subtypes (other CRFs and unique recombinant forms.	3. All reasonable attempts shall be made to test rare subtypes. 4. For IVDs that include a claim for detection of HIV Ag, appropriate specimens for the same subtypes shall also be included in the testing panel. The use of panels of virus-like-particles (VLPs) or viral cultures may be considered acceptable – however their use in place of characterized specimens shall be justified.	
1.4.2 Mixed titre panels	Testing of a panel of specimens with a range of analyte concentrations (for example, antibody "mixed-titre" panel).		
1.5 Validation of reading times			
1.5.1 Validation of reading times	For IVDs for which a reading interval is specified (that is, time when result can first be read; time beyond which result should not be read), validation of critical time points shall be provided.	1. The ranges of humidity tested for shall be risk based, taking into consideration the likely operational settings. 2. The intended operating temperature, upon which reading time has been validated, shall be clearly stated in the instructions for use.	WHO Prequalification – Diagnostic Assessment (*8*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
	Performance studies shall be conducted at each of 3 temperatures (at the mid-point and two extremes of the claimed operating range); the effect of humidity on reading times shall also be investigated.	3. Some of these aspects could be evaluated within the flex studies (see section 1.11.1 below).	
1.6 Analytical sensitivity			
1.6.1 Seroconversion	A minimum of 25 commercial or well-characterized seroconversion panels shall be tested: • test at least 40 early seroconversion specimens (see Note 2); • all seroconversion specimens shall be reactive (see Note 3); • start with a negative bleed(s) and should have narrow bleeding intervals.	1. Specimens should have been collected at short intervals to cover the seroconversion period and should also cover the whole window period. 2. Early seroconversion: – p24 Ag and/or HIV RNA-positive; – not recognized by all Conformité Européenne (CE)-marked third-generation enzyme immunoassays; – indeterminate or negative by confirmatory assays. 3. Seroconversion: – p24 Ag and/or HIV RNA-positive; – recognized by all CE-marked third-generation enzyme immunoassays; – indeterminate or positive by confirmatory assays.	European Commission (*2*) Health Products and Food Branch, Health Canada (*7*) CLSI EP12-A2 (*6*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.6.2 Limit of detection for HIV-1 p24 Ag, where appropriate	Analytical sensitivity estimated as the concentration of HIV-1 p24 Ag at the assay cut-off. The determination shall comprise a minimum of 15–20 replicate tests of an 8-member dilution panel of a suitable biological reference material – for example, the First WHO International Reference Reagent for HIV-1 p24 antigen (NIBSC code 90/636).	4. Seroconversion sensitivity shall be reported to the user in the instructions for use. 5. Optimally, testing should be conducted using more than 1 lot of the final design (locked-down).	
1.7 Prozone/high-dose hook effect			
1.7.1 Prozone/high-dose hook effect	For each claimed analyte, the potential for a prozone/high-dose hook effect shall be determined: • using multiple highly reactive specimens (minimum of 20); • using at least 2 different concentrations (diluted by at least a factor of 10); • by the testing of several replicates by the same operator on the same day.	1. Specimens shall be chosen that have a high analyte concentration, as determined using an IVD method other than the IVD intended to be prequalified (for example, enzyme immunoassay). This second method shall be of a design not subject to prozoning. 2. An increase in signal upon dilution of a specimen implies a hook effect.	Health Products and Food Branch, Health Canada (*7*) Butch, AW (*9*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.8 Analytical specificity			
1.8.1 Potentially interfering substances	The potential for false results (false negatives and false positives) arising from interference from at least the substances/conditions listed below in sections 1.8.1.1 and 1.8.1.2 (see Note 1) shall be determined using: • a minimum of 100 specimens (either naturally occurring or spiked to a low reactivity); • each substance/condition represented, where possible, by at least 3–5 specimens from different individuals. Testing shall be undertaken using both HIV-negative and HIV-positive specimens (unspiked or spiked) with each potentially interfering substance at physiologically relevant dosages.	1. The risk assessment conducted for an IVD shall identify substances where the potential for interference can reasonably be expected for the analyte being detected (for example, HIV-1/2 antibodies and/or HIV-1 p24 Ag). 2. Where either the scientific literature and/or risk analysis identifies the potential for false results in co-infected individuals (for example, decreased sensitivity or specificity), further investigation shall be undertaken using both HIV-negative and HIV-positive specimens. 3. In addition to the substances listed here, IVDs that are used to test oral fluid shall take into account the effect of oral infections, such as Candida, as well as tobacco, mouthwash, concomitant medications, dental fixtures, toothpaste, food or drink (consumed immediately prior to testing), consumption of alcohol and teeth brushing. 4. Any observed interference shall be investigated and performance limitations of the IVD reported in the instructions for use. Results shall be reported with respect to each condition and not be reported as an aggregate of the total number of specimens tested in the study.	Health Products and Food Branch, Health Canada (*7*) European Commission (*2*) CLSI EP07-A2 (*10*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.8.1.1 Endogenous	• human antibodies to the expression system (for recombinants), for example, anti-*Escherichia coli* or human anti-mouse antibody (HAMA); • recipients of multiple blood transfusions, and pregnant (including multiparous) women; • haemoglobin, lipids, bilirubin and protein; • elevated immunoglobulin G and immunoglobulin M; • rheumatoid factor; • sickle-cell disease; • other autoimmune conditions including systemic lupus erythematosus (SLE) and anti-nuclear antibodies (ANA).		

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.8.1.2 Exogenous	• relevant medicines, including: antiparasitic, antimalarial, antiretroviral and anti-tuberculosis medications; • common over-the-counter anti-inflammatory medications (aspirin, paracetamol and ibuprofen); • ethanol and caffeine.		
1.8.2 Cross-reactivity	The potential for false-positive results arising from cross-reactivity (see Note 1) shall be determined for a minimum of 100 specimens, including, where possible, at least 3–5 specimens representing each of the following: • non-HIV viral infections, including: hepatitis B, C infection and acute hepatitis A infection, cytomegalovirus, acute Epstein–Barr virus, varicella zoster virus, yellow fever virus post-immunization, measles, influenza A and B and tick-borne encephalitis;	1. The types of interferences tested for shall be risk based, taking into consideration the operational setting as well as the intended users for the analyte being detected (for example, HIV-1/2 antibodies and/or HIV-1 p24 Ag). 2. Any observed interference shall be investigated and performance limitations of the IVD reported in the instructions for use.	

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
	• other retroviruses, including human T-lymphotropic cell virus-1 and -2; • bacteria/parasites, including: malaria, visceral leishmaniasis, tuberculosis and human African trypanosomiasis; • influenza vaccine recipient; • vaccine-induced HIV seropositivity; • other unrelated conditions known to cause cross-reactivity in HIV IVDs.		
1.9 Metrological traceability of control material values			
1.9.1 Metrological traceability of control material values	The traceability of an assay-specific quality control specimen to a validated reference material shall be demonstrated – for example, the First WHO International Reference Panel for anti-human immunodeficiecy virus tests (NIBSC code 02/210) or the First WHO International Reference Reagent for HIV-1 p24 antigen (NIBSC code 90/636).	1. HIV RDT kits may not include external quality control specimens, but the IVD shall have a procedural control. The extent to which a control band corresponds to a valid test (identification of and traceability to a suitable reference) should be demonstrated. Comment 1: the nature of the procedural control (specimen addition or only reagent addition) shall be explained. Comment 2: an external control specimen is one that is run in conjunction with the IVD, but is physically separate from it – for example, an RDT cassette.	WHO Prequalification – Diagnostic Assessment (*8*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
		2. In some jurisdictions there is a requirement for the use of a "National Testing Panel" for lot release and IVD validation. Such a national requirement does not remove the need for evidence of traceability to a validated reference material as described here.	
1.10 Stability			
	Replicate testing shall be undertaken using a panel (for each claimed pathogen/analyte) consisting of at least: • 1 non-reactive specimen; • 2 low-reactivity specimens, near assay cut-off (see Note 2); • 1 medium-reactivity specimen. Wherever possible, specimens chosen for the testing panel shall reflect the main specimen types intended for use with the IVD (for example, capillary whole blood and/or oral fluid, as appropriate).	1. The testing panel shall include all claimed analytes and include whole blood specimens and/or oral fluid specimens, as appropriate, in accordance with intended use (for example to verify proper flow and absence of background interference, and to account for other variables). 2. Where detection of multiple genotypes and/or subtypes is claimed, equivalent performance (for example, sensitivity and specificity) shall have been demonstrated; otherwise evidence of stability in these genotypes/subtypes will need to be provided. 3. Ideally, the stability testing panel shall be composed of natural (that is, undiluted) specimens. Where this is not feasible, stock specimens to be diluted should represent a range of stages of infection (antibody maturation) so as to take into account the limitations of mimicking low IVD reactivity with a high-avidity specimen.	ISO 23640:2011 (*11*) CLSI EP25-A (*12*) Technical Guidance Series for WHO Prequalification – Diagnostic Assessment (*13*) ASTM D4169 - 14 (*14*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.10.1 Shelf-life (including transport stability)	Real-time, minimum of 3 lots of final design product and: • transport stressed (simulated) before real-time studies are undertaken; • IVD in final packaging subjected to drop-shock testing.	4. Lots shall comprise different batches of critical components. 5. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled. 6. Claims for stability shall be based on the second-last successful data point from the least-stable lot – with (where lots are different) a statistical analysis showing that the bulk of lots will be expected to meet the claimed life. For example, for testing conducted at 3, 6, 9, 12 and 15 months where stability was observed at 15 months, then the maximum stability claim shall be 12 months. 7. Accelerated studies do not replace the need for real-time studies. 8. In-use stability of labile components shall be determined using components in their final configuration.	
1.10.2 In-use stability	• minimum of 1 lot, using panel(s) compiled as above; • testing of all labile components (for example, buffers vials, sealed cartridges, etc. – see Note 8).		

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
1.11 Flex studies			
1.11.1 Flex studies	The influence of the following factors on expected positive and negative results shall be considered: • specimen and/or reagent volume; • buffer pH (measure of robustness – for example, as affected by evaporation of the buffer); • reading time (that is, the interval between when the first and last readings can be taken); • IVD sturdiness, including robustness of packaging and labelling lighting and humidity (see Note 3); • operating temperature.	1. Refer to WHO document PQDx_018 "Instructions for compilation of a product dossier" for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use. 2. The factors listed opposite should be investigated in ways that not only reflect but also exceed likely operating conditions in low- and middle-income countries, so that the limitations of the device can be understood. For example, in addition to investigating deviations of temperature within those claimed in the instructions for use, temperature ranges should be investigated that exceed those of claimed operating conditions and which cause test failure (incorrect/ invalid results). 3. The impact of lighting can be two-fold – that is, the impact of lighting on packaging (for example, fading) and on the sufficiency of lighting to read the test lines.	WHO Prequalification – Diagnostic Assessment (*8*)

Part 2 Establishing clinical performance characteristics (professional use and/or self-testing)

Aspect	Testing requirements	Notes on testing requirements	Source documents
2.1 Diagnostic sensitivity and specificity			
	Diagnostic sensitivity and specificity shall be determined for each claimed specimen type. Testing should be conducted: • at different geographical settings (minimum of 2 regions); • by a variety of intended users; • using more than 1 lot.	1. Prequalified HIV RDTs are generally used by lay providers and health-care workers. For WHO prequalification purposes, these should be considered as the intended user rather than a trained laboratory professional. 2. Where an IVD is intended to detect multiple analytes without differentiating which analyte is detected, the testing panel shall comprise specimens that are reactive only for each individual analyte (that is, not dual HIV-1/HIV-2-positive, etc). 3. A separate specimen shall be collected prior to testing to establish the reference result. The testing algorithm used to determine the reference results shall include a state-of-the-art fourth-generation immunoassay, with all initially reactive specimens reflexed for full characterization of HIV status. 4. Problematic specimens – that is, those with unexpected results but which otherwise meet the selection criteria for a study – shall not be systematically excluded from analysis.	European Commission (*2*) Health Products and Food Branch, Health Canada (*7*)
2.1.1 Diagnostic sensitivity	Testing of: • at least 400 specimens confirmed to be HIV-1 antibody positive; • at least 100 specimens confirmed to be HIV-2 antibody positive (where HIV-2 detection is claimed; see Note 2); • at least 50 specimens confirmed to be HIV p24 Ag positive (where Ag detection is claimed; see Note 2).		

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
2.1.2 Diagnostic specificity	Testing of: • at least 1000 HIV antibody/ antigen negative specimens.	5. Consideration shall be given to the influence of antiretroviral medications present in a specimen on the serostatus of such specimens, and to how this might affect specimen selection. 6. Lots (locked-down design) shall comprise different batches of critical components. 7. Where possible, all tests that produce a discrepant result (between the assay under evaluation and the reference results) shall be repeated using the same lot, and then on all available lots and the variability noted. Performance characteristics shall be reported using initial results only. The results of further testing of specimens with discrepant results shall be reported separately as additional information on IVD performance. 8. All indeterminate results shall be included in the denominator data for analysis. 9. All invalid test results shall be recorded. 10. Estimates of diagnostic/clinical sensitivity and specificity shall be reported with 95% confidence intervals. 11. Results shall be expressed separately for each specimen type and for each specimen type per intended use (no aggregation of results).	

Part 3 Qualification of usability (self-testing)

PURPOSE: Assessment of product design, instructions for use and usability of RDTs for self-testing by analysis of the following:

- Results of a questionnaire to assess whether the key messages and instructions from packaging and labelling would be understood and easily followed by untrained intended users (that is, self-testers).
- Test results obtained by untrained users (that is, self-testers) of simulated RDTs (for example, pre-made and with contrived results).
- Test results and interpretations when the assay is performed by untrained intended users (that is, self-testers) (*15–18*).

ADDITIONAL POINTS:

- For each type of study summarized below, the study group shall comprise untrained subjects whose age, gender, level of education, literacy and additional supplementary skills may challenge the usability of the IVD by its intended users, including in unfavourable operational settings (for example, poor lighting).
- These assessment activities will determine the changes needed to optimize the IVD for use by self-testers. Changes may range from minor (simplification of instructions for use) to major. The impact of any change on safety and performance shall be determined.
- Results from any one of the stages summarized below may indicate that assay redesign is necessary. This may in turn result in a need to revalidate the IVD or to perform additional specific performance studies and to update the risk analysis.

Aspect	Testing requirements	Notes on testing requirements	Source documents
3.1 Qualification of usability (self-testing)			
3.1.1 Labelling comprehension study	Questionnaire-based testing of subjects representative of intended users, to assess the ability of such users to correctly comprehend key messages from packaging and labelling with regard to: • proper self-selection (whether or not users understand if it is appropriate for them to undertake testing); • understanding key warnings, limitations and/or restrictions; • proper test procedure; • test result interpretation. Questionnaire shall be administered to at least 200 subjects, representative of intended users, in order to demonstrate comprehension of key messages.	1. Instructions for use and labelling shall be clear and easy to understand; use of pictorial instructional material is encouraged.	European Commission (*2*) ISO 18113-1:2009 (*19*) ISO 15197:2013 (*20*) IEC 62366-1:2015 (*21*) MHRA (*22*) Poffenberger, K (*23*) FDA (*24*) European Parliament and European Council (*25*) Center for Devices and Radiological Health, FDA (*26*) WHO (*27*) USAID and WHO (*28*) Center for Devices and Radiological Health, FDA (*29*)

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
3.1.2 Results interpretation study	A minimum of 400 subjects to interpret the results of contrived IVDs (for example, static/pre-made tests) to assess their ability to correctly interpret pre-determined test results. Contrived tests shall be made to demonstrate the following potential test results: • non-reactive • range of invalid results • reactive • weak reactive. Study group to consist of at least 200 self-testers from 2 high-prevalence (> 5%) and geographically diverse populations, and at least 200 self-testers from a low-prevalence (< 5%) population to demonstrate correct interpretation of simulated test results.	1. The study group may include subjects recruited as part of the labelling comprehension study.	

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
3.1.3 Observed untrained user study	Testing by at least 900 self-testing subjects comprising at least 200 self-testers in each of 2 high-prevalence (> 5%) geographically diverse populations, and at least 500 self-testers from a low-prevalence (< 5%) population. • Each subject to self-collect test specimen and perform test according to only those materials provided with the IVD (for example, instructions for use, labels and other instructional materials).	1. A separate venous whole blood specimen shall be collected prior to testing to establish the reference results for HIV-1 status (and HIV-2 where detection is claimed). The testing algorithm used to determine the reference results shall include use of a state-of-the-art fourth-generation immunoassay, with all initially reactive specimens reflexed for full characterization of HIV status. 2. For WHO purposes, the term "professional use" encompasses a diversity of skills, training and experience, and does not necessarily imply "highest standard of skills, training and experience". It may be a useful step in the evaluation of usability to compare the performance of self-testers with that of health-care workers, lay providers and laboratory technicians. However, concordance observed between the different types of users may mask poor performance within each user group. Consequently, such comparisons do not replace the need for comparisons to "clinical truth" based on the establishment of reference results for each subject.	

Table *continued*

Aspect	Testing requirements	Notes on testing requirements	Source documents
	• Each such test to be observed by a trained laboratory or health-care professional. The observing professional does not tutor or interact with the subject conducting the test, but notes errors and other observations about the self-tester. Observation may also be conducted by viewing a video recording of self-testing. • The observing professional also interprets the test result, in a blinded fashion and within the validated reading time stated in the instructions for use.	3. There may be a high likelihood of bias at the community level when simple study population sample methodologies are applied. Efforts shall be made to avoid convenience sampling of people (participants) who already know they are HIV positive.	

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   Note: the above guidance does not apply to HIV test kits which are used for patient management, or HIV test kits intended to be used outside the laboratory, that is, at the point of care and/or for home use. Separate guidance titled "Draft Guidance Document – HIV Simple/Rapid Test Kits" is available for manufacturers of near-patient HIV test kits upon request from Health Canada's Medical Devices Bureau, Health Canada; email: device_licensing@hc-sc.gc.ca
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Annex 5

Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment

Establishing stability of in vitro diagnostic medical devices TGS–2

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Preface

WHO Prequalification – Diagnostic Assessment: Technical Guidance Series

WHO prequalification of IVDs

WHO prequalification is coordinated through the Department of Essential Medicines and Health Products. WHO prequalification of in vitro diagnostic medical devices (IVDs) is intended to promote and facilitate access to safe, appropriate and affordable IVDs of good quality in an equitable manner. The focus is on IVDs for priority diseases and on their suitability for use in resource-limited settings. WHO prequalification is based upon a comprehensive assessment of individual IVDs using a standardized procedure that is aligned with international best regulatory practice. It also involves post-qualification activities for IVDs to ensure their ongoing compliance with prequalification requirements.

Procurement of prequalified IVDs

Products that are prequalified by WHO are eligible for procurement by United Nations agencies. The products are then commonly purchased for use in low- and middle-income countries.

Prequalification requirements

IVDs prequalified by WHO are expected to be accurate, reliable and able to perform as intended for the lifetime of the IVD under conditions likely to be experienced by a typical user in resource-limited settings. Countries in which WHO-prequalified IVDs are procured often have minimal regulatory requirements, and the use of IVDs in these countries presents specific challenges. For example, IVDs are often used by health-care workers who do not have extensive training in laboratory techniques, in harsh environmental conditions, in the absence of extensive pre- and post-test quality assurance capacity, and for patients with a disease profile that differs from the profiles encountered in high-income countries. Therefore, the requirements of WHO prequalification may differ from the requirements of high-income countries, or those of the regulatory authority in the country of manufacture.

About the Technical Guidance Series

The Technical Guidance Series (TGS) was developed following a WHO working group consultation held on 10–13 March 2015 in Geneva, Switzerland. The consultation was attended by experts from national regulatory authorities, national reference laboratories, and WHO prequalification dossier reviewers and inspectors. The guidance series is a result of the efforts of this and other international working groups.

Audience and scope

This guidance is intended for manufacturers interested in WHO prequalification of their IVD. It applies in principle to all IVDs that are eligible for WHO prequalification for use in WHO Member States. This guidance should be read in conjunction with relevant international and national standards and guidance.

The TGS documents are freely available on the WHO web site.

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The draft technical guidance document was posted on the WHO Prequalification website for public consultation on 14 December 2015. Various stakeholders, including manufacturers submitting to WHO prequalification of IVDs, IVD manufacturing industry associations, various national and international regulatory bodies, and IVD standards organizations, were informed of the consultation in order to solicit feedback. A two-month response period was provided.

Second-round public comments were received from the following: Ms A Asahina, Alere Medical Co., Ltd, Chiba, Japan; Dr J Budd, Beckman Coulter Inc., Chaska, the USA; Dr C Candia Ibarra, Ministerio de Salud Pública y Bienestar Social, Asunción, Paraguay; Dr NA Carrington, Roche Diagnostics, Indianapolis,

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Following incorporation of the second-round public comments, a revised draft was published on the WHO Biologicals website for a final round of public consultation between 18 June and 18 September 2017. The comments received were incorporated to produce the document WHO/BS/2017.2304. The document was adopted by the WHO Expert Committee on Biological Standardization as a WHO written standard on 20 October 2017.

1 Abbreviations

ASTM	ASTM International
CE	Conformité Européenne (European Conformity)
CLSI	Clinical and Laboratory Standards Institute
EIA	enzyme immunoassay
HBsAg	hepatitis B surface antigen
HBV, HCV	hepatitis B virus, hepatitis C virus
IFU	instructions for use
IgG, IgM	immunoglobulin G, immunoglobulin M
ISO	International Organization for Standardization
IVD	in vitro diagnostic medical device
NAT	nucleic acid test
NIBSC	National Institute for Biological Standards and Control

NS3, NS4, NS5	HCV non-structural proteins
OD	optical density
PEI	Paul-Ehrlich-Institut
QA	quality assurance
QC	quality control
QMS	quality management system
RDT	rapid diagnostic test
RPM	revolutions per minute
R&D	research and development
SOP	standard operating procedure(s)
TGS	WHO Technical Guidance Series
TP	*Treponema pallidum*

2 Definitions

The definitions given below apply to the terms used in this document. They may have different meaning(s) in other contexts. Common English dictionary definitions apply to non-defined concepts, such as device, constituent, equipment, evaluation, part, product, reaction, signal, substance, etc.

Accelerated stability evaluation: Study designed to increase the rate of chemical and/or physical degradation, or change, of an IVD reagent by using stress environmental conditions to predict shelf-life.

Note: The design of an accelerated stability evaluation can include extreme conditions of temperature, humidity, light or vibration (*1*).

Acceptance criteria: A defined set of conditions that must be met to establish the performance of a system (*2*, *3*).

Numerical limits, ranges or other suitable measures for acceptance of the results of analytical procedures (*2*, *3*).

Accuracy of measurement: Closeness of the agreement between the result of a measurement and a true value of the measurand.

Note 1: Accuracy of measurement is related to both trueness of measurement and precision of measurement.

Note 2: Accuracy cannot be given a numerical value in terms of the measurand, only descriptions such as "sufficient" or "insufficient" for a stated purpose (*4*).

Arrhenius plot: Mathematical function that describes the approximate relationship between the rate constant of a chemical reaction and the temperature and energy of activation (*2*).

Batch/Lot: Defined amount of material that is uniform in its properties and has been produced in one process or series of processes.

Note: The material can be either starting material, intermediate material or finished product (*5*).

Biocidal products: Active substances and preparations containing one or more active substance intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means (*6*).

Characteristic: Distinguishing feature.

Note 1: A characteristic can be inherent or assigned.

Note 2: A characteristic can be qualitative or quantitative.

Note 3: Characterization: a description of the distinctive nature or features of something (*7*).

Component: Part of a finished, packaged and labelled IVD (*5*).

Note: Typical kit components include antibody solutions, buffer solutions, calibrators and/or control materials (*5*).

Constituent: Raw materials used to make a component.

Control material: Substance, material or article intended by its manufacturer to be used to verify the performance characteristics of an IVD (*5*, *8*).

Design input: The physical and performance requirements of an IVD that are used as a basis for IVD design (*9*).

Drift: Characteristic slow change of a metrological value from a measuring instrument (*10*).

Environmental factors: Variables that might affect the performance or efficacy of IVD reagents – for example, temperature, airflow, humidity and light (*2*).

WHO note: For WHO purposes, this also includes altitude and microorganisms.

Evidence: Information which can be proved true based on facts obtained through observation, measurement, testing or other means (modified from (*7*).

Independent lots: lots with different production (or manufacturing, purification, etc.) runs of critical reagents (for example, biological reagents prepared in different syntheses, growths or purifications or other risk-defined critical reagents from different manufactured lots or from different suppliers if applicable).

Instructions for use (IFU): Information supplied by the manufacturer to enable the safe and proper use of an IVD.

Note: Includes the directions supplied by the manufacturer for the use, maintenance, troubleshooting and disposal of an IVD, as well as warnings and precautions (*5*).

WHO note: In order to avoid confusion, please note that, in the USA, the acronym IFU also stands for "Indications for use", and the acronym IU stands for "Intended use" or "Indications for use" (the acronym PI is often used in the USA to indicate the package insert, which may contain IFU). The International Organization for Standardization (ISO) definition and requirements (*5*) for IFU cover the intended use and the precise method of use and is the definition used by WHO and throughout this and other TGS documents.

In-use stability: Duration of time over which the performance of an IVD reagent within its expiration date remains within specified limits after opening of the container system supplied by the manufacturer and use under standard operation conditions (for example, storage on the instrument).

WHO note: For the purpose of this guidance document, WHO considers that it includes the number of times the reagents can be removed, used and returned to the storage condition without impact on test kit performance. It must reflect the routine conditions of use (for example, on-board stability, reconstitution and open-vial/bottle stability). A single product may have several different types of in-use stability claim, each reflecting different aspects of its usage. For example, an IVD reagent may have one in-use stability claim for unopened storage on board its associated instrument system and another stability claim once it is opened and put into active use. Another type of in-use life is the calibration interval of an IVD reagent (2).

In vitro diagnostic medical device (IVD): A medical device, whether 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.

Note 1: IVDs include reagents, calibrators, control materials, specimen receptacles, software, and related instruments, 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.

Note 2: In some jurisdictions, certain IVDs may be covered by other regulations (*11*).

IVD reagent: Chemical, biological or immunological components, solutions or preparations intended by the manufacturer to be used as an IVD (*5*).

WHO note: This document uses the terms IVD and IVD reagent interchangeably.

Life-cycle: All phases in the life of a medical device, from the initial conception to final decommissioning and disposal (*12*).

Metrological traceability: Property of the result of a measurement or the value of a standard whereby it can be related to stated references (usually national or international standards) through an unbroken chain of comparisons, all having stated uncertainties.

Note: Each comparison is affected by a (reference) measurement procedure defined in a calibration transfer protocol (*4*).

Performance claim: Specification of a performance characteristic of an IVD as documented in the information supplied by the manufacturer.

Note: This can be based upon prospective performance studies, available performance data or studies published in the scientific literature (*5*).

WHO note: "Information supplied by the manufacturer" includes but is not limited to: statements in the IFU, in the dossier supplied to WHO and/or regulatory authorities, in advertising and on the internet.

Referred to simply as "claim" or "claimed" in this document.

Precision: The closeness of agreement between independent test results obtained under stipulated conditions (*4*).

Real-time stability evaluation: Study designed to establish or verify the shelf-life of the IVD reagent when exposed to the conditions specified by the manufacturer.

Note: Conditions that can affect the stability of an IVD reagent include temperature, transport conditions, vibration, light and humidity (*1*).

Risk management: The systematic application of management policies, procedures and practices to the tasks of analysing, evaluating, controlling and monitoring risk (*12*).

Risk-management plan: For the particular IVD being considered, the manufacturer shall establish and document a risk-management plan in accordance with the risk-management process (*12*).

Shelf-life: Period of time until the expiry date, during which an IVD reagent, in its original packaging, maintains its stability under the storage conditions specified by the manufacturer.

Note: Stability and expiry date are related concepts (*5*).

WHO note: In this document "Labelled life" is considered to be the time up to the expiry date printed on the label of an IVD or IVD component.

Stability: Ability of an IVD reagent to maintain its performance characteristics within the limits specified by the manufacturer.

Note 1: Stability applies to:

- IVD reagents, calibrators and controls, when stored, transported and used under the conditions specified by the manufacturer;
- reconstituted lyophilized materials, working solutions and materials removed from sealed containers, when prepared, used and stored according to the manufacturer's IFU;
- measuring instrument or measuring system after calibration.

Note 2: Stability of an IVD reagent or measuring system is normally quantified with respect to time:

- in terms of the duration of a time interval over which a metrological property changes by a stated amount;
- in terms of the change of a property over a stated time interval.

WHO note: because definition restricts IVD **reagent** only. Refer to (*1*) definition 3.10.

Stability monitoring: Real-time stability testing at certain points in time during shelf-life (or in-use life) to assure that an IVD reagent performs within specified claims (*2*).

Note: A continuing stability monitoring programme (ongoing stability monitoring) is required to verify that the stability claim is maintained over the

life-cycle of the product. Data on stability must be obtained at end of shelf-life (see (*1*); section 4.1) and ideally at the halfway point of assigned shelf-life so that any problems that do occur can be dealt with in a timely fashion.

Trueness of measurement: Closeness of agreement between the average values obtained from a large series of results of measurements and a true value (*4*).

Validation: Confirmation by examination and provision of objective evidence that the requirements for a specific intended use or application have been fulfilled (*7*).

Verification: Confirmation by examination and provision of objective evidence that specified requirements have been fulfilled (*7*, *13*).

3 Introduction

3.1 Key concepts

Stability is the ability of an IVD reagent to maintain its performance characteristics over a defined time interval (*12*). The purpose of most stability studies is to establish or verify the time interval, and the storage conditions that can maintain stable IVD performance characteristics.

3.2 Rationale of stability studies

The stability of an IVD is fundamental to its reliable performance over a defined period of time. It is a regulatory requirement for the manufacturer to provide objective, scientifically sound evidence to support all claims made regarding the stability of an IVD. In addition, a manufacturer can use stability studies to demonstrate the probability that lots manufactured up to the end of the life-cycle of the IVD will meet predetermined user needs (as identified in design inputs).

3.3 Purpose of this document

The purpose of this document is to provide IVD manufacturers with guidance on possible approaches to determine stability. It also describes the expectations of WHO prequalification in relation to stability studies.

3.4 Standards

WHO recommends the following standards for use in establishing stability claims: International Organization for Standardization (ISO) 23640:2011 (1); Clinical and Laboratory Standards Institute (CLSI) EP25-A (*2*) and ASTM International D4169 - 14 (*14*). It is recommended that manufacturers be familiar with these standards and consider them when designing and planning their

stability studies. For other relevant standards see *TGS–1: Standards applicable to the WHO Prequalification of in vitro diagnostic medical devices*[1].

3.5 Limitations of this guidance

This guidance document should not be taken as a prescriptive checklist of the stability testing that must be performed, but as a guide on how to improve processes and generate the evidence needed to ensure a comprehensive and systematic procedure with an appropriate risk-management plan.

Depending on the particular categorization of the product and on the particular jurisdiction, additional regulatory and/or legal requirements, beyond the scope of this document, may apply.

The examples included throughout the document are not exhaustive and apply to the principles outlined in this document only. Manufacturers must still perform their own product-specific risk assessment for each of their IVDs, which may identify other critical characteristics (for example, physical measurements).

4 Considerations when applying for WHO prequalification

WHO requires that reports of studies used in establishing the stability claims for the product be submitted as part of the prequalification application.[2] As part of the WHO prequalification assessment, manufacturers must describe the rationale, the study methods, the stability monitoring programme followed and the testing algorithms used, with references to the relevant standard operating procedures (SOP). The information provided must demonstrate the link to the predetermined user requirements and product development.

The expectations of WHO prequalification may be different from the inputs of the users and from the requirements of the regulatory authority in the country of manufacture. In addition, the expectations set out in this guidance document may be additional to the requirements of ISO 23640 (*1*) and the expectations of CLSI EP25-A (*2*). Wherever possible, this guidance document explains the reasons for these additional expectations. Other approaches to meeting these additional expectations, supported by rigorous risk assessment or other evidence, may also be acceptable in dossiers submitted for WHO prequalification.

[1] Available at: http://www.who.int/diagnostics_laboratory/guidance/170808_tgs1_standards_2.0.pdf?ua=1

[2] WHO documents PQDx_049 *Product dossier checklist and PQDx_018 Instructions for compilation of a product dossier* are available on the WHO Prequalification – Diagnostic Assessment website: http://www.who.int/diagnostics_laboratory/evaluations/en/

4.1 Manufacturer responsibility

It is a manufacturer's responsibility to ensure that the evidence supporting performance claims regarding the end of the IVD shelf-life is objective and scientifically rigorous.

4.2 Suitability for use in WHO Member States

The stability studies submitted to WHO prequalification shall accurately reflect the expected environmental conditions and the normal usage conditions/methods encountered by users in WHO Member States, such as:

- extremes of temperature under in-use conditions and during transportation;
- extremes of humidity encountered under in-use conditions and during transportation and storage;
- any affects that light may have on IVD functionality, especially on the length of time for which a result is claimed to be stable; and
- prevalence of certain microorganisms.

4.3 Meeting customer requirements

By undertaking well-designed stability studies (including periodic verification activities) the manufacturer can demonstrate that the product meets input requirements (that is, customer requirements), as required by ISO 13485 (see (*15*) section 7.2: Customer-related processes). Meeting predetermined user expectations, not merely evaluating the capability of an IVD, is a fundamental aspect of IVD development (see (*9*) definition (f); and (*15*) section 7.3.4). It is a proactive means for the manufacturer to prevent quality problems at lot release and in the post-production and marketing phase.

5 Basic principles for stability testing

5.1 Critical characteristics or metrics of the IVD

A well-designed stability study must generate evidence of the stability of each of the critical constituents in the IVD (**risk-evaluated critical constituents**), evidence of stability for each of the claimed analytes, and evidence for any particular level of performance, including the precision, sensitivity and specificity of the kit. A documented risk-based approach should be taken to determine which claims and constituents must be evaluated over the stated shelf-life.

Examples:

1. *A hepatitis C virus (HCV) assay containing the critical constituents related to detection of NS3 or core proteins must have the stability of all such constituents proven for the shelf-life of the IVD.*
2. *For an assay designed to detect both immunoglobulin G (IgG) and immunoglobulin M (IgM) by use of protein A and protein L, the stability of both protein A and protein L must be proven in the IVD.*
3. *For an IVD to quantitate CD4, all the constituent antibodies used (for example, anti-CD3 and anti-CD4) must be shown to be stable in the IVD.*
4. *For an IVD claimed to detect particular seroconversion specimens or genotypes, or to have specified precision at particular analyte concentrations, or a particular specificity, each of these claims at risk or that change over time must be proven over the stated shelf-life (see TGS–4: Guidance on test method validation for in vitro diagnostic medical devices (16).*

Other critical characteristics (also called critical metrics) identified in the risk assessments may include physical measurement (for example, volume, pH, flow rate, legibility and adhesion). These characteristics must be shown to meet their specifications for the shelf-life of the IVD but are outside the scope of this document.

5.2 Finalized product presentation

During stability testing, all IVD components (including the IVD, calibrator and/or control material, etc.) must be made and tested to the finalized manufacturing specifications and in the finalized packaging, including intended labels and containers (see section **10.4**). In most circumstances, all presentations (for example, different buffer volumes used for different kit sizes) must be used during stability testing. Where some presentations are not tested, the manufacturer should document the rationale, justifying why all presentations have not been tested.

5.3 Environmental conditions

The stability study must subject the IVD to a combination of conditions that define, with predetermined confidence limits, the stability for lots marketed during the life-cycle of the IVD. The combination of conditions and durations of exposure and number of lots to be used will be driven by a manufacturer's risk assessment for the IVD and by research and development (R&D) data. The risk assessment should, at a minimum, take into account the following:

- the variability of the constituent materials (identifying the most important sources of variation);
- an understanding of the nature of user environments; and
- the extremes of conditions (temperature, humidity, ambient pressure and vibration) potentially occurring during transportation to those users (see also section **4.2**).

Boundary conditions for stability studies must reflect realistic extreme conditions that are consistent with the design input requirements for the IVD. The subsequent stability studies will prove the IVD capable of meeting performance requirements up to the end of its stated shelf-life, after transportation to the users.

5.4 Minimum number of lots

The design of stability studies must take into consideration lot-to-lot variability, with a risk assessment conducted to identify the most important sources of variability. The degree of variation of individual lots affects the confidence that a future production lot will remain within specification throughout its shelf-life. Lot variability is most often caused by minor differences in the biological reagents rather than by lack of reproducibility of the manufacturing process. Although existing standards (*1*, *2*) recommend the use of a single lot for certain stability studies, the impact of lot-to-lot variability must be taken into consideration and the use of additional lots may be necessary. Three lots, at a **minimum**, must be used to establish or verify shelf-life; in-use claims require testing on a **minimum** of one lot. To ensure that the potential for lot-to-lot variability is addressed, independent lots must be used – that is, lots containing different batches of critical constituents such as nitrocellulose membranes, recombinant antigens, peptides, nucleic acids and the enzymes used in nucleic acid test-based (NAT-based) testing technologies.

Example:

For NAT-based testing technologies, it is crucial to use independent lots of enzyme for stability studies, as the manufacturing process can affect them. Other components (including primer, probe and buffer) can also be affected by the manufacturing process (for example, in terms of purity, pH, and DNase and RNase contamination). Thus for these other components, the use of independent lots that represent both material and process variability are also recommended.

5.5 Assessment of liquid components

The orientation of the product during storage (that is, upright versus inverted or horizontal) may need to be included in a protocol where contact of the product with the different parts of the container (such as the closure system or the body

of the container) may be expected to affect the stability of the products contained (for example, liquid component). This is sometimes referred to as "inverted container stability". The product orientation may need to be moved occasionally during the stability study to ensure that there is direct contact between the liquid contents and all parts of the container. This aspect requires particular attention during in-use stability studies of components that are diluted or reconstituted from a freeze-dried state before use.

5.6 Specimens for the stability testing panel[3]

The specimens used in the stability testing panel(s) must reflect the performance claims related to the IVD. The specimen types most likely to be used in those WHO Member States in which the IVD is intended to be used must be considered and, as appropriate, included in the specimen panels used throughout the stability studies (see **Appendix 2**). If a variety of specimen types (for example, serum, plasma, whole blood and saliva) are claimed as being suitable for use in the IFU, the stability study plan must be designed to provide evidence that the IVD will meet its claims (for example, for sensitivity, specificity, proportion of valid runs and precision) for each of the specimen types for the whole of the claimed shelf-life, including during transport to the final users, unless an alternative approach can be justified using a documented rationale. Evidence must be statistically valid (see section **11.5**). Regulatory requirements may also dictate the addition of specified panel members.

5.7 Validation of stability testing panel

The stability testing panel(s) must be validated, and rejection and replacement criteria must be established. The validation of the panel members used is crucial. Panel members themselves must be stable and they must monitor parameters that are useful in controlling the characteristic being tested.

Storage of a validated panel for testing stability is not always feasible. For example, this is often the case for assays requiring fresh and/or whole blood specimens (for example, assays for counting CD4 cells). When replacing panel members, particularly for CD4 monitoring, the accuracy of results generated using the replacement material must be confirmed using an appropriate reference method (for example an instrument validated for use in an ISO 15189 (*17*) accredited laboratory). Replacement criteria for unstable panel members must include the duration for which a critical member will give valid results.

[3] A panel is a collection of well-characterized specimens and other materials that are used to monitor aspects of IVD and component function during stability studies, for in-process control, for some aspects of design validation and at release to sale. The same materials might be used for each of these purposes but be assigned different acceptance criteria for the different functions.

5.8 Panel member selection and value assignment criteria

Panel members are chosen specifically to ensure that each member has an attribute relevant to the intended use. The goal of stability testing is to ensure that the test method appropriately monitors functionality at the end of the assigned life (shelf-life or in-use life) of the antigens, epitopes and antibodies, along with any physical specifications relevant to the intended use.

For example, an intended use claim may be that early seroconversion specimens are detected. To show that this claim is true at the end of the product's shelf-life, a stability panel member representative of a very early seroconversion specimen could be included. This might be a weakly reactive IgM specimen, or some other specimen that has been shown to closely mimic the behaviour of the IVD with the critical specimens. Rare and valuable specimens would not be expected to be tested at all time points of stability studies. However, evidence must be provided that key performance claims made in the IFU, published material (including advertising) and dossiers submitted to WHO prequalification are met at the end of the assigned shelf-life and in-use life.

Each panel member is assigned an expected value and this is used to assign the acceptance criteria for that panel member. The expected value for each panel member is assigned in a measurable manner that is relevant to the outputs of the particular methodology. For example, the acceptance criteria for each panel member may be assigned in terms of sample-to-cut-off ratio, cycle time (CT) values or band intensity measured quantitatively/semi-quantitatively.

In the example of a weakly reactive IgM seroconversion specimen, the specimen at the start of shelf-life may have an RDT reading of 1+ out of 4 assigned as its expected value using a semi-quantitative value based on band intensity. The acceptance criteria assigned as a result may be that "all reactive specimens remain reactive, and all non-reactive specimens do not react in the assay".

Panel members must be chosen so that they will not only be relevant in demonstrating the intended use but will also have values that will appropriately detect, and therefore monitor, any deleterious effects of storage. A strong positive specimen that has a 4+ out of 4 semi-quantitative reading may continue to give this reading despite decay in the assay, whereas a specimen with a reading of 1+ out of 4 (with an assigned acceptance criteria of "remaining positive") is more likely to give an indication of the ongoing stability of the assay.

Thus it is essential to know (and document) that whenever a panel member meets the acceptance criteria, this is a true reflection of the stability of the product and not due to the inability of the specimen result output to reflect any change in the IVD.

5.9 Time points

A simple study design requires a minimum of three testing intervals (*2*):

1. an initial baseline test;
2. a test at the time point beyond the claimed stability limit (see section **5.9.1**);
3. one point in between.

This simple study design is acceptable for submission to WHO prequalification under some circumstances and for some IVDs based on:

- the manufacturer's risk analysis;
- whether the manufacturer has prior-objective documented experience of the stability of the product; and
- whether the statistical confidence in the result is sufficiently great for all lots tested.

The benefits of a simple study design are that a small number of testing intervals and fewer resources are required. However, such a simple design represents a high-risk approach that has the potential to waste time and resources if the IVD does not meet the acceptance criteria with an appropriate margin of statistical confidence at the end of testing. If the acceptance criteria would have been met at another intermediate time point then that might have been acceptable as an assigned shelf-life.

A more effective and well-established approach routinely used is to test at a number of additional predetermined intermediate time point intervals (between 1 and 2 above). Typically, testing is carried out at relatively short intervals (every 10 or 14 days) for the first 3 months, and then at monthly intervals until at least one month beyond the design input-specified shelf-life. This protocol provides information on whether the IVD ages more rapidly in the period just after manufacture than later on in the shelf-life, and usually provides sufficient data to enable the assignment of a confidence interval to the shelf-life.

The manufacturer could identify the most practical intermediate test points from a risk evaluation of a specific IVD and include them in the stability study plan/protocol. Such planning will also help manufacturers to estimate the resources required to implement the testing.

Testing of all panel members is not expected at each of the test/time points. However, testing with all stability testing panel members is expected at the initial, the second to last and the last test/time point for all of the study types.

The manufacturer should consider and document the rationale for the selection of intermediate test points, and choose panel members to be tested at these intermediate test points (for example, representative members, specimens that are close to the medical decision points and those at the extremes of the assay range tested).

5.9.1 Duration of testing

Testing conducted in stability studies should extend beyond the shelf-life determined from user needs. At a minimum, testing should extend at least one time point (one testing interval) beyond the predetermined user requirement to provide a margin for uncertainty. The length of the time periods chosen will depend on risk assessment, but should provide a safeguard in the event of unexpected IVD failure during the testing period, where extrapolation from an earlier time point would not be considered acceptable.

It is recommended that the standard relevant units of measurement are used for the entire study (for example, unopened kit shelf-life is normally measured in months; opened IVD/reagent stability in days or weeks; and allowed reading times for enzyme immunoassay (EIA) and RDT in minutes or hours after performing the assay).

5.10 "Zero time" values and variance

The value of each measured characteristic at the beginning of the stability study and its variability over the course of the study are important pieces of information. They should be measured independently for each lot of material in the stability study. Analysis of the data will indicate if a statistically significant change has occurred to any measured parameter from any lot during the course of the study. A statistically significant change may not be of practical significance. Relevant practical limits will have been predetermined in IVD or process development. However, all statistically significant changes must be thoroughly evaluated to decide whether they represent some important change that would otherwise be undetected.

Zero time values could be obtained by evaluating each measured characteristic for each lot on five or more occasions to establish the value and its variance with freshly made materials. A definition of "occasion", following appropriate consideration, could be specified, for example, as involving a different day, a different operator and a different set of equipment in order to investigate potential sources of analytical variation. Later in the study, apparent differences in the values of the characteristics can be detected reliably, relative to the "zero time" value.

6 Shelf-life studies

6.1 Requirements for determination of shelf-life

The stated shelf-life of an IVD must normally be based on real-time experimental results. Accelerated stability studies are usually not sufficient to support a claimed shelf-life, although they may be used in situations where experience already exists with similar products (see (*1*) section 4.1) or when the stability of very similar products is already known (see (*2*) section 7.3.1).

Note: If at the time of dossier submission for WHO prequalification the real-time study outcome is not available, accelerated studies might be considered. The manufacturer must justify why the accelerated study is acceptable as supportive evidence until real-time experimental results become available. In these cases, the results of real-time stability studies will be requested as a condition of WHO prequalification. The shelf-life of the IVD could be extended upon WHO review of real-time data.

6.1.1 Real-time stability studies

Real-time stability is determined using storage temperatures derived from user requirements, over a period longer than the required life of the IVD.

Where a broad range of storage temperature is claimed (for example, "Store at 4–40 °C") WHO expects the studies will provide evidence for stability over the whole of the temperature range for at least the length of the claimed shelf-life. However, where claimed stability is restricted to a limited range (for example, "Store at 2–8 °C") it is acceptable for stability studies to be conducted at a single temperature within this range.

It is recommended that a sequential approach be used (*2*) in which IVDs are first submitted to stresses simulating transport before they are placed into a shelf-life or in-use study. This approach best simulates the real-life situation, where products will first be transported to the end user and then stored under the recommended conditions before use, possibly until almost the end of their labelled shelf-life.

It may be routine practice to store IVDs for an extended period after manufacture before shipping. In this case, the IVDs would be kept first for a defined period of time under recommended storage conditions, then taken through the transport stress condition sequences, and finally put back into the recommended storage conditions for the duration of the study (*2*).

6.1.2 Accelerated stability studies

Accelerated stability studies are designed to predict the shelf-life of an IVD using increased rates of chemical and/or physical degradation caused by

extreme environmental conditions (for example, elevated temperature at higher humidity).

Accelerated stability studies provide results in a relatively short time. However, the results of these studies are reached using assumptions about the degradation of reagents and other IVD components that may not reflect their observed performance under actual conditions of storage and use.

If the Arrhenius equation is used to calculate the expected life at temperatures other than those actually used then the parameters of the equation must be derived from the experimental data and not assumed (*2*). Manufacturers must ensure that there are sufficient data (for example, for different temperatures and test intervals) to allow for reliable extrapolation.

7 Component stability studies

7.1 General principles

7.1.1 Testing on final specifications

Component stability studies, including antimicrobial and desiccant studies, must be performed using components made according to finalized and approved manufacturing specifications (ideally to validated manufacturing scale) on qualified manufacturing equipment and meeting finalized and approved in-process quality control (QC) specifications.

7.1.2 Considering component stability

IVD components are sometimes prepared in bulk and stored before being used in several different lots of a completed IVD. The design-input documentation should define how long components are likely to be stored before use. With that information, component stability studies should be planned to provide evidence that component shelf-lives will not restrict IVD shelf-life, since **an IVD cannot have a shelf-life beyond that of any of its dependent components**.

The shelf-lives of components manufactured in bulk and used in several different lots of an IVD can be verified using three lots of the component as a minimum for shelf-life studies and, depending on documented risk assessment related to variability, one or more lots subsequent to changes made to the component. It is possible there will be two shelf-lives to evaluate: that of the bulk material stored prior to transferring to the final packaging and that of the component in its final packaging. The final contents of the evaluated lots of the component must differ with regard to the batches of critical constituents used (independent lots) but, subject to documented risk assessment, may all be tested in their final presentation with a single set of the other components that will be used together to constitute the IVD.

Examples of stored components:

Wash solutions and substrates for EIA, amplification reagents for NAT and calibrators for quantitative tests; all manufactured and stored in their final labelled vials ready to be put into a kit.

Component stability can be assessed from the functionality of the lot and also by factors related to the component that might change over time, such as turbidity, colour, microbial contamination and the pH of liquid components. Depending on the IVD and the conditions it is subjected to, it may be necessary to distinguish between turbidity that arises from heat/cold denaturation and turbidity that arises from microbial contamination.

7.1.3 Considering constituent stability

The stability study plan should consider whether components made from freshly made constituents (for example, antigens, recombinant antigens, enzymes, antibodies and membranes) will have the same shelf-lives as components made from stored raw materials. Evidence should be provided to support the use of stored constituents and detailing the lot-to-lot variability of critical constituents.

The stability study plan should also consider the choice of reagents or methods to ensure that the most appropriate are used to measure the performance of the component being studied (whether made from freshly made constituents or from constituents with an already proven shelf-life).

Examples of stored constituents:

Purified recombinant antigens and monoclonal antibodies stored in aliquots ready for use.

7.2 Stability of control materials

Assay-specific control materials provided by the manufacturer are used to show that an IVD has performed as intended during use. These are often referred to as "run controls" and are provided with some IVDs, along with an IFU statement that if the control meets a certain criterion then the IVD will have functioned as expected. "Control materials" does not refer to controls such as international calibrators or those used in external quality assurance (QA) programmes.

The manufacturer must be able to demonstrate that the loss of signal from control materials does not occur at a different rate from the loss of signal from a validated panel member or from genuine, critical specimens; otherwise a failing IVD might be regarded as still functional. Thus, the stability of control materials must accurately reflect the stability of the IVD. The use of a control material that is apparently more stable than the IVD and other components, or the use of incorrectly assigned values for the control material, must be avoided (*18*).

Example:

It is frequently seen in dossiers submitted for WHO prequalification that a positive run control will produce a signal of > 2.0 optical density (OD) in a freshly manufactured lot, and the IFU will state that an OD > 0.8 for the same control qualifies a run. Thus the IVD may have lost more than half its activity and still appear functional, even though some critical specimens are shown in the dossier to have very weak signals on freshly made IVDs. This is not considered appropriate unless data can be provided that demonstrate that the critical specimens will still be detected at the end of shelf-life and with a control material signal of 0.8 OD.

7.3 Biocidal stability and efficacy

7.3.1 Rationale

Bacterial and fungal organisms relevant to the environment of use must be identified in the design input risk assessment, and antimicrobial preservatives should be chosen, based on risk assessment, to prevent contamination of the product in storage and in use. Antimicrobial preservative effectiveness must be demonstrated throughout the shelf-life of the IVD.

If a new or modified preservative (for example, a different concentration) is used as a result of further information on the conditions of intended use, the manufacturer must obtain evidence that the new antimicrobial preservative or concentration chosen does not negatively affect the stability of the IVD.

7.3.2 Study conditions

The studies should reflect expected in-use conditions for opened containers – the stability of the IVD in the user environment, as intended by the manufacturer, must be proven. On-board stability must be tested for an IVD used with an instrument.

See (*18*) sections 51, 61 and 62; and (*19*) Appendix XI for suggested study methods. Examples of bacterial groups to consider are spore-forming bacteria, fungi, indigenous bacteria, bacteria found in the environment of the country of manufacture and those found in the countries of intended use. Specific examples outlined in references (*18*) and (*19*) include *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, Salmonella species, *Pseudomonas aeruginosa*, *Clostridium sporogenes* and *Staphylococcus aureus*.

7.4 Desiccant functionality

Desiccants affect the stability of the entire IVD. Stability studies must show that the desiccant will support the product over the whole claimed shelf-life within the predetermined extremes of transport, storage and in-use conditions.

Note: For WHO prequalification purposes:

1. It is recommended that a self-indicator (a humidity indicator that changes colour upon saturation) be part of the desiccant design. However, WHO strongly recommends against the use of cobalt dichloride, the most commonly used humidity indicator, as it is a carcinogenic substance.
2. Sachets are preferred to tablets, since the labelling instruction "Do not eat" is more visible. There have been reports of desiccants in a tablet formulation being mistaken for antimalarial medicine.

8 Stability during transport

8.1 Rationale

Transport stability studies evaluate the tolerance of an IVD to the types of environmental conditions (for example, temperature and humidity) and physical conditions (for example, inversion, vibration, physical handling and stacking) to which it is likely to be subjected during and after shipping from the manufacturer to the end user. These studies should provide evidence that there will be no impact on IVD performance over the whole of its stated shelf-life as a result of the transportation of the IVD by the recommended methods.

The manufacturer should assess the potential impact of multiple factors and justify and document whether or not to include them in the evaluation. Final transport conditions recommended by the manufacturer should reflect (and the stability study plan document) the assessment of the conditions expected to be encountered in the areas of use. The manufacturer should address any issues that arise as a result of the transportation studies (for example, failing the stressed conditions), and address these limitations in the manufacturer documentation (for example, shipping documents and IFU if applicable).

WHO expects that a transportation challenge would precede the real-time determination of shelf-life, and in-use studies. This will serve to determine that transportation conditions do not reduce the shelf-life of the IVD (see section **6.1.1**).

In some cases it may be acceptable for the product to undergo transportation-stability studies without a subsequent long-term real-time stability study. In this case, shelf-life must be established under specified storage conditions along with a stringent and evidence-based risk assessment of the probabilities of extreme transport stress affecting IVD performance at the end of the claimed life (see (*2*) section 4.2.3).

8.2 Challenge conditions

Determination of the stability of an IVD during transportation should take into consideration the local routes and means of transport used to supply the IVD, which are usually defined in the design input risk assessment. It is not necessary to test the IVD to the point where it is no longer usable, but merely to validate the window of transport conditions within which the IVD will retain its claimed performance to the end of its stated shelf-life. However, knowledge of the possible limitations of an IVD and at what point the IVD becomes unusable is useful to a manufacturer when trouble-shooting post-marketing problems. WHO expects the manufacturer to take into consideration the possibility that the product might continue to be subjected to suboptimal storage conditions by the end user.

> ***Example:***
>
> *A static challenge of 45 °C for 3 days may represent conditions seen during the actual transportation of an IVD – however, a more stringent challenge of cyclical high and low temperatures (including freezing) for a longer period of time, and followed or preceded by exposure to vibration might better cover a "worst-case scenario" of shipment, storage and subsequent transportation to the end user.*

8.3 Number of lots

Where transport stability studies are incorporated into studies to establish shelf-life, as recommended in this guidance document, a minimum of three lots of the IVD must be used. For transport studies alone, a minimum of one lot of the IVD may be used, however, as with shelf-life studies, more lots may be required depending on lot-to-lot variability (see section **10.1**).

8.4 Simulated versus actual challenge

An actual shipping challenge can be used to verify the conditions found in the simulated transportation challenges. However, it may only replace a simulated shipping challenge where there is an appropriate risk evaluation and where experience and data have been actively collected for similar products and documented in detail (for example, it is not sufficient to note "no complaints").

In the R&D phase, actual data from shipping can be used to define the conditions needed for an appropriate simulation of extremes. However, in the post-production phase, actual shipping challenges often do not explore the full range of shipping conditions that could be encountered, including extreme values.

8.5 Multiple stress test sequences (simulated transport challenges)

Proof of IVD performance after actual shipment is generally not sufficient evidence of stability under all conditions and delay hazards. Multiple stress test sequences are typically needed to address the range of transport conditions used for global product delivery. Relevant guidance (*14*) recommends the evaluation of several extreme conditions.

Appropriate stress test sequences may be developed on the basis of data from actual product transport studies. Testing multiple stress sequences allows a manufacturer to identify the most cost- and/or resource-effective transport conditions from a set of alternatives, while ensuring adequate product stability protection (see (*2*) section 4.2.3).

Note: For WHO prequalification, the environmental conditions investigated as part of a stability study must reflect those likely to be encountered in resource-limited WHO Member States. For example, temperatures at some airport tarmacs in sub-Saharan Africa can exceed 40 °C, while temperatures encountered during air transport fall below 0 °C. Significant delays can be encountered at any time and especially during wet season transport to remote health centres.

See Appendix 1 for an example of a protocol for simulated transport challenges.

8.6 Physical conditions

Physical handling can be both manual and mechanical. The relevant user and commercial factors should be identified as part of the design input risk assessment and the packaging and shipping methods developed accordingly. Reference (*14*) describes a number of factors to be considered, and their evaluation: drop, impact, compression, vibration, repetitive shock, longitudinal shock, cyclic exposure, vacuum, impact and inversion; along with the size, weight and composition of the packaging. This should be regarded as part of stability testing.

9 In-use stability studies

9.1 Rationale

In-use stability of an IVD is the period of time over which components retain adequate performance, after transport to the users, once they are opened, reconstituted and/or diluted and exposed to the environmental conditions in which they will be used.

As far as possible, the study should be designed to simulate the use of the product in practice. If a range of conditions for use is stated in the IFU (for

example, "use at 15–40 °C") evidence must be provided to prove the stability over that range with all the specimen types claimed (for example, serum, whole blood and oral fluid), unless a documented rationale is provided. It is considered best practice for the manufacturer to claim a stability range that includes an appropriate safety margin (for example, test range 2–35 °C, claimed 4–30 °C) to ensure that that the claimed stability range is acceptable. However, where claimed in-use stability is restricted to a limited range (for example, "use at 35–37 °C") it is acceptable for in-use stability studies to be conducted at a single temperature within this range, subject to evidence from documented robustness studies or risk assessments.

It is good practice to perform the in-use stability testing at both the start and end of the shelf-life of the IVD (or with components at the start and end of their shelf-lives if any of the components have a longer shelf-life than the complete IVD) and after simulated transport challenge (see section 8). This will confirm that the IVD will have the claimed in-use life throughout its whole shelf-life.

All studies should support precisely defined periods of in-use stability claims.

Example:

An RDT test cassette may be labelled "Use immediately on opening". However, it is still necessary to determine the interval (one hour, one day, etc.) over which IVD performance remains stable after the component is opened.

9.2 Conditions of use

Determination of the in-use stability of an IVD and/or its components must reflect the routine conditions of use of the IVD. Freeze-thaw stability should be considered to address situations in which reagents may be exposed to multiple freeze-thaw cycles during use.

Note: For WHO prequalification, in-use stability studies should take into account the environmental conditions and usage conditions encountered in WHO Member States and by users, such as exposure to extreme temperature, humidity and light and to microorganisms.

9.3 Multiple in-use stability claims

Depending on the way in which the IVD is used it may be necessary to have several in-use stability claims. In situations where multiple stability claims are made, a manufacturer must provide evidence (from testing that investigates routine use) supporting each of the claims.

***Examples*:**

1. *A reagent may have a stated period of stability once it has been placed on board an instrument and another period of stability once it is in active use (that is, during actual use/testing).*
2. *Multiple-use reagents (for example, buffers) may repeatedly be exposed to high temperatures during the day while in use and exposed to lower temperatures when not in use and stored in the refrigerator. The actual use of the multiple-use reagent – squeezing of bottles, exposure of the lid and tip to working surfaces and hands, and exposure to dust and light – may also affect stability. Stability studies and associated risk assessments should take all of these factors into account.*

10 Production lots used in stability studies

10.1 Considering variability

As noted in section **12.3** below, planning for stability studies must take into consideration all possible sources of variation within and between manufactured lots. For most IVDs it is likely that differences between batches of the biological reagents will cause the most variation. Factors to consider include apparently minor and technically uncontrollable differences in the culture and purification of recombinant antigens and antibodies; synthesis and purification of primers, probes and peptides; undocumented production changes of an outsourced buffer component; and lot variability of nitrocellulose membrane used in lateral-flow IVDs.

At a minimum, lots chosen for stability studies must be independent lots – that is, they must differ in the source lot of their critical constituents, for example, different purification and/or culture batches for all recombinant antigens and monoclonal antibodies. If pilot or small-scale lots are chosen, special attention must be paid to the potential for variability (see also section **12.3**). However, the sources of variation will depend on the particular process, product and component, and should be identified during product development risk analyses.

Use of different batches of critical components ensures that the stability evidence obtained is more likely to be representative of long-term manufacture. Any variability found can be taken into consideration when assessing the outcome of the studies against the design input requirements and when making claims. This minimizes user problems and hence complaints.

10.2 Testing the final configuration

Shelf-life, in-use and transport stability must be determined for the finalized approved product in terms of:

- manufacturing specifications
- release-to-market QA criteria
- packaging and labelling (see section **10.4**)
- validated manufacturing scale on qualified manufacturing equipment.

Note 1: For WHO prequalification, it is important that the stability studies have been conducted using the IVD intended to be prequalified, and not surrogates and/or closely related products. Changes perceived as small (for example, change in production scale, bulk container materials, supplier of a critical biological or vial stopper) can have unexpected effects on stability and other performance characteristics. After such changes, a new documented risk assessment and, if necessary, a stability plan and study, is needed. Manufacturers should have change-control procedures in place compliant with ISO 13485 (*15*).

Note 2: Stability studies undertaken in the R&D phase of the product life-cycle provide an important understanding of how to design the product so that it will meet the final stability requirements identified in the input documentation. However, these studies are usually not sufficient for submission to WHO prequalification assessment since they may not reflect the final design and manufacture of the IVD.

10.2.1 Exceptions

If any of the above criteria are not met (for example if "pilot lots" or small-scale lots are used, or if the method of use described in the IFU is not finalized), strong evidence must be provided that the materials that were evaluated will perform exactly the same as the final commercial product.

Note: In some exceptional circumstances, where it is not possible to sample from actual production lots, samples from pre-production or development lots might be used. If this is the case, manufacturers should justify why production lots were not used, and provide robust evidence that the lots chosen are expected to behave identically to the production lots. Data concerning lot-to-lot variability must still be submitted. Although WHO will consider the available evidence on its merits, this preliminary information must be followed by stability claims conducted on fully qualified production lots.

10.3 Number of lots required for testing

Current guidance (*1*, *2*) recommends that three product lots at a **minimum** must be used to establish or verify shelf-life; in-use claims require testing on a **minimum** of one lot. The actual minimum number of lots to be used must be determined by a stringent risk assessment based on evidence of variability

obtained during R&D (see section **10.1**). However, the minimum will never be less than three lots for shelf-life verification.

WHO note: It is **not** acceptable to sample IVDs from a single production lot but to label them so that they appear to have been taken from three separately manufactured production lots. This is true for all performance evaluation and regulatory submission purposes. WHO prequalification investigates batch records during on-site inspections. Non-compliance with this requirement may result in a critical non-conformity grading.

10.4 Components of lots required for testing

Current guidance (*1*, *2*) recommends that stability work be performed using materials in their final packaging. Labelling is a significant factor of packaging and is known to present stability issues in some cases. For example, some label adhesives diffuse through some plastics, enter vials and affect the function of the reagents over time. Other label types lose adhesion over time; while some printing inks fade. The physical stability of packaging requires the same degree of risk evaluation and subsequent experimental verification as its chemical stability, with attention given to the countries of intended use. This is most important for primary packaging but must also be considered for secondary packaging, particularly for transport stability studies.

If there is more than one configuration or version of the IVD (for example, pack size differences, or Conformité Européenne (CE) marked and non-CE marked) then any potential effects on performance, including stability, must be assessed. In particular, if different reagent-container sizes are used in packs with different volumes of reagent (for example, different volumes for single use and multiple use), stability evidence should be obtained on all variants, even if the contents of the containers are identical, unless stringent risk evaluation supported by physical or chemical evidence indicates otherwise.

Once component shelf-lives are assigned, it is expected that both relatively fresh components and components which have progressed into their assigned shelf-life will be used when selecting the different production lots for use in studies to establish the product shelf-life (*1*, *2*).

11 Stability study plan

Stability studies should be well designed, scientifically sound, well implemented, well recorded and able to deliver meaningful conclusions concerning IVD performance. This will minimize the time and resources required by the manufacturer to generate appropriate evidence and by the regulatory authority to assess it.

It is good practice to prepare, within the mechanisms of a quality management system (QMS), a plan for the investigation of each characteristic

of IVD stability. A well-developed study plan, with clearly defined objectives, responsibilities and pass/fail criteria, should be developed, reviewed and internally approved in advance of testing. The plan should be based on the design input requirements.

It is essential that the stability study plan takes into account the intended use of the product to ensure that the relevant critical characteristics are all captured by the plan. The results of the stability studies should support the claims made in the IFU.

Careful forward planning will help to ensure that sufficient resources are made available, effective experiments are performed, and both experimental results and associated documentation are recorded in an appropriate manner.

11.1 Responsibilities

The study plan should outline the responsibilities and applicable training for all staff involved in the study. The responsibilities for implementing the study plan must be assigned to appropriately qualified and trained staff. Responsibilities to be allocated include study set up, testing, monitoring, validation of equipment and/or processes, sample selection, risk assessment and corresponding documentation.

In addition, the manufacturer must nominate a person responsible for investigating failures and a person responsible for conducting risk assessments if the IVD fails to meet the requirements of the design inputs.

11.2 Preparing the testing plan

A complete, detailed description should be prepared that documents all of the required testing and procedures to be undertaken and the expected outcomes. Authorization of the plan should be obtained internally in advance of commencing work. The plan should include the following details:

- the qualification and training of technical staff performing the work;
- any biohazard issues identified with reagents;
- aspects of instrumentation, including storage facilities or rooms, validation, calibration, monitoring and servicing;
- the lot/batch numbers of kits to be used, with justification for any manufacturing anomalies or deviations from documented procedures;
- the expected life of the kit from the input documentation;
- any proposal, with justification, to launch a kit with a shelf-life based on accelerated data, or to launch with a shorter shelf-life than in the input documentation while awaiting the conclusion of real-time testing;

- documentation of the nature and extent of in-use testing;
- the justification for the selection of lots and components, taking into account lot-to-lot variability and the critical characteristics;
- the number of units (test cassettes, bottles, tablets, etc.) of each component to be collected and stored under each condition;
- the nature of the panel to be used, justifying each panel member's inclusion and defining the volume and characterization of the bulk specimen to be used, and the aliquot size and number to be stored for the testing;
- the expected criteria for each panel member at the beginning and end of the product's proposed shelf-life;
- the statistical methods to be used for data analysis, including those used to identify outlying values and to establish criteria (see section **11.5**); and
- the methods for approval and justification of any deviations from the plan.

11.3 Product storage

A sufficient number of product components from the identified lots should be reserved and stored separately to ensure that the study will be completed with identified products. Sufficient numbers of the testing IVDs should be retained to allow for additional testing, calculated from estimated invalid result rates.

11.4 Documentation

The plan should make reference to the preparation of a study report that will be used to summarize the interim, and ultimately final, study findings and conclusions. The study plan, the testing protocol, the study report and all associated documentation (worksheets, etc.) should be controlled within the manufacturer's QMS. At the end of the study, the manufacturer should be able to confirm whether or not the design input requirements have been met.

Any changes from the methods identified in the plan must be recorded and undergo risk assessment. The plan should refer to the development of a detailed and valid testing protocol that includes all information and material relevant to testing.

11.5 Statistical methods

Statistical methods are used to support stability claims by providing estimates of the probability of results being as stated. For example, prior to the stability studies on an EIA, it has been documented that if a panel member has at least a particular

OD then the IVD will meet a particular claim. Given the results of the stability study using that panel member and showing the variability within and between lots of the IVD, the probability of future similar production of the IVD meeting claims at the assigned life can be estimated. The derivation of valid criteria and the probability of maintenance of all claims can be estimated by appropriate statistical methods.

There is a wealth of information available on the statistical methods used in the R&D of IVDs, from both ISO (*20*–*22*) and CLSI (*2*, *23*–*26*). Although most of these methods apply to quantitative assays, information on statistical methods for qualitative assays is also available (*27*).

The fundamental considerations for stability testing are the number of replicates required at each time point and the number of different production lots required which together will produce an "acceptable overall probability estimate" of the likelihood of future production lots meeting claims (and hence user input requirements) at the end of the shelf-life.

However, consideration must also be given to what represents "an acceptable overall probability limit". "Acceptability" is a decision critical to quality and must be decided upon in advance based on the input requirements (for example, 80% confidence that 95% of lots will meet the claims). This is a tolerance interval as described in ISO 16269-6:2014 (*22*). The consideration can then be phrased as: "How many replicates and how many different production lots can then be derived from the tolerance interval required?"

It is strongly recommended that manufacturers seek advice from a professional statistician once the quality-critical requirements have been defined and before beginning any experimental work.

The statistical methods to be used must be documented in the plans and protocols of any stability study and consideration given to the treatment of unexpected and atypical results. In general, all results must be used unless there is a documented physical reason that the result can be ignored – for example, known operator error, too little volume, incorrect timing or use of an unqualified instrument (one lacking maintenance or calibration). Any ignored results must nevertheless be recorded and included in the report of the stability study.

11.6 Stability testing protocol

As part of an approved study plan for the determination of IVD stability, a detailed testing protocol should be prepared as appropriate (examples of stability protocols are provided in Appendix 1: Examples of stability protocols). The protocol should include the following as a minimum:

- QMS identifiers (for example, experiment name, document references, etc.) that allow traceability to both the overarching study plan and to the records/documents generated, such as result worksheets.

- The training requirements for operator(s).
- The expected dates and times when the data will be collected.
- The objectives of the study (that is, determination of shelf-life, determination of in-use stability of a component, etc.).
- The name and lot number of the IVD and/or components to be investigated.
- Specification of how the components will be sampled from the production department.
- The panel members to be used and their characterization, including valid test methods which reflect the IFU claims.
- The experimental method that will be used for testing. This must follow the finalized testing method from the IFU where appropriate. It must describe clearly how the experiment is to be performed in terms of:
  - required storage and/or challenge conditions
  - duration of storage/challenge
  - schedule of testing intervals (see (*2*) section 4.3)
  - stability testing panel
  - numbers of replicate tests performed for each panel member.
- How and where results are to be recorded.
- The acceptance criteria.
- How aberrant, discordant or invalid results will be dealt with.
- How storage/challenge conditions are to be applied:

***Example*:** *For determination of stability during transportation it should be made clear that each IVD will be subjected to a sequence of stated temperatures.*

- How actual storage/challenge conditions are recorded:

***Example*:** *Recording of temperature not as "room temperature" but as an actual numerical value obtained from calibrated instrumentation.*

Note: Statements of a general nature can be unclear to a regulatory or WHO reviewer. For example: "Sample buffer was stored at the required temperature and tested each month". This statement raises questions such as: (a) were the bottles of sample buffer stored open at the required temperature for the entire testing period; or (b) were the bottles stored capped and refrigerated, and only reopened briefly at the required temperature at each schedule test point?

To avoid confusion, the details of actual storage and use procedures are required in the testing report.

11.7 Reading and recording results

11.7.1 Avoiding reader bias

It is good practice to use approaches that make the reading of results as objective as possible, such as using a documented scoring system. For IVDs for which a subjective element forms part of the result (for example, reading the intensity of an RDT band within a specified time frame) the results should always be reviewed by both a first and a second reader to avoid operator bias. Both readers must be blinded to the expected results and the second reader must also be blinded to the first reader's results. If a validated band intensity scoring tool is to be included in the final RDT kit, this should be used to record results.

11.7.2 Recording actual individual results

The results of a test (not only the test interpretation) should be recorded. An interpretation on its own provides insufficient detail to detect the degradation of a signal over time. Photographic records of qualitative tests are recommended, as appropriate.

Some IVDs (for example, line-blots) may require the presence of particular band patterns to allow an interpretation to be reached, and several different patterns may yield the same final result. Recording only the final interpretation of a test specimen may cause the failure of particular bands to go unnoticed, while allowing the IVD to pass stability assessment.

Quantitative assays, for example EIAs or NATs, should be tested with sample panels containing concentrations of analyte across the quantitative range of the assay. Numerical results should be reported and statistical methods should be applied to ensure that the assay is measuring the analyte appropriately across the quantitative range.

Qualitative assays, for example EIAs and NATs, should also be tested with samples at several different analyte concentrations, including samples at low concentration near the cut-off level of the assay. Results should be recorded as positive or negative according to the predetermined cut-off level of the assay.

> ***Example*:**
>
> *Some RDTs may stipulate that the strength of test band is not correlated with the strength of antibody titre. Nevertheless, the following should be recorded: (a) the intensity of observed patterns according to a predetermined and validated intensity scoring system with as fine a gradation as possible; and (b) the final result interpretation.*

11.7.3 Retention of records

WHO recommends the retention of photographic records, machine printouts and electronic data, or physical retention of membranes from opened test cassettes, as appropriate. Records should be retained for the period of time equivalent to the commercial lifetime of the IVD but not less than two years (modified from (*15*) section 4.2.4).

11.8 Instability versus imprecision

Testing at more than two time points can be important for avoiding confusion between imprecision and instability. For example, if a 10% decrease (compared to the zero time value) is recorded from testing at the end of the shelf-life, it may not be possible to judge if the difference was due to imprecision or instability. The inclusion of additional test points (for example one or more between the zero time and the end of the shelf-life) allows for fluctuation caused by imprecision to be distinguished from drift due to instability.

Increased clarity between instability and imprecision can be gained by increasing the number of replicates and runs, primarily with reference to the zero time values (see sections **5.9** and **5.10**).

11.9 Testing schedule

Testing intervals should be selected to detect any trending of results over the testing period. Different testing intervals may be required for different components. For example, it may be appropriate to test an IVD test cassette against a panel on a monthly or quarterly basis, but to test for open vial stability on a weekly basis.

11.9.1 Acceptance criteria for results

The acceptance criteria to establish what is acceptable or not acceptable should be defined according to the panel criteria for both qualitative and quantitative test methods. Results from failed (invalid) test runs must not be used in the determination of the stability claim. However, the invalid results should be recorded and included in the report of the stability testing.

12 Stability study report

12.1 General

After testing has been completed, the findings should be summarized in a stability study report. The report should clearly identify the IVD that was tested, the objectives of the study, the conditions under which the IVD was tested and the conclusions that were drawn from the findings. The report should be traceable

to the study plan, testing protocol and input requirements. It should make clear references to other supporting documentation (for example, result worksheets).

12.2 Link to claims

The results and conclusions of stability studies presented in the report must support the claims of IVD stability reported in the IFU and elsewhere in the WHO prequalification dossier.

12.3 Consider variability

An overall stability claim (whether for shelf-life, in-use stability or stability during transportation) must be based on the expected stability when taking into account inter-lot variability.

> ***Example:***
>
> *The manufacturer should evaluate the variability between the different lots studied (see section 10.1) and assume that any differences in shelf-life are inherent to the manufacturing process. The claimed life should be calculated so that a known and stated proportion of all lots (usually > 95%) will meet the claimed shelf-life. Frequently, more than three lots are needed to obtain a realistic idea of the variability of the results.*

12.4 IVD stability versus component stability

A claim of stability for an IVD as a whole must not exceed any individual component stability.

> ***Example:***
>
> *For an IVD claimed to detect HIV-1 and HIV-2 antibodies – if detection of HIV-1 antibodies is stable to 24 months but that of HIV-2 to only 18 months then the shelf-life must be based on the shorter time of 18 months.*

13 Changes to a WHO prequalified IVD

13.1 Dealing with change

Any critical or major modification to a WHO prequalified IVD or to its process of manufacturing will require the provision of new direct evidence of stability.

An appropriate risk assessment and an accelerated stability study comparing the original product and the modified product for usability, performance and lot-to-lot variability may serve to assess the impact of the changes to product formulation or manufacture.

It would be necessary to validate the stability of the modified IVD on a **minimum** of one lot of the IVD (subject to risk assessment) in order to

demonstrate equivalence between the original and modified IVDs. Testing of further lots may be appropriate depending on the product nature, variability of components and failure risk (see (*2*) section 7.1.2). WHO expects the results of accelerated testing to be confirmed by real-time studies.

If there are different presentations, evidence of the stability of each one must be provided (see also section **10.4**).

The following examples illustrate the scope for considering the performance evidence from one IVD as support for the performance of another. It should be noted that the observations discussed here refer specifically to IVD stability. Other aspects of IVD performance should still be validated as appropriate.

Examples:

1. *An HIV RDT uses an identical test cassette and physical components as a manufacturer's existing, fully validated, HCV RDT, but the reagent formulations are different (antigen/antibodies, buffers, conjugates, etc.) – evidence of stability of the HCV RDT would not suffice for the HIV RDT. Even if the manufacturer claims that both IVDs have been sold in a number of countries for several years and no adverse feedback has been reported, this would not constitute evidence in support of the stability of either IVD.*
2. *From an HIV RDT that has been fully validated for detection of HIV-1 antibodies, a new product is developed that includes detection of HIV-2 antibodies. The stability of any sample buffers that are identical between the two IVDs would, most likely, not need to be validated. However, other components (conjugates, antigens or antibodies) that are different between the two IVDs would need to be tested; it would not be sufficient to assume that HIV-1 reagents will have the same stability in the new IVD. An IVD modification of this nature is likely to require substantial new validation of stability.*
3. *An HIV RDT previously intended for testing serum/plasma has a claim added for detection of HIV-1 in whole blood. The only substantive design change associated with the new claim is the addition of a small filter pad near the sample port which acts as a filter for whole blood specimens. Depending on the nature of the material, it may be reasonable to argue that the pad material would not be expected to age; that it is not, in any practical sense, chemically labile. Consequently, shelf-life and in-use stability may not necessarily need to be retested in full. However, stability during transportation may need to be determined to provide confidence that the modification is able to withstand likely shipping conditions (for example, that the extra square*

of filter pad material does not dislodge when packages are jostled and bumped in transit).

4. *Based on an HIV RDT that has been fully validated for the detection of HIV-1 antibodies, a new IVD is developed which includes detection of antibodies to Treponema pallidum (TP). Detection of TP-specific antibodies occurs on a completely separate membrane (and associated architecture) to that of HIV-antibody detection. Additional handling steps may have an impact on the stability of the HIV-1 antibodies and retesting may be required. It may be necessary to review evidence of stability during transportation to ensure that new components are not affected by transit (for example, where a new packaging concept is used).*
   - *If a new machine is used for striping of the HIV-1/TP IVD, validation of the new machine (installation qualification, operational qualification and performance qualification) would be required to show that the stability studies are still valid.*
   - *If the IVD is designed in a way that HIV and TP detection occurs either on the same membrane and/or using most of the same architecture (and assuming that sample buffers are identical between IVDs) it is likely that this new IVD would need to be fully validated.*

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Appendix 1

Examples of stability protocols

This appendix uses the example of a wholly fictitious IVD to illustrate the kinds of experimental design that would be required to adequately determine:

1. the stability of whole kits during transport followed by the stability of whole kits during shelf-life; and
2. the in-use stability of whole kits including reagents.

The information provided in these examples should be used as a guide to possible approaches for generating evidence of a standard sufficient to satisfy the expectations of WHO prequalification. Further examples can be found in the WHO Prequalification: Sample Product Dossiers available on the WHO Prequalification website[1].

WHO expects that a transportation challenge would precede the real-time determination of shelf-life and in-use studies.

Description of the fictitious IVD

The fictitious IVD used in the examples below is an RDT for the detection of antibodies to HIV-1, HIV-2 and *Treponema pallidum* (TP) in serum, plasma and whole blood, and is referred to as the HIV/TP RDT.

The IVD kit components are: a test cassette sealed in a foil pouch (with desiccant) and a bottle of specimen buffer/diluent for use.

It is recommended that the kit be stored at 8–40 °C and brought to 15–30 °C before use.

It is recommended that once the sealed foil pouch of the test cassette is opened that the test cassette be used immediately.

The specimen buffer is expected to have similar stability to the sealed and pouched test cassette. The stability of the opened bottle of specimen buffer is determined below (see **Example 2: In-use stability protocol**).

[1] http://www.who.int/diagnostics_laboratory/guidance/sample_product_dossier/en/

Stability study plan:

The manufacturer has developed a stability study plan to determine the stability of the HIV/TP RDT. As part of this plan, a preliminary determination of accelerated stability has been made at several extremes of temperature, which suggests that the IVD would be stable to an equivalent of 12 months following manufacture. The plan calls for the development of real-time stability protocols that will form the basis of subsequent testing of the IVD.

Preliminary work has shown that the variability between lots is minimal. As a result, three independent lots (with no critical constituents in common) will suffice to enable a reasonable estimation of shelf-life, taking lot-to-lot variability into account.

Example 1: Evaluation of transport stability followed by real-time stability

Objective

To determine the stability after transportation of the HIV/TP RDT in real-time using simulated shipping conditions, and to generate components that have already undergone stress testing to be used in real-time shelf-life studies as proposed in Stability Study Plan XZY00001.

Preparation

Acquire sufficient numbers of the IVD kits from three independent production lots using a predetermined sampling protocol (for example, random, first X number of kits in first box, every 100th kit, etc.). Allow at least 10% overage for unexpected requirements and re-testing.

> *Note 1*: To provide security against unforeseen events, duplicate tests should be performed as a minimum. However, testing in triplicate will provide more statistical confidence in the observed test result.

The IVD kits chosen for testing must be in their final packaging including all labelling (see section **10.4**).

The IVD kits are stored so that the reagents are in contact with all elements of the packaging (for example, the bottles in the IVD kits are stored horizontally, lying flat on their sides, allowing liquids to remain in contact with the bottle closures).

Acquire sufficient volume of each panel member for the duration of the testing schedule (see testing schedule below).

The protocol for these studies specifies the number of IVD kits to be picked, the statistical sampling plan to be used and the required panel members and their volumes.

Documentation

In Worksheet XYZ00001 record the following:

- the lot numbers from which the IVD kits were sampled;
- the number of IVD kits sampled from each lot; and
- details (including manufacturing/lot information) for each of the IVD kit components that will be tested as part of this protocol (test cassette and specimen buffer).

Testing schedule: for transport simulation

Testing will be conducted at 0, 3, 6, 9, 12 and 13 months.

> *Note 1*: Testing beyond 13 months will allow for an understanding of when, in real-time, the IVD is likely to "fail" and may allow for an extension of the proposed shelf-life.
>
> *Note 2*: For determination of shelf-life, a fresh bottle of specimen buffer must be opened at each testing point – though there may be circumstances in which multiple sampling could be taken from the same bottle after it has been opened.

The IVD kits will be divided into two groups. One group will be stored at 40 ± 5 °C, the other at 8 ± 2 °C. IVD kits from each group will then be subjected to the following conditions:

***Condition 1*:** Temperature and humidity sequence; all IVD kits will be taken through a temperature and humidity sequence consisting of:

i) Ambient humidity (X% RH)

- Put at IFU storage temperature for 24 ± 4 hours followed by
- 30 ± 5 °C for 24 ± 4 hours, followed by
- 45 ± 5 °C for 24 ± 4 hours, followed by
- 8 ± 5 °C for 24 ± 4 hours, followed by
- IFU storage temperature for 24 ± 4 hours

Followed by

ii) Desert humidity (30% RH)

- Put at IFU storage temperature for 24 ± 4 hours followed by
- 30 ± 5 °C for 24 ± 4 hours, followed by
- 45 ± 5 °C for 24 ± 4 hours, followed by
- 8 ± 5 °C for 24 ± 4 hours, followed by
- IFU storage temperature for 24 ± 4 hours

Followed by

iii) Tropical humidity (85% RH)

- Put at IFU storage temperature for 24 ± 4 hours followed by
- 30 ± 5 °C for 24 ± 4 hours, followed by
- 45 ± 5 °C for 24 ± 4 hours, followed by
- 8 ± 5 °C for 24 ± 4 hours, followed by
- IFU storage temperature for 24 ± 4 hours

Followed by

iv) Ambient humidity (X% RH)

- Put at IFU storage temperature for 24 ± 4 hours followed by
- 30 ± 5 °C for 24 ± 4 hours, followed by
- 45 ± 5 °C for 24 ± 4 hours, followed by
- 8 ± 5 °C for 24 ± 4 hours, followed by
- IFU storage temperature for 24 ± 4 hours.

Note 1: It is important to make clear that the above complete sequence of temperatures will be used, as opposed to separate IVD kits being held at individual temperatures. The actual temperatures, durations and the nature of the sequence will depend on the IVD and on the kinds of conditions expected to be encountered during shipping.

Note 2: Freezing temperatures are not considered in this example but should be included if the IVD kits could be exposed to freezing temperatures during transport.

Note 3: If transport by air is anticipated, the effect of reduced pressure should be included in the protocol for a period of time at least 10% longer than the longest anticipated flight, and at a pressure expected in aircraft holds.

Note 4: The protocol should call for testing of at least five individual IVD kits after each stress condition, using the stability panel members giving the most informative results. This approach will enable verification that the IVD kits are sufficiently stable to progress to the next condition – though this should already be known from preliminary experiments and R&D work.

***Condition 2*:** Transport stress conditions – shaking; each IVD kit will be placed on a shaking table at X revolutions per minute (rpm) for X hours/days at 42 ± 5 °C as defined by ASTM D4169 section 12.[2]

After the simulated shipping challenge, each IVD kit will be returned to its corresponding storage temperature (40 ± 5 °C or 8 ± 2 °C).

Testing schedule for real-time stability studies

Testing will be conducted at 0, 3, 6, 9, 12 and 13 months. At each scheduled time point, the allotted number of IVD kits will be brought to 15–30 °C and used to test each member of the panel in triplicate.

Note 1: The test at 0 months will provide evidence that the IVD kit is stable under extreme conditions of shipping (but similar to those likely to be experienced); the testing at later time points will provide evidence to support the claimed shelf-life after transport; and testing beyond the claimed shelf-life will provide evidence that the IVD kit is stable and not close to a failure point.

Documentation for transport stress conditions

In Worksheet XYZ00001 record the following:

- the lot numbers of the IVD kits used to conduct the test;
- the operator(s) name(s);
- the dates of testing;
- identifying details for each member of the panel being tested;
- the temperature at which the IVD kits are stored;
- the values of temperature and humidity for each of the challenge conditions;
- instrument settings for the shaking apparatus and duration of operation for challenge conditions;

[2] See: Standard practice for performance testing of shipping containers and systems. ASTM D4169 - 14. West Conshohocken (PA): ASTM International; 2014.

- the ambient temperature and humidity during testing;
- each test result as an interpretation according to the IFU;
- each test result as a band intensity – band intensity should be scored using the calibrated scale described in Protocol ZXY00001 (for example, 0; faint/trace; +1; +2; +3;…+10) even though the IFU do not give scores to results);
- any aberrations or deviations from the protocol, the reason for the deviation and any remedial action undertaken. Results from invalid assays must be recorded but not included in the calculations of shelf-life. Apparently aberrant results, unless the underlying cause can be positively identified as not related to a problem with the IVD, must be included in the calculations of shelf-life.

Panel for monitoring stability

See the suggestions in **Appendix 2: Suggested specimens for stability testing panels**.

Acceptance criteria

Each panel member should show a band intensity result that matches its expected result at each tested time point. The expected result must be validated so that if the IVD fails to meet the claims (for example, fails to detect critical specimens, has unacceptable performance at medical decision concentrations or has unacceptable specificity) the panel member would also fail to meet its specified result.

The stability after transportation of the IVD kit will be taken as the time point before the last time point to have met the acceptance criteria – for example, if the IVD is stable to 13 months, the stability after transportation will be deemed to be 12 months.

The stability after transportation should be identical to the claimed shelf-life of the IVD kit – that is, the extremes of possible conditions to which the IVD kit is likely to be subjected during transport must not affect the shelf-life of the IVD.

Calculation of results

Detailed statistical instruction must be obtained from a professional statistician with an understanding of the expectations of the stability study plan and outcome. Professional statistical input is particularly recommended when calculating confidence limits for discrete data such as readings from a graduated scale.

Each of the following applies at each time point:

The variance of the results for all replicates within and between all the lots must be calculated for each panel member. From the overall variance between lots, the confidence with which future lots of the IVD kit will detect the panel member at that time point after manufacture and transport can be calculated. If the confidence that the panel member will meet its specification is less than some pre-defined value (normally 95%), it must be deemed to have failed at that time point and the shelf-life of the IVD kit should be restricted accordingly.

If regression analysis is used to define the time point at which a panel member would not meet its criterion, then lot-to-lot variability must be included when setting the confidence limits around the regression line. However, real-time data must extend beyond the claimed shelf-life so that the intercept of the regression confidence limit and the expected value must be at a time period longer than the claim. It is usually more appropriate to calculate as discussed in the previous paragraph, particularly if the regression cannot be proven to be linear.

Example 2: In-use stability protocol

Objective

To determine the stability of opened bottles of the specimen buffer used in the IVD kit in real-time when stored at 15–30 °C as proposed in Stability Study Plan XYZ00001.

> *In this example, the manufacturer recommends that the test cassette be used immediately upon opening; this claim should also be validated in a separate experiment, so that it can be confirmed that the IVD will still perform satisfactorily after the test cassette has been removed from its pouch and left at room temperature for 1, 2, 6 and 24 hours, etc., as appropriate.*

Acquire sufficient numbers of IVD kits from one production lot using a predetermined sampling protocol (for example, random, first X number of kits in the first box, every 100th kit, etc.).

Acquire sufficient volume of each panel member for the duration of the testing schedule. Establish a method for randomizing the panel for testing.

In Worksheet XYZ00001 record the following:

- the lot numbers from which the IVD kits were sampled;
- the number of IVD kits sampled from each lot; and
- details (including manufacturing/lot information) for each of the IVD kit components that will be tested as part of this protocol (test cassette and specimen buffer).

Preparation

Two lots of specimen buffer are to be tested. One lot of the component must be freshly made, while the other should be towards the end of the assigned shelf-life of the IVD kit.

The component is to be tested in its final packaging.

The IVD kits are stored so that the reagents are in contact with all elements of the packaging (for example, the bottles in the IVD kits are stored horizontally, lying flat on their sides, allowing liquids to remain in contact with the bottle closures).

Half of each lot will be stored at 30 ± 5 °C, the other half at 15 ± 5 °C. At the start of testing, each bottle will be brought to room temperature (20 ± 2 °C), opened, used for testing and then recapped and returned to the stated storage temperature.

> *Note*: It is important that the components under test are opened and used under circumstances likely to occur in users' laboratories (that is, not in rooms with HEPA-filtered air) thus mimicking, as far as possible, genuine use.

Testing schedule

At each subsequent scheduled time point the allotted number of bottles will be brought to room temperature and used to test each panel member in triplicate. Testing will be conducted at 0, 1, 2, 3, 4 weeks, etc., up to the end of the claimed in-use life.

Documentation

In Worksheet XYZ00001 record the following:

- the lot number of the IVD kit used to conduct the test;
- the operator(s) name(s);
- the dates of testing;
- the temperature at which the IVD kits are stored;
- the ambient temperature during testing;
- identifying details for each member of the panel being tested;
- each test result as a band intensity – band intensity should be scored using the calibrated scale described in Protocol ZXY00001 (for example, 0; faint/trace; +1; +2; +3;…+10);
- each test result as an interpretation according to the IFU;

- any aberrations or deviations from the protocol, the reason for the deviation and any remedial action undertaken.

Panel for testing stability

See the suggestions in **Appendix 2: Suggested specimens for stability testing panels**.

Acceptance criteria

Each panel member should show a band intensity result that matches its expected result at each tested time point. The in-use stability of the sample buffer will be taken as the time point before the last time point to have met the acceptance criteria – for example, if the IVD kit is observed to be stable to 5 weeks, the in-use stability will be deemed to be 4 weeks.

Appendix 2

Suggested specimens for stability testing panels

Examples in this section

Not all of the specimens in the examples that follow will be necessary for all IVDs, and nor is the list exhaustive. Panels must be composed according to strict risk-management principles and all decisions must be documented and traceable.

The minimum set of specimens recommended for inclusion in a testing panel for different types of products are outlined below.

1. Specimens to monitor NAT-based tests

If a proprietary nucleic acid preparation/extraction system is provided, the recovery must be shown to meet claims for each genotype from each of the specimen types claimed (for example, dried blood spots, whole blood and plasma). Successful removal of inhibitory substances, if intended, must be demonstrated for appropriate specimen types. Unless potentially variable biological reagents are involved, this system would be expected to be verified in manufacture and not necessarily tested at release.

Specimens	Remarks
Specimens to demonstrate maintenance of sensitivity and/or limit of detection, and/or accuracy, and precision	Traceability is required to one of the WHO international standards[1] if available – for example, the Third WHO International Standard for HIV1-RNA for NAT-based assays (National Institute for Biological Standards and Control (NIBSC) code 10/152); or the Fourth WHO International Standard for hepatitis C virus RNA for NAT-based assays (NIBSC code 06/102). More than one genotype may be required to validate these claims: see the First WHO International Reference Panel for hepatitis B virus genotypes for NAT-based assays (Paul-Ehrlich-Institut (PEI) code 5086/08). This may be required on each of the claimed specimen types.

[1] The catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/

Table *continued*

Specimens	Remarks
Specimens to demonstrate specificity and validity of runs	Sufficient negative specimens should be included to ensure that the claims will be met at end of shelf-life.
Specimens (or reagents) to demonstrate stability of each of the critical components of the IVD	If more than one part of the genome is to be detected, both systems must be shown to be stable. If both DNA and RNA are measured the complete system must be shown to be stable.

2. Specimens to monitor tests that measure CD4 cells

Rationale

CD4 measurements are quantitative, and accuracy at the clinical decision point is crucial. The design input should have information on the accuracy and other parameters required, and the panel must be designed to provide evidence that these parameters are maintained over the assigned life of the reagent and measuring IVD.

Parameters

The panel used in stability work must be able to demonstrate the following:

- stability of all the antibodies used in the IVD (frequently anti-CD4 and anti-CD3 antibodies; any other critical components must also be covered);
- accuracy and trueness of measurement maintained at the critical level (at least five specimens required);
- claimed linearity over the required range of CD4 count (at least five specimens required); and
- measure drift.

Specimens

Artificial specimens (such as stabilized blood specimens) can be used if a risk assessment based on R&D work indicates that they are effective. Fresh specimens are usually required. Measurements should be compared to an approved reference system.

Examples of approaches

Aged or in-use lots may be compared with a reference – for example, a new lot. Precision studies can be performed as described elsewhere.[2]

3. Specimens to monitor tests for HIV antibodies

Specimens	Remarks
IgM first seroconversion specimens and IgG first seroconversion specimens	Possible approaches to obtain samples: • Study the early data from commercial seroconversion panels where the seroconversion was frequently monitored by IgM and IgG blots. • Study the responses to second and third generation assays or protein A and protein L assays (this approach is less useful).
All other parts of the HIV proteome included – for example, reverse transcriptase (RT)	
Late stage specimens – usually a high-dilution set near the sample-to-cut-off ratio	This might serve to monitor any kit run control. *Note*: HIV serology is not particularly genotype dependent. It is usually not necessary to include controls for genotype detection unless risk assessment or experiment shows that it is required for a particular IVD.
HIV-2, diluted to near the sample-to-cut-off ratio	Seroconversion specimens are very rare.
HIV-1 (O), if claimed	
Difficult specimens to monitor specificity and invalidity rate	100 negatives at release subject to risk analysis and statistical analysis of the allowable (relative to the claimed) false-reactive rate and invalidity rate.

[2] Evaluation of precision of quantitative measurement procedures; approved Guideline EP05-A3. Third edition. Wayne (PA): Clinical and Laboratory Standards Institute; 2014.

4. Specimens to monitor tests for HIV-1/2 and *Treponema pallidum* (TP) antibodies

Specimens	Remarks
Specimens to detect HIV	See the above section 3. Specimens to monitor tests for HIV antibodies.
Specimens to detect all the critical epitopes in the IVD – for example, TpN47, TpN17 and TpN15	*Note*: Each of these epitopes plays a role in detecting syphilis in different stages of the infection. It is necessary to have a panel member to monitor each epitope system present (and possibly each stage of infection) even if poly-fusion proteins are used. This can be avoided if the manufacturer can demonstrate that each epitope system is equally stable.
Specimens able to show that the invalidity and specificity rates do not fall outside the claims, particularly if whole blood is a claimed specimen type	*Note*: It would not be sufficient for WHO prequalification to extrapolate to the stability of HIV-2/TP detection by testing only HIV-1-positive specimens.

5. Specimens to monitor tests for HCV antibodies

Specimens	Remarks
NS3 first seroconversion specimens and core first seroconversion specimens	
Specimens to monitor any other antibodies claimed (frequently against NS5 and NS4)	Results can be obtained from line immunoassays that differentiate antibody responses to the different proteins.
A late-stage dilution near the sample-to-cut-off ratio	*Note*: HCV serology is not particularly genotype dependent in terms of anti-core and anti-NS3, but it is possible to make serotyping assays based on NS4 that mimic genotyping reasonably well. It is usually not necessary to include controls for genotype detection unless risk assessment or experiment for a particular IVD show otherwise.

Table *continued*

Specimens	Remarks
Difficult specimens to monitor specificity and invalidity rate	100 negative specimens subject to risk analysis and statistical analysis of the allowable (relative to the claimed) false-reactive rate and invalidity rate.

6. Specimens to monitor tests for HBsAg

Specimens	Remarks
Specimens to define sensitivity relative to the claim	Traceability is required to one of the WHO international standards[1] – for example, the Third WHO International Standard for hepatitis B virus surface antigen (genotype B4; HBsAg subtypes *ayw1/adw2*); NIBSC code 12/226) for one or more specimens and probably also to the *ad* and *ay* standards available from a commercial supplier. Commercially available seroconversion specimens are almost all of the *adw2* subtype – different from the Third WHO International Standard – so claims of critical threshold specimen detection must be proven by specimens in the panel.
Specimens to monitor the maintenance of the claims for a variety of serotypes/genotypes and mutant forms	These will almost certainly be traceable to the First WHO International Reference Panel for hepatitis B virus genotype for HBsAg assays (PEI code 6100/09).
Specimens to control against prozone/high dose hook effect if found or if theoretically an issue	
If detection of HBsAg in the presence of anti-HBsAg is claimed (current best practice) proof of maintenance of the claim is required	

[3] The catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/

Table *continued*

Specimens	Remarks
Specimens to monitor the critical components of the IVD	If the monoclonal antibodies used have a particular function or bias, such as against the *ayr* or *adr* subtypes (not controlled by the standards) or are used to detect mutant forms of the antigen, then each must be monitored to ensure viability at end of shelf-life. These may be the same specimens as mentioned in the previous paragraphs. If there are critical dissociation chemicals or red-cell capture or rupture agents used then these must also be monitored.
Difficult specimens to monitor specificity and invalidity rate	100 negative specimens subject to risk analysis and statistical analysis of the allowable (relative to the claimed) false-reactive rate and invalidity rate.

Appendix 3

Summary table of standards relevant to stability studies

Recommendation	Comment	Standard
Studies must be compliant with CLSI EP25-A and ISO 23640:2011	The minimum expected standards.	CLSI EP25-A ISO 23640:2011
Studies must be fully documented with risk evaluations, plans and protocols prior to initiation	Risk assessment must be specific to the analyte, type of physical device and assay format, and previous manufacturing experiences, not generic nor by rote.	CLSI EP25-A (many sections) ISO 23640:2011 (section 2) ISO 14971:2007
Studies and risk management must take into consideration the conditions likely to be encountered in the geographical and health-care settings in which the IVD is intended to be used	This is particularly important for transport stress where extreme conditions must be evaluated.	
IVDs must be subjected to simulation of transport stress before being used to establish any form of stability	This is particularly important to WHOPQ as transport will always be involved before use of an IVD, and transport conditions cannot be guaranteed nor predicted.	CLSI EP25-A (section 4.2.3) ISO 23640:2011 (section 5.2)
Transport simulation must cover the extremes of environmental conditions ascertained during risk evaluations	It is most unlikely that actual transport will involve all extreme conditions that might occur during the marketing life of the IVD, or that the conditions during actual transport can be adequately documented.	CLSI EP25-A (section 4.2.3)
IVDs used in any stability studies must be made to finalized manufacturing specifications, to final scale and in the packaging (including labelling) in which the IVDs will be made available	If IVDs are not made to final validated and documented manufacturing scales, stringent proof must be presented that the scale change will not affect any parameters of the IVD, nor any of the manufacturer's claims. Pre-production lots can only be used for stability work if these conditions are met.	Good manufacturing practice (GMP) CLSI EP25-A

Table *continued*

Recommendation	Comment	Standard
If several presentations of the IVD are to be presented, all aspects of stability must be shown for each	If, for example, two pack sizes are to be provided then each pack size must be evaluated completely, even though the contents are identical except for vial size.	CLSI EP25-A
Sufficient numbers of independent lots of the IVD must be evaluated to enable each form of stability to be evaluated in terms of inter-lot variability	"Independent lots" means lots with different production (or manufacturing, purification, etc.) runs of critical reagents (for example, biological reagents prepared in different syntheses, growths or purifications or other risk-defined critical reagents from different manufactured lots or from different suppliers if applicable). CLSI EP25-A and ISO 23640:2011 specify **minimum** numbers of lots to be used but give no guidance to recommended numbers beyond documented risk evaluation.	CLSI EP25-A (section 4.4)
If critical components of the IVD are assigned lives independently of the life of the IVD, the various forms of stability of the IVD must be proven with those reagents at different stages of their lives	It must be documented that stored materials (for example, freeze-thawed biological reagents) operate as expected during the whole of the assigned shelf-life.	CLSI EP25-A (section 4.4)
Each form of stability must be defined statistically with respect to any inter-independent lot variability, not just assigned to the minimum stability found among the lots that happened to be evaluated experimentally	If any lot-to-lot variability is found, the manufacturer must provide evidence that subsequent lots will not have worse stability than that claimed.	

Table *continued*

Recommendation	Comment	Standard
If any control material with a claim to prove the functionality of the IVD is provided to users that claim must be justified in stability studies in addition to any other studies	If the analytic function of the IVD is out of specification from any cause, including stability failure, the control material must be demonstrated to be able to alert the user to that fact.	
Use of accelerated stability, even to provide interim life assignments, must be justified scientifically	Accelerated stability is acceptable in providing interim life if the parameters of the Arrhenius equation, or any other method used, are adequately proven and documented.	CLSI EP25-A (section 7.3 & Appendix B) ISO 23640:2011 (section 5.3.1; notes 1 & 2)

WHO/EMP/RHT/PQT/TGS2/2017.02

The Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment is intended to assist manufacturers in meeting WHO prequalification requirements for their IVD. For further information on this guidance and other TGS documents email: diagnostics@who.int

Annex 6

Biological substances: WHO International Standards, Reference Reagents and Reference Panels

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

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

A list of current WHO International Standards, Reference Reagents and Reference Panels for biological substances is available at: http://www.who.int/biologicals.

At its meeting in October 2017, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list.

[1] 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%202_Inter%20_biol%20ef%20standards%20rev2004.pdf?ua=1, accessed 27 March 2018).

Additions[2]

Preparation	Activity	Status
Biotherapeutics other than blood products		
Parathyroid hormone 1-34 (recombinant, human)	0.914 mg/ampoule 9140 IU/ampoule	Second WHO International Standard
Rituximab	In vitro biological activities: 1000 IU/ampoule (CDC activity) 1000 IU/ampoule (ADCC activity) 1000 IU/ampoule (cell-binding activity) 1000 IU/ampoule (apoptotic activity)	First WHO International Standard
Infliximab	500 IU/ampoule (TNF-neutralizing activity) 500 IU/ampoule (binding activity) 50 µg/ampoule for use in therapeutic drug monitoring	First WHO International Standard
Blood products and related substances		
Activated blood coagulation factor IX (human)	10.5 IU/ampoule	Second WHO International Standard
Blood coagulation factor XII (plasma, human) (via assignment of additional analytes to the current Second WHO International Standard for blood coagulation factor XI)	FXII:C = 0.86 IU/ampoule FXII:Ag = 0.80 IU/ampoule	First WHO International Standard
Activated blood coagulation factor X (human)	6.7 U/ampoule	First WHO Reference Reagent

[2] Unless otherwise indicated, all materials are held and distributed by the National Institute for Biological Standards and Control, Potters Bar, Herts, EN6 3QG, the United Kingdom. Materials identified by an * in the above list are held and distributed by the Paul-Ehrlich-Institut, 63225 Langen, Germany.

Preparation	Activity	Status
In vitro diagnostics		
anti-cytomegalovirus immunoglobulin G	46.4 IU/vial	First WHO International Standard*
Chikungunya virus RNA for NAT-based assays	2.5 x 10^6 IU/ml	First WHO International Standard*
Lupus anti-dsDNA serum	100 U/ampoule	First WHO Reference Reagent
Genomic KRAS codons 12 and 13 mutations	Consensus mutation %; consensus mutant KRAS copy number; and consensus total KRAS copy number provided for KRAS mutations p.G12A; p.G12C; p.G12D; p.G12R; p.G12S; p.G12V; and p.G13D	First WHO Reference Panel
Human herpes virus 6B DNA for NAT-based assays	7.75 $\log_{10}$ IU/ml	First WHO International Standard
Hepatitis A virus RNA for NAT-based assays	4.42 $\log_{10}$ IU/ml	Third WHO International Standard
HIV-1 RNA for NAT-based assays	5.10 $\log_{10}$ IU/ml	Fourth WHO International Standard
Ebola virus antibodies (plasma, human)	1.5 IU/ml	First WHO International Standard
Ebola virus antibodies (plasma, human)	[no assigned units]	First WHO Reference Panel
Plasmodium falciparum antigens	HRP2 = 1000 IU/ampoule pLDH = 1000 IU/ampoule	First WHO International Standard
Vaccines and related substances		
mOPV type 1	7.32 $\log_{10}$ $TCID_{50}$/ml	First WHO International Standard
mOPV type 2	6.74 $\log_{10}$ $TCID_{50}$/ml	First WHO International Standard
mOPV type 3	6.66 $\log_{10}$ $TCID_{50}$/ml	First WHO International Standard

Preparation	Activity	Status
bOPV type 1+3	7.19, 6.36 and 7.32 log10 TCID50/ml for serotypes 1 and 3 and total virus content, respectively	First WHO International Standard
Pertussis toxin	1881 IU/ampoule (histamine sensitization test) 680 IU/ampoule (CHO cell clustering assay)	Second WHO International Standard
Vi polysaccharide of *Citrobacter freundii*	1.94 ± 0.12 mg/ampoule	First WHO International Standard
Vi polysaccharide of *Salmonella* Typhi	2.03 ± 0.10 mg/ampoule	First WHO International Standard
Anti-typhoid capsular Vi polysaccharide immunoglobulin G (human)	100 IU/ampoule	First WHO International Standard
Antiserum to respiratory syncytial virus	1000 IU/vial	First WHO International Standard

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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)

WHO Expert Committee on Biological Standardization
Sixty-second report.
WHO Technical Report Series, No. 979, 2013 (xiii + 366 pages)

WHO Expert Committee on Biological Standardization
Sixty-first report.
WHO Technical Report Series, No. 978, 2013 (xi + 384 pages)

WHO Expert Committee on Biological Standardization
Sixtieth report.
WHO Technical Report Series, No. 977, 2013 (viii + 231 pages)

WHO Expert Committee on Biological Standardization
Fifty-ninth report.
WHO Technical Report Series, No. 964, 2012 (viii + 228 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 substances, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of general issues brought to the attention of the Committee. The next part of the report, of particular relevance to manufacturers and national regulatory authorities, outlines the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, WHO Guidelines on the quality, safety and efficacy of Ebola vaccines, and WHO Guidelines on procedures and data requirements for changes to approved biotherapeutic products were adopted on the recommendation of the Committee. In addition, the following two WHO guidance documents on the WHO prequalification of in vitro diagnostic medical devices were also adopted: (a) Technical Specifications Series (TSS) for WHO Prequalification – Diagnostic Assessment: Human immunodeficiency virus (HIV) rapid diagnostic tests for professional use and/or self-testing; and (b) Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment: Establishing stability of in vitro diagnostic medical devices.

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

A series of annexes are then presented which include an updated list of all WHO Recommendations, Guidelines and other documents on biological substances used in medicine (Annex 1). The above four WHO documents adopted on the advice of the Committee are then published as part of this report (Annexes 2–5). Finally, all additions and discontinuations made during the 2017 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 6. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.

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