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, a WHO guidance document on the Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine was adopted along with WHO Guidelines on procedures and data requirements for changes to approved vaccines and revised WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated).

Subsequent sections of the report provide information on the current status and proposed development of international reference materials in the areas of antibiotics; biotherapeutics other than blood products; blood products and related substances; in vitro diagnostic device reagents; 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) followed by the three WHO documents adopted on the advice of the Committee (Annexes 2–4). All additions and discontinuations made during the 2014 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 5. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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

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SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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 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)

WHO Expert Committee on Biological Standardization
Fifty-eighth report.
WHO Technical Report Series, No. 963, 2011 (viii + 244 pages)

WHO Expert Committee on Biological Standardization
Fifty-seventh report.
WHO Technical Report Series, No. 962, 2011 (viii + 206 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)
WHO Expert Committee on Biological Standardization

Sixty-fifth report

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.
Contents

Abbreviations  xiii

1. Introduction  1

2. General  4

  2.1 Current directions  4
    2.1.1 Strategic directions in biological standardization: WHO priorities  4
    2.1.2 Vaccines and biotherapeutics: recent and planned activities in biological standardization  5
    2.1.3 Therapeutic biological medicines: current developments and challenges  7
    2.1.4 Blood products and related in vitro diagnostics: recent and planned activities in biological standardization  8
    2.1.5 Overview of the international response to the Ebola epidemic, including accelerated development of vaccines and novel therapies  10

  2.2 Reports  11
    2.2.1 Report from the WHO Blood Regulators Network  11
    2.2.2 Report from the WHO collaborating centres for biological standards  13

  2.3 Feedback from custodian laboratories  14
    2.3.1 Developments and scientific issues highlighted by custodians of WHO biological reference preparations  14

  2.4 Cross-cutting activities of other WHO committees and groups  17
    2.4.1 Proposed WHO Guidelines on good review practices  17
    2.4.2 Proposed technical supplements to WHO guidance on the storage and transport of time- and temperature-sensitive pharmaceutical products  18
    2.4.3 Proposed WHO Guidelines on good regulatory practices  18
    2.4.4 Collaborative procedure for facilitating the licensing of WHO-prequalified medicinal products  19
    2.4.5 Update of matters arising from the Expert Group on International Nonproprietary Names  19
    2.4.6 Proposal to revise the procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies  20
    2.4.7 A WHO and EDQM collaborative study on the determination of saccharide content of the Haemophilus influenzae type b component in liquid vaccine presentations  21
    2.4.8 Update on the WHO global action plan to minimize poliovirus facility-associated risk  21

3. International Recommendations, Guidelines and other matters related to the manufacture and quality control of biological substances  23

  3.1 Vaccines and related substances  23
    3.1.1 Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine  23
    3.1.2 Recommendation to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)  24
    3.1.3 Guidelines on procedures and data requirements for changes to approved vaccines  25
    3.1.4 Regulatory written standards pipeline  26
3.1.5 Clinical evaluation of dengue vaccines
3.1.6 Biotherapeutic products including similar biotherapeutic products
3.1.7 Multilateral activities relating to biotherapeutic products including similar
biotherapeutic products
3.2 Blood products and related substances
3.2.1 Strengthening production capacity for blood components including plasma
for fractionation
3.2.2 Shortage of anti-diphtheria and other specific immunoglobulins
3.2.3 MERS coronavirus serum panel
3.2.4 Use of convalescent sera to respond to emerging infectious disease threats
3.2.5 Overview of the biological standards endorsed by the ISTH for WHO approval

4. International reference materials – antibiotics
4.1 WHO International Standards and Reference Reagents – antibiotics
4.1.1 Second WHO International Standard for bleomycin complex A2/B2
4.2 Proposed new projects and updates – antibiotics
4.2.1 Proposed Third WHO International Standard for amphotericin B

5. International reference materials – biotherapeutics other than
blood products
5.1 WHO International Standards and Reference Reagents – biotherapeutics other
than blood products
5.1.1 Third WHO International Standard for luteinizing hormone (human pituitary)
5.1.2 First WHO International Standard for proinsulin (human)
5.2 Proposed new projects and updates – biotherapeutics other than blood products
5.2.1 Proposed First WHO Reference Reagent for Rituximab for use in complement-
dependent cytotoxicity assays
5.2.2 Proposed First WHO Reference Reagent for Batroxobin

6. International reference materials – blood products and
related substances
6.1 WHO International Standards and Reference Reagents –
blood products and related substances
6.1.1 First WHO International Standard for activated blood coagulation factor XI
6.1.2 First WHO Reference Panel for lupus anticoagulant
6.1.3 First WHO International Standard for A Disintegrin And Metalloprotease
with ThromboSpondin type 1 motifs 13 (ADAMTS13)
6.1.4 Fourth WHO International Standard for plasmin
6.2 Proposed new projects and updates – blood products and related substances
6.2.1 Proposed Second WHO International Standard for blood coagulation factor XI
6.2.2 Proposed Second WHO International Standard for activated blood
coaulation factor IX
6.2.3 Proposed second WHO reference reagents for anti-A and anti-B in
intravenous immunoglobulin

device reagents
7.1 WHO International Standards and Reference Reagents – in vitro diagnostic
device reagents
7.1.1 Third WHO International Standard for hepatitis B virus surface antigen
7.1.2 First WHO International Standard for Toxoplasma gondii DNA for NAT-based assays  
7.1.3 First WHO International Standard for hepatitis C virus core antigen  
7.2 Proposed new projects and updates – in vitro diagnostic device reagents  
7.2.1 Proposed First WHO Reference Panel for vCJD  
7.2.2 Proposed first WHO international standards for herpes simplex virus DNA type 1 and 2  
7.2.3 Proposed replacement WHO international standards for prostate-specific antigen (free) and prostate-specific antigen (90:10)  
7.2.4 Proposed First WHO International Standard for anti-Müllerian hormone  
7.2.5 Proposal to assign a holotranscobalamin value to the First WHO International Standard for vitamin B12 and folate in human serum

8. International reference materials – vaccines and related substances  
8.1 WHO International Standards and Reference Reagents – vaccines and related substances  
8.1.1 First WHO Reference Reagent for anti-malaria (Plasmodium falciparum) human serum  
8.1.2 Second WHO International Standard for Haemophilus influenzae type b capsular polysaccharide  
8.1.3 First WHO International Standard for anti-typhoid capsular Vi polysaccharide immunoglobulin G (human)  
8.2 Proposed new projects and updates – vaccines and related substances  
8.2.1 Proposed Second WHO International Standard for Bordetella pertussis toxin  
8.2.2 Proposed Third WHO International Standard for tetanus toxoid for use in flocculation test  
8.2.3 Proposed Seventh WHO International Standard for rabies vaccine  
8.2.4 Proposed First WHO International Standard for meningococcal serogroup X polysaccharide  
8.2.5 Proposed First WHO International Standard for antibody to A(H7N9) influenza virus

Annex 1  
WHO Recommendations, Guidelines and other documents related to the manufacture and quality control of biological substances used in medicine

Annex 2  
Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine

Annex 3  
Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)  

Annex 4  
Guidelines on procedures and data requirements for changes to approved vaccines

Annex 5  
Biological substances: WHO International Standards, Reference Reagents and Reference Panels
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13 to 17 October 2014

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WHO Expert Committee on Biological Standardization
Sixty-fifth report

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Dr T.Q. Zhou, TSN/EMP
Abbreviations

ACT  α1-chymotrypsin
ADAMTS13  A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs 13
AEFI  adverse event following immunization
AMH  anti-Müllerian hormone
APEC  Asia-Pacific Economic Cooperation
BBio  Bilthoven Biologicals B.V.
BGTD  Biologics and Genetic Therapies Directorate
bOPV  bivalent OPV
BRN  WHO Blood Regulators Network
BRP  biological reference preparation
BSE  bovine spongiform encephalopathy
BTP  biotherapeutic product
CBER  Center for Biologics Evaluation and Research
CCID$_{50}$  cell-culture infectious dose 50%
CNS  central nervous system
CTD  common technical document (Ectd, electronic CTD)
cVDPV  circulating vaccine-derived poliovirus
DBSQC  Division of Biological Standards and Quality Control
DCVMN  Developing Countries Vaccine Manufacturers Network
DNA  deoxyribonucleic acid
EDQM  European Directorate for the Quality of Medicines & HealthCare
ELISA  enzyme-linked immunosorbent assay
FRET  fluorescence resonance energy transfer
FIX  factor IX
FIXa  activated factor IX
FXI  factor XI
FXIa  activated factor XI
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>GAP III</td>
<td>WHO global action plan to minimize poliovirus facility-associated risk after eradication of wild polioviruses and cessation of routine OPV use</td>
</tr>
<tr>
<td>GCV</td>
<td>geometric coefficient of variation</td>
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<tr>
<td>GMP</td>
<td>good manufacturing practice</td>
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<tr>
<td>GMT</td>
<td>geometric mean titre</td>
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<tr>
<td>GPEI</td>
<td>Global Polio Eradication Initiative</td>
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<tr>
<td>HBsAg</td>
<td>hepatitis B surface antigen</td>
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<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
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<tr>
<td>HCV</td>
<td>hepatitis C virus</td>
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<tr>
<td>Hib</td>
<td><em>Haemophilus influenzae</em> type b</td>
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<tr>
<td>holoTC</td>
<td>holotranscobalamin</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>herpes simplex virus</td>
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<td>herpes simplex virus type 1</td>
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<tr>
<td>HSV-2</td>
<td>herpes simplex virus type 2</td>
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<td>IBPC</td>
<td>Institute for Biological Product Control</td>
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<tr>
<td>ICDRA</td>
<td>International Conference of Drug Regulatory Authorities</td>
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<tr>
<td>ICH</td>
<td>International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
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<tr>
<td>IFPMA</td>
<td>International Federation of Pharmaceutical Manufacturers &amp; Associations</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
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<td>INN</td>
<td>International Nonproprietary Names</td>
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<td>IPV</td>
<td>inactivated poliomyelitis vaccine</td>
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<td>IQ</td>
<td>installation qualification</td>
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<tr>
<td>IRP</td>
<td>International Reference Preparation</td>
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<tr>
<td>ISA</td>
<td>international standard for antibiotics</td>
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<td>ISTH</td>
<td>International Society on Thrombosis and Haemostasis</td>
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<tr>
<td>IU</td>
<td>International unit(s)</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>IVIG</td>
<td>intravenous immunoglobulin</td>
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<tr>
<td>Lf</td>
<td>limit for flocculation</td>
</tr>
<tr>
<td>LH</td>
<td>luteinizing hormone</td>
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<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
</tr>
<tr>
<td>LMIC</td>
<td>low- and middle-income countries</td>
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<tr>
<td>LOD</td>
<td>limit of detection</td>
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<tr>
<td>MA</td>
<td>marketing authorization</td>
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<tr>
<td>MAPREC</td>
<td>mutant analysis by polymerase chain reaction and restriction enzyme cleavage</td>
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<tr>
<td>MCB</td>
<td>master cell bank</td>
</tr>
<tr>
<td>MEF</td>
<td>Mediterranean Expeditionary Force</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Middle East respiratory syndrome coronavirus</td>
</tr>
<tr>
<td>MFDS</td>
<td>Ministry of Food and Drug Safety</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and Health Products Regulatory Agency</td>
</tr>
<tr>
<td>MSL</td>
<td>master seed lot</td>
</tr>
<tr>
<td>NAT</td>
<td>nucleic acid amplification technique</td>
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<tr>
<td>NCL</td>
<td>national control laboratory</td>
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<tr>
<td>NIBSC</td>
<td>National Institute for Biological Standards and Control</td>
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<tr>
<td>NIFDS</td>
<td>National Institute of Food and Drug Safety Evaluation</td>
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<tr>
<td>NITAG</td>
<td>National Immunization Technical Advisory Group</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
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<tr>
<td>OLSS</td>
<td>Office of Laboratories &amp; Scientific Services</td>
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<tr>
<td>OPV</td>
<td>oral poliomyelitis vaccine</td>
</tr>
<tr>
<td>OQ</td>
<td>operational qualification</td>
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<tr>
<td>PAS</td>
<td>prior approval supplement</td>
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<tr>
<td>PATH</td>
<td>Program for Appropriate Technology in Health</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PCV</td>
<td>porcine circovirus</td>
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<tr>
<td>PEI</td>
<td>Paul-Ehrlich-Institut</td>
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<tr>
<td>PERT</td>
<td>product-enhanced reverse transcriptase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PQ</td>
<td>performance qualification</td>
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<tr>
<td>PSA</td>
<td>prostate-specific antigen</td>
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<td>QC</td>
<td>quality control</td>
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<td>RHSC</td>
<td>Regulatory Harmonization Steering Committee</td>
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<td>RIVM</td>
<td>Rijksinstituut voor Volksgezondheid en Milieu</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>RT</td>
<td>reverse transcriptase</td>
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<tr>
<td>SAGE</td>
<td>Strategic Advisory Group of Experts</td>
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<tr>
<td>SBP</td>
<td>similar biotherapeutic product</td>
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<tr>
<td>sIPV</td>
<td>Sabin-based IPV</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operating procedure</td>
</tr>
<tr>
<td>SRID</td>
<td>single radial immunodiffusion</td>
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<tr>
<td>SSC</td>
<td>Scientific and Standardization Committee (of ISTH)</td>
</tr>
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<td>SSI</td>
<td>Statens Serum Institute</td>
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<tr>
<td>TAL</td>
<td>WHO Technical Assistance and Laboratory Services</td>
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<td>tOPV</td>
<td>trivalent OPV</td>
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<tr>
<td>TSE</td>
<td>transmissible spongiform encephalopathy</td>
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<td>USP</td>
<td>United States Pharmacopeial Convention</td>
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<td>USP–India</td>
<td>United States Pharmacopeial Convention–India</td>
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<tr>
<td>VAPP</td>
<td>vaccine-associated paralytic poliomyelitis</td>
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<tr>
<td>vCJD</td>
<td>variant Creutzfeldt-Jakob disease</td>
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<tr>
<td>Vi</td>
<td>Vi capsular polysaccharide</td>
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<tr>
<td>VLP</td>
<td>virus-like particle</td>
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<tr>
<td>VMS</td>
<td>virus master seed</td>
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<tr>
<td>VWS</td>
<td>virus working seed</td>
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<tr>
<td>WCB</td>
<td>working cell bank</td>
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<tr>
<td>WHOCC</td>
<td>WHO collaborating centre</td>
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<tr>
<td>wIPV</td>
<td>IPV derived from wild-type strains</td>
</tr>
<tr>
<td>WPV</td>
<td>wild poliovirus</td>
</tr>
<tr>
<td>WSL</td>
<td>working seed lot</td>
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1. Introduction

The WHO Expert Committee on Biological Standardization met in Geneva from 13 to 17 October 2014. The meeting was opened by Mr Kees de Joncheere, Director of the Department of Essential Medicines and Health Products (EMP).

Mr de Joncheere welcomed the Committee, meeting participants and observers, and reminded the Committee that it has a mandate to review developments in the field of biological substances that include vaccines, biological therapeutics, blood products and related in vitro diagnostic devices. He pointed out that during its past 64 meetings, the Committee had established approximately 70 written standards and approximately 300 international biological reference preparations essential for the quality control, regulation and clinical dosing of biological products.

Mr de Joncheere then brought attention to the importance of access to safe, quality and affordable medicines and health technologies for public health care systems. Equitable access to medical products is one of the cornerstones of universal health care. The development and adoption of norms and standards to regulate the quality, safety, efficacy and cost-effective use of medical products is a crucial foundation on which this aspiration is built. As part of this, an inclusive standards-development process can facilitate global technical consensus and represents an important step towards convergent regulatory decision-making among countries. The proactive technical support provided by WHO, together with WHO collaborating centres (WHOCCs) and partner organizations, remains key to facilitating the consistent application of standards.

Mr de Joncheere went on to suggest that expectations for reduced regulatory and policy burdens, and for greater transparency, will continue to grow. Although new norms and standards will need to reflect these aspirations, this must not be to such an extent that quality suffers. The convergence of international norms and standards will thus be increasingly recognized as a key driver in addressing the needs for standards-setting expertise and associated resources, which are likely to be insufficient to cover all demands in any one Member State. There is now an opportunity through the appropriate use of WHO norms and standards as the benchmark to achieve the desired global regulatory and policy convergence.

Mr de Joncheere reminded the Committee that the standardization of biological substances was now high on the agenda of Member States. In May 2014, the World Health Assembly had adopted two resolutions of particular relevance to this area, namely: (a) Access to biotherapeutic products including similar biotherapeutic products and ensuring their quality, safety and efficacy; and (b) Regulatory system strengthening for medical products. Many countries recognized that they do not have the regulatory capacity or expertise to evaluate
biotherapeutic products (BTPs) in general and similar biotherapeutic products (SBPs) in particular. WHO will enhance its work with countries to strengthen the capacities of national regulatory authorities (NRAs), quality-control laboratories and national pharmacovigilance centres to regulate the safety, efficacy and quality of the entire spectrum of medical products before authorization for use, and to monitor their safety and use after authorization. Mr de Joncheere pointed out that one example of WHO efforts in this area was the convening of the biennial International Conference of Drug Regulatory Authorities (ICDRA), which had met in August 2014, and the outcomes of which had included recommendations on BTPs.

Mr de Joncheere then drew attention to the Ebola epidemic and to the pressing need for the parallel development, testing, licensure and use of currently experimental interventions. Ebola is considered to be a global public health emergency that requires high-priority responses from all countries. Biological substances are amongst the most promising candidate interventions, and the strong engagement of the Committee, and the support of all experts and organizations represented at this meeting, was of the utmost importance.

Mr de Joncheere pointed out the typically full agenda of the Committee, outlined the organizational aspects of the meeting and moved on to the election of meeting officials. In the absence of dissent, Dr Elwyn Griffiths was elected as Chairman and Dr John Petricciani was elected as Rapporteur for the plenary sessions, and for the track considering vaccines and biotherapeutics. Dr Harvey Klein was elected as Chairman and Dr Anthony Hubbard and Dr Micha Nübling as Rapporteurs for the track considering blood products and in vitro diagnostic device reagents. Dr Klein was also elected as Vice-Chairman for the plenary sessions of the Committee.

Mr de Joncheere expressed his thanks on behalf of WHO to the Committee, to WHOCcS, and to all the experts, institutions and professional societies working in this area whose efforts provide vital support to WHO programmes. He concluded by reminding participants that Committee members acted in their personal capacities as experts and not on behalf of their organizations or countries.

The Secretary to the Committee, Dr David Wood, then presented an overview of the function of WHO Expert Committees, and of the important and greatly valued role they play in providing assistance to Member States. Established by the World Health Assembly or Executive Board, WHO Expert Committees act as official advisory bodies to the Director-General of WHO and were governed by formal rules and procedures. Dr Wood then outlined the proposed meeting agenda and the major issues to be discussed. Declarations of Interests made by four members of the Committee, one Temporary adviser and one participant were presented to the meeting. Following an earlier evaluation, WHO had concluded that none of the declarations made constituted a significant conflict
of interest, and the individuals concerned would be allowed to participate fully in the meeting.

In recognition of the significance of the work of the Committee, Dr Wood announced that the Director-General of WHO would be meeting with the chairs of the Expert Committees on Biological Standardization and on Specifications for Pharmaceutical Preparations, and the chair of the Expert Group on International Nonproprietary Names.

Following participant introductions, the Committee adopted the proposed agenda (WHO/BS/2014.2250).
2. General

2.1 Current directions

2.1.1 Strategic directions in biological standardization: WHO priorities

Dr Wood outlined the three major components of the current WHO norms and standards paradigm, namely: (a) the production of global written standards; (b) the development of global measurement standards; and (c) the conducting of regulatory science in areas such as assay standardization, the further development and refinement of quality-control tests, and establishing the scientific basis for setting specifications. In recent years, a series of workshops to support the implementation of standards had been held and found to be extremely valuable by participants.

Key strategic public health drivers for WHO activities in this area include the need to respond to public health emergencies (such as Ebola and polio outbreaks), ensure access to BTPs and strengthen regulatory systems. These issues had been the subject of detailed discussion at the 2014 ICDRA meeting and a number of corresponding recommendations had been made. Other recommendations from ICDRA included providing assistance on capacity-building for national blood systems, and collaborating in efforts to identify, monitor and prevent or better manage medicines shortages. It was also recommended that WHO should become more involved with advanced therapies, such as cell and gene therapies, and with the associated clinical trials.

Dr Wood then outlined opportunities for the WHO Technologies Norms and Standards group to review its focus and priorities for 2015–2020, taking into account the above recommendations. Intended approaches include: (a) strengthening the WHO norms and standards portfolio in several key respects (including in the area of high-risk in vitro diagnostics; (b) strengthening the provision of technical support in implementing WHO norms and standards; and (c) encouraging the use of WHO norms and standards to shape markets for products of public health importance.

The Committee supported the future focus and priority areas presented for WHO activities during 2015–2020. It was highlighted, however, that the proposed developments would have unavoidable resource implications which would need to be addressed if the strategy were to succeed. One recurring theme for a number of the proposed WHO activities was the crucial importance of the enhanced utilization of networks of existing key stakeholders. Recent improvements in networking activities in areas such as vaccine and biotherapeutics development and production now needed to be replicated and enhanced in the other strategic WHO activity areas outlined.

During subsequent discussion, attention turned to the precise role and remit of the Committee, and to its scope of work, work process and priority-
setting approach in the context of increasing requests for support in areas that were currently not part of the Committee agenda. Concern was expressed, for example, that the Committee was increasingly being asked to address general issues of capacity building in Member States. Such issues are not well aligned with the role of the Committee in biological standardization, and might be addressed elsewhere within WHO. Following consideration it was agreed that a subgroup of the Committee should be established to review the best approach for responding to requests from Member States in the most efficient manner.

Dr Wood concluded by drawing attention to the fact that 2014 marked the 60th year that the National Institute for Biological Standards and Control (NIBSC) had been a WHOCC, during which period it had contributed greatly to the field of biological standardization, and to public health in general. The Committee acknowledged the important role that NIBSC had played, and expressed its thanks for its continuous efforts and contributions over such an extended period.

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

Dr Ivana Knezevic outlined WHO activities in the areas of the standardization and regulatory evaluation of vaccines and biotherapeutics, while also highlighting a number of key strategic issues.

In relation to standardization and regulatory evaluation, three measurement standards for vaccines had been prepared for consideration by the Committee in 2014, five were under development for presentation to the Committee in 2015 (or 2016) and three were planned for consideration by the Committee in 2016. In the area of biotherapeutics, two measurement standards had been prepared for consideration by the Committee in 2014, two were under development for presentation to the Committee in 2015 and three were planned for consideration by the Committee in 2016.

As outlined below in sections 3.1.1–3.1.4, one WHO Recommendations document, one WHO Guidelines document and one WHO guidance document had been prepared for consideration by the Committee in 2014, four documents were under development for presentation to the Committee in 2015 and two were planned for consideration by the Committee in 2016, including revised WHO Guidelines on the clinical evaluation of vaccines. A further eight documents were in the early stages of consideration with as yet unclear timelines for completion. During the period 2005–2013, a total of 20 WHO Guidelines or Recommendations for specific vaccines had been adopted by the Committee and published, along with numerous WHO guidance documents covering a broad range of standardization activity areas.

Seven implementation activities related to standards had been carried out during 2013–2014, two were planned for 2015 and one for 2016. Dr Knezevic
highlighted the publication of implementation workshop reports and the importance of illustrative case studies as a key element in promoting the application of WHO guiding principles. The development of E-learning tools was also under way in a number of key subject areas and consideration was being given to their development and use for other topics.

Dr Knezevic then discussed a range of strategic issues, including the two recent World Health Assembly resolutions outlined above, the scope of work and priority setting, survey feedback from key stakeholders, improving synergies in vaccine standardization through the network of WHOCCs for the standardization and evaluation of vaccines, and the provision of support to global, regional and inter-country networks. With specific reference to the 2014 World Health Assembly, resolution WHA67.21 requested WHO:

...to convene the WHO Expert Committee on Biological Standardization to update the 2009 guidelines [on SBPs], taking into account the technological advances for the characterization of BTPs and considering national regulatory needs and capacities, and to report on the update to the Executive Board.

Progress made in the implementation of this resolution will be reported, through the Executive Board, to the Sixty-ninth World Health Assembly in 2015. In addition, resolution WHA67.20 on regulatory system strengthening requested WHO:

...to increase support and guidance for strengthening the capacity to regulate increasingly complex biological products ... and, where appropriate, on new medicines for human use based on gene therapy, somatic-cell therapy and tissue engineering.

Dr Knezevic went on to report that stakeholder feedback in relation to the strategic aims of WHO had, in general, been constructive and positive, with the recent expansion of the scope of work having been well received.

During subsequent discussion, a range of specific issues was discussed including the vital importance of WHO support to regulatory, manufacturing and other networks, the crucial role of the implementation workshops, and the need to harmonize efforts to replace the use of animal models in the quality control of biological substances, including through the application of the 3R principles (Refine, Reduce, Replace). Further consideration was then given to the development of guidance, and suggestions were provided on how best to proceed. The Committee agreed that in all the activity areas outlined, the primary determining factor of progress would be the availability of resources, including in some cases of additional resources, and requested that it be kept updated of developments and progress.
2.1.3 **Therapeutic biological medicines: current developments and challenges**

Given the activities under way worldwide to develop SBPs, there is a crucial need to consider the current and future requirement for WHO standards and reference materials for therapeutic biological substances. At present, the need for standards is assessed primarily according to medical importance. In the past, the activities of biological agents with therapeutic potential were usually defined by and traced to WHO biological standards before the development of drug products. For “traditional” agents such as insulin, WHO standards with defined unitages were established even before the structure of the active principle was known. Even where the discovery and identification of the active principle was based on molecular cloning and recombinant expression, WHO bioassay standards were usually established at the time of identification of the active principle. Both biological substances isolated from tissues and the first wave of licensed recombinant biological substances had natural sequences, and as a consequence the determination of biological activity was carried out with reference to WHO international biological standards which already existed.

More recently, however, the biotechnology used to develop drugs has moved into a new phase in which the biological substances being developed are not naturally existing molecules. These substances include monoclonal antibodies, sequence or other structural variants of natural proteins, pegylated or other secondarily derived structures, and hybrid or fusion proteins. The development and licensing process for such drugs is undertaken in the absence of an international standard for the product being developed. As products approach the end of patent protection, the possibility of various SBPs being developed arises, along with a corresponding need for international standards. In relation to such developments, it will be important to emphasize the different functions and non-overlapping roles of the comparator product and the bioassay standard.

Derivatized and fusion proteins pose specific challenges for standardization, and selecting the appropriate material is not always obvious. The reluctance of some innovator companies to support standardization programmes through the donation of candidate materials has also become a significant issue, and may be based upon a misunderstanding of the intended use of the international standard.

The provision of standards for physicochemical methods has been primarily a pharmacopoeial activity. However, recent developments have highlighted a need for WHO to increasingly become collaboratively involved with partner pharmacopoeias and, for example, to consider the undertaking of joint standardization activities and projects, for which there has been some degree of precedence already. This would require the development of guidelines and principles for the conducting of such projects. Other areas in which the importance of standards and reference materials is increasingly being recognized include the measurement of clinical efficacy or adverse drug reactions, and the
identification of so-called bad batches of products. Reference materials to assess method performance (“performance indicators”) have also been valuable in limited instances in the past, and are becoming increasingly necessary.

Historically, WHO international standards have typically been value-assigned in International Units (IU) with no formal equivalence in milligrams or formal ampoule content in milligrams being stated. However, the determination of content in milligrams is increasingly becoming an important component in the quality control of biological substances, and the purpose of assigning IU values for at least some standards should be reconsidered.

The Committee agreed that these are important and complex issues, and should be discussed more fully with various stakeholders in the near future. In addition to the points outlined above, consideration should also be given to developing an overall standards-development policy and prioritization strategy. The Committee also agreed that a reasonable starting point would be for WHOCCs involved in the development of reference materials to formulate a plan of action for considering these issues, and to recommend specific follow-up steps. For example, a series of WHO-coordinated focused meetings could be organized to address a range of issues. Such meetings would need to involve multiple stakeholders such as pharmacopoeias, industry and representatives of other WHO committees with an interest in this field. It would also be necessary to take into account the views of developing countries in which SBPs are now being, or will be, produced.

The Committee expressed its interest in being kept informed of progress in this area and looked forward to a report at its next meeting.

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

Dr Ana Padilla outlined a number of activities undertaken during 2014 in the following four areas:

- the current shortage of supplies of specific life-saving human and equine blood-derived immunoglobulins listed in the WHO Model List of Essential Medicines;
- posting on the WHO web site of urgently requested WHO Blood Regulators Network (BRN) position papers (see sections 2.2.1 and 3.2.4 below) on: (a) Collection and use of convalescent plasma or serum as an element in Middle East Respiratory Syndrome Coronavirus response; and (b) Collection and use of convalescent plasma or serum as an element in filovirus outbreak response;
- improving access to safe blood products through the local production of quality plasma in blood establishments;
convening of ICDRA workshops in 2014 on: (a) regulatory models for minimizing risks in blood and blood products; and (b) the current status and potential future directions of advanced therapies regulation.

In relation to the shortage of life-saving immunoglobulins Dr Padilla drew the attention of the Committee to the combination of factors that had resulted in the present crisis and outlined a range of short-, mid- and long-term actions that had been identified by WHO through internal discussions. In the short term, the mapping of national stockpiles and of the quality-assurance approaches used was an agreed-upon key step. Potential longer-term approaches included exploring, with potential donors including the Gavi Alliance and countries holding national stockpiles, the possibility of financing a prequalification process and creation of a WHO stockpile.

Dr Padilla then updated the Committee on the progress made during 2014 by the WHO project to improve access to safe blood products through the local production of quality plasma in blood establishments in low- and middle-income countries (LMICs) (see section 3.2.1). The overall aims of this project, for which Indonesia had previously been selected as a pilot country for implementation, included the optimizing of blood donation use and reducing plasma wastage as part of helping countries to use their own plasma for the manufacture of essential plasma derivatives. Another main objective was to help build technical and regulatory capacity to ensure the maintaining of quality standards in the production of blood components and plasma for fractionation in blood establishments. Dr Padilla outlined the rationale and objectives of two WHO training workshops held in Indonesia in 2014 on: (a) blood-testing technologies and risk assessment; and (b) enforcement and implementation of good manufacturing practice (GMP) in blood establishments.

In relation to the 2014 ICDRA workshops, Dr Padilla highlighted the detailed range of recommendations which had emerged in the two areas of regulatory models for minimizing risks in blood and blood products, and the current status and potential future directions of advanced therapies regulation.

Dr Padilla concluded by highlighting the marked increase in the number of WHO biological reference preparations established in this area in recent years, and set out a number of next steps in furthering the response of WHO to the 2010 resolution WHA63.12 on the Availability, safety and quality of blood products. These would include the development of new guidance documents and tools on residual risk in blood components, and on the preparation and calibration of secondary reference materials for the regulation of in vitro diagnostic devices, particularly those for use in high-risk contexts such as blood safety. In addition, increased capacity-building efforts would be made in line with the pilot project under way in Indonesia, including in relation to the implementation and regulatory oversight of blood-safety measures and production systems.
2.1.5 Overview of the international response to the Ebola epidemic, including accelerated development of vaccines and novel therapies

Dr Wood presented an overview of the current Ebola epidemic, and recent information on its epidemiology. Approximately 700 cases per week had been reported in West Africa during the first 3 weeks of September 2014, with a total of 401 cases (resulting in 232 deaths) having been reported among health care workers as of 5 October 2014. Given that the primarily affected countries were Guinea, Liberia and Sierra Leone, a ministerial meeting was convened by WHO in July 2014 to accelerate actions on Ebola-related disease in West Africa.

Dr Wood then presented an overview of the major WHO actions taken in response to the epidemic, which had included a consultation held in early September 2014 on potential Ebola prevention and treatment approaches based upon whole blood therapies and convalescent plasma, candidate vaccines and novel therapeutic drugs. The significance of the epidemic was further highlighted by the convening of the International Health Regulations Emergency Committee in August 2014 and of a United Nations Security Council Emergency Session on Ebola in September 2014, and the establishment of a United Nations mission headquartered in Accra, Ghana.

In terms of the potential prevention and treatment approaches, there was broad consensus that the use of whole blood therapies and convalescent blood sera was an area that warranted priority consideration. Dr Wood informed the Committee that the BRN had prepared a position paper on the collection and use of convalescent plasma or serum as part of the response to the Ebola epidemic (see sections 2.2.1 and 3.2.4 below), and that WHO had developed a subsequent interim guidance document on the empirical use of convalescent whole blood or plasma. After highlighting the broad range of guidance provided in the interim document in areas such as donor selection, and the screening, handling and transfusion of donated blood and plasma, Dr Wood noted the very high degree of cooperation that had already taken place between numerous stakeholders, including in the effective and open sharing of information. Dr Wood at the same time stressed the importance of ensuring that efforts in this area did not interfere with other ongoing interventions, and emphasized the importance of effective social-engagement and communications activities in overcoming potential obstacles to the implementation of such an approach. Dr Wood updated the Committee on the progress made in developing strategies to recruit

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convalescent patients as donors, in identifying the infrastructural requirements needed to support plasma collection and in the designing and implementing of clinical studies. To support such studies, consortia of investigators had been formed involving collaborators worldwide, including investigators from the three most affected countries of Guinea, Liberia and Sierra Leone.

The Committee was also informed that the two candidate vaccines currently under clinical evaluation had been selected on the basis of protection in non-human primates following lethal challenge, and the availability of GMP-grade vaccine. Their safety in humans was now being assessed. About 15,000 doses of one of the vaccines should be available by the end of 2014, along with at least 1,500 doses of the other. An overview of the respective Phase I studies was provided, with safety and immunogenicity data expected to be available in early 2015. Phase III clinical trials in highly exposed populations may be initiated in early 2015 should Phase I data prove supportive. A general near-term development plan on pre-exposure use of vaccines in both non-affected and affected areas was then presented. The feasibility and design of such trials were currently under discussion. The GAVI Alliance is considering the potential role it could play in accelerating Ebola vaccine development. In addition to vaccines, there are also at least eight experimental therapies under consideration, including those based upon the use of monoclonal antibodies.

Dr Wood then presented a range of ICDRA recommendations to WHO and WHO Member States on the development and evaluation of potential treatments, and emphasized that additional standards would be needed to calibrate antibody levels in Ebola survivors, convalescent blood donations, convalescent plasma and vaccine recipients, as well as to calibrate Ebola viral load.

The Committee noted the report and recognized the complexity of the Ebola epidemic, and the urgent need for interventions ranging from basic biohazard-related precautions to vaccines, convalescent whole blood or plasma, and therapeutic drugs. The Committee endorsed the preparation of reference materials to calibrate: (a) anti-Ebola antibody levels; and (b) Ebola viral load. It was further recognized by the Committee that regulatory preparedness will be vital in ensuring rapid access to potentially safe and effective vaccines. The Committee endorsed the preparation of WHO Guidelines on the regulatory evaluation of products intended to be used in public health emergencies, such as Ebola virus disease.

2.2 Reports

2.2.1 Report from the WHO Blood Regulators Network

Dr Christian Schäerer updated the Committee on the activities of the BRN during 2013–2014, and began by reminding the Committee of the objectives of the BRN, namely:
WHO Expert Committee on Biological Standardization   Sixty-fifth report

- to identify issues and share expertise and information;
- to promote the science-based convergence of regulatory policy, including by fostering the development of international consensus on regulatory approaches;
- to propose solutions to specific issues, especially emerging public health challenges such as the vulnerability of countries to communicable disease threats.

As of October 2014, a wide range of regulatory agencies from Australia, Canada, France, Germany, Japan, Switzerland and the USA were BRN members. Additional organizational issues highlighted included the assuming of the BRN Chair by Swissmedic in October 2013, and the unsuccessful application, following an evaluation process, for BRN membership made by the Korean Ministry of Food and Drug Safety.

Following on from its previous face-to-face meeting on 24 October 2013, the BRN had held eight teleconferences with a face-to-face meeting scheduled during the current session of the Committee.

During 2013–2014, BRN activities had included the preparation and review of the 2013 BRN workplan and discussions on a wide range of blood regulatory and safety topics. In addition, in response to urgent requests from WHO, BRN position papers had been developed and posted on the WHO web site2 on: (a) Collection and use of convalescent plasma or serum as an element in Middle East Respiratory Syndrome Coronavirus response; and (b) Collection and use of convalescent plasma or serum as an element in filovirus outbreak response. Since the posting of the latter on the WHO web site in August 2014, a range of BRN work products and other inputs had also been provided covering specific aspects of the collection and use of convalescent plasma as an empirical treatment during Ebola outbreaks.

Following on from its review and discussion of the scientific basis for donor exclusion for men who have sex with other men, a paper prepared with BRN involvement had now been published,3 setting out the collective views of a representative NRA working group.

After outlining BRN inputs into a number of key workshops held in 2014, Dr Schaerer concluded by highlighting the range of topics that had been identified for discussion by BRN members during the current session of the Committee.

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2 http://www.who.int/bloodproducts/brn/en/
2.2.2 **Report from the WHO collaborating centres for biological standards**

An overview was presented by Dr Stephen Inglis of the network of WHO collaborating centres (WHOCCs) for the standardization and evaluation of vaccines. This network currently consisted of eight WHOCCs:

- National Institute for Biological Standards and Control (NIBSC), Medicines and Healthcare Products Regulatory Agency, Potters Bar, England;
- Center for Biologics Evaluation and Research (CBER), Food and Drug Administration, Silver Spring, MD, USA;
- Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases (NIID), Tokyo, Japan;
- Immunobiology and Biochemistry Group, Office of Laboratories & Scientific Services (OLSS), Therapeutic Goods Administration, Woden, Australia;
- National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety (MFDS), Chungcheongbuk-do, Republic of Korea;
- Biologics and Genetic Therapies Directorate (BGTD), Health Canada, Ottawa, Canada;
- Institute for Biological Product Control (IBPC) of the National Institutes for Food and Drug Control (NIFDC), Beijing, China;
- Division of Virology, Paul-Ehrlich-Institut (PEI), Langen, Germany.

The first meeting of the network was held in April 2012, with the second hosted by PEI on 17–19 March 2014. The main focus of the latter meeting was on regulatory science and the role of the network. In that context, a specific emphasis was placed on collaborative projects with added value and public health importance. Despite acknowledged resource and capability restrictions, the need for a common vision for success for the next 5 years was recognized along with a need to define the criteria for success. Representatives from seven of the WHOCCs attended along with WHO and other organization representatives. Sessions were co-chaired by WHOCC partnerships. It was agreed that the degree of success of the network would be assessed retrospectively after 5 years to evaluate if more had been achieved collaboratively than would have been achieved individually by each WHOCC. The maximizing of efficacy was identified as a clear objective of the collaborative approach.

A number of priority areas were suggested by the WHOCCs and other stakeholders. Following vigorous discussion it was agreed that some projects would clearly be beyond the resources or remit of the group, including direct involvement in national licensing processes. It was further agreed that network
activities should focus on a small number of key priorities that would not in any way hinder ongoing activities of individual WHOCCs. Three key priorities were identified: (a) improving the implementation of WHO standards; (b) making the process of developing standards as efficient as possible; and (c) identifying gaps where standards are needed.

A number of next steps were identified, which included setting up working groups to address:

- the development of agreed network approaches and priorities
- the assignment of responsibilities
- the setting of goals and timelines.

Discussions centred on the recognized importance of harmonizing the activities of the network with the existing priorities and workplan of WHO. The Committee welcomed the presentation given by Dr Inglis and requested that it be kept updated of the progress of the network.

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, England

Dr Inglis reminded the Committee that in 2013 NIBSC had successfully become part of the Medicines and Health Products Regulatory Agency (MHRA), with its location remaining unchanged. Furthermore, NIBSC had established a new division to focus on advanced therapies such as cell and gene therapy, and strong collaboration with WHO was envisaged.

During 2013 and 2014 the volume of distributed standards had remained typical at approximately 29,000 and 28,000 ampoules, respectively. Over the same period, five replacement standards were established, and 16 such projects were ongoing. Two new standards had also been established, with 36 such projects ongoing. In addition to its work on standards, NIBSC had also made contributions in the areas of guideline development and provision of training.

Dr Inglis suggested that the time is now right for a refreshed WHO strategy for the development of measurement standards and proposed that a series of meetings be held starting in 2015 at NIBSC, with SBPs as the focus. The outcomes of these meetings would then be reported to the Committee.

Dr Inglis also discussed genomics as an important emerging tool for diagnosis and treatment decisions, and advised that this is an area that required
close monitoring of where physical reference materials may be required. He concluded with comments on the implications of the recently ratified Nagoya protocol on the timely availability of seasonal influenza samples for the development of vaccines.

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

Dr Karl-Heinz Buchheit outlined a range of recent EDQM activity areas in biological standardization, including the European Pharmacopoeia, international standards for antibiotics (ISAs), the Official Medicines Control Laboratory network, the European Committee on Blood Transfusion, and the biological standardization programme. In several of these undertakings it was noted that WHO has Observer status.

Dr Buchheit then reminded the Committee that EDQM is the custodian centre for ISAs – a responsibility that had been taken over from NIBSC in 2006. One proposed ISA replacement and one request for the establishment of another would be considered by the Committee during the current meeting. As with other international standards, ISAs required distribution, replacement as needed and the conducting of international collaborative studies. Dr Buchheit also drew attention to the 2014 World Health Assembly resolution (WHA67.25) which emphasized the importance of effective antimicrobial agents, and which requested WHO to ensure uninterrupted access to them and other essential medicines. Towards that aim, Dr Buchheit reaffirmed the willingness of EDQM to continue in its role as the custodian centre for ISAs.

Dr Buchheit then went on to outline the goals of the biological standardization programme which included: (a) the establishment of European Pharmacopoeia biological reference preparations; (b) the standardization of test methods for the quality control of biological substances; (c) the elaboration of alternative methods in support of the 3Rs concept (Refine, Reduce, Replace) to minimize the use of animals in research; and (d) the provision of support to international harmonization efforts, including through collaboration with WHO and non-European partners. Programme achievements to date included the initiation or conclusion of 139 projects on reference standards and method development (including 21 projects on the 3Rs concept). Projects of potential interest to the Committee included the development and evaluation of alternative in vitro tests for both pertussis toxin and pertussis vaccine, and of a standardized in vitro assay for hepatitis A vaccine. A number of other new and ongoing standardization projects of potential interest to the Committee were outlined.

Dr Buchheit reiterated that the development of alternatives to animal experiments remained a major EDQM commitment in line with European Union directives. WHO was requested to consider incorporation of the 3R initiative into its written standards and other guidance, where appropriate. The inclusion
of 3R methods in WHO guidelines was viewed by EDQM as being of paramount importance in promoting their global acceptance.

Dr Buchheit concluded by highlighting a number of key harmonization and other implementation issues for regional standard-setting bodies when no international standard or other WHO guidance was available. In the absence of such guidance there was a potential risk of differences emerging in the direction or rate of implementation of approaches, including differences in the speed of implementation of the 3R approach. Over time, it appeared that the process for establishing standards was becoming increasingly difficult due to a range of shipment, procurement and other factors. The use of international standards and reference reagents in the veterinary field was also highlighted as a specific issue requiring clarification.

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

Dr Klaus Cichutek reviewed the activities of both of the PEI WHOCCs in their respective activity areas. Selected ongoing activities of the WHOCC for Quality Assurance of Blood Products and In Vitro Diagnostic Devices included the development of a hepatitis C standard for core antigen, and a genotype panel for hepatitis E virus RNA for nucleic acid amplification technique (NAT)-based assays, along with follow-up studies on the current international standards for hepatitis D RNA and mycoplasma DNA. Studies were also being conducted on the proposed enlargement of the First WHO International Reference Repository for platelet transfusion relevant bacterial strains. Ongoing collaborative work in this area included participation in the BRN, contributing to the development of WHO technical documents and provision of support to the European Commission-funded WHO project on improving access to safe blood products through local production and technology transfer in blood establishments.

Recent activities of the PEI WHOCC for the standardization and evaluation of vaccines included participation in the activities of the WHO Strategic Advisory Group of Experts, supporting the Developing Country Vaccine Regulators’ Network and the provision of training and technical assistance in a range of relevant areas.

A number of PEI activities related to the following scientific issues were then highlighted by Dr Cichutek: (a) development of vaccines and treatments for Ebola; (b) epidemiological study of narcolepsy; (c) methods, particularly novel methods, for product testing; (d) illegal and falsified medicines; and (e) research activities. Dr Cichutek concluded by outlining the detailed recommendations of an ICDRA workshop on current trends in regulating blood and cell therapies.

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

Dr Jay Epstein reported on the successful completion of the third year of the CBER-WHO Cooperative Agreement to enhance regulatory capacity to support
influenza vaccine introduction in low-middle income countries. Funding had specifically been used to support NRA assessments and staff training, and an international proficiency study of the single radial immunodiffusion (SRID) assay. Under another cooperative agreement, CBER was also supporting the strengthening of global pharmacovigilance capacities for vaccines.

After outlining the working approach of the Division of Biological Standards and Quality Control (DBSQC), Dr Epstein informed the Committee of a wide range of ongoing or proposed activities in the further development of potency standards, reference preparations, international standards, reference panels and reagents. Highlighted activities included work on facilitating influenza vaccine manufacturing and the development of a factor Xla-calibrated thrombin generation test as a quality control for immunoglobulins. In the area of reference development, a broad range of completed and upcoming activities included the proposed development of a variant Creutzfeldt-Jakob disease (vCJD) reference panel in non-human primate blood. Dr Epstein then outlined the range of ongoing United States Food and Drug Administration activities undertaken in collaboration with multiple organizations in response to the Ebola epidemic.

Dr Epstein concluded by highlighting a number of recent and planned workshops sponsored by CBER which focused upon the successful implementation of regulatory policy and the importance of regulatory science research.

2.4 Cross-cutting activities of other WHO committees and groups

2.4.1 Proposed WHO Guidelines on good review practices

At its previous meeting in 2013, the Committee had been informed of a partnership between WHO and the Asia-Pacific Economic Cooperation (APEC) Regulatory Harmonization Steering Committee (RHSC) in the development of a draft document that was intended to evolve into WHO Guidelines on good review practices. The Committee had agreed that this would be a useful resource.

Consequently, in February 2014, a draft document was completed and endorsed by the RHSC for formal submission to WHO. Following its initial acceptance by WHO, this draft had been subjected to a process of parallel consultation by both this Committee and by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The Committee was informed that the document focused primarily on the provision of high-level guidance on the principles, processes and other elements of good regulatory review practice, and would be applicable to both drugs (including biological substances) and higher-risk medical devices. As one building block in a set of tools, the document was intended to help regulatory authorities to review safety, efficacy and quality data for medical product applications filed with regulatory authorities for marketing authorization (MA).
The Committee noted the document and agreed that a decision on its adoption be deferred to the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The latter subsequently agreed to the adoption of the Guidelines.4

2.4.2  **Proposed technical supplements to WHO guidance on the storage and transport of time- and temperature-sensitive pharmaceutical products**

The WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products was developed in consultation with the WHO Task Force on Regulatory Oversight on Pharmaceutical Cold Chain Management and was published in 2011 as Annex 9 of the forty-fifth report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The intention was that the guidance should be directly applicable in both developed and less developed countries since experience with vaccine supply-chain assessments in the latter had demonstrated that the mandatory standards set out could be achieved, with some countries also being capable of meeting many of the optional requirements.

As previously reported to the Committee, a WHO Secretariat had worked with a number of experts in order to develop a set of 18 technical supplements, each following the same structural format. The intended purpose of these supplements was to provide the technical detail in each of the subject areas necessary for achieving the standards set out in the main guidance document.

In line with previous meeting recommendations, and following external expert inputs, these supplements had now been subjected to an editorial review process. Prior to their finalization, publication and distribution to regulatory agencies, ministries of health, relevant international organizations, public and private pharmaceutical industry sectors, and supply-chain professionals, final drafts were prepared for consideration by both this Committee and by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The Committee noted the draft supplements and agreed that a decision on their adoption be deferred to the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The latter subsequently agreed to the adoption of the technical supplements.4

2.4.3  **Proposed WHO Guidelines on good regulatory practices**

The Committee was informed that the proposal to develop WHO Guidelines on good regulatory practices emerged following a series of ICDRA recommendations

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made in 2010. Three workshops had then been planned to discuss the required conceptual basis and proposed content. In 2014, the first of these workshops was held and recommendations made on the nature, scope and target audience of the proposed resource. As part of the process of developing a draft outline, a framework of key points to consider was also developed and this framework was presented to the Committee for its views.

Following discussion and clarification of the overall aims and intended benefits of the proposed Guidelines, agreement was reached on the concept in principle. The Committee expressed its interest in being informed of further progress and looked forward to reviewing the draft document in due course.

2.4.4 **Collaborative procedure for facilitating the licensing of WHO-prequalified medicinal products**

The Committee was provided with an overview of the collaborative procedure for the assessment and accelerated national registration of WHO-prequalified pharmaceutical products and vaccines. The strategic priorities of the prequalification system were described along with the basic elements of accelerated registration of WHO-prequalified products.

The Committee was informed that the procedure had now been revised and published on the WHO web site, and was currently undergoing evaluation. The revised procedure (*Collaborative procedure between the World Health Organization Prequalification of Medicines Programme and national medicines regulatory authorities in the assessment and accelerated national registration of WHO-prequalified pharmaceutical products and vaccines*) incorporated key aspects of the previous procedure used for the expedited review of imported prequalified vaccines for use in national immunization programmes. In 2010, this procedure had successfully been used to facilitate the licensing of meningococcal A conjugate vaccine for use in 26 countries of the African meningitis belt. This facilitated procedure incorporated the sharing of assessment reports, laboratory-testing results and inspection reports, which were all components of the revised procedure.

It was expected that submission of the revised procedure to the Committee for endorsement would occur in 2015. The Committee agreed that the revised document would be useful and looked forward to an update in 2015.

2.4.5 **Update of matters arising from the Expert Group on International Nonproprietary Names**

The Committee had been informed in 2013 that discussions on an International Nonproprietary Names (INN) proposal for SBPs had taken place, and that there was no consensus on creating nomenclature qualifiers to distinguish between one SBP and another and between the SBP and its reference product. Given the
mandate of the WHO INN programme to ensure the clear identification of all pharmaceutical substances, both chemical and biological, WHO had been asked to develop a system for preventing the assignment of non-unified qualifiers to SBPs by individual regulatory bodies.

The Committee heard that following INN Expert Group review of the range of issues to be addressed, work had begun on the development of a Biological Qualifier scheme that would be voluntary and distinct from the existing INN programme. Two important elements in the proposed scheme were the assignment of a unique code specific to the site of manufacture, and the centralized and secure hosting of the resulting database by WHO. A draft scheme had been adopted in April 2014 which, following its circulation, had generated a large response. The development of a revised draft was now planned for 2015, at which point additional public comment will be sought.

The Committee was informed that more than 45% of applications for new international nonproprietary names in 2014 had been for biological products, and that monoclonal antibodies are the largest class of biological substances considered by the INN Expert Group. The Committee was reminded that a system is already in place for establishing new international nonproprietary names for monoclonal antibodies, and that it appeared to be working satisfactorily.

The Committee was further informed that the establishment of a nomenclature for cell therapies and novel vaccines comprised of biological substances such as mRNA, peptides and proteins was being considered by the INN Expert Group. The Committee was invited to express its views on these potential initiatives. Following discussion, and taking into consideration that a key element in accepting a product for naming in the INN system is its uniformity, the Committee considered that the fields of cell therapy and novel vaccines were in an early stage of development, and that it was too early to begin formulating an INN naming system for them.

2.4.6 Proposal to revise the procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies

The Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies had been adopted by the Committee in 2010 (Annex 6, WHO Technical Report Series, No. 978). In its original wording, section 3.4 of the procedure on the initial testing of vaccine samples prohibits the manufacturer from being informed of where the testing is performed while also discouraging the use of the national control laboratory (NCL) of the producing country.

The Committee was informed that accumulated experience with the initial testing of vaccine samples revealed that the conditions stipulated under section 3.4 lead to an extension of the time needed for testing, to an increased risk of quality deterioration during shipment, and to additional costs and WHO workload. In order to improve the efficiency of the process and decrease testing
costs, the WHO Technical Assistance and Laboratory Services group (TAL) had initiated a pilot study in which manufacturers directly delivered vaccine samples and related materials to the WHO-contracted testing laboratory. Study results indicated that this approach shortened the transportation time and led to a shorter application process, a reduced risk to the shipped goods and cost savings for all affected parties. In addition, allowing the use of direct shipments to WHO-contracted laboratories appeared to have no negative implications for the quality and impartiality of the testing conducted. TAL had therefore proposed a revision of section 3.4 that allowed for both direct shipment and the use of the NCL of the producing country.

Following discussion on a range of issues, and further clarification of a number of aspects of the proposal, the Committee recommended that the revised section 3.4 be adopted.

2.4.7 A WHO and EDQM collaborative study on the determination of saccharide content of the *Haemophilus influenzae* type b component in liquid vaccine presentations

The Committee was reminded that the quality of the *Haemophilus influenzae* type b (Hib) component in vaccine combinations is usually controlled by determining the total polysaccharide content and the free unconjugated saccharide content. The Committee was informed that because of noncompliant results following testing of the Hib content of a pentavalent vaccine, the WHO Prequalification of Vaccines Programme had initiated a project to establish a methodology using a single-test protocol for the quantitative determination of the saccharide content of the Hib conjugate component of liquid vaccine presentations (suspensions). Results indicated that one test appeared to offer an efficient means of determining Hib saccharide content in different liquid vaccine presentations produced by different manufacturers.

The Committee was informed that a WHO and EDQM international collaborative study was to be conducted in order to confirm these results across a broader range of participating laboratories. Ten laboratories from seven countries would be participating in the study, including five NRA control laboratories and five manufacturer laboratories.

The Committee agreed that the study would provide useful information, and looked forward to reviewing the results in 2015.

2.4.8 Update on the WHO global action plan to minimize poliovirus facility-associated risk

The Committee was updated on WHO activities related to the withdrawal of trivalent oral poliomyelitis vaccines (tOPVs) by the end of 2015 in the context of the overarching WHO poliovirus containment policy. Each of the three phases
of this policy was outlined and containment requirements during the period
2014–2020 described. In addition, a number of facility, population immunity and
environment and location safeguards were listed.

A key activity in Phase I of the containment policy would be the revision
of the 2009 WHO global action plan to minimize poliovirus facility-associated risk
after eradication of wild polioviruses and cessation of routine OPV use (GAP III)
and its alignment with the WHO Polio Eradication and Endgame Strategic Plan

It was expected that following further review by various WHO groups,
the revised GAP III would be presented to the World Health Assembly in 2015.
It was also noted that the adoption of GAP III may necessitate the revision of
Annex 2 of the WHO Technical Report Series, No. 926. A range of comments,
suggestions and other inputs were then made by the Committee for consideration,
particularly in relation to the extent to which consultations with appropriate
government agencies had taken place and to the categorization of viral strains.
The Committee requested that its specific comments on the draft document be
taken into account during its further revision, and asked to be kept informed of
future progress.
3. International Recommendations, Guidelines and other matters related to the manufacture and quality control of biological substances

All WHO Recommendations, Guidelines and guidance documents established at the meeting are listed in Annex 1, which provides an updated listing of all current WHO Recommendations, Guidelines and other documents related to the manufacture, evaluation and quality control of biological substances used in medicine.

3.1 Vaccines and related substances

3.1.1 Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine

The Committee was reminded of the background and development history of this WHO guidance document. The finding of an adventitious agent in a biological medicinal product has been of concern to regulatory agencies, manufacturers and public health officials since the early 1900s when the issue first arose. Since then, there have been several instances of a signal being detected for a potential adventitious agent as a contaminant of a marketed product; the most recent of which occurred in 2010. Although a broad pre-licensure regulatory framework existed, a number of aspects associated with the discovery of adventitious agents subsequent to MA were not well defined in terms of regulatory actions and decision-making. A number of requests had therefore been made for WHO to provide guidance to countries on the development and implementation of regulatory risk-evaluation strategies during the post-marketing period.

In 2010, in response to such concerns, both the Committee and ICDRA had recommended that WHO take the lead in providing the relevant guidance to its Member States. In 2012, the Committee reviewed the initial draft document and recommended a number of changes. The subsequently revised draft document was then discussed at several WHO meetings and underwent a second round of public consultation in 2013. The current document (WHO/BS/2014.2232) took into consideration all the comments received and was intended to provide guidance to regulators regarding the principles of risk evaluation when a signal for a potential adventitious agent or novel endogenous agent is detected in an already licensed or registered vaccine.
In addition, a review paper\(^5\) had been published in 2014 of four previous cases in which an agent or signal of an agent was found in a licensed vaccine. By illustrating how such situations were addressed and what lessons were learnt, the paper is intended to supplement the WHO guidance document. In addition, publication of the paper in the broader scientific literature will facilitate wider access by all interested parties.

Discussion took place on a range of issues, with clarifications sought in a number of key areas. Specific suggestions, as well as comments and submissions received, were also taken into consideration. The Committee recommended that the revised WHO guidance document be adopted and annexed to its report (Annex 2).

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

Since the WHO Recommendations for the production and control of poliomyelitis vaccine (inactivated) were last revised in 2000 and supplemented in 2003, there have been several changes in the production of such vaccines – including the use of seed viruses derived from Sabin strains – which make further revision of the Recommendations necessary. To facilitate this process, a meeting was convened by WHO in March 2012 to discuss the international specifications for inactivated poliomyelitis vaccine (IPV). During discussion a number of key issues were considered, both in terms of the quality control and evaluation of IPVs – including Sabin-based IPV (sIPV) – and the revision of the WHO Recommendations.

In October 2012, a drafting group was established and a Technical Working Group meeting was held in May 2013 to further discuss and reach consensus on key issues relevant to the revision of the existing WHO Recommendations. WHO then organized an informal consultation in March 2014 to review the recommendations prepared by the drafting group and to seek consensus on a number of outstanding important technical and regulatory issues. The document (WHO/BS/2014.2233) subsequently underwent two rounds of public consultation prior to its submission for consideration by the Committee.

Major issues addressed in the revised WHO Recommendations included:

- an updated “General considerations” and other sections to reflect the future development of IPV in accordance with global programmatic needs;
- an updated history of the different virus seed strains used by manufacturers for IPV production (Appendix 1);

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International Recommendations, Guidelines and other matters

- an updated section on international standards and reference preparations;
- an updated section on general manufacturing recommendations and control tests;
- updated terminology;
- inclusion of specific tests for sIPV and IPV made from strains derived by recombinant DNA technology;
- updated appendices;
- inclusion of new sections on the nonclinical and clinical evaluation of IPV.

Additional changes had also been made to bring the document into line with other WHO Recommendations published since the last revision.

Following discussion of the comments and submissions received, and after making a number of corresponding changes to the text, the Committee recommended that the revised WHO Recommendations be adopted and annexed to its report (Annex 3).

3.1.3 Guidelines on procedures and data requirements for changes to approved vaccines

For a number of reasons, changes to the vaccine manufacturing process or product labelling information often need to be implemented after a new vaccine has been approved. However, it is important to recognize that some manufacturing changes may impact upon the quality, safety and efficacy of an already approved vaccine, while changes to the labelling information may affect its safe and effective use.

The regulation of changes to approved vaccines is one of the most important elements in ensuring that vaccines of consistent quality, safety and efficacy are distributed after they receive authorization or licensure. As a result, regulators in numerous WHO Member States had requested further guidance from WHO on the data needed to support changes to approved vaccines to ensure their comparability with respect to the quality, safety and efficacy of the vaccines manufactured prior to the change.

Following a process of consultation and development, WHO Guidelines (WHO/BS/2014.2238) had been produced to support the establishment of national requirements for the regulation of post-approval changes. The reporting categories for major, moderate and minor quality changes and their respective reporting procedures are provided in the main body of the document with the data requirements needed to support the proposed changes provided in the appendices.
Detailed discussion took place on a range of issues, with clarifications sought in a number of key areas. Specific suggestions, as well as comments and submissions received, were also taken into consideration. After making a number of corresponding changes to the draft text, the Committee recommended that the revised WHO Guidelines be adopted and annexed to its report (Annex 4).

3.1.4 **Regulatory written standards pipeline**

The Committee was informed that four documents on the following subjects were scheduled for submission to the Committee in 2015:

- GMP for biological products
- human papillomavirus (HPV) vaccines
- regulatory risk assessment for BTPs
- regulatory expectations in the context of controlled temperature chains.

In addition, written standards were scheduled for consideration by the Committee in 2016 in the following two areas: (a) regulation of influenza vaccines in non-producing countries; and (b) clinical evaluation of vaccines. Written standards for which there was currently no specific timeline included those on influenza vaccines for pregnant and lactating women; the safe production of IPV; meningitis vaccines; vector-based vaccines; respiratory syncytial virus vaccines; hepatitis E vaccines; and product-specific guidance on SBPs.

In relation to the first of the above items scheduled for submission in 2015, the Committee was informed of the history of the current range of WHO guidance on GMP for pharmaceuticals, biological products and blood establishments. The current WHO Guidelines on GMP for biological products had been published in 1992 (Annex 2, WHO Technical Report Series, No. 822). Following strong requests from national regulators, a process of revision in this area had first been initiated in 2007 and had culminated in 2014 in a consultation involving a broad range of stakeholders.

It was intended that a draft document would be made available for public consultation in late 2014 that would eventually become a supplement to the 2014 WHO good manufacturing practices for pharmaceutical products: main principles published by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.6

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The Committee provided a number of comments and suggestions in relation to the proposed revision process and looked forward to considering the document at its next meeting.

3.1.5 **Clinical evaluation of dengue vaccines**

The Committee was informed that subsequent to the adoption of the WHO Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) (Annex 2, WHO Technical Report Series, No. 979) three technical questions had arisen in light of recent clinical trial data from one manufacturer. The Committee was asked for its guidance on the most appropriate mechanism for responding to the issues raised by this development.

To facilitate discussion, three options were presented to the Committee: (a) update the entire WHO Guidelines; (b) amend the existing WHO Guidelines; or (c) develop a separate text to be read in conjunction with the Guidelines using a question-and-answer format.

The Committee was then provided with a detailed account of each of the issues raised prior to discussion of the three options presented for its consideration. It was concluded that the most appropriate course of action would be for an expert group on dengue to provide advice. As a first step, a summary of the consultations of such a group would provide useful interim information that could be made publically available.

3.1.6 **Biotherapeutic products including similar biotherapeutic products**

The Committee was provided with an overview of recent and upcoming WHO activities in the area of BTPs (including SBPs). These activities included the development of both written and measurement standards and the holding of implementation workshops. In this respect, the Committee was also reminded of resolution WHA67.21 on access to BTPs (including SBPs) and on ensuring their quality, safety and efficacy. Resolution WHA67.21 had specifically requested that WHO update its current 2009 Guidelines in this area. The Committee was informed that a 2014 WHO survey on the regulation of BTPs had been well received and following final analysis the survey results would be published by the end of the year. The survey findings would be used to assist WHO in identifying key areas, such as regulatory convergence, in which to focus its future activities.

The Committee was then informed of the outcomes of pre-ICDRA and ICDRA meetings held in Brazil in 2014. Key recommendations made to WHO included the updating of its norms, standards and tools to facilitate the further development of expertise in the regulatory evaluation of biological products, and to promote the reaching of consensus on the nomenclature to be used for SBPs. Other important activity areas identified for further attention included
the holding of implementation workshops, provision of an information-sharing platform and the development of tools to measure the progress made in achieving regulatory convergence.

Following a brief overview and discussion of upcoming WHO events and other activities in this area, the Committee considered a number of key points and suggestions in relation to specific priority activities that could most beneficially be pursued by WHO.

3.1.7 Multilateral activities relating to biotherapeutic products including similar biotherapeutic products

The Committee was provided with an overview of the global BTP landscape, including the manufacturing, regulatory and other challenges faced, and the diversity of approaches taken in different countries. The standardization of vaccines and blood products was highlighted as a model for regulatory convergence and harmonization in this area. In this respect, WHO, APEC and the International Pharmaceutical Regulators Forum had each developed and implemented a range of initiatives related to regulatory convergence. In the case of APEC economies, a roadmap for promoting such convergence had now been developed and endorsed.

Nevertheless, the issue of inefficiency caused by overlapping and redundant activities remained a priority issue that needed to be resolved. The Committee was informed of the potentially key role of the International Coalition of Medical Regulatory Authorities in identifying potential synergies and opportunities for collaboration. In addition, attention was drawn to the participation of the Ministry of Food and Drug Safety, Republic of Korea, in a number of global initiatives in this area.

A number of potential principles and proposals for further collaborative efforts, along with key conclusions, were presented to the Committee for their consideration, and in turn the Committee emphasized the crucial importance of pharmacovigilance programmes and post-marketing surveillance in assessing the safety and efficacy of BTPs including SBPs.

3.2 Blood products and related substances

3.2.1 Strengthening production capacity for blood components including plasma for fractionation

The attention of the Committee was drawn to two WHO reports which set out the conclusions and follow-up actions of a WHO/European Commission project to support low- and middle-income countries (LMICs) worldwide in raising production standards and strengthening the regulatory oversight of blood components and plasma for fractionation. This project was in line with resolution WHA63.12, which addressed the need to improve regulatory oversight
and quality assurance systems in blood establishments to improve the availability, quality and safety of blood products in LMICs.

During Phase I of the project an assessment was made of the needs, challenges and opportunities that were likely to be associated with efforts to improve production standards in LMIC blood establishments. This involved reviewing the volume of plasma separated from whole blood that is currently wasted worldwide and identifying key steps needed to improve the situation. Analysis indicated that the volume of recovered plasma wasted worldwide was of the order of 9.3 million litres each year. In order to prevent wasting such a valuable resource there was a need to build and improve local capacity for the collection of blood/plasma which could then be made available for the manufacture of life-saving derivatives. Improving local production standards would then lead to a general improvement in public health at both national and international level by increasing access to essential, safe and effective blood components and plasma derivatives.

Following the completion of Phase I and in line with key stakeholder inputs on how best to build upon its findings, WHO initiated a 2-year programme of Phase II activities in Indonesia in order to demonstrate “proof of concept” in a selected pilot country. Information obtained through situation assessments and gap analysis at various points was used to identify the main challenges to be addressed and to inform the development of project activities. In full collaboration with relevant partners, WHO undertook the development, management and monitoring of a series of activities which addressed both the legal framework required to enhance the regulation of blood products and the need to strengthen the technical capacity of the regulatory authority and blood establishments. Two WHO training workshops were held to cover: (a) a blood testing strategy and related assessment of residual risk of transfusion-transmitted infectious diseases; and (b) the implementation of GMP, including mock inspections in three different blood establishments.

The efforts made by Indonesia in strengthening the regulation of blood products were acknowledged, including the imminent finalizing and formal adoption of a National Decree through which sole responsibility for regulation of the entire national blood system and blood products was to be conferred upon the National Agency of Drug and Food Control. WHO will provide further support to this process through the focused development and capturing of written GMP and standard operating procedures (SOPs) in a single blood establishment, and by the holding of further workshops and other training in the area of national blood-screening policy and regulation involving key stakeholders directly involved in the definition of national standards for blood products in Indonesia. Other follow-up steps would include the finalization of WHO guidelines on risk assessment based on donor epidemiology and different testing strategies, and on the design and calibration of secondary standards.
Such guidelines were considered to be a high-priority requirement in supporting associated decision-making processes in LMICs.

The Committee recognized the substantial progress that had been achieved to date and concurred with the proposed next steps. These would appear to specifically involve targeted capacity building in the blood centre selected as the model for the implementation of national standards and GMP along with the cooperative development of a training module for each of the main steps in the plasma-production process. In this way, a structured capacity-building programme for qualifying a blood establishment could be put in place, in parallel with the development and promulgation of national blood standards and implementation of strengthened regulatory oversight.

The Committee additionally recommended that the next steps should also include an assessment of the functionalities and competencies of the blood regulator and of the selected pilot blood establishment subsequent to completion of programme building and training. For example, questionnaires provided by candidate plasma fractionators, blood centre accreditation audits and evaluation against the published WHO BRN assessment criteria would all be valuable in identifying the further areas to be addressed.

3.2.2 Shortage of anti-diphtheria and other specific immunoglobulins

The Committee was informed that there was now a severe shortage of a number of specific immunoglobulins, namely anti-diphtheria, anti-tetanus, anti-rabies and anti-botulism and of a number of snake antivenoms. These products were typically requested through the WHO procurement channels and primarily used in LMICs.

A proposal had now been made to map national stockpiles in countries where secure stocks and reliable regulatory oversight existed, and to enquire whether these countries would be willing to maintain a WHO stockpile subject to the requisite agreements among participating countries, manufacturers and relevant international organizations.

The Committee noted the report.

3.2.3 MERS coronavirus serum panel

The Committee was informed that, in close cooperation with WHO, NIBSC was currently involved in the manufacture and intended future distribution of a Middle East respiratory syndrome coronavirus (MERS-CoV) serum panel for use in anti-MERS-CoV antibody testing. Currently there are a number of in-house antibody assays used for the diagnosis of past MERS-CoV infection. However, these antibody assays are not standardized and the proposed panel would provide valuable insights into their key features.
Heat-inactivated samples (25-mL volume) collected during the convalescent phase of patients whose infection was confirmed by NAT-based assay had been requested by WHO. Such a volume from each convalescent case would be sufficient for the production of approximately 50 panels. A number of previous problems in relation to the required Material Transfer Agreements now seem to have been resolved by WHO, and NIBSC is now ready to begin the process of heat inactivation and aliquoting of samples prior to the coordinating of panel dispatch.

During discussion, it was suggested that white blood cells be obtained during further sample collections in the ongoing outbreak as this could then allow for the cloning of antibodies for characterization.

The Committee noted the report.

3.2.4 Use of convalescent sera to respond to emerging infectious disease threats

The Committee was reminded (see section 2.2.1) that following an urgent request from WHO a BRN position paper on Collection and use of convalescent plasma or serum as an element in filovirus outbreak response had been posted on the WHO web site in August 2014. This position paper sets out a range of recommendations on the use of convalescent plasma or serum as part of a treatment response. Convalescent plasma could, based on theoretical considerations, be used for the treatment of Ebola virus disease. The position paper therefore recommends that studies into the feasibility, safety and effectiveness of convalescent plasma in this area should be undertaken, while highlighting the key role of regulatory authorities in facilitating progress. Such progress will depend upon countries establishing regulatory conditions for the collection of plasma, for clinical studies and for reporting patient outcomes. In the longer term the possibility of large-scale immunoglobulin production could be considered. It would also be important to identify partners in outbreak areas, avoid conflicting initiatives and establish the feasibility of a controlled trial.

To date, transfusion with blood from convalescent patients had been used empirically as an Ebola therapy, but its effectiveness was currently unknown. Although well designed studies of convalescent plasma could lead to the identification of criteria for clinical use, a significant assessment of the feasibility of clinical trials was needed as a first step. If such an approach proved to be effective, investment in blood programme infrastructures capable of plasma separation may then be warranted as part of a strategy for responding to both the current situation and to potential future outbreaks, above and beyond the existing

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7 http://www.who.int/bloodproducts/brn/en/
need to improve the availability of safe blood for transfusion. The document also identified gaps in scientific knowledge that needed to be addressed as well as the critical elements in assessing the feasibility of clinical studies.

The Committee noted the report.

3.2.5 **Overview of the biological standards endorsed by the ISTH for WHO approval**

The Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) provides expert review of proposed WHO international standards in the haemostasis area. This activity is coordinated through the WHO-ISTH Liaison Group, and in 2014 the following four proposed WHO international standards were endorsed by the SSC/ISTH: First WHO International Standard for activated blood coagulation factor XI; First WHO Reference Panel for lupus anticoagulant; First WHO International Standard for A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs 13 (ADAMTS13) plasma; and the Fourth WHO International Standard for plasmin.

The Committee noted the report.
4. International reference materials – antibiotics

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

4.1 WHO International Standards and Reference Reagents – antibiotics

4.1.1 Second WHO International Standard for bleomycin complex A2/B2

Bleomycin is a glycopeptide antibiotic active against both Gram-positive and Gram-negative bacteria and is used as an anticancer agent in the treatment of Hodgkin's lymphoma, squamous cell carcinomas and testicular cancer. The chemotherapeutical forms are primarily bleomycin A2 and B2. Bleomycin appears on the WHO Model List of Essential Medicines.

As stocks of the International Reference Preparation of Bleomycin established in 1980 were becoming exhausted, EDQM, in its capacity as the custodian of WHO ISAs, had taken appropriate steps for its replacement. Following the donation of bulk material by Nippon Kayaku, Japan, a total of 500 vials of candidate material had been produced by EDQM.

An international collaborative study was then conducted to establish the Second WHO International Standard for bleomycin complex A2/B2. The candidate material provided by EDQM (EDQM code ISA_46290) was analysed by eight laboratories from eight countries, each using their own in-house diffusion assay. Within- and between-laboratory repeatability was generally very good in terms of determining the potency of the candidate sample. The results of accelerated thermal degradation studies at 1–6 months indicated that the candidate would be stable for long-term use.

The Committee considered the report of the study (WHO/BS/2014.2236) and recommended that the candidate material ISA_46290 be established as the Second WHO International Standard for bleomycin complex A2/B2 with an assigned anti-microbiological activity of 12,500 IU/vial.

4.2 Proposed new projects and updates – antibiotics

4.2.1 Proposed Third WHO International Standard for amphotericin B

Amphotericin B is used globally as an important antibiotic and appears on the WHO Model List of Essential Medicines. The current Second WHO International Standard for amphotericin B was established in 2007 and is used by pharmacopoeias, and national and regional regulatory authorities to establish their secondary standards. Where appropriate, the international standard is also used by manufacturers to establish their in-house working standards.

As stocks of the current standard were expected to be exhausted before the end of 2017, a replacement was now needed. EDQM, in its capacity as the
custodian of WHO ISAs, was now taking appropriate steps for its replacement. It was intended that following the donation of bulk material by a major manufacturer, EDQM would formulate and process a suitable candidate material.

A collaborative study involving around 12 laboratories was proposed which would include pharmacopoeias, official medicines control laboratories and others skilled in antibiotic titration. An appropriate statistical evaluation would then be made taking the current international standard lot as the primary standard.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a Third WHO International Standard for amphotericin B, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2015.
5. International reference materials – biotherapeutics other than blood products

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

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

5.1.1 Third WHO International Standard for luteinizing hormone (human pituitary)

The glycoprotein hormone, luteinizing hormone (LH), produced in the anterior pituitary gland, plays a major role in the regulation of reproductive processes. Determinations of the LH concentration in samples of serum and plasma by immunoassays have a number of clinical applications, contributing to clinical diagnoses regarding infertility, menstrual irregularities, and precocious or delayed puberty, and distinguishing primary and secondary ovarian/testicular failure.

The Second WHO International Standard for pituitary luteinizing hormone had been established by the Committee in 1988 and had been used widely for the calibration of immunoassays to measure LH in serum and plasma. Stocks of this international standard were now low, and in 2011 the Committee had recognized the need for its replacement. In the absence of a source of human pituitary glands from which to purify LH, a batch of ampoules was filled using the same bulk material that had been used for the current international standard. Of these, the Committee was informed that 2900 ampoules could be made available for use as an international standard.

An international collaborative study was conducted to establish the Third WHO International Standard for luteinizing hormone (human pituitary). The candidate material (NIBSC code 81/535) was calibrated against the current standard (NIBSC code 80/552) by 11 laboratories from seven countries, each using their own in-house immunoassay. Within- and between-laboratory repeatability was generally very good in terms of determining the LH content of the candidate material. The study included a partial assessment of the impact of the new standard on the routine measurement of LH in human serum samples. In this and other respects, the candidate material was found to be suitable for use as an international standard.

The results of stability studies indicated that the candidate material was sufficiently stable, on the basis of an accelerated thermal degradation study at 2 years and 26 years, to serve as an international standard. The Committee was informed that real-time stability studies were in progress.

The Committee considered the report of the study (WHO/BS/2014.2240) and recommended that the candidate material 81/535 be established as the
Third WHO International Standard for luteinizing hormone (human pituitary) with an assigned potency of 33 IU/ampoule.

5.1.2 **First WHO International Standard for proinsulin (human)**

Human proinsulin is synthesized by pancreatic beta cells before being enzymatically cleaved to insulin and C-peptide. Elevated serum concentrations of proinsulin are indicative of insulinomas, diabetes mellitus and other disorders of glucose metabolism. Measurements of proinsulin in serum and plasma by immunoassays thus contribute to the diagnosis of insulinoma and are used to monitor beta-cell dysfunction.

Stocks of the current WHO International Reference Reagent for proinsulin (human) established in 1986 were now exhausted. In 2010, the proposed development of a First WHO International Standard for proinsulin (human) had been endorsed by the Committee. The proposed standard was to be prepared through the calibration of a previously filled batch of 2972 ampoules of human proinsulin candidate material (NIBSC code 09/296).

An international collaborative study was therefore conducted to establish the First WHO International Standard for proinsulin (human). The candidate material was provided by a single manufacturer and was analysed by 17 laboratories from nine countries, each using their own in-house immunoassay. Within- and between-laboratory repeatability was generally very good in terms of determining the proinsulin content of the candidate material. In this and other respects, the candidate material was found to be suitable for use as an international standard.

The results of accelerated thermal degradation studies indicated that the candidate was stable at temperatures used for storage (−20 °C) and laboratory manipulation (20 °C) and would be suitable for long-term use.

The Committee considered the report of the study (WHO/BS/2014.2237) and recommended that the candidate material 09/296 be established as the First WHO International Standard for proinsulin (human) with an assigned mass content of 7.0 µg/ampoule.

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

5.2.1 **Proposed First WHO Reference Reagent for Rituximab for use in complement-dependent cytotoxicity assays**

Monoclonal antibodies derived by recombinant DNA technology are the fastest growing group of innovative biotherapeutics, with over 28 products already approved for use in humans and hundreds more in clinical development. However, concerns have been raised with WHO over the quality and safety of
biotechnology products, including monoclonal antibodies and associated SBPs intended for clinical use in humans. There have also been numerous cases of the marketing of counterfeit and falsified products. The availability of validated potency standards for important monoclonal antibodies should help to address these concerns.

Rituximab is an important monoclonal antibody that has been approved for the treatment of a wide range of important diseases in oncology and rheumatology. It is also the first therapeutic monoclonal antibody to lose market exclusivity and several manufacturers have already produced, or are developing, Rituximab SBPs or standalone versions that aim to match the innovator product as closely as possible.

A potency standard would be of global importance in ensuring the consistent safety and efficacy of these new products. An international collaborative study was therefore proposed to develop a First WHO Reference Reagent for Rituximab for use in complement-dependent cytotoxicity assays. An SBP or standalone version of Rituximab, either approved or in clinical development, may be used as the source of the candidate material depending upon availability.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a First WHO Reference Reagent for Rituximab for use in complement-dependent cytotoxicity assays, and agreed that the collaborative study should proceed in 2015 with subsequent submission of its outcome to the Committee in 2016.

5.2.2 Proposed First WHO Reference Reagent for Batroxobin

Batroxobin is a snake venom thrombin-like enzyme used as a reagent for measuring Batroxobin clotting time – a measurement of fibrinogen in plasma that, unlike thrombin, is insensitive to heparin. In addition, there is ongoing research to develop Batroxobin as a therapeutic agent for the treatment of ischaemic stroke and sudden hearing loss.

The Second British Standard for Batroxobin was prepared in 1993 and there are now fewer than 200 ampoules remaining of the 1000 originally filled. It was proposed that in order to maintain a consistent unit of activity for Batroxobin the current standard should now be replaced by a WHO reference reagent.

Since work was already under way to replace the First International Reference Preparation of Ancrod (a snake venom with similarities to Batroxobin) it would be helpful to calibrate both Batroxobin and Ancrod together in parallel collaborative studies involving the same laboratories. It was envisaged that the study would likely involve fewer than five laboratories as not many laboratories were familiar with calibrating the activity of snake venoms. To maintain continuity, the unit for the Batroxobin reference reagent would be derived from the existing Second British Standard for Batroxobin, with methods developed
and provided where needed. The Committee was informed that Pentapharm in Switzerland had offered Batroxobin harvested from snakes, which was the same material provided for the establishment of the existing British standard.

The Committee endorsed the proposal (WHO/BS/2014.2243) to develop a First WHO Reference Reagent for Batroxobin, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2015.

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

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

6.1.1 First WHO International Standard for activated blood coagulation factor XI

Activated factor XI (FXIa) has been implicated as a major contributor to thromboembolic events associated with the infusion of intravenous immunoglobulins (IVIGs). In 2012 the First WHO Reference Reagent for activated blood coagulation factor XI (human) (NIBSC code 11/236) was established to support the measurement of FXIa in immunoglobulin products. The Committee was informed that demand for this preparation had been significant and that current stocks would be exhausted by the end of 2014.

Value assignment of a proposed replacement preparation (NIBSC code 13/100) relative to the current reference reagent was undertaken through an international collaborative study involving 17 laboratories from 11 countries. Fifteen of the participating laboratories used chromogenic methods specific for FXIa, whereas two used clotting methods which detected both FXI zymogen and FXIa. Value assignment was based upon the results obtained from the specific chromogenic methods only and resulted in an overall mean of 9.8 IU/ampoule with low inter-laboratory variability (geometric coefficient of variation (GCV) = 3.3%). Accelerated thermal degradation studies indicated that the candidate material 13/100 was extremely stable with < 0.001% loss per year at −20 °C. Samples of four immunoglobulin preparations were also included in the study. Estimates for two of these four samples were associated with lower inter-laboratory variability (GCVs = 6.9% and 13.9%) compared to previous estimates made in the reference reagent study, suggesting improved laboratory testing capabilities.

Although value assignment for the candidate material was based only upon the chromogenic methods, further studies based upon the use of non-activated partial thromboplastin time and thrombin generation assays will be conducted to assess its broader commutability. A new study was also under way involving CBER, NIBSC and the United States Pharmacopeial Convention (USP) to investigate the commutability of the material in relation to immunoglobulin preparations from a larger number of manufacturers.

The Committee considered the report of the study (WHO/BS/2014.2245) and recommended that the candidate material 13/100 be established as the First...
WHO International Standard for activated blood coagulation factor XI with an assigned value of 9.8 IU/ampoule. The Committee further recommended that the Instructions for Use should clearly indicate that value assignment was based only upon chromogenic assay results, and that users should also be aware that matrix effects may occur with some products.

6.1.2 First WHO Reference Panel for lupus anticoagulant

Lupus anticoagulant syndrome is characterized by the presence of antiphospholipid antibodies in the circulation. The condition is associated with recurrent thrombotic events and diagnosis includes clotting-based laboratory tests. Misdiagnosis is common, with quality assurance schemes indicating high levels of both false-negative and false-positive diagnoses. Lupus anticoagulant testing guidelines recommend the use of appropriate reference materials to improve the quality of testing. The First British Reference Panel (Plasma) for Lupus Anticoagulant had been exhausted within 5 years of its establishment by NIBSC in 1999.

In order to develop a reference panel of well characterized lupus anticoagulant plasmas to support the establishment and maintenance of test methods, 19 laboratories from 11 countries participated in an international collaborative study. During the study the following three individual candidate plasma preparations for use in the proposed panel (NIBSC code 13/172) were evaluated: (a) lupus negative (NIBSC code 12/148); (b) moderate positive (NIBSC code 12/150); and (c) strong positive (NIBSC code 12/152). Most data were obtained using Russell’s viper venom time and the activated partial thromboplastin time. Inter-laboratory variability was reduced overall when the ratios were recalculated relative to the candidate negative plasma 12/148. All methods confirmed the negative status of 12/148, the positive status of both 12/150 and 12/152 and the higher reactivity of 12/152 compared to 12/150. Stability studies based on the accelerated thermal degradation of key coagulation factors (FV, FVII and FVIII) as surrogates indicated that the candidates were suitably stable with predicted loss per year of < 0.2% at −20 °C. Additionally, a small in-house study indicated comparability between the British panel and the proposed WHO reference panel.

The Committee considered the report of the study (WHO/BS/2014.2244) and recommended that the candidate panel 13/172 be established as the First WHO Reference Panel for lupus anticoagulant with the following components: lupus negative (12/148), lupus moderate positive (12/150) and lupus strong positive (12/152). Although values would not be assigned to the panel components, it was recommended that information on the methods used be provided in the Instructions for Use.
6.1.3 **First WHO International Standard for A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs 13 (ADAMTS13)**

Acquired or congenital deficiency of ADAMTS13 leads to the persistence of ultra-large multimers of von Willebrand factor in the circulation and the development of thrombotic thrombocytopenic purpura. The measurement of ADAMTS13 function and antigen is therefore an important component in the diagnosis of this blood disorder. An international collaborative study involving 32 laboratories from 14 countries was undertaken to assign values for ADAMTS13 function and antigen to a candidate pooled normal plasma (NIBSC code 12/252). Value assignment was based on assays of 12/252 conducted by laboratories relative to their local pooled normal plasma (arbitrarily assigned a value of 1.0 unit/mL).

The majority of laboratories used a Fluorescence Resonance Energy Transfer (FRET) assay and/or activity enzyme-linked immunosorbent assay (ELISA) to measure function, and all used ELISA methods for the measurement of antigen. No significant difference was observed between estimates of function obtained by FRET and by activity ELISA, with a combined overall mean of 0.91 units/mL and low inter-laboratory variability (GCV = 12.4%). Combining all estimates for antigen gave a mean value of 0.92 units/mL and inter-laboratory variability of 16.3%. Stability estimates for 12/252 obtained using an accelerated thermal degradation protocol indicated function and antigen losses of < 0.1% per year for ampoules stored at −20 °C.

Results for two patient samples (ADAMTS13 deficiency caused by autoantibody) were associated with greater inter-laboratory variability for both functional activity and antigen, and the function/antigen ratio was much reduced when compared to the normal candidate 12/252. Inter-laboratory variability for the antigen was not improved when calculated relative to 12/252. Estimates for a preparation of recombinant ADAMTS13 relative to the local plasma pools and 12/252 were variable, indicating that the proposed international standard may not be an appropriate reference for this purified material.

The Committee considered the report of the study (WHO/BS/2014.2246) and recommended that the candidate material 12/252 be established as the First WHO International Standard for A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs 13 (ADAMTS13) with assigned values of 0.91 IU/ampoule for functional activity and 0.92 IU/ampoule for antigen.

6.1.4 **Fourth WHO International Standard for plasmin**

The WHO international standard for plasmin is used to standardize plasmin potency measurements, and future levels of demand are likely to increase given
renewed interest in the possible use of plasmin as a thrombolytic drug. As stocks of the current Third WHO International Standard for plasmin were almost exhausted, a candidate purified replacement preparation had been lyophilized in ampoules (NIBSC code 13/206) and value assignment undertaken through an international collaborative study involving 15 laboratories from 12 countries.

Combined laboratory mean values obtained from 52 independent chromogenic assays gave an overall mean value of 8.0 IU/ampoule, with low inter-laboratory variability (GCV = 7.8%). As only three laboratories performed fibrinolytic assays in the study, the value assignment did not include these results. Stability studies based on the accelerated thermal degradation of a trial fill with identical formulation indicated that the proposed candidate material would exhibit suitable stability. Active site titration performed by a single laboratory indicated a concentration of 1.5 µM after reconstitution.

The Committee considered the report of the study (WHO/BS/2014.2249) and recommended that the candidate material 13/206 be established as the Fourth WHO International Standard for plasmin with an assigned value of 8.0 IU/ampoule. The Committee further recommended that the Instructions for Use should indicate that the assigned value was obtained using only chromogenic methods and that the molar value for the active site was for research guidance only as it was based solely on the results from one laboratory.

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

6.2.1 Proposed Second WHO International Standard for blood coagulation factor XI

The current WHO international standard is used in the laboratory diagnosis of factor XI (FXI) deficiency, the potency labelling of FXI therapeutic concentrates and the quality control of other plasma-derived products. Given present levels of demand, a replacement for the current standard will be required in approximately 2 years. The candidate material for the proposed Second WHO International Standard for blood coagulation factor XI will be prepared from pooled normal plasma donations made to the United Kingdom national blood service. Value assignment for functional activity will be performed relative to the current WHO international standard. The collaborative study will also address the value assignment of an extra analyte (FXI antigen) since this is required for the differential diagnosis of type I and type II FXI deficiency and for the accurate quantification of FXI in immunoglobulin preparations.

The Committee endorsed the proposal (WHO/BS/2014.2242) to develop a Second WHO International Standard for blood coagulation factor XI.
6.2.2 Proposed Second WHO International Standard for activated blood coagulation factor IX

The current WHO international standard is used to measure activated factor IX (FIXa), which is a process-related contaminant in therapeutic FIX concentrates. As it is highly thrombogenic the level of this impurity is controlled during the production process. With the development of new-generation FIX products demand for this standard has now increased and a replacement standard will be required within the next 2 years. The candidate material will be donated by a manufacturer of therapeutic FIX and value assignment carried out relative to the current WHO international standard.

The Committee endorsed the proposal (WHO/BS/2014.2242) to develop a Second WHO International Standard for activated blood coagulation factor IX.

6.2.3 Proposed second WHO reference reagents for anti-A and anti-B in intravenous immunoglobulin

These reference reagents are used to control haemagglutination assays for anti-A and anti-B in IVIG products. The current WHO reference reagents for anti-A and anti-B were established in 2008 and consist of a positive control (NIBSC code 07/306), a negative control (NIBSC code 07/308) and a limit preparation (NIBSC code 07/310) to define the pharmacopoeial limit where applicable.

Level of demand for these reagents is high and in recent years has risen following increased reports of haemolysis following IVIG infusion. This appears to be associated with new-generation IVIG products with high anti-A and anti-B titres. Following heavy usage of both the positive and negative controls, and greater than anticipated demand for the limit preparation, stocks of 07/308 and 07/310 in particular are running low and replacements are required.

Candidate materials will be prepared from IVIG products and, as was the case for the current reagents, a collaborative study will be performed involving NIBSC, EDQM and CBER.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2242) to develop second WHO reference reagents for anti-A and anti-B in intravenous immunoglobulin. Among the issues raised was the ongoing international discussion on whether the detection limits used in the batch testing of immunoglobulins using such reference reagents should be lowered. Any such future change would need to be reflected in the Instructions for Use.
7. International reference materials –
In vitro diagnostic device reagents

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

7.1 WHO International Standards and Reference
Reagents – in vitro diagnostic device reagents

7.1.1 Third WHO International Standard for hepatitis B virus surface antigen

More than 2 billion people worldwide are estimated to be infected with the hepatitis B virus (HBV), with approximately 600 000 dying each year from acute infections or from cirrhosis and hepatocellular carcinoma caused by chronic infection. Despite the availability of safe and effective prophylactic vaccines, HBV infection remains a major public health problem worldwide. As laboratory diagnosis of infection centres on the detection of the hepatitis B surface antigen (HBsAg) sensitive screening and accurate diagnostic HBsAg assays are an essential element in disease prevention and management.

An urgent proposal to replace the now almost depleted current international standard was endorsed by the Committee in 2012. The candidate material for the putative replacement standard donated to WHO (NIBSC code 12/226) comprised a non-adjuvanted HBsAg vaccine bulk derived from human plasma which had been inactivated by heat and formaldehyde treatment and diluted in negative plasma. Further analysis revealed B4 (two different strains) to be the underlying genotype, which was different from the A2 used in the current standard. However, further analysis indicated suitable antigenicity and potency of the candidate material.

An international collaborative study involving 12 laboratories from nine countries generated 22 data sets using both quantitative and qualitative HBsAg assays. Study samples included the current and previous WHO international standards, a blinded duplicate of the candidate replacement material and several samples of different genotypes (D and E) for an analysis of commutability. The mean potency of the candidate material across the different assays was 47.3 IU/mL. The relative harmonization of laboratory assay results was best for the current second standard and worst for the first with the candidate material being comparable to the former. Accelerated thermal degradation studies after storage for 1 year and additional reconstitution studies indicated suitable stability for the candidate material.

The Committee considered the report of the study (WHO/BS/2014.2241) and recommended that the candidate material 12/226 be established as the Third WHO International Standard for hepatitis B virus surface antigen. The Committee agreed that the exact potency value of 47.3 IU/ampoule obtained in
the collaborative study should be assigned without rounding. The Committee further agreed that the remaining small stocks of the second international standard should be retained for potential future studies.

### 7.1.2 First WHO International Standard for *Toxoplasma gondii* DNA for NAT-based assays

Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*. Transmission can occur through ingestion of raw meat or contaminated water, vertically during pregnancy and through transplanted organs. The real-time polymerase chain reaction (PCR) assays used to detect *T. gondii* are sensitive but suffer from high inter-assay variability.

The proposed international standard was value assigned in arbitrary IU during an international collaborative study involving 17 laboratories from 14 countries. The study used different NAT-based assays to test five samples comprising a freeze-dried candidate (NIBSC code 10/242) and four liquid preparations. The candidate material consisted of *T. gondii* tachyzoites harvested from mice and inactivated. Different trial fills were performed to determine the most suitable reagent mix for lyophilization of the parasites. The mean *T. gondii* DNA content of each sample was determined and a mean log_{10} “equivalent” per millilitre of 6.0 obtained for the candidate material. No degradation of the candidate material was observed during accelerated thermal degradation studies. The use of 10/242 as a reference significantly reduced inter-laboratory assay variability for concentrated samples, more so in quantitative than in qualitative assays.

The Committee considered the report of the study (WHO/BS/2014.2248) and recommended that the candidate material 10/242 be established as the First WHO International Standard for *Toxoplasma gondii* DNA for NAT-based assays with an assigned value of $1 \times 10^6$ IU/mL.

### 7.1.3 First WHO International Standard for hepatitis C virus core antigen

Infection with hepatitis C virus (HCV) causes an enormous global health care burden with disease severity ranging from mild illness lasting several weeks to a serious lifelong condition that can eventually lead to liver cirrhosis or hepatocellular carcinoma. HCV is primarily transmitted by blood transfusion, haemodialysis, intravenous drug use and tattooing. An estimated 150 million individuals worldwide are chronically infected with HCV with more than 350 000 people dying each year from related liver damage.

HCV core antigen determination is a quantitative marker used in monitoring HCV therapy and a qualitative infection marker for screening or diagnosis when used either singly or in combination assays which incorporate antibody detection. Combination assays are intended to be used to shorten the diagnostic window phase. As a result, candidate materials for a WHO
international standard for HCV core antigen should ideally be obtained from the early infection phase.

During an international collaborative study 12 laboratories from nine countries characterized a lyophilized plasma candidate preparation (PEI code 129096/12) obtained from a donor infected with HCV genotype 1a. Characterization of the candidate material was conducted in parallel with the corresponding liquid-frozen bulk material and four liquid-frozen HCV core antigen-positive plasma specimens. Six assays (two quantitative antigen, one qualitative antigen and three qualitative antigen/antibody combination assays) were used in the study.

Considerable differences were observed in analytical sensitivity between the various assays, yielding an endpoint titre range from 1:2 for the least sensitive assay to 1:3200 for the most sensitive assay. Intra-laboratory and inter-laboratory variability for the candidate material and bulk material were in an expected range for immunoassays, indicating that the candidate material remained homogenous following lyophilization.

A complementary study was performed, using selected representative assays, on a range of low-, medium- and high-HCV core antigen-positive clinical samples from early HCV infection (“seroconversion panels”). This additional study demonstrated that the analytical sensitivity of the assays correlated with clinical sensitivity of detection based on the proportion of pre-seroconversion samples detected across the panels. Accelerated and ongoing real-time stability studies indicated that the candidate material was stable and suitable for long-term use when stored as recommended.

As the measured potency of the candidate material was dependent upon the sensitivity of the test kit used, unit determination was based upon the value obtained using the most sensitive assay. It was noted that very low level antibody was present in the reference material based on testing with an experimental assay.

The Committee considered the report of the study (WHO/BS/2014.2247) and recommended that the candidate material 129096/12 be established as the First WHO International Standard for hepatitis C virus core antigen with an assigned potency of 3200 IU/mL and clear indication of its recommended use in HCV core antigen assays only.

7.2 Proposed new projects and updates – in vitro diagnostic device reagents

7.2.1 Proposed First WHO Reference Panel for vCJD

Variant Creutzfeldt-Jakob disease (vCJD) is a rare and invariably fatal transmissible spongiform encephalopathy (TSE) and there remains a potential risk of secondary exposure through blood transfusion and blood-derived products. Based on archived appendices it is possible that up to 1 in 2000 people
in the United Kingdom may have depositions of abnormal prion protein in lymphoid tissues. A number of blood tests now under development for donor screening will require reference materials for their validation. These reference materials could also be used to validate prion-removal devices. As there is insufficient material from vCJD-infected individuals to prepare the required reference materials, it was proposed that the blood of macaques infected with vCJD be used as an alternative.

To prepare such reference materials, serial bleedings of three macaques inoculated with macaque-adapted vCJD were obtained at multiple time points prior to and during the onset of the earliest symptoms and terminal stage of disease, and the bleedings for each time point pooled. Bleedings were also obtained from a single uninfected macaque.

Infectivity, as determined using bovinized transgenic mice, was demonstrated at the time of terminal disease and at low titre at the time of first symptoms. A protein misfolding cyclic amplification assay was positive in the blood obtained at the stage of terminal disease. Further data were due to be obtained from the transfusion of blood from the final stage of disease into negative macaques to confirm the relevance of this animal model for transfusion risk. Different materials (including whole blood, plasma and buffy coat) from each of the different infection phases had been produced for use in the proposed candidate reference panel.

During discussion of the proposal (WHO/BS/2014.2242) the potentially vital importance of the materials was highlighted. However, with only three assays known to be under development and limited knowledge of the form, concentration and infectivity of the target vCJD agent in the different materials, it was considered premature to assign the materials the status of a WHO reference panel. It was suggested that the reactivity of available human blood specimens from vCJD patients be compared to the results obtained with the macaque bleeding at terminal disease using the same assays and in collaboration with the similar NIBSC programme. The Committee noted the willingness of CBER to share the materials as appropriate pending their further characterization.

7.2.2 Proposed first WHO international standards for herpes simplex virus DNA type 1 and 2

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are two related viruses that cause facial lesions in the form of cold sores (predominantly HSV-1) and genital ulcers (predominantly HSV-2). HSV is also an important pathogen of the central nervous system (CNS) manifesting either as meningitis or encephalitis. The use of molecular techniques to detect HSV is now recognized as the reference method for the diagnosis of patient infection, predominantly the specific diagnosis of CNS infection. The early and accurate diagnosis and treatment of viral CNS infections caused by HSV decreases morbidity and mortality rates.
Although most molecular diagnostic assays allow for the simultaneous detection of HSV-1 and HSV-2, with most assays being of qualitative design, proficiency-testing schemes indicate high variations in their sensitivity. There is thus a clear need for international standards for use in the accurate determination of assay sensitivity for both HSV-1 and HSV-2. It was proposed that strains of HSV-1 and HSV-2 be obtained from cell culture and formulated at a concentration of approximately $1 \times 10^7$ genomes/mL in “universal” buffer. The well characterized cell-cultured materials would then be evaluated in a collaborative study, with investigators encouraged to use their normal testing matrices to test clinical samples obtained from the different tissue sources typically used in testing.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2242) to develop first WHO international standards for herpes simplex virus DNA type 1 and 2.

7.2.3 Proposed replacement WHO international standards for prostate-specific antigen (free) and prostate-specific antigen (90:10)

The current standards are used by diagnostics manufacturers to calibrate prostate-specific antigen (PSA) immunoassays and are therefore crucial in harmonizing the diagnosis and management of prostate cancer. Prostate cancer is the most common cancer in men, with, for example, approximately 35 000 newly diagnosed cases and 10 000 deaths occurring per year in the United Kingdom. Stocks of the current standards are extremely low and replacements are urgently needed.

It is intended that two candidate materials will be prepared from a purchased commercial source purified from seminal fluid: free PSA and PSA complexed to α1-chymotrypsin (ACT) at a ratio of 90 free PSA to 10 ACT. A stakeholder meeting has been planned at NIBSC in 2015 to discuss the formulation and characterization of the proposed standards. It is expected that the two candidates will be value assigned by ultraviolet absorbance and quantitative amino acid analysis, and then further characterized by immunoassay and compared to a range of clinical samples in immunoassay systems to determine their commutability.

The Committee endorsed the proposal (WHO/BS/2014.2242) to develop replacement WHO international standards for PSA and for PSA (90:10).

7.2.4 Proposed First WHO International Standard for anti-Müllerian hormone

The measurement of anti-Müllerian hormone (AMH) in serum by immunoassay is used as a marker of testicular tissue in paediatric males, to determine ovarian reserve in connection with in vitro fertilization and in the detection of polycystic ovarian syndrome and hypogonadism. Several commercial ELISA kits are available including four CE-marked kits, and requests for assay standardization had been made.
The proposed WHO international standard would be used by manufacturers, proficiency-testing schemes and clinical laboratories to calibrate and control immunoassays for AMH. Two manufacturers had now provided recombinant AMH for formulation and evaluation studies with the option of providing bulk material later. Pilot stage 1 of the project will include small-scale trial fills, stability testing and commutability evaluation in 3–4 laboratories. Stage 2 would then include the large definitive fill and the participation of 8–10 laboratories in value assignment. The four possible approaches to calibration of the international standard were IU labelling, consensus mass calibration, physicochemical evaluation and a primary calibrant approach. The pilot stage would involve significant developmental work.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2242) to develop a First WHO International Standard for anti-Müllerian hormone on the condition that the outcome of pilot stage 1 of the project would be reviewed by the Committee in 2015 prior to initiation of stage 2.

7.2.5 Proposal to assign a holotranscobalamin value to the First WHO International Standard for vitamin B12 and folate in human serum

Holotranscobalamin (holoTC) represents the biologically available form of vitamin B12. The measurement of holoTC is thus an alternative to the traditional approach of using total serum B12 assays. The advantages of holoTC measurement include fewer indeterminate results, particularly among those over 65 years of age; greater utility in pregnant women or patients taking an oral contraceptive; and no requirement for serum pre treatment. There is also some evidence that holoTC level is a better marker for early B12 deficiency than total B12.

As assays for holoTC were now becoming commercially available it was proposed that a holoTC value be assigned to the current WHO international standard (NIBSC code 03/178). It was suggested that the three assay methods currently available for holoTC should be harmonized using 03/178 as soon as possible. An international collaborative study was therefore planned which would involve two to three patient samples. A consensus value would then be assigned to the current WHO international standard and be submitted to the Committee for establishment in 2015.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2242) to assign a holoTC value to the current WHO international standard 03/178, with the suggestion that the number of patient samples to be used in the study be increased.
8. International reference materials – vaccines and related substances

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

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

8.1.1 First WHO Reference Reagent for anti-malaria (*Plasmodium falciparum*) human serum

Malaria is the world’s most common parasitic disease and kills more people than any other communicable disease with the exception of tuberculosis. A WHO-sponsored meeting in 2005 had identified the urgent need to develop a suitable immunoassay standard for *Plasmodium falciparum* malaria antibodies as an important element in the development of malaria vaccines. The Committee was informed that there was currently no human antiserum international standard or reference reagent for any species of malaria.

The Committee was reminded that at its 2013 meeting it had considered the findings of an international collaborative study conducted to evaluate and establish the First WHO Reference Reagent for anti-malaria (*Plasmodium falciparum*) human serum. The Committee had deferred a decision to establish the proposed material pending obtaining additional information on the cross-reactivity of the candidate sample to pre-erythrocytic antigen of *P. falciparum* and pending further clarification of its intended use.

The candidate anti-malaria (*P. falciparum*) reference reagent (NIBSC code 10/198) was a single multivalent preparation that had been developed using diluted human defibrinated plasma. The material had been prepared from pooled plasma collected in Kenya and filled into approximately 5400 glass ampoules and freeze dried. Sixteen laboratories from 12 different countries participated in the collaborative study with each using their own in-house ELISA. The overall objectives of the study were to determine whether the candidate material was fit for purpose and to assign a unitage to it.

Within- and between-laboratory repeatability had been found to be generally very good for potency determinations of the candidate sample. Stability studies had further demonstrated that the candidate material was very stable at the temperatures used both for storage (−150 °C to −20 °C) and laboratory manipulation (4 °C to −56 °C). The results from both accelerated thermal degradation and reconstitution studies indicated that the candidate was stable for long-term use. The Committee was informed that approximately 4000 ampoules would be available for use as a reference reagent.

The intended uses of the proposed reference reagent were: (a) to allow for cross-comparison of the results of vaccine clinical trials performed in...
different centres and/or using different products, (b) to facilitate standardization and harmonization of immunological assays used in epidemiology research and (c) to allow for optimization and validation of immunological assays used in malaria vaccine development.

The Committee considered the report of the study (WHO/BS/2014.2235) and recommended that the candidate material 10/198 be established as the First WHO Reference Reagent for anti-malaria (*Plasmodium falciparum*) human serum with an assigned unitage of 100 units/ampoule.

8.1.2 **Second WHO International Standard for *Haemophilus influenzae* type b capsular polysaccharide**

*Haemophilus influenzae* type b (Hib) can cause numerous invasive diseases worldwide, with the burden of Hib disease being most significant in resource-poor countries. Hib conjugate vaccines exist either as monovalent vaccines or in combination with a range of other vaccines. The WHO recommendation that Hib conjugate vaccines are included in all routine infant immunization programmes has resulted in a dramatic reduction in invasive Hib disease in young children worldwide.

The supply of effective vaccine is dependent upon confirmation of vaccine potency and safety through quality-control testing. A wide variety of assays are used for the quantification of the capsular polyribosylribitol phosphate polysaccharide in purified polysaccharide, bulk conjugates and final lot vaccines. The First WHO International Standard for *Haemophilus influenzae* type b capsular polysaccharide was established by the Committee in 2005. The continuing development of new Hib vaccines, the implementation of global Hib vaccine distribution, and the increasing number of NCLs testing and releasing Hib vaccines around the world had increased the demand for this standard preparation. As a result, the current stock level was less than 20 ampoules.

An international collaborative study was therefore conducted to establish the Second WHO International Standard for *Haemophilus influenzae* type b capsular polysaccharide. Two candidate materials were provided by two different vaccine manufacturers. Following preliminary trial fill studies at NIBSC, both candidate material 1 and candidate material 2 were confirmed to be suitable for evaluation as candidate replacement standards and assigned the NIBSC codes 12/218 and 12/306, respectively. An international collaborative study was then initiated with the primary aim of calibrating the candidate standards in SI units, using the ribose assay. The two candidate samples were analyzed by 13 laboratories from nine countries, each using their own in-house assay. Within- and between-laboratory repeatability was generally found to be very good.

The Committee was further informed that three stability studies were currently ongoing (real-time stability, accelerated thermal degradation and stability following reconstitution in water). The results obtained to date from
both the accelerated thermal degradation study and use after reconstitution study indicated that candidate material 2 in particular appeared to be stable for long-term use. The Committee was informed that approximately 4000 ampoules would be available for use as an international standard.

The Committee considered the report of the study (WHO/BS/2014.2239) and recommended that the candidate material 12/306 be established as the Second WHO International Standard for *Haemophilus influenzae* type b capsular polysaccharide with an assigned content of 4.904 ± 0.185 mg/ampoule.

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

Typhoid fever is caused by infection with *Salmonella enterica* subspecies enterica serovar Typhi (S. Typhi). S. Typhi expresses a Vi capsular polysaccharide (Vi) which is considered to be the main virulence factor and a protective antigen. Although the disease is responsible for considerable morbidity and mortality in developing countries, the true incidence of typhoid fever remains unknown.

In 2009, the Committee had endorsed the replacement of the First International Reference Preparation of anti-typhoid serum (equine). WHO then organized a meeting in 2012 to develop guidelines on the quality, safety and efficacy of typhoid conjugate vaccines. It was concluded that reference materials were needed for Vi and for anti-Vi antibodies for the calibration of immunoassays, for comparison of clinical trial studies, and for the analysis of typhoid conjugate vaccines by immunoassays and physicochemical assays.

A candidate international standard for anti-Vi immunoglobulin G (IgG) in human serum (NIBSC code 10/126) was prepared from pooled sera taken from nine immunized volunteers. In addition, a CBER reference reagent for human anti-Vi IgG (Vi-IgG<sub>R1, 2011</sub>) was made available to facilitate evaluation of the immunogenicity of Vi conjugate vaccines. These preparations differed in terms of their composition, the vaccines used for immunization and other aspects. As a result, it was felt that the comparison of studies that had used either one or the other material was likely to lead to confusion. An international collaborative study was therefore conducted based upon the head-to-head comparison of both materials.

The primary aims of the study were: (a) to assess the suitability of the candidate material 10/126 as an international standard for human anti-Vi antibody; (b) to assess the potency of 10/126 relative to Vi-IgG<sub>R1, 2011</sub>; and (c) to assess the reactivity of both materials in currently used Vi ELISAs. Ten laboratories from seven countries participated in the study, each using both standard and locally developed ELISAs.

The Committee was informed that although the within-laboratory repeatability was generally good, agreement between laboratories was poor,
with the exception of the standard kit ELISA results used by three of the 10 participant laboratories. The data obtained did not allow for calibration of the candidate material 10/126 relative to the CBER reference reagent. It was therefore not possible to determine if the proposed candidate standard would be suitable for use as an international standard. Several issues were identified that may have contributed to the variability of ELISA results, including the quality of the Vi preparations used, and the presence of anti-lipopolysaccharide IgG in candidate 10/126.

Stability studies demonstrated that candidate 10/126 was stable at temperatures used for storage (−20 °C). In addition, accelerated thermal degradation studies conducted for almost 4 years indicated that candidate 10/126 would remain stable for long-term use.

The Committee considered the report of the study (WHO/BS/2014.2234) and recommended that further studies be conducted to determine if candidate material 10/126 would be suitable for establishment as the First WHO International Standard for anti-typhoid capsular Vi polysaccharide Immunoglobulin G (human). The Committee also expressed concern about the use of commercial ELISA kits given the lack of information on their consistency of composition and future availability.

8.2 Proposed new projects and updates – vaccines and related substances

8.2.1 Proposed Second WHO International Standard for \textit{Bordetella pertussis} toxin

The presence of antibodies against pertussis toxin is an indication of pertussis infection, which remains the cause of significant mortality and morbidity worldwide. As the toxoid form of pertussis toxin is the essential component of all currently licensed acellular pertussis vaccines, native pertussis toxin standards are required as working references by manufacturers, control laboratories and research organizations for both in vivo and in vitro assays and research development work. Following average sales of around 170 ampoules per year there was currently a controlled demand for approximately 100 ampoules per year of the existing First International Standard for pertussis toxin (JNIH-5) with 800 ampoules currently in stock. A replacement international standard was therefore needed.

Native pertussis toxin in liquid formulation was prepared by the Serum Institute of India and kindly donated to NIBSC in March 2014 where it will be freeze dried if deemed suitable following initial characterization. Sufficient quantity of material was available for the production of around 10 000 ampoules.

Approximately 10 laboratories will be recruited to participate in a collaborative study to assign an IU value to this material. Values will be determined
using the histamine sensitization assay against JNIH-5. The possibility of assigning unitage for different assay systems such as CHO-cell, and enzymatic activity and carbohydrate binding activity will also be assessed.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a Second WHO International Standard for \textit{Bordetella pertussis} toxin, and agreed that the collaborative study should proceed in 2015 with subsequent submission of its outcome to the Committee in 2016.

8.2.2 Proposed Third WHO International Standard for tetanus toxoid for use in flocculation test

Tetanus vaccines are among the most widely used and successful of human vaccines. They form an essential component of the primary childhood immunization schedule, as well as being used for the reinforcement of immunity in adults and adolescents. In addition, many polysaccharide conjugate vaccines essential for the prevention of invasive bacterial diseases contain tetanus antigen as the carrier protein.

The measurement of limit for flocculation (Lf) content is a critical step in the production process of tetanus vaccines since the final product is formulated based on the number of Lf units in the bulk purified tetanus toxoid. The measurement of Lf content is also used to determine antigenic purity where the Lf content is expressed per milligram of protein nitrogen.

Following its establishment in 2007, the Second WHO International Standard for tetanus toxoid for use in flocculation test had been widely used, with an average demand of 500–600 ampoules per year. As this rate of use was likely to continue and might even increase, the currently estimated 2500 remaining ampoules represented around 4–5 years supply. There was thus a need to initiate replacement of this standard before stocks were depleted. A second objective of the proposed project was to prepare a pharmacopoeial reference standard for the USP–India. To promote harmonization the same material will be used to establish both the WHO and USP standards.

Bulk purified tetanus toxoid (>1500 Lf/mg protein nitrogen) will be sourced from a vaccine manufacturer. Sufficient quantity for a batch size of up to 20,000 ampoules will be requested. It was estimated that up to 5000 ampoules of the final product will be made available to the USP for establishment of the reference standard. This will allow a batch size of up to 15,000 ampoules for establishment of the WHO international standard.

An international collaborative study will be conducted to calibrate the candidate standards in Lf units using the WHO recommended flocculation (Ramon) method. The study design will be based upon that used to calibrate the Second WHO International Standard for tetanus toxoid for use in flocculation test.
Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a Third WHO International Standard for tetanus toxoid for use in flocculation test and agreed that the collaborative study should proceed in 2015 with subsequent submission of its outcome to the Committee in 2016.

8.2.3 **Proposed Seventh WHO International Standard for rabies vaccine**

Rabies is a neglected zoonosis with substantial public health and economic impact with an estimated 60,000 people dying of rabies every year, mostly in Asia and Africa. Rabies vaccines for human and veterinary use are produced in many countries and the minimum potency requirements are expressed in IU. The Committee was informed that restrictions had been placed on the distribution of the remaining 700 ampoules of the Sixth WHO International Standard for rabies vaccine. The vaccine manufacturer who donated the material used to develop the current and previous standards will now be approached for material for a replacement standard. Should the previous donor be unable to provide such replacement material, donations from other human vaccine manufacturers will be solicited.

An international collaborative study will then be conducted involving 10–20 manufacturer and national control laboratories worldwide performing a range of rabies vaccine assays. Study samples will include the candidate replacement standard, the Sixth WHO International Standard for rabies vaccine and, if possible, vaccines from other manufacturers.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a Seventh WHO International Standard for rabies vaccine and agreed that the collaborative study should proceed in 2015 with subsequent submission of its outcome to the Committee in 2016.

8.2.4 **Proposed First WHO International Standard for meningococcal serogroup X polysaccharide**

Meningococcal disease caused by serogroup X is emerging in Africa. Following on from the 2006–2010 outbreaks of serogroup X meningitis in Niger, Uganda, Kenya, Togo and Burkina Faso, there had been an impetus to develop an effective polysaccharide-based vaccine for controlling group X disease. To address this issue, vaccine manufacturers were now developing glycoconjugate vaccines, with GMP batches undergoing preclinical testing. Efforts to develop a quantitative standard were thus timely.

It was proposed that a candidate meningococcal group X polysaccharide be included in the previously agreed upon collaborative study for the establishment of unitage and fitness for purpose of a candidate First WHO International
Standard for meningococcal serogroup A polysaccharide, since the quantification assays used are similar. The Committee was informed that the Finlay Institute in Cuba had donated material sufficient to make approximately 1000 ampoules of the candidate material.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a First WHO International Standard for meningococcal serogroup X polysaccharide, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2015.

8.2.5 Proposed First WHO International Standard for antibody to A(H7N9) influenza virus

H7N9 influenza continues to cause severe respiratory disease in humans in China and there is the possibility that the virus will spread to other countries. H7N9 vaccine trials were now taking place and the serology assays used needed to be standardized. Previous international standards for antibodies to A(H5N1) and A(H1N1)pdm09 viruses had demonstrated the value of international standards in this area. The proposed collaborative study would involve 15–20 laboratories worldwide, using human sera from vaccine trials and a range of serology assays from various geographical areas.

Following discussion and further consideration, the Committee endorsed the proposal (WHO/BS/2014.2243) to develop a First WHO International Standard for antibody to A(H7N9) influenza virus, and agreed that the collaborative study should proceed with subsequent submission of its outcome to the Committee in 2015.
Annex 1

WHO Recommendations, Guidelines and other documents related to the manufacture and quality control 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 used in medicine 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.

All WHO Recommendations, Guidelines and other documents on 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 also available on request from the World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

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

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

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

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

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\(^1\) Abbreviated in the following pages to “TRS”.\n
<table>
<thead>
<tr>
<th>Recommendations, Guidelines and other documents</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitious agents, regulatory risk evaluation on finding an adventitious agent in</td>
<td>Adopted 2014, TRS 993 (2015)</td>
</tr>
<tr>
<td>a marketed vaccine: scientific principles</td>
<td></td>
</tr>
<tr>
<td>Animal cells, use of, as in vitro substrates for the production of biologicals</td>
<td>Revised 2010, TRS 978 (2013)</td>
</tr>
<tr>
<td>BCG vaccines (dried)</td>
<td>Revised 2011, TRS 979 (2013)</td>
</tr>
<tr>
<td>Biological standardization and control: a scientific review commissioned by the</td>
<td>Unpublished document</td>
</tr>
<tr>
<td>UK National Biological Standards Board (1997)</td>
<td>WHO/BLG/97.1</td>
</tr>
<tr>
<td>Biotherapeutic protein products prepared by recombinant DNA technology</td>
<td>Revised 2013, TRS 987 (2014)</td>
</tr>
<tr>
<td>Biotherapeutic products, similar</td>
<td>Adopted 2009, TRS 977 (2013)</td>
</tr>
<tr>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Diphtheria, tetanus, pertussis (whole cell), and combined (DTwP) vaccines</td>
<td>Revised 2012, TRS 980 (2014)</td>
</tr>
<tr>
<td>Diphtheria vaccines (adsorbed)</td>
<td>Revised 2012, TRS 980 (2014)</td>
</tr>
<tr>
<td>Recommendations, Guidelines and other documents</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Haemorrhagic fever with renal syndrome (HFRS) vaccines (inactivated)</td>
<td>Adopted 1993, TRS 848 (1994)</td>
</tr>
<tr>
<td>Hepatitis B vaccines prepared from plasma</td>
<td>Revised 1987, TRS 771 (1988)</td>
</tr>
<tr>
<td>Hepatitis B vaccines made by recombinant DNA techniques</td>
<td>Revised 2010, TRS 978 (2013)</td>
</tr>
<tr>
<td>Human interferons prepared from lymphoblastoid cells</td>
<td>Adopted 1988, TRS 786 (1989)</td>
</tr>
<tr>
<td>Influenza vaccines (inactivated)</td>
<td>Revised 2003, TRS 927 (2005)</td>
</tr>
<tr>
<td>Influenza vaccines (live)</td>
<td>Revised 2009, TRS 977 (2012)</td>
</tr>
<tr>
<td>Influenza vaccines, human, pandemic, regulatory preparedness</td>
<td>Adopted 2007, TRS 963 (2011)</td>
</tr>
<tr>
<td>Japanese encephalitis vaccines (inactivated) for human use</td>
<td>Revised 2007, TRS 963 (2011)</td>
</tr>
<tr>
<td>Japanese encephalitis vaccines (live, attenuated) for human use</td>
<td>Revised 2012, TRS 980 (2014)</td>
</tr>
<tr>
<td>Malaria vaccines (recombinant)</td>
<td>Adopted 2012, TRS 980 (2014)</td>
</tr>
<tr>
<td>Measles, mumps and rubella vaccines and combined vaccines (live)</td>
<td>Adopted 1992, TRS 848 (1994); Note TRS 848 (1994)</td>
</tr>
<tr>
<td>Meningococcal A conjugate vaccines</td>
<td>Adopted 2006, TRS 962 (2011)</td>
</tr>
<tr>
<td>Meningococcal C conjugate vaccines</td>
<td>Adopted 2001, TRS 924 (2004); Addendum (revised) 2007, TRS 963 (2011)</td>
</tr>
<tr>
<td>Recommendations, Guidelines and other documents</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Pertussis vaccines (acellular)</td>
<td>Revised 2011, TRS 979 (2013)</td>
</tr>
<tr>
<td>Pneumococcal conjugate vaccines</td>
<td>Revised 2009, TRS 977 (2013)</td>
</tr>
<tr>
<td>Poliomyelitis vaccines (oral)</td>
<td>Revised 2012, TRS 980 (2014)</td>
</tr>
<tr>
<td>Regulation and licensing of biological products in countries with newly developing regulatory authorities</td>
<td>Adopted 1994, TRS 858 (1995)</td>
</tr>
<tr>
<td>Synthetic peptide vaccines</td>
<td>Adopted 1997, TRS 889 (1999)</td>
</tr>
<tr>
<td>Tetanus vaccines (adsorbed)</td>
<td>Revised 2012, TRS 980 (2014)</td>
</tr>
<tr>
<td>Thromboplastins and plasma used to control oral anticoagulant therapy</td>
<td>Revised 2011, TRS 979 (2013)</td>
</tr>
<tr>
<td><strong>Recommendations, Guidelines and other documents</strong></td>
<td><strong>Reference</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Tuberculins</td>
<td>Revised 1985, TRS 745 (1987)</td>
</tr>
<tr>
<td>Typhoid vaccines, conjugated</td>
<td>Adopted 2013, TRS 987 (2014)</td>
</tr>
<tr>
<td>Vaccines, changes to approved vaccines: procedures and data requirements</td>
<td>Adopted 2014, TRS 993 (2015)</td>
</tr>
<tr>
<td>Vaccines, lot release</td>
<td>Adopted 2010, TRS 978 (2013)</td>
</tr>
<tr>
<td>Vaccines, nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines</td>
<td>Adopted 2013, TRS 987 (2014)</td>
</tr>
<tr>
<td>Vaccines, prequalification procedure</td>
<td>Adopted 2010, TRS 978 (2013)</td>
</tr>
<tr>
<td>Vaccines, stability evaluation</td>
<td>Adopted 2006, TRS 962 (2011)</td>
</tr>
<tr>
<td>Varicella vaccines (live)</td>
<td>Revised 1993, TRS 848 (1994)</td>
</tr>
<tr>
<td>Yellow fever vaccines</td>
<td>Revised 2010, TRS 978 (2013)</td>
</tr>
<tr>
<td>Yellow fever vaccines, laboratories approved by WHO for the production of</td>
<td>Revised 1995, TRS 872 (1998)</td>
</tr>
<tr>
<td>Yellow fever virus, production and testing of WHO primary seed lot 213-77 and reference batch 168-736</td>
<td>Adopted 1985, TRS 745 (1987)</td>
</tr>
</tbody>
</table>
Annex 2

Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine

1. Introduction 65
2. Background 66
3. Scope 68
4. Terminology 69
5. Roles and responsibilities 72
6. Regulatory risk evaluation 74
   6.1 How was the signal detected? 75
   6.2 What is already known about the product concerned? 75
   6.3 Where was the signal detected? 75
   6.4 What exactly was detected? 78
   6.5 New risk–benefit assessment 80
7. Summary and conclusions 81
8. Authors and acknowledgements 83
9. References 86
Guidance documents published by 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.
1. Introduction

The finding of an adventitious agent in a biological medicinal product has been of concern to regulatory agencies, manufacturers and public health officials since the early 1900s when the issue first arose (1). Since then, there have been several instances of a signal being detected for a potential adventitious agent as a contaminant of a marketed product, for example in measles-mumps-rubella vaccine (2). The most recent examples are the finding of porcine circovirus (PCV) nucleic acid sequences or infectious circovirus in rotavirus vaccines in 2010 (3, 4).

In response to such developments, and recognizing the scientific advances made in the detection of adventitious agents in biological medicinal products, the 2010 WHO Expert Committee on Biological Standardization and the International Conference of Drug Regulatory Authorities (ICDRA) (5) recommended that WHO take the lead in providing guidance to its Member States on carrying out national regulatory risk evaluation strategies when an adventitious agent is detected in a vaccine that has already been licensed.

This WHO document is intended to provide guidance to regulators on the scientific principles of risk evaluation when a signal for a potential adventitious agent or novel endogenous agent is detected in an already licensed or registered vaccine. Risk evaluation may support potential regulatory actions by the national regulatory authority (NRA) and/or national control laboratory (NCL), the relevant inspectorate (for example, the Good Manufacturing Practice (GMP) inspector) and/or relevant public health officials such as the National Immunization Technical Advisory Group (NITAG). However, specific guidance on the national decision-making process for regulatory actions is beyond the scope of this document.

Manufacturers routinely manage risk to assure the quality of their products in the manufacturing procedures and environment as part of their compliance with GMP. In some countries, quality-by-design principles have also been applied. Public health officials make decisions on the basis of risk–benefit assessments and, often, on the basis of cost–benefit balances. These established practices are assumed to remain in place when a potential new adventitious agent is found, and are also beyond the scope of this document.

It is important to note that, in the context of this guidance document, it is understood that regulatory risk evaluation is an independent evaluation process performed by regulatory authorities on the basis of data provided by the manufacturer and that it differs from quality risk management principles, as outlined for example in guidelines published by WHO (6) and by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (7). Depending on the capability and capacity of the NRA and/or NCL, the independent evaluation process may include its own laboratory investigations.
2. Background

Although the principles of drug regulation are generally consistent internationally, the legislation, duties, responsibilities and structure of the institutions responsible for translating the principles into laws, regulations and guidance may vary substantially from country to country. Nevertheless, a number of functions are generally considered essential for an acceptable regulatory system in a producing country, namely: (a) marketing authorization and licensing; (b) post-marketing surveillance (including for adverse events); (c) lot release; (d) access to laboratory facilities; (e) GMP inspections of manufacturing sites and distribution channels; and (f) authorization and monitoring of clinical trials. Countries take these elements into consideration and adapt the principles to their structure. In addition to these regulatory functions, some countries have a NITAG (8) that helps to guide national immunization policies and that usually works closely with the relevant regulatory agencies when safety concerns arise.

To help assure that biological products and the biological starting materials from which they are manufactured are free of adventitious agents, NRAs and/or NCLs require a variety of tests to be performed by manufacturers at relevant stages of the production process. Further, it is important to ensure that the risk of potential contamination with adventitious agents, including those that cause transmissible spongiform encephalopathies (TSEs), is reduced by ensuring the quality of starting materials and/or of the production process for a biological medicinal product (for example, aseptic processing, viral clearance during purification processes and TSE risk assessment). In addition to traditional tests, new technologies for detecting adventitious agents are being developed and are coming into use. These new detection technologies may have higher sensitivity than methods used previously and may detect agents that earlier methods were not capable of detecting, or may detect nucleic acid sequences attributed to such agents. Until now (2014), screening for adventitious agents has relied upon the use of transmission electron microscopy, in vitro infectivity or biochemical assays, in vivo assays and specific polymerase chain reaction (PCR) tests. New methods and technologies, such as next-generation sequencing or microarrays, are powerful tools for the detection and identification of sequences from viruses and other adventitious agents without prior knowledge of the nature of the agent. In the future such new technologies may uncover the presence of other, as yet unrecognized, adventitious agents. Furthermore, new agents are emerging and being discovered. Therefore, the situation may arise where, subsequent to marketing authorization of a product, it is discovered that the cell substrate from which it was produced or the raw materials used in its production are contaminated with a previously undetected or unknown adventitious agent (9).
Adventitious agents and the viral safety of biological medicinal products are governed by a broad pre-licensure regulatory framework that includes: (a) evaluation by regulators of the manufacturer’s control of the manufacturing environment; (b) compliance with current GMP; (c) testing of starting materials, intermediates and the final product; and (d) requirements for the validation of viral testing and for inactivation and/or removal procedures. Detailed WHO recommendations are available in relation to the use of animal cell substrates for the manufacture of biological medicinal products (10). The risk associated with TSEs in general, and bovine spongiform encephalopathy (BSE) in particular, is addressed primarily through precautionary measures set out in WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (11). The latest version of WHO tables on tissue infectivity distribution in transmissible spongiform encephalopathies – which is periodically updated as new data become available – should also be consulted (12).

Nevertheless, the discovery of a signal for a potential adventitious agent in a product subsequent to marketing authorization raises concerns that are not well addressed in existing guidance in terms of regulatory actions and decision-making. For example, a clear evaluation strategy was not in place to support regulatory decision-making when nucleic acid sequences for PCVs or infectious circovirus were reported in rotavirus vaccines (3, 4). Similar situations have occurred in the past, including findings in the 1960s of SV40 in poliomyelitis vaccines (13) and of avian leukosis virus in yellow fever vaccines (14, 15). In the 1970s, the discovery of bacteriophages in commercial sera and live viral vaccines led to the need for regulatory actions (16, 17). The development of product-enhanced reverse transcriptase (PERT) and related PCR-based reverse transcriptase (RT) assays led to the discovery of RT activity at levels not detectable by the conventional assay used in the control of avian cell-derived vaccines in the mid-1990s, thus suggesting the possible presence of a contaminating retrovirus (18–22).

These examples illustrate that both conventional and new methods have led to the discovery of infectious agents or the marker of a viral agent in vaccines. Recent advances in technology have the potential for other types of findings to be made that are suggestive of contamination with an adventitious agent. Such findings could include the discovery of a structure suggestive of a viral particle by visualization technologies such as enhanced electron microscopy, or the discovery of a nucleic acid sequence suggestive of an adventitious agent by modern amplification or sequencing technologies. These sequencing technologies may involve assessing genomes (free or encapsidated) or RNA transcripts. Further, the technologies may entail either positive selection against a curated database of known sequences of adventitious agents or negative selection.
to eliminate host cell sequences followed by analysis of what remains. In either case, regulators may be faced with making risk evaluations and decisions about the safety of licensed vaccines on the market in their country on the basis of incomplete data on whether an adventitious agent is present or not.

3. Scope

This WHO document provides guidance to regulators on the scientific principles of risk evaluation when evidence (a “signal”) for a potential adventitious agent is detected in a vaccine that is already licensed or registered. The regulatory implications of such a finding are also considered.

While the same principles may apply to all biological products, this document focuses on vaccines, for several reasons. Vaccines are used globally in national immunization programmes and are given to whole populations of healthy individuals who are often children. As a result, there could be a major global impact if a serious safety issue were to arise involving the finding of a signal for a potential adventitious agent in a childhood vaccine. On the other hand, the premature withdrawal of a vaccine due to suspected contamination by an adventitious agent could lead to major outbreaks of vaccine-preventable disease in both immunized and non-immunized populations. An understanding of regulatory risk evaluation principles is therefore critically important in the context of vaccines. Nevertheless, if an adventitious agent were to be identified in a biological product other than a vaccine (including biotherapeutics prepared by recombinant DNA technology) the scientific principles described here could apply in the regulatory risk evaluation. However, it is beyond the scope of this document to provide further specific guidance since each case will have unique characteristics that will require judgements to be made by NRAs and manufacturers.

The regulatory risk evaluation process can, and often does, lead to other regulatory actions and considerations. For example, as a result of a regulatory risk evaluation, safety information may need to be revised. While such actions are to be expected when appropriate, they are beyond the scope of this document. In addition, a regulatory risk evaluation may indicate that a change in the production process could improve the ability to remove the agent from (or inactivate it in) future lots of the product, thus reducing the risks. However, changes to the manufacturing process may have impacts on the established quality, purity, potency, safety and efficacy of a licensed or registered product. These considerations are also beyond the scope of this document.

The document also does not cover any aspect of risk management or steps to be taken by manufacturers, nor does it provide guidance on decisions that may be taken by public health officials such as NITAGs. In the context of
complaints about pharmaceuticals and other incident-based risks to health, GMP inspectorates usually have their own risk-assessment or risk-management procedures, including a risk classification. Such risk-assessment or risk-management procedures are also not included in the scope of this document.

4. Terminology

The definitions given below apply to the terms as used in this WHO guidance document. They may have different meanings in other contexts.

Adventitious agent: contaminating microorganism of the cell culture or starting/raw materials, including bacteria, fungi, mollicutes (mycoplasmas or spiroplasmas), mycobacteria, rickettsia, protozoa, parasites, agents causing TSEs and viruses that have been unintentionally introduced into the manufacturing process of a biological product. The source of the contaminant may be the legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production or in their legacy), the environment, personnel, equipment or elsewhere.

Cell substrate: cells used to manufacture a biological product. The cells may be primary or cell lines, and may be grown in monolayer or suspension culture conditions. Examples of cell substrates include primary monkey kidney, MRC-5, CHO, and Vero cells. Cells used to generate essential components that will be used to make a final product, such as Vero cells used for “reverse genetics” of an influenza virus to seed vaccine production, are considered to be “pre-production” cell substrates. Cells used to manufacture the bulk product (for example, packaging cell lines for gene therapy vectors, Vero cells for vaccine production, and CHO cells for recombinant protein expression) are considered to be “production” cell substrates.

Dedicated facility: a manufacturing establishment or suite within the establishment that is used solely for the production of one product and is not used to manufacture any other product at any time. In contrast, a multi-use facility is one in which multiple products may be made either on a campaign basis (one at a time, in series) or simultaneously.

Environmental risk: the risk to public health and the environment. It does not include the risk to the intended recipient of the vaccine, which is assessed through clinical studies of the vaccine. It also does not include the risk to laboratory workers.

Inspectorate: a civil agency charged with inspecting and reporting on manufacturing facilities to ensure compliance with regulatory requirements.

Intermediate: partly processed product that must undergo further manufacturing steps before it becomes a bulk product.
**Investigational and action plan:** a documented approach to undertaking a risk-reduction and risk-management strategy through root-cause investigations and application of corrective and/or preventive actions.

**Marketing authorization:** an official document issued by the competent NRA for the purpose of marketing or free distribution of a product after evaluation for safety, efficacy and quality. In some countries, the term “licensing” or “registration” is used.

**Microarrays:** a collection of spots of nucleic acids attached to a solid surface. Each spot contains picomoles of a specific nucleic acid sequence that serves as a probe. A sample may be hybridized to the spots and detected by fluorophore, silver or chemiluminescence labelling. The solid support may be in the form of a chip or beads made from silicon or glass.

**National Immunization Technical Advisory Group (NITAG):** a technical resource providing guidance to national policy-makers and programme managers to enable them to make evidence-based immunization-related policy and programme decisions.

**Next-generation sequencing:** high-throughput sequencing technology that processes sequences in parallel, producing thousands or millions of sequences at once from a sample. Examples of methods and technologies include 454 pyrosequencing, Illumina and Ion Torrent. Each method has different attributes, such as length of a typical sequence read, accuracy, number of reads per run, time for a run and costs. As a result, the choice of method should take into account the purpose for which the data are to be generated. Significant bioinformatics using curated (trusted) databases are needed to analyse the considerable amount of data generated in each sequencing run.

**Quality by design:** a systematic approach to product development or manufacturing that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. A means to build into a product or process the inherent set of characteristics needed to fulfil quality requirements.

**Regulatory risk evaluation:** a systematic process of evaluating information to support a risk–benefit decision within a regulatory review and evaluation framework. It consists of an independent evaluation of the risk assessment performed by the manufacturer, taking into consideration all relevant and available information and data.

**Risk:** the combination of the probability of occurrence of harm and the severity of that harm. In the context of pharmaceutical quality it is the probability and severity of any kind of negative impact (hazard) on the quality of the product.

**Risk assessment:** a systematic process of organizing information to support a risk decision to be made within a risk-management process. It consists
of the identification of hazards, and the analysis and evaluation of risks associated with exposure to those hazards.

Risk evaluation: the comparison of the estimated risk to given risk criteria, using a quantitative or qualitative scale to determine the significance of the risk.

Risk-reduction strategy: a plan or method for achieving a decrease in the probability of occurrence of harm and/or the severity of that harm.

Risk management: a systematic process for the assessment, control, communication and review of risks. Risk management in the context of pharmaceutical quality is often referred to as quality risk management – a systematic process for the assessment, control, communication and review of risks to the quality of the medicinal product across the product’s life-cycle. A model for quality risk management is outlined in the relevant WHO and ICH guidelines (6, 7).

Root-cause investigation: a problem-solving method that involves systematic investigation of deviations or out-of-specification results in order to identify an underlying root cause of the faults or problems that caused them. Generally, the analysis aims to identify the factors that resulted in the nature, magnitude, location and timing of hazards or adverse outcomes. In this way, necessary behaviours, actions, inactions or conditions that require changing to prevent the deviation or out-of-specification results from recurring in future may be detected, corrected and/or prevented.

Sensitivity: the lower limit of quantification (LLOQ) or, for a non-quantitative assay, the limit of detection (LOD) of an assay. The LLOQ is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The LOD is the lowest amount of the analyte in a sample that can be reliably detected, but not necessarily quantified as an exact value.

Specificity: the ability of a method to detect a specific microorganism or range of microorganisms that might be present in the test sample.

Starting material: biological starting materials include the cell substrate or cell banks, a cell seed in the case of bacterial vaccines, or a viral seed in the case of viral vaccines. In the case of primary cell substrates, starting material considerations should also include the source, such as species, tissue or organ, from which the cell substrate was derived.

Test or assay: an analytical procedure or method – used for example for the identification of an analyte, for measuring the content or presence of impurities, or for the quantification of active ingredients.

Upstream: relating to activities that occur at or near the beginning of a process or manufacturing flow – such as cell culture and harvest, or establishment
of seeds or cell banks – as distinct from downstream activities such as purification, concentration, formulation and filling.

**Validity:** an expression of the degree to which a measurement performed actually measures the characteristic which the investigator wishes to measure. This degree may be ascertained by a combination of analytical validation of the measurement method (23) and scientific validation that the method being used for a given purpose actually measures the intended characteristic and that the characteristic is scientifically meaningful.

### 5. Roles and responsibilities

Regulatory oversight is the responsibility of the NRA and/or NCL. Whenever there are new findings concerning adventitious agents with the potential to have a negative impact on the quality, safety or efficacy of a marketed vaccine, it is the responsibility of the manufacturer to provide the NRA and/or NCL with all relevant data and information, plus, when requested, currently available materials (samples or specimens). All this information is critical for regulatory investigation and decision-making, which should include: (a) confirmation and evaluation of the findings; (b) the manufacturer’s own risk assessment, and risk-reduction and risk-management strategy; and (c) an investigational and action plan in order to facilitate any regulatory action that might be necessary.

On the basis of the manufacturer’s data and any other reliable and credible data that are available, the NRA and/or NCL will evaluate the risk of the potential adventitious agent. If a regulatory agency is in a position to perform its own independent investigations on available biological material, this may help in assessing the risk.

The main areas to consider in a risk evaluation performed by a regulatory agency relate to the following questions:

- How was the signal detected?
- What is already known about the product concerned?
- Where was the signal detected?
- What exactly was detected?

These areas are described in detail in section 6 below. The sequence of the questions is of no significance. Moreover, regardless of whether sufficient data are already available to answer the questions, each of these areas should be considered prior to a new risk–benefit assessment. Each time new data emerge, a new risk–benefit assessment may be necessary.

Regulatory risk evaluation should be carried out on the basis of current science and technology. Regulators should conduct an independent evaluation
of the manufacturer’s new data in the context of the risk–benefit assessment of
the licensed or marketed vaccine. The potential impact of regulatory decisions
on public health should be discussed with public health officials. The evaluation
should take into account the country-specific risk–benefit assessment of public
health officials, if available. The whole evaluation process should lead to an
updated risk–benefit assessment to be used as the basis for any regulatory action
that may be necessary. In addition, the updated assessment will be important for
helping public health officials to decide on current recommendations regarding
the use of the product in their country.

Since the process is likely to be dynamic and new data for evaluation will
continue to emerge during the process, the assessment should include feedback
loops at each step. There is a need for transparent communication between the
NRA and/or NCL and the manufacturer, and potentially between NRAs, as well
as between NRAs and other groups such as the NITAG, GMP inspectorate and
relevant experts from the scientific community.

Depending on national practice or due to the characteristics of the
finding, it may also be necessary to include and/or facilitate direct communication
between the manufacturer and public health officials such as the NITAG. If the
marketed product is still being evaluated in ongoing clinical trials (for example,
Phase IV studies) then ethics review committees will need to be informed and
involved in the decision-making process.

Importantly, the role of WHO in the global coordination of responses
and communication between the NRAs of different countries and regulatory
regions has been, and continues to be, pivotal in such situations – as for example
in the case of the discovery of RT activity in chicken-cell derived measles vaccine
(19). This is especially the case for licensed vaccines that may be in use and
are marketed in many countries. Coordinated efforts will be required in such
situations to avoid public confusion. When a company discovers – and/or an
NRA receives a report of – a signal of a potential adventitious agent in a marketed
vaccine, communication with WHO should be undertaken immediately. For
vaccines that are prequalified by WHO, communication with the unit responsible
for prequalification\(^1\) would be an appropriate entry point (24, 25). Different
regulators may have different risk–benefit considerations for their country on
the basis of, among other factors, vaccine supply, disease prevalence and severity,
and the specific epidemiological situation. Nonetheless, for the purposes of risk
communication and public transparency, a globally coordinated response is
warranted whenever a potential adventitious agent is found in a vaccine that is
marketed in countries across the world.

\(^1\) Prequalification Team, World Health Organization, 1211 Geneva 27, Switzerland.
6. Regulatory risk evaluation

Fig. 1 outlines the process for conducting a regulatory risk evaluation.

Fig. 1
Regulatory risk evaluation process

Any finding of a potential adventitious agent previously unrecognized

Risk management performed by the manufacturer: confirmation/evaluation/risk assessment

Information to be provided to NRAs for an independent regulatory risk evaluation:
Investigational and action plan

Points to consider for regulatory risk evaluation

How was the signal detected?
• Sensitivity, specificity and validity of the assay used to identify the agent including appropriate control materials
• Laboratory investigations performed by NRA/NCL (if applicable)

What is already known about the product concerned?
• Data provided in the original file for licensing (if appropriate)
• Post-marketing data, pharmacovigilance (if available)

Where was the signal detected? (Risk associated with the product)
• type of vaccine
• starting material, intermediate, final product
• purification/inactivation
• route of administration
• environmental risk
• others.

What exactly was detected? (Risk associated with the agent)
• kind of signal (e.g. DNA, virus, …)
• origin (e.g. animal, …)
• infectious/pathogenic
• potential long-term effects
• environmental risk
• others.

New risk–benefit assessment
(consider already vaccinated persons and those to be vaccinated)
6.1 **How was the signal detected?**
This question addresses all the issues related to sensitivity, specificity and validity of the methodology used to detect and/or identify the potential agent. The question indicates the need for reliable confirmatory data provided by the manufacturer and, if applicable, by laboratory investigations performed by the NRA and/or NCL.

6.2 **What is already known about the product concerned?**
Manufacturer data relevant to the finding, and all other reliable data available, should be evaluated together with relevant information from the initial marketing authorization. This is of particular interest if the newly detected agent or potential agent was likely to have been present at the time of marketing authorization. Manufacturing and quality-control data, including control over raw-material suppliers, should be assessed in close cooperation and communication with the responsible GMP inspectorate. It is especially important to assess potential GMP failures, strategies to avoid or mitigate the newly detected agent, and the root-cause investigation.

In cases where the detected potential agent is a previously unrecognized adventitious agent, nonclinical and clinical data, including post-marketing and/or pharmacovigilance data if available, will be vital in evaluating the safety of the contaminated vaccine and any potential risks associated with the contaminating agent. The assessment of these data should consider risks that may be unique to a specific patient population – such as immunocompromised individuals, infants or the elderly – if they are included in the clinical indication for the product or often receive the product off-label. Moreover, the epidemiology of the agent may inform the assessment of the risk. The evaluation of potential long-term effects will depend upon the type and amount of data available.

6.3 **Where was the signal detected?**
The type of vaccine concerned has an important impact on the potential risk. Other factors that are linked to the type of product should also be considered. For example: Was the signal found in the starting materials, reagents, intermediates or the final product? Do any of the manufacturing steps, including purification and/or inactivation processes on the starting materials or on the product, have a positive or negative impact on the signal? What is the route of administration of the product, and how does this influence the risk to the product recipient from the potential agent?

Other parameters, such as the dose, the schedule of administration and characteristics of the recipient population, may be usefully considered in the risk evaluation. It may be vitally important for the manufacturer to investigate
retained materials (raw or starting materials, intermediates or bulks, and final containers) in order to define a root cause, and for regulators to review and evaluate such investigations. This may be especially significant if an adventitious agent of an unexpected species is found (such as an equine virus being found when no equine materials were thought to have been used in manufacturing). Furthermore, the potential risk to the environment is influenced by features of the product concerned (for example, by the way in which it is excreted by the product recipients, or the way manufacturing wastes are handled) as well as by the type of agent found.

Within this context, it is appropriate to distinguish the characteristics of the three main stages in vaccine production – namely, starting materials, intermediates and final product. The following questions are applicable once an adventitious agent has been detected or the finding is suggestive of the presence of an agent:

- How was the agent introduced (that is, what were the results of the root-cause investigation)? If the agent was introduced by starting materials then most (or all) lots could be implicated, including clinical lots used in product development. This means that the agent could have been consistently present during clinical investigations of the vaccine. If the agent was introduced by the environment, by personnel or by specific batches of raw materials then a more limited number of lots may be implicated. Assessment of whether those specific lots were used in humans, and clinical follow-up of humans exposed to the implicated lots, could provide valuable data on the actual risk to humans. In terms of consistency, it is crucial to characterize the genetic sequence and load of the agent between different lots.

- Are other products affected? If the agent was introduced in the cell substrate or raw materials that are used by the manufacturer in multiple products then products other than just the implicated product may be affected. If the agent was introduced during production of a specific lot (or lots) then other products manufactured concurrently in the same facility as the affected lot could be implicated. A thorough investigation should be undertaken to evaluate whether other products or lots could be contaminated and, if so, these should be treated in the same way as the vaccine lot that was originally implicated. However, if the affected cell substrate or raw material batches are used for the sole purpose of producing one product, and/or if the facility is a dedicated facility, the risk to other products from that manufacturer may be limited. If product lots are infected by an upstream contamination, the frequency of the contamination
should be addressed. In this case, clinical lots used in product development may provide particularly useful information in terms of the potential impact of the contamination on the health of human subjects in clinical trials, as available in the clinical safety databases. A retrospective analysis of the clinical lots may be beneficial to show whether the integrity and loads of the agent are comparable between the clinical lots and the marketed product (commercial lots).

- Especially for a live viral vaccine that may be given by normal routes of infection, what is the impact of the route of administration of the product? Viruses vary in their routes of normal infection and may establish productive infections only when the host is exposed by a particular route. However, a product may be administered by a route other than the normal routes of viral exposure. In the case of parenteral products, which bypass the normal defence mechanisms of the host (such as skin, saliva and stomach acids), infection may occur more readily. Of course, it may also be the case that exposure through an abnormal route may preclude exposure of susceptible target cells, thus reducing risk. Alternatively, if a product such as a live viral vaccine is delivered by a normal viral infection route (such as orally or intranasally) then information about normal virus exposure (such as epidemiology data) may be more relevant to the situation.

- Is there a risk to the environment? It may be determined that the agent does not pose a significant risk to the recipients of the product. However, the agent to which they were exposed may be shed by recipients through normal excretory processes. For example, polioviruses can replicate in the human gut and be shed in faeces, thereby exposing the close contacts of recipients of oral poliomyelitis vaccine through the faecal-to-oral route. If the adventitious agent is shed by the product recipients, then human and animal contacts of these recipients could be placed at risk of exposure to the agent. In the case of shedding of the agent from product recipients, it will be valuable to evaluate whether any variation can be detected in the genetic sequence of the agent isolated from excreta.

- Is there a risk of dissemination at the manufacturing plant? Even if the product itself is not contaminated, but an upstream material such as a cell substrate is found to be contaminated, then there may be a risk to the environment from the manufacturing process and/or disposal of manufacturing waste products, particularly if the agent found is not already endemic to the geographical region in which the facility is located. It would be expected, in line with current GMP, that waste is decontaminated before release into the
environment, but procedures should be reviewed regarding their effectiveness in relation to the agent concerned.

6.4 What exactly was detected?
The risk associated with the agent detected depends primarily on its physical nature (for example, whether what was found was nucleic acid or an intact virus). In addition, thorough consideration should be given to the normal host species of the agent (for example, whether it is animal-, plant- or human-derived) and to whether or not it has the potential to be infectious or even pathogenic to humans or animals. Any potential long-term effects, or other effects that can be linked to the agent, will also need to be evaluated. As noted above, the potential risk to the environment will depend upon the characteristics of the agent and the product concerned.

The following series of questions and potential answers is intended to help decision-makers to evaluate the risks to humans associated with the discovery, through the use of new detection technology, of the nucleic acid of an adventitious agent in the starting materials, intermediates or final product of a licensed vaccine. The series may be modified for other types of findings, such as structures suggestive of viral particles, microbial agents or enzymatic activities suggestive of enzymes encoded by viruses.

Reasonable questions following a finding based upon nucleic acid detection might include, but would not necessarily be limited to:

- Is the agent a known agent, a member of a known family or a novel agent?
- Are the nucleic acids that were found simply fragments or are they full-length intact genomes?
- Are the nucleic acids that were found free or particle-associated?
- If the nucleic acids are associated with particles, have these particles the potential to infect cells of the suspected normal host species?
- Are these particles infectious in the suspected normal host species?
- Are the particles infectious in cell cultures, including human cells?
- Is the agent known to be infectious in humans?
- Is the agent pathogenic or an opportunist pathogen in humans?
- Is the agent transmissible from human to human, from animal to human or from human to animal?

It should be borne in mind that, as in all scientific investigations, the evaluation will be complex and likely to be more complicated than answering a
series of questions. Each situation will likely be unique, and addressing all steps in the algorithm may not be possible. Any of the individual elements of such an algorithm may also not apply in some cases, or may need to be adapted depending on each particular situation. Where nucleic acids are found that might indicate the presence of an adventitious agent, the following illustrative general approach may be taken to address the above questions.

If the viral nucleic acids are full-length and intact but free (that is, not particle-associated), then they have the potential to be infectious if they are taken up by susceptible cells; thus they may still represent a risk, depending upon whether or not the conditions favour or disfavour infectivity. These conditions include, but are not limited to, those described here. For example, if the route of inoculation exposes the free nucleic acids to nucleases (for example, by oral administration), they would be eliminated or fragmented before they could be taken up by cells. The route of administration could also influence the availability of susceptible target cells to take up the free nucleic acids.

Similarly, if the nucleic acids are particle-associated but fragmented, consideration should be given to whether or not they infect cells in the vaccine recipient. Also, when the nucleic acids are uncoated in the cell, the possibility must be considered that they could be repaired by natural cellular repair mechanisms, thus leading to a productive infection in the recipient despite the nucleic acids being fragmented inside the viral particle.

In either of these cases, or in the case of infectious particles that lead only to an abortive infection in human cells, concern may still exist if the agent is one that is known to result in pathology following abortive infection. In particular, oncogenic viruses could still represent a risk even if they result only in abortive infections.

For some viruses it has been shown that, while the host species does not display disease, infection of humans (or another non-host species) by the virus may result in significant morbidity or mortality. A significant example of this is simian herpes B virus which usually does not cause disease in monkeys, which are the natural hosts, but can cause fatal disease in humans.

If it is unknown whether an adventitious agent causes disease in humans but it causes disease in the host species, this may also represent a potential risk. In such a situation, if the adventitious agent was found in a starting material (lot) that was used in clinical trials a re-evaluation of the existing data should be considered. The existing databases generated from post-marketing safety studies or surveillance, as well as from clinical trials, should be searched for signals from the clinical data reflective of the known pathology in the host species in order to determine if a similar disease syndrome might be occurring in product recipients. However, symptoms and pathology may be quite different in humans, and this must be kept in mind during the evaluation of clinical safety databases. As part
of assessing the potential limitation of existing information, consideration should also be given to the possibility that existing databases may not have captured the relevant signal (symptom or pathology).

One indicator that the agent may be able to infect humans, even in the absence of a well described disease syndrome or recognized zoonosis, would be the occurrence of antibodies in product recipients and naturally exposed individuals. In addressing the issue of whether the agent infects humans, one question that should be asked is whether there is evidence of immunity in humans – such as the presence of antibodies in veterinarians or individuals involved in the husbandry of the animal species associated with the agent (for example, pig farmers in the case of PCVs). In addition, if serum samples had been saved from human subjects from the clinical trials of the product, these could be screened for antibodies to the suspected agent. This may require a review of the informed consent forms from the original trial to determine if this additional use of the sera is covered by the consent given by the subjects. If not, subjects may need to be requested to give informed consent for their sera to be used for this purpose. When clinical trials are being designed, the potential need for such future uses of stored sera should be considered, along with the required storage conditions, in order to facilitate the rapid and ethical future use of existing samples.

6.5 New risk–benefit assessment

In principle, a new risk–benefit assessment is needed whenever the magnitude and scope of benefits, or of previously unrecognized risks, are elucidated and confirmed. The risk–benefit assessment should therefore be updated periodically, especially after process changes, in accordance with the principles of risk assessment. Methods and principles for a systematic approach for risk–benefit assessments are provided elsewhere (26) and are not within the scope of this document.

Within the health care community there may be different perspectives on benefit and risk. In the case of GMP and/or pharmaceutical technical issues, the risk–benefit assessment falls within the responsibility of the GMP inspectorate, or the NRA and/or NCL. Each (new) risk–benefit assessment of a vaccine may also have public health implications. The risk–benefit assessment of public health officials such as NITAGs is usually performed separately and may differ from the regulatory assessment due to additional considerations, such as population versus individual health considerations and the need for cost–benefit analyses. As with the GMP inspectorate, close collaboration and communication between the licensing authority and public health officials is considered to be crucial. In addition, communication with ethics committees should be considered if the product is still being investigated in clinical studies (for example, Phase IV studies).
Risk–benefit assessments of vaccines depend not only on scientific and biological considerations but also on regional considerations, and on the particular circumstances (for example, epidemiology, availability of alternative vaccines, and regulatory or legal framework) in those areas. Nevertheless, in an increasingly global environment, communication and the exchange of information on a global level is of utmost importance. Furthermore, there is a need for public transparency, and for risk communication to be clear, credible and consistent. As a result of these and other factors, WHO has a key role to play in the global coordination of communication efforts in this area.

7. Summary and conclusions

Regulatory risk evaluation is a dynamic process both in terms of how it has evolved over the past 60 years and in the way in which information is accumulated and evaluated in any given instance. Much has been learnt since the discovery of SV40 as a contaminant of poliomyelitis vaccines in the 1960s, and it is hoped that the lessons of past cases of finding an adventitious agent in vaccines will provide useful guidance for the future.

The four case studies discussed by Petricciani et al. (22) cover a broad range of possible contaminants of viral vaccines. In each case the initial response was to determine whether the finding posed an unacceptable risk to public health in light of the proven or expected benefits of the vaccine in question, and whether the signal really was indicative of a live infectious virus contaminant with serious adverse effects on recipients. In all four cases, and after consideration of the scientific advice, the vaccines concerned were not removed from the market, or were only temporarily suspended, since the benefits of immunization were believed to significantly outweigh the risk of any potential adverse effects. After further evaluation, that initial assessment proved to be correct.

In each case, the response to the initial findings benefited from knowledge gained from past experiences. It is important to make adjustments based on lessons learnt from experience. These events highlighted that in order to respond effectively, it is essential to have access to expert scientific advice, good communication, public transparency, international collaboration and effective global coordination.

The manner in which the international scientific community dealt with the case of RT in measles and mumps vaccines should serve as a model for dealing with similar issues in the future. During the scientific investigation and response to the RT finding, excellent coordination and collaboration allowed for the reaching of a global consensus on what regulatory actions were appropriate, which in turn facilitated clear communication of the issues.
Despite similar good collaboration in addressing the PCV issue, this incident also highlighted the complexities involved in arriving at a global consensus when local and/or regional considerations have to be taken into account. Different NRAs and NCLs may have different risk–benefit considerations for their country based on factors such as vaccine supply, disease prevalence and severity, and their specific epidemiological situation. All of these factors must be taken into consideration during the decision-making process, which should always be based on sound science. In all cases, the potential impact of regulatory decisions on public health should be discussed with public health officials, with a clear need for transparency in the decision-making process.

The PCV case also highlighted the same issue previously identified during the bacteriophage case, namely that the quality of reagents such as sera and trypsin used in vaccine production, and in the preparation of important biological starting materials such as cell banks and viral seeds, must be well controlled in order to have a final product that is free from contamination. Indeed, concern about the quality of starting materials has recurred periodically (for example, RT in eggs and BSE in bovines). The risk of such microbial contamination can be minimized by limiting the use of animal-derived materials in manufacturing, applying GMP, and conducting stringent testing and control of raw materials, manufacturing intermediates and final product.

The issue of the safety of vaccines for global use is an area where WHO collaborating centres and other expert advisors may, when requested, provide advice to WHO on cell substrate viral safety. Such expert advice, based on state-of-the-art technologies developed by manufacturers, NRAs, NCLs and academic institutions, would provide valuable support to WHO in facilitating timely responses to unexpected findings of adventitious agents in vaccines.

The final general observation from the four case studies relates to the key role that WHO has played in coordinating a global response to the finding of an adventitious agent (or signal of an agent). The role of WHO in coordinating the global actions to be taken and facilitating communication among the NRAs of various countries and regulatory regions continues to be pivotal in such situations, as for example in the case of the discovery of RT activity in chicken cell-derived measles vaccine. This is especially the case for licensed vaccines that may be in use in many countries. Although WHO coordinates efforts directed towards achieving international consensus, it is the NRA that will ultimately make decisions based upon the risk–benefit assessment for their own populations. As a result, there may be different decisions reached in different countries or regions. In order to avoid confusion, it is important that NRAs clearly communicate the rationale and basis for their decision. In addition, in the interest of global public health, any signal of a potential adventitious agent in a marketed vaccine should be reported promptly to WHO. NRAs and manufacturers have a responsibility
to immediately inform WHO of such a finding and to take appropriate follow-up actions, particularly in the case of prequalified vaccines.

Since the finding of SV40 in poliomyelitis vaccines there have been numerous scientific and technological advances, along with the routine use of more efficient methods of communication and information exchange. Such developments have led to an increasingly comprehensive and transparent response to the finding of an adventitious agent (or signal) in a marketed vaccine. Manufacturers and regulators continue to investigate new technologies, and when new methods prove to be superior to existing ones then updated practices should be introduced.

A central element of the regulatory risk evaluation process is that the assessment needs to be updated each time new significant data emerge; thus, it is an iterative process. Nevertheless, it is often the case that there is a need for immediate decisions at an early stage of the evaluation when many of the answers to the questions outlined in this guidance document will not be available. This presents particularly challenging situations for all interested and affected parties. Due to the potential complexity of future events, simple universally applicable guidance or a fully comprehensive list of priorities to consider cannot be provided. Among the most important lessons from the past is the desirability of transparency and open communication. When all parties with a vested interest in the outcome of a regulatory risk evaluation are aware of – and understand – the basis upon which decisions are made, the probability of miscommunication and error are minimized. WHO will therefore continue to play a vital role not only in the global coordination of responses to the discovery of a signal for a potential adventitious agent but also in the public communication of the outcomes of regulatory decision-making.

8. Authors and acknowledgements

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9. References


2. Brown D. Unexpected protein found in measles-mumps vaccine; entity said to be from chicken, harmless to humans. The Washington Post, 9 December 1995.


Annex 3

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


Introduction

Scope

General considerations

Terminology

Part A. Manufacturing recommendations

A.1 Definitions

A.2 General manufacturing recommendations

A.3 Control of source materials

A.4 Control of vaccine production

A.5 Filling and containers

A.6 Control tests on the final lot

A.7 Records

A.8 Retained samples

A.9 Labelling

A.10 Distribution and shipping

A.11 Stability, storage and expiry date

Part B. Nonclinical evaluation of poliomyelitis vaccines (inactivated)

B.1 Characterization of poliovirus seed lots derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology)

B.2 Antigenic profile

B.3 D-antigen content of IPV derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology)

B.4 Evaluation of immunogenicity in animal models

B.5 Nonclinical safety studies

Part C. Clinical evaluation of poliomyelitis vaccines (inactivated)

C.1 General considerations

C.2 Immunogenicity studies

C.3 Concomitant administration with other vaccines

C.4 Pre-licensure safety data

C.5 Post-marketing studies and surveillance
Recommendations published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments or examples intended to provide additional guidance to manufacturers and NRAs.
Introduction

The Requirements for inactivated poliomyelitis vaccine (IPV) were first formulated in 1959 (1) and revised in 1965 (2). Following several advances in vaccine production technology, the Requirements were further updated in 1981 (3) and amended in 1985 (4). At that time, the introduction of continuous cells for the manufacture of IPV was a novel development. Therefore, when the regulatory control of products manufactured in continuous cells had been standardized, the Requirements were again updated in 2000 (5). An addendum was then developed in 2003 (6), specifying the measures to be taken to minimize the accidental risk of reintroducing wild-type poliovirus from a vaccine manufacturing facility into the community after global certification of polio eradication.

Since the Recommendations for the production and control of poliomyelitis vaccine (inactivated) were last revised in 2000 (5) and 2003 (6), there have been several changes in vaccine production, including the use of seed viruses derived from Sabin strains, which make further revision of the Recommendations necessary. To facilitate this process, a meeting to discuss the international specifications for IPV – attended by experts from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry involved in the research, manufacture, authorization and testing and/or release of IPV around the world – was convened by WHO on 29 March 2012. During the discussions, critical issues were considered in relation to both the quality control and evaluation of IPV, including Sabin-based IPV (sIPV), and the revision of the Recommendations described in Annex 2 of WHO Technical Report Series 910 (5). WHO then convened an international Technical Working Group meeting in Geneva on 14–15 May 2013 (7) – attended by experts from academia, NRAs, NCLs and industry involved in the development, manufacture, authorization and testing and/or release of IPV (including sIPV and other new developments of novel IPV) – to further discuss and reach consensus on critical issues relevant to the revision of Annex 2 of WHO Technical Report Series 910. WHO additionally organized an informal consultation at its headquarters in Geneva on 25–26 March 2014 – attended by academics, researchers, vaccine manufacturers and regulators involved in IPV development, production, evaluation and regulatory licensure – to review the draft Recommendations prepared by a drafting group and to seek consensus on key technical and regulatory issues.

Major issues addressed in these revised WHO Recommendations include:

- updating of “General considerations” and other sections to reflect the future development of IPV in accordance with global programmatic need (for example, the use of Sabin strains and strains derived by recombinant DNA technology);
• inclusion of a new Appendix 1 to update the history of the different virus seed strains used by manufacturers for IPV production;
• updating of the section on international standards and reference preparations;
• updating of the section on general manufacturing recommendations and control tests;
• updating of terminology;
• inclusion of specific tests for sIPV and IPV made from strains derived by recombinant DNA technology;
• updating of the appendices;
• inclusion of new sections on nonclinical and clinical evaluation of IPV.

Additional changes were also made to bring this document into line with other WHO Recommendations published since the last revision.

**Scope**

These WHO Recommendations provide guidance to NRAs and manufacturers on the quality and nonclinical and clinical aspects of IPV in order to ensure the quality, safety and efficacy of the vaccines. The scope of the present document encompasses IPV derived from: (a) the wild-type strains that have been used in the manufacture of IPV for many years; (b) the attenuated Sabin strains that have been used in the manufacture of oral poliomyelitis vaccine (OPV); and (c) new alternative poliovirus strains currently under development, including those derived by recombinant DNA technology.

This document does not cover vaccines which are based on virus-like particles (VLPs) and replicons. However, some of the aspects discussed may be relevant to these types of seeds and should be taken into consideration during vaccine development using such seeds.

The document should be read in conjunction with other relevant WHO guidelines such as those on the nonclinical (8) and clinical (9) evaluation of vaccines.

Among the most significant changes in production has been the increasing use of IPV in combination with other vaccines, such as those based upon diphtheria toxoid and tetanus toxoid (“DT-based”) combined vaccines, which raises considerations – such as interaction of the poliovirus antigens with other antigens and/or adjuvants – that do not apply when IPV is used as a standalone product. These considerations are dealt with in a separate WHO document (10) but not in these revised WHO Recommendations. However,
to provide further guidance on control of the vaccine, key tests that may be influenced by other antigens and/or adjuvants present in combined vaccines are identified.

**General considerations**

Poliomyelitis is an acute communicable disease of humans \((11)\) caused by three distinct poliovirus serotypes – types 1, 2 and 3 – which can be distinguished by neutralization tests. Poliovirus is classified as a species C human enterovirus of the *Picornaviridae* family and is composed of a single-stranded, positive-sense RNA genome and a protein capsid.

Where sanitation is poor, faecal-to-oral transmission predominates, whereas oral-to-oral transmission may be more common where standards of sanitation are high. In most settings, mixed patterns of transmission are likely to occur. In the pre-vaccine era, when poliovirus was the leading cause of permanent disability in children, virtually all children became infected with polioviruses. On average, in the absence of protection by humoral or maternal antibody, 1 in every 200 susceptible individuals develops paralytic poliomyelitis \((11)\).

Progress in polio control (and, since 1988, in polio eradication) has been due mainly to the widespread use of vaccines. An IPV (Salk vaccine, wIPV) \(^1\) was first licensed in 1955; live, attenuated OPV (Sabin vaccine) was licensed in the USA in 1961 as a monovalent (mOPV) vaccine, followed by a trivalent OPV (tOPV) licensed for use in 1963 \((11)\). In May 1988, the World Health Assembly resolved to eradicate poliomyelitis globally by the year 2000 and the Global Polio Eradication Initiative (GPEI) was established. The sustained use of poliomyelitis vaccines worldwide since 1988 has led to a precipitous drop in the global incidence of poliomyelitis by more than 99\% \((11)\). Globally, the last case of poliomyelitis caused by naturally circulating wild poliovirus (WPV) type 2 (WPV2) occurred in India in 1999. No case due to WPV type 3 (WPV3) has been detected globally since 10 November 2012. Despite the overall success of the GPEI, Afghanistan, Nigeria and Pakistan remained endemic in 2014 for the transmission of WPV type 1 (WPV1). The Horn of Africa, Cameroon, and parts of the Middle East (Egypt, Israel and the Syrian Arab Republic) also reported WPV1 circulation associated with imported WPV1 in 2013, resulting in clinical cases following a period of elimination \((11)\).

Given the progress made towards polio eradication, countries have increasingly switched from using OPV to wIPV in routine immunization programmes, primarily in order to eliminate the burden of vaccine-associated

\(^1\) In this document the use of the abbreviation “IPV” refers to IPV derived from any strain. “wIPV” indicates IPV derived from wild-type strains only, and “sIPV” represents IPV derived from Sabin strains only.
paralytic poliomyelitis (VAPP), a rare adverse event associated with OPV. The incidence of VAPP has been estimated at two to four cases per million birth cohort per year in countries using OPV (11). The sole use of wIPV successfully eradicated polio in some countries, notably the Netherlands and in Scandinavia. In most of the countries that have introduced wIPV as the only poliomyelitis vaccine over the past decade, there has been no evidence of continued circulation of poliovirus strains, thus indicating that wIPV is able to inhibit the community transmission of poliovirus. However, Israel, which switched to an all-IPV routine immunization schedule in 2004, reported the detection of WPV1 in sewage samples from February 2013 onwards. However, no clinical cases of paralytic poliomyelitis have been reported in Israel, the West Bank or Gaza as of 31 December 2013 (11, 12).

In addition to VAPP, the live poliovirus strains in OPV, currently predominantly Sabin type 2, can occasionally revert to a transmissible form termed circulating vaccine-derived poliovirus (cVDPV) (13, 14), which acts essentially the same as the wild type in causing poliomyelitis. This is an obvious threat to polio eradication that is not posed by the use of IPV.

The WHO GPEI, in conjunction with its partners, developed the comprehensive Polio Eradication and Endgame Strategic Plan 2013–2018 with the goal of achieving a polio-free world by 2018 (15). This plan involves the detection of poliovirus, the interruption of spread, immunization strengthening, withdrawal of OPV, containment, certification and legacy planning, and provides a timetable of events following identification of the last wild-type poliovirus. Three of the key features of the GPEI strategic plan are: (a) the withdrawal of the type-2 OPV strain from tOPV and the introduction of bivalent (types 1 and 3) OPV (bOPV); (b) the introduction of routine use of IPV for managing long-term poliovirus risks, including type-2 cVDPV; and (c) the cessation of all OPV use following the global certification of total WPV serotype eradication (15).

Although the last WPV2 was detected in 1999, eight countries reported cases of paralytic poliomyelitis in 2013 associated with cVDPVs, most of them derived from Sabin type 2 (11). There are 250–500 VAPP cases per year with 40% of these due to type-2 Sabin poliovirus. The need to synchronize OPV cessation was identified in 2008, and withdrawal of the use of type-2 OPV and the introduction of bOPV began in 2009 (15) in supplementary immunization activities or for outbreak control. In 2012 the WHO Strategic Advisory Group of Experts (SAGE) on Immunization recommended that all countries using OPV should introduce at least one dose of IPV in their routine immunization programmes to mitigate the risks of withdrawal of type-2 OPV (16). One of the prerequisites for type-2 OPV cessation is the availability of an affordable IPV option for all OPV-using countries. This may include full dose, fractional dose and adjuvanted IPV, and the intradermal use of IPV in addition to intramuscular or subcutaneous administration.
When poliomyelitis due to wild-type polioviruses and cVDPVs is eradicated (17), laboratories and manufacturers that store or use wild-type or vaccine-derived polioviruses, or any other related viruses, will become an important potential source of reintroduction of these viruses into the community. To manage and control this risk, WHO is finalizing a Global Action Plan that requires the implementation of: (a) appropriate primary safeguards of biorisk management with poliovirus facility containment specifications; (b) secondary safeguards of population immunity in the country approving manufacturing operations; and (c) tertiary safeguards of facility location in countries with demonstrated good personal, domestic and environmental hygiene standards, including closed sewage systems with effective effluent treatment (18).

To mitigate biosafety and biosecurity concerns associated with virulent wild-type viruses used in the manufacture of wIPV, the use of attenuated strains for IPV production has been proposed (19). Production of IPV from live-attenuated Sabin poliovirus seeds has been shown to be technically feasible (20–24), and the first Sabin IPVs have been licensed in Japan in the form of two combination vaccines. Manufacturers in various countries are establishing the production of IPV from live-attenuated Sabin strains or IPV using strains derived by recombinant DNA technology, and are at different stages in the development and licensing process. Additional new manufacturers and IPV manufacturers that currently use wild-type poliovirus strains may wish to consider evaluating the potential offered by a Sabin-based IPV or IPV using strains derived by these alternative means.

Wild-type polioviruses are both transmissible and virulent. They will have to be grown under appropriate and strict containment if they are to be used to produce IPV after the elimination of circulating WPV, according to defined timelines, beginning with type 2 (18). The Sabin vaccine strains are attenuated, and transmission from vaccine recipients is limited. However, they are unstable on passage in cell culture and the human gut, and can revert to give cVDPVs.

Given these uncertainties, assurance is required in relation to the characteristics of the live-attenuated Sabin virus before inactivation in order to justify the implementation of containment measures that may be different from those required for wIPV production (18). Production conditions should be validated by the full range of tests including in vivo and in vitro testing of the master seed and working seed and successive monovalent bulks (with the number to be approved by the NRA) to ensure that the attenuated phenotype of the Sabin strains in monovalent pools is maintained. Subsequently, a limited range of tests, such as mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency. The number of pools of each type tested each year should be justified and agreed by the NRA. Furthermore, it is important that, at intervals to be agreed with the
NRA, pools should be tested with the full range of tests to ensure that production conditions remain satisfactory.

In addition to the Sabin strains that are used in the manufacture of OPV, alternative attenuation methods utilizing recombinant DNA technology are being investigated (25–29). Strains derived by such a methodology may have properties specifically designed to be suitable for the safe production of vaccine (for example, the inability to replicate in the human gut). Such strains should be considered as they become available and may require specific characterization. Biocontainment requirements for such strains will need to be determined on a case-by-case basis. Only virus strains that are approved by the NRA should be used.

An overview of the history of virus seeds that are currently used in IPV production is shown in Appendix 1.

The in vivo potency assay in rats has been standardized and shown to have advantages over those previously described in vivo tests for IPV (30). When the in vivo assay is required for routine production batches, it should be performed at the level of the final bulk. The assay in rats is described in detail in Appendix 2 of this document. The in vivo assay should be used to characterize the vaccine after any changes in the manufacturing process that may influence the quality and immunogenicity of the vaccine, unless otherwise justified and agreed by the NRA.

The in vivo potency test described in these WHO Recommendations requires the assay of neutralizing antibodies to each of the three poliovirus types. This test requires the use of live poliovirus and, for historical reasons, many laboratories use wild-type strains of poliovirus. The attenuated Sabin strains of poliovirus have been shown to be suitable for the assay of neutralizing antibodies in the in vivo test, in principle, by a collaborative study (30) and should be used, but validation of the use of the Sabin strains by each manufacturer should be provided.

Immunization with OPV will cease at some point in the future when poliomyelitis has been eradicated. After that time, the biocontainment levels for use of the Sabin strains for laboratory work will be reviewed. Laboratories are therefore encouraged to investigate the use of alternatives to live viruses for the assay of poliovirus neutralizing antibodies in order to comply with future biocontainment requirements.

The development of transgenic mice that express the human poliovirus receptor (TgPVR mice) (31, 32) has led to the development of an in vivo immunization/challenge model (33, 34) that may be useful for assessing the vaccine efficacy of new poliovirus strains. This test is not proposed for lot release. Any work with transgenic mice should comply with WHO guidelines for the maintenance, containment and transport of transgenic animals (35).

The manufacturer of the final lot must be responsible for ensuring conformity with all the recommendations applicable to the final vaccine (Part A,
sections A.5–A.11) even where manufacturing involves only formulating the final bulk from trivalent bulks supplied by another manufacturing establishment and filling the final containers. The manufacturer of the final lot must also be responsible for any production and control tests performed by an external contract laboratory, if applicable, with the approval of the NRA.

If an immunization schedule combining both IPV and OPV is to be claimed which could potentially achieve both high serum antibody levels and intestinal protection (11), clinical studies designed to establish such a combination (sequential) schedule should also examine patterns of virus excretion following poliovirus challenge (with OPV), in addition to serum neutralizing antibodies, in different sequential schedules.

Terminology

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

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

Adventitious agents: contaminating microorganisms of the cell culture, or source materials used in its culture, that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced into the manufacturing process.

Cell-culture infectious dose 50% (CCID$_{50}$): the quantity of a virus suspension that will infect 50% of cell cultures.

Cell bank: a cell bank is 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.

The individual containers (for example, ampoules or vials) should be representative of the pool of cells from which they are taken, and should be frozen on the same day by following the same procedure and by using the same equipment and reagents.

Cell seed: a quantity of well characterized cells derived from a single tissue or cell of human or animal origin and stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which may be used for the production of a master cell bank.

D-antigen: refers to the antigen found in the sucrose gradient fraction that contains native virus particles which are the target of neutralizing antibodies (36). D-antigen units were originally defined on the basis of an agar precipitin
test performed with D-antigen-specific polyclonal sera. A vaccine preparation that produced a precipitin line at the distance of 25 mm from the centre was arbitrarily assigned a value of 600 D-antigen units using a particular antibody at a particular concentration. This test was used in the initial calibration of reference materials. The D-antigen content of IPV is currently determined by enzyme-linked immunosorbent assay (ELISA).

**Final bulk:** the finished vaccine present in the container from which the final containers are filled. The final bulk may be prepared from one or more trivalent bulks.

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

**Inactivated purified monovalent pool:** a filtered and purified monovalent pool that has been inactivated through the use of a validated method.

**Master cell bank (MCB):** a quantity of well characterized cells of human or animal origin derived from a cell seed at a specific population doubling level or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions, such as the vapour or liquid phase of liquid nitrogen in aliquots of uniform composition. The MCB is prepared from a single homogeneously mixed pool of cells and is used to derive all working cell banks. The testing performed on a replacement MCB (derived from the same clone or from an existing master or working cell bank) is the same as for the initial MCB, unless a justified exception is made.

**Monovalent pool:** a pool of a number of single harvests of the same virus type processed at the same time.

**Production cell culture:** a collection of cell cultures derived from one or more ampoules of the working cell bank used for the production of IPV.

**Purified monovalent pool:** a concentrated and purified pool of a number of single harvests of the same virus type processed at the same time.

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

**sIPV:** inactivated poliomyelitis vaccine derived from Sabin strains only.

**Trivalent bulk:** a pool of a number of inactivated purified monovalent pools processed at the same time and containing all three virus types, blended to achieve a defined D-antigen content for each type.

**Virus master seed lot:** a quantity of virus suspension that has been processed at the same time to ensure a uniform composition, and passaged for a specific number of times that does not exceed the maximum approved by the NRA. It is characterized to the extent necessary to support development of the virus working seed lot.
Virus sub-master seed lot (only applicable for Sabin master seed supplied by WHO): a quantity of virus suspension produced by a single passage from the Sabin virus master seed supplied by WHO and made at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate time frame, and that has been processed at the same time to ensure its uniform composition. The virus sub-master seed lot should be characterized to the extent necessary to support the development of the virus working seed lot. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.1).

Virus working seed lot: a quantity of virus of uniform composition derived from the virus master seed lot made at a multiplicity of infection, ensuring that cytopathic effect develops within an appropriate time frame and used at a passage level approved by the NRA for the manufacturing of vaccine.

wIPV: inactivated poliomyelitis vaccine derived from wild-type poliovirus strains only.

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 would be used for vaccine production. All containers are treated identically and once removed from storage are not returned to the stock.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be poliomyelitis vaccine (inactivated). The proper name should be equivalent to the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 Descriptive definition

Poliomyelitis vaccine (inactivated) should consist of a sterile aqueous suspension of poliovirus types 1, 2 and 3 grown in cell cultures, concentrated, purified and inactivated. The antigen may be formulated with a suitable adjuvant. The preparation should satisfy all the recommendations formulated below.

A.1.3 International reference materials

An International Standard of IPV is available for use in in vitro assays to measure the D-antigen content of IPV containing classical wild-type strains. It is stored frozen in ampoules containing 1 mL of trivalent IPV. This material is for use
in calibrating secondary reference preparations of IPV, which are then used in potency tests to calculate D-antigen content. International standards and reference reagents for the control of in vivo potency assays are under investigation. The need for an International Standard for IPV based on the Sabin or other strains is also being investigated.

An International Reference Preparation (IRP) of poliomyelitis vaccine (inactivated) was established by the WHO Expert Committee on Biological Standardization in 1962 (37). This preparation was a trivalent blend prepared in 1959 in primary monkey kidney cells from type-1 (Mahoney), type-2 (MEF) and type-3 (Saukett) strains of poliovirus. After preparation of the IRP, significant advances in the production and control of IPV occurred and vaccines of increased potency and purity were developed. An enhanced potency IPV (PU78-02) from the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) was widely used as a reference preparation for control purposes. When stocks of this reagent were almost exhausted, a new reference material (91/574) was established by the WHO Expert Committee in 1994 as the second WHO International Reference Reagent for in vivo and in vitro assays of IPV (38). Potencies of 430, 95 and 285 D-antigen units per millilitre were assigned, respectively, to poliovirus types 1, 2 and 3 of this preparation. A separate aliquot of the preparation, established by the European Pharmacopoeia Commission as the Biological Reference Preparation (BRP) batch 1, has an identical assigned titre (39). Material from a concentrated trivalent bulk from a commercially available IPV vaccine was established as the BRP batch 2 in 2003, with assigned potencies of 320, 67 and 282 D-antigen units per millilitre for types 1, 2 and 3, respectively (40). Following inconsistency in the performance of some vials of 91/574, the use of this reference was discontinued in 2010. In 2013, the Third WHO International Standard for inactivated poliomyelitis vaccine (12/104) was established by the WHO Expert Committee using BRP batch 2 as the reference in the study. Potencies of 277, 65 and 248 D-antigen units per millilitre were assigned to poliovirus types 1, 2 and 3, respectively (41).

There are still gaps in the scientific knowledge of biological standardization of IPV, and some inconsistency has been found in results obtained by different laboratories and methods. The validation of international references suitable for vaccines produced from different poliovirus strains will be required.

An International Standard for anti-poliovirus types 1, 2 and 3 antibodies (human) is available for the standardization of neutralizing antibody tests for poliovirus (42).

The International Standards listed above are available from the NIBSC, Potters Bar, England.
A.2  **General manufacturing recommendations**

The general manufacturing requirements contained in the WHO Good manufacturing practices for pharmaceutical products: main principles (43) and Good manufacturing practices for biological products (44) should apply to establishments manufacturing IPV.

WHO guidance has also been developed on the safe production and quality control of IPV prepared using wild-type poliovirus (6) with the same document also providing guidance on vaccines produced from attenuated poliovirus strains (such as Sabin strains). In addition, facilities that manufacture IPV should comply with the current global recommendations for poliovirus containment appropriate to the particular poliovirus strains used in both vaccine production and quality-control departments (18). WHO guidance on the safe production and quality control of IPV manufactured from wild-type polioviruses (6) may require updating in order to better align such guidance with WHO global recommendations for poliovirus containment (18).

Attenuated strains derived by recombinant DNA technology that are used in IPV production should not be readily transmissible from person to person. The applicable containment conditions will depend on the phenotype and production conditions and should be assessed on a case-by-case basis to ensure an acceptable level of phenotypic stability. In any case, any biocontainment arrangement should comply with the global recommendations for poliovirus containment that are current at the time of production (18).

Staff involved in the production and quality control of IPV should be shown to be immune to all three types of poliovirus.

The manufacturer should also be able to demonstrate the availability of appropriate means to respond adequately to and manage an inadvertent release of unfinished product containing live viruses.

A.3  **Control of source materials**

A.3.1  **Virus strains and seed lot system**

A.3.1.1  **Virus strains**

Strains of poliovirus used in the production of IPV should be identified by historical records, which should include information on the strains’ origin and subsequent manipulation (for example, wild, attenuated or manipulated by recombinant DNA technology). The strain identity should be determined by infectivity tests and immunological methods. In addition, Sabin strains and strains derived by recombinant DNA technology should be identified by nucleotide sequence analysis.

Only virus strains that are approved by the NRA and that yield a vaccine complying with the recommendations set out in these WHO Recommendations should be used.
A.3.1.2 Virus seed lot system

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

If Sabin virus master seeds are supplied by WHO, a virus sub-master seed lot should be prepared by a single passage from the WHO master seed at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate time frame. The virus sub-master seed lot should be characterized to the extent that is necessary to support the development of the virus working seed lot. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.2.2 and Part B of WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (45). The virus sub-master seed lot should be subject to the same tests as a virus master seed lot.

Virus master and working seed lots should be stored in dedicated temperature-monitored freezers at a temperature that ensures stability on storage (for example, \( \leq -60^\circ C \)).

A.3.1.3 Tests on virus master and working seed lots

Each virus master and working seed lot used for the production of vaccine batches should be subjected to the tests listed in this section and certain tests applicable to single harvests listed in sections A.4.3.1–A.4.3.3 below.

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

A.3.1.3.1 Tests in rabbit kidney cell cultures (only for virus master seeds derived from strains which have previously been passaged on primary monkey kidney cells)

Virus master seeds that have previously been passaged on primary monkey kidney cells should be tested in rabbit kidney cell cultures for the presence of herpes B virus and other viruses. A sample of at least 10 mL of virus seeds should be tested. Serum used in the nutrient medium of the cultures should have been shown to be free from B virus inhibitors using herpes simplex virus as an indicator virus. The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm²/mL of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.
The inoculated and control cultures should be incubated at a temperature of 37 °C and should be observed at appropriate intervals for a period of at least 2 weeks.

For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period. The sensitivity of each batch of rabbit kidney cells should be demonstrated by challenge with a validated amount of herpes simplex virus. The challenge test should be approved by the NRA.

A.3.1.3.2 Tests for adventitious viruses and freedom from detectable SV40 sequences
A.3.1.3.2.1 Tests for adventitious viruses in cell cultures

The virus master and working seed lot used for the production of vaccine batches should be free from adventitious viruses in cell culture assays.

A sample of at least 40 mL of each virus master and working seed lot should be tested for the presence of adventitious agents. The sample should be neutralized against the specific type of poliovirus by a high-titred antiserum.

Monoclonal antibodies may be useful in this test.

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

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

The sample should be tested in primary Cercopithecus sp. kidney cell cultures, or cells that have been demonstrated to be of equal susceptibility to SV40 virus, and in human diploid cells. The tissue cultures should be incubated at 37 °C and observed for 2 weeks. At the end of this observation period, at least one subculture of supernatant fluid should be made in the same tissue culture system. The sample should be inoculated in such a way that the dilution of the supernatant fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm²/mL of supernatant fluid. At least one culture vessel of the cell cultures should remain uninoculated and should serve as a control. The cells inoculated with the supernatant fluid and the uninoculated control cultures should be incubated at 37 °C and observed at appropriate intervals for an additional 2 weeks.

If necessary, serum may be added to the primary cultures at this stage, provided that the serum does not contain SV40 antibody or other inhibitors.
The virus master and working seed lot passes the test if there is no evidence of the presence of adventitious agents. For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the observation period.

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: (a) degenerate nucleic acid amplification technique (NAT) for whole virus families, with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and (c) high-throughput sequencing. These methods might be used in the future to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and with the approval of the NRA (46).

The theoretical risk of the presence of potential human, simian, bovine or porcine adventitious agents in the seed lots, which may be derived from the use of bovine serum or porcine trypsin, should be assessed. If necessary, viruses such as bovine polyomavirus, porcine parvovirus or porcine circovirus (PCV) may be screened by using specific assays, such as molecular NAT-based assays (46).

A.3.1.3.2.2 Tests for freedom from detectable SV40 sequences

The virus master seed lot should be shown to be free from detectable SV40 sequences by using specific validated assays which are approved by the NRA, such as molecular NAT-based assays (46).

DNA of SV40 is widely used as molecular biological reagent, and contamination of polymerase chain reaction (PCR) assays is potentially a major problem. One approach is to identify separate genomic regions of SV40 for amplification, and to use one region for screening purposes and the other for the confirmation of repeatedly positive samples. It is useful if the second genomic region used for confirmation varies between isolates from different sources, as it is then possible to show that it has a unique sequence and that positive results are not due to contamination with laboratory strains of SV40. The sensitivity of the PCR assays should be established for the genomic regions used.

A.3.1.3.3 Additional tests on seeds from Sabin strains and other attenuated strains derived by recombinant DNA technology

If live-attenuated Sabin strains are used for vaccine production, established master seeds should be used and additional tests should be performed. The virus master seed lots used for the production of vaccine batches should be tested to
monitor virus molecular characteristics – for example, by MAPREC – and should meet the specifications established in agreement with the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (45). Tests to monitor virus molecular characteristics are described in section A.3.2.4 of those Recommendations (see also section A.4.4.2.7 of this document).

Suitable in vitro tests should be performed on the master seed from attenuated strains derived by recombinant DNA technology. The tests may include full genome characterization by nucleotide sequencing or deep sequencing techniques and demonstration of genetic and phenotypic stability on passage under production conditions. Such tests should be validated for this purpose by using appropriate standards and materials, and should be approved by the NRA.

The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology in in vivo neurovirulence tests should be considered and scientifically justified, in agreement with the NRA.

Any new virus working seed derived from an established master seed, including Sabin strains and other attenuated strains derived by recombinant DNA technology, and at least three consecutive monovalent pools should be analysed in tests to monitor virus molecular characteristics such as MAPREC, when relevant (see section A.4.4.2.7.1).

A.3.2 Cell lines
The general production precautions, as formulated in Good manufacturing practices for biological products (44), should apply to the manufacture of IPV, with the additional requirement that, during production, only one type of cell should be introduced or handled in the production area at any one time. Vaccines may be produced in a human diploid cell line or in a continuous cell line.

A.3.2.1 Master cell bank (MCB) and working cell bank (WCB)
The use of a cell line for the manufacture of IPV should be based on the cell bank system. The cell seed and cell banks should conform to WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (46). The MCB should be approved by the NRA. The maximum number of passages (or population doublings) by which the WCB is derived from the MCB and the maximum number of passages of the production cultures should be established by the manufacturer and approved by the NRA.

Additional tests may include, but are not limited to: (a) examination for the presence of retrovirus and tumorigenicity in an animal test system (46); and (b) propagation of the MCB or WCB cells to or beyond the maximum in vitro passage for production.
The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB (47) and is available to manufacturers on application to the Coordinator, Technologies Standards and Norms, Department of Essential Medicines and Health Products (EMP), Health Systems and Innovation (HIS) Cluster, World Health Organization, Geneva, Switzerland.

A.3.2.2 Identity test

Identity tests on the MCB and WCB are performed in accordance with WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (46) and should be approved by the NRA.

The WCB should be identified by means of tests such as biochemical tests (for example, isoenzyme analysis), immunological tests, cytogenetic marker tests and DNA fingerprinting or sequencing. The tests should be approved by the NRA.

A.3.3 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacterial, fungal and mycoplasmal contamination – as specified in Part A, sections 5.2 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances – and freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of 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 (46).

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

The source(s) of animal components used in the culture medium should be approved by the NRA. The components should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50). The serum protein concentration should be reduced by rinsing the cell cultures with serum-free medium and/or purification of the virus harvests.

In some countries, control tests are carried out to detect the residual animal serum content in the final vaccine (see section A.6.6).

Human serum should not be used. If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. As a minimum, it
should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (51). 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 (50).

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

Penicillin and other beta-lactams should not be used at any stage of manufacture because of their nature as 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.

Nontoxic pH indicators may be added, such as phenol red at a concentration of 0.002%.

Only substances that have been approved by the NRA may be added.

Bovine or porcine trypsin used for preparing cell cultures should be tested and found to be free of cultivatable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate (46). The methods used to ensure this should be approved by the NRA.

In some countries, irradiation is used to inactivate potential contaminant viruses. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all 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 but also high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process (46).

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free from risk of contamination and should be subject to the usual considerations for any reagent of biological origin (46).

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 (50).

A.4 Control of vaccine production

A.4.1 Control cell cultures

When human diploid or continuous cell lines are used to prepare cultures for the production of vaccine, a fraction equivalent to at least 5% of the total or 500 mL of cell suspension, or 100 million cells, at the concentration and cell passage level
employed for seeding vaccine production cultures, should be used to prepare control cultures.

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

A.4.1.1 Tests of control cell cultures

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

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

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

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

A.4.1.2 Tests for haemadsorbing viruses

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

Some NRAs require, as an additional test for haemadsorbing viruses, that other types of red blood cells, including cells from humans (blood group IV O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells.

A reading should be taken after incubation at 2–8 °C for 30 min, and again after a further incubation for 30 min at 20–25 °C.

If a test with monkey red blood cells is performed, readings should also be taken after a final incubation for 30 min at 34–37 °C.

In some countries the sensitivity of each new batch of red blood cells is demonstrated by titration against a haemagglutinin antigen before use in the test for haemadsorbing viruses.
A.4.1.3 Tests for other adventitious agents in cell supernatant fluid

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

A second indicator cell line should be used to test an additional 10-mL sample of each pool. When a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line (46).

The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm²/mL of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated cultures should be incubated at a temperature of 35–37 °C and observed at appropriate intervals for a period of at least 14 days.

Some NRAs require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least an additional 14 days. Furthermore, some NRAs require that these cells should be tested for the presence of haemadsorbing viruses.

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

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

Some selected viruses may be screened using specific validated assays which are approved by the NRA, such as molecular NAT-based assays (46).

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

A.4.1.4 Identity tests

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

Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), immunological tests, cytogenetic tests (for example, for chromosomal markers) and tests for genetic markers (for example, DNA fingerprinting or sequencing).
A.4.2  Cell cultures for vaccine production

A.4.2.1  Observation of cultures for adventitious agents

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

If animal serum is used for cell cultures before the inoculation of virus, the medium should be removed and replaced with serum-free maintenance medium after the cells have been washed with serum-free medium, if appropriate.

A.4.3  Control of single harvests

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

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

In some countries, samples are taken after storage and filtration with the agreement of the NRA.

A.4.3.1  Sterility test for bacteria, fungi and mycoplasmas

A volume of at least 10 mL of each virus master and working seed lot (see section A.3.1.3) and single harvest should be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, sections 5.2 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances, or by a method approved by the NRA. If the test is performed outside the production facilities, adequate containment procedures (18) should be used according to the virus strain used for production.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and the agreement of the NRA (46).

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

A.4.3.2  Virus titration

The virus concentration of each virus master and working seed lot (see section A.3.1.3) and single harvest should be determined by titration of infectious virus
using tissue culture methods. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of Hep-2C or Vero cells in microtitre plates is suitable for this purpose (45). The same cells should be used for virus titrations on monovalent pools throughout the production process.

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

In some countries the virus titration may be carried out on the purified, pooled monovalent harvest after demonstration of consistency of production at the stage of the single harvest.

A.4.3.3 Identity test

The poliovirus in each virus master and working seed lot (see section A.3.1.3) and single harvest should be tested for serotype and strain identity by neutralization with specific antiserum or molecular methods approved by the NRA.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

The strain identity of each of the three serotypes may be determined by standard or deep nucleotide sequence analysis or by a suitable molecular technique.

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

A.4.4 Control of purified monovalent pools

A.4.4.1 Purification of monovalent pools

Each monovalent pool of virus, consisting of several single harvests of the same serotype, should be purified before inactivation. Removal of host cell protein should be assessed during process validation (46).

An acceptable method is to clarify the virus suspension by filtration, to concentrate the virus by ultrafiltration and, thereafter, to collect the virus peak after passing it through a gel-filtration column. Further purification is achieved by passing the virus through an ion-exchange column. Other purification procedures resulting in acceptable release criteria may be used – for example, passing the preparation through an immobilized DNA-ase column.
A.4.4.2 Tests on purified monovalent pools

A.4.4.2.1 Residual cellular DNA

For viruses grown in continuous cells, the purified monovalent pools should be tested for residual cellular DNA (46). The purification process should be shown by calculation to reduce consistently the level of cellular DNA to less than 10 ng per human dose.

In some countries, IPV produced in mammalian cells is required to contain less than 100 pg DNA per human dose.

This test may be omitted from routine testing, with the agreement of the NRA, if the manufacturing process is validated to achieve this specification (46).

If assessed, the size distribution of the DNA may be considered as a characterization test, taking into account the amount of DNA detectable using appropriate methods, as approved by the NRA (46).

In some countries this test is performed on the trivalent bulk following validation, and with the agreement of the NRA.

A.4.4.2.2 Virus titration

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

The use of Hep-2C or Vero cells in microtitre plates is suitable for this purpose (45).

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

A.4.4.2.3 Identity test

The poliovirus in each purified monovalent pool should be tested for serotype and strain identity by neutralization, using specific antiserum or molecular methods approved by the NRA.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

The strain identity of each of the three serotypes may be determined by nucleotide sequence analysis or a suitable molecular technique.
A.4.4.2.4  **D-antigen content**

The D-antigen content of each purified monovalent pool should be determined by use of a validated immunochemical method and should be calculated by using a reference vaccine calibrated against the WHO International Standard (see section A.1.3).

A.4.4.2.5  **Protein content**

The purified monovalent pool should be shown to contain no more than 0.1 µg of protein per D-antigen unit of poliovirus or should be within the limits approved for that particular product by the NRA.

A.4.4.2.6  **Filtration before inactivation**

Each purified monovalent pool should be filtered before inactivation.

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

Filters containing asbestos should not be used.

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

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

A sample of the filtered purified monovalent pool should be retained and its virus titre determined as described in section A.4.4.2.2.

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

A.4.4.2.7  **Additional tests for purified monovalent pools produced from Sabin vaccine seeds or from other attenuated seeds derived by recombinant DNA technology**

Production conditions should be validated by the full range of tests, including in vivo and in vitro testing of the master and working seed and successive monovalent bulks (the number to be approved by the NRA), to ensure that the attenuated phenotype of the Sabin strains in monovalent pools is maintained. Subsequently, a limited range of tests, such as MAPREC, may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency.
The number of pools of each type tested each year should be justified and should be agreed with the NRA. Furthermore, it is important that, at intervals to be agreed with the NRA, pools should be tested with the full range of tests to ensure that production conditions remain satisfactory.

The use of the rct40 test is discouraged as it requires the use of WPV controls.

In vitro tests to monitor virus molecular characteristics (consistency) and in vivo neurovirulence tests which could be used for this purpose are described in sections A.4.4.2.7.1 and A.4.4.2.7.2, respectively.

Suitable in vitro tests should be performed on purified monovalent pools derived from attenuated strains derived by recombinant DNA technology. Tests may include full genome characterization by nucleotide sequencing or deep sequencing techniques. Such tests should be validated for this purpose by the use of appropriate standards and materials, and should be approved by the NRA.

An in vitro test (described above) for the molecular consistency of production may be performed on single harvests before preparing the purified monovalent pool. If performed, the acceptance/rejection criteria should be updated periodically and should be approved by the NRA.

A.4.4.2.7.1 Tests to monitor virus molecular characteristics (consistency)

In vitro tests such as MAPREC, which are used to determine the molecular consistency of production of monovalent pools, should meet the specifications for the test used (45).

Results from MAPREC tests should be expressed as ratios relative to the relevant type-specific International Standard for MAPREC analysis of poliovirus (Sabin). The acceptable variation of mutant content from batch to batch should be agreed with the NRA in the light of production and testing experience.

For type 3 (472-C) a purified monovalent pool should be rejected if the level of mutations is more than 1.0% when normalized against the International Standard. The limits for types 1 and 2 should be approved by the NRA.

Levels of mutations obtained by manufacturers who have implemented the test for types 1 and 2 virus have been less than 2.0% for type-1 Sabin (for the sum of both mutations 480-A, 525-C) and 1.5% for type-2 Sabin (481-G) (45, 52).

The test(s) used should be approved by the NRA. The MAPREC assay provides a sensitive and quantitative measure of consistency for monovalent pools derived from Sabin viruses.
A.4.4.2.7.2 *Neurovirulence tests*

Appropriate in vivo tests which may be used to evaluate the phenotype of virus in purified monovalent pools produced from the Sabin vaccine strains are described in section A.4.4.7.2 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (45).

For other attenuated strains derived by recombinant DNA technology, the need for testing purified monovalent pools by in vivo neurovirulence tests should be considered and should be scientifically justified with the agreement of the NRA.

A.4.5 *Control of inactivated purified monovalent pools*

A.4.5.1 *Inactivation procedure*

The virus in the filtered purified monovalent pools should be inactivated by a method approved by the NRA. Prior to inactivation, the concentration of the filtered monovalent pool, based on viral titre or D-antigen content, should be adjusted to the acceptable range established during the process validation. The acceptable range should be established during validation studies.

Most manufacturers during the past 40–50 years have used formaldehyde as the method for inactivation.

The method of inactivation should be shown to give consistent inactivation for the production of acceptable vaccine. The progress of inactivation should be monitored by suitably spaced determinations of virus titres. The inactivation period should exceed the time taken to reduce the titre of live virus to undetectable amounts by a factor of at least 2.

A second filtration should be made during the process of inactivation.

This step is made after the virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

The kinetics of viral inactivation should be established by each manufacturer and should be approved by the NRA. Adequate data on viral inactivation kinetics should be obtained and consistency of the inactivation process should be monitored. For this purpose, the virus titre and D-antigen content of each filtered purified monovalent pool before, during and at the end of inactivation should also be determined, as specified in sections A.4.4.2.2 and A.4.4.2.4, respectively.

A record of consistency (effective inactivation and kinetic of inactivation) should be established by the production of at least five consecutive lots and, if broken, a root-cause analysis should be performed and a further five consecutive filtered purified monovalent pools should be prepared and shown to be satisfactory for establishing this record.
A.4.5.2 Test for effective inactivation

Two samples should be taken of a volume equivalent to at least 1500 human doses of each inactivated purified monovalent pool. One sample should be taken at the end of the inactivation period and the other not later than three quarters of the way through this period. After removal or neutralization of the inactivating agent, the samples should be tested for the absence of infective poliovirus by inoculation into tissue cultures. Kidney cells from some monkey species, such as those of the genera *Macaca*, *Cercopithecus* and *Papio* sp., appear to be more sensitive than others. If other tissue culture systems, including continuous cell lines (for example, L20B), are used, they should have been shown to possess at least the same sensitivity to poliovirus as those specified above by inoculating with partially formalin-inactivated virus (as opposed to infectious, untreated virus) as formalin treatment changes the biological properties of poliovirus (see below). When primary monkey kidney cells are used for this test, the two samples should be inoculated into culture vessels of tissue cultures derived from different batches of cells.

The dilution of the sample in the nutrient fluid should not exceed 1 in 4 and the area of the cell sheet should be at least 3 cm²/mL of sample. One or more culture vessels of each batch of cultures should be set aside to serve as uninoculated control culture vessels with the same medium.

The formaldehyde in samples of vaccine for tissue culture tests is generally neutralized at the time of sampling by the addition of bisulfite. Usually, the samples are subsequently dialysed.

It is possible to conduct tissue culture tests on non-dialysed material. However, this is often found to be toxic to cells, even with a dilution of 1 in 4. If in such tests nonspecific degeneration of cells occurs, or if the sensitivity of the tissue culture system is reduced, the test should be repeated on dialysed material. The virus D-antigen content after dialysis should be determined to ascertain whether any D-antigen was lost during the dialysis process.

The tissue culture vessels should be observed for at least 3 weeks. Not less than two subcultures should be made from each original culture vessel. The first subculture from each culture vessel should be made prior to the first medium change, and the second subculture should be made at the end of the observation period. The subcultures should be observed for at least 2 weeks.

If infectious poliovirus is detected in samples taken at the end of inactivation, or in the samples taken no later than three quarters through the inactivation process, the inactivated purified monovalent pool should not be used for further processing. The isolation of live poliovirus from an inactivated purified monovalent pool must be regarded as a break in the manufacturing
consistency record and a production process review and revalidation should be undertaken.

If primary monkey kidney cells are used in this test, they may contain adventitious agents that could interfere with the test result. It is important to demonstrate that each test retains sensitivity to detect partially inactivated polioviruses. At the end of the observation period, the cell culture used for the detection of residual live virus should be challenged with a validated amount of live Sabin virus of the same type as that of the inactivated purified monovalent pool. The details of the challenge procedure should be approved by the NRA.

If continuous cell lines are used in this test, the ability to detect infectious virus should be checked concurrently for each test by introducing a positive control at the beginning of each test. Positive control flasks should be inoculated with a low quantity of virus close to the detection limit of the method. Alternatively, if no positive control is used, a challenge test should be performed as described above for primary monkey kidney cells.

The problem of detecting residual active poliovirus in an inactivated vaccine is not the same as that of measuring infective virus in untreated suspensions. Poliovirus that has been exposed to the action of formaldehyde without becoming inactivated has been shown to require a much longer period to produce cytopathic changes than untreated virus. For this reason it is desirable that tissue cultures in tests for the presence of residual active virus are observed for as long a time as is technically possible. A satisfactory tissue culture system for this purpose depends, therefore, not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

The serum added to the nutrient fluid should be tested for inhibitors to poliovirus at serum concentrations up to 50%. Only serum free from inhibitors to all three types of poliovirus should be used.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that early changes of fluid may result in unadsorbed virus being removed and the validity of the test would thus be impaired. Therefore, the fluid should be changed no earlier than 5–7 days after inoculation.

**A.4.5.3 Sterility test for bacteria and fungi**

Each inactivated purified monovalent pool should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

**A.4.5.4 D-antigen content**

The D-antigen content of each inactivated purified monovalent pool should be determined by use of a validated immunochemical method and should be
calculated by use of a reference vaccine calibrated against the WHO International Standard (see section A.1.3). The results obtained should be within the required limits established by the NRA.

A.4.6 Control of trivalent bulk

Only those inactivated purified monovalent pools that have been shown to be satisfactory should be blended to form a trivalent bulk.

A.4.6.1 Test for absence of infective poliovirus

A sample of at least 1500 mL or, if purified and concentrated vaccine is prepared, the equivalent of at least 1500 human doses of each trivalent bulk should be tested in cell cultures for the absence of infective poliovirus by the procedure described in section A.4.5.2. If infective poliovirus is isolated, this trivalent bulk, or product derived from it, should not be used.

In some countries this test may be omitted on the trivalent bulk if, following a review of manufacturing records, the test for inactivation has been performed with satisfactory results on the inactivated purified monovalent pool, subject to approval by the NRA.

When a trivalent bulk is supplied by one manufacturer to another, the validation of inactivation may rely on the inactivation tests performed by the bulk supplier.

A.4.6.2 Sterility test for bacteria and fungi

The trivalent bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.4.6.3 Residual formaldehyde

The content of free residual formaldehyde in the trivalent bulk should be determined by a method approved by the NRA. The limits should be approved by the NRA.

A.4.6.4 D-antigen content

The D-antigen content of each trivalent bulk should be determined by use of a validated immunochemical method and should be calculated by use of a reference vaccine calibrated against the WHO International Standard (see section A.1.3). The results obtained should be within the required limits established by the NRA.
A.4.7 **Control of final bulk**

Preservatives, excipients or other substances that might be added to or combined with the trivalent bulk to form the final bulk should have been shown, to the satisfaction of the NRA, to have no deleterious effect on the immunizing potency and the safety profile of the poliovirus antigens. Preservative efficacy should be demonstrated during product development using a method approved by the NRA.

The operations necessary for preparing the final bulk from trivalent bulk should be conducted in such a manner as to avoid contamination of the product. In preparing the final vaccine bulk, any substances such as diluents, stabilizers or adjuvants that are added to the product should have been shown, to the satisfaction of the NRA, not to impair the safety and efficacy of the vaccine in the concentration used. Until the final bulk is filled into containers, the final vaccine bulk suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity.

A.4.7.1 **Sterility test for bacteria and fungi**

The final bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.4.7.2 **Potency tests**

Each final bulk should be tested in an in vivo assay for immunogenicity by tests approved by the NRA. An in vivo potency assay in rats has been standardized and shown to be a suitable test for IPV. Product-specific reference preparations may be used in these tests (see Appendix 2).

The D-antigen content of each final bulk should be determined using a validated immunochemical method and calculated using a reference vaccine calibrated against the WHO International Standard (see section A.1.3). The results obtained should be within the required limits established by the NRA. This test may be omitted on the final bulk if conducted on the final lot.

When consistency of production has been established on a suitable number of consecutive final bulks, the in vivo assay may be omitted with the agreement of the NRA. This can occur once it has been demonstrated that the acceptance criteria for the D-antigen determination are such that the in vitro test yields a comparable result to the in vivo assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary by heat treatment or other means of diminishing the immunogenic activity.

Where there is a change in the manufacturing process of the antigens or in their formulation that might impact on the quality and immunogenicity of the
vaccine, the in vivo test should be performed to demonstrate the comparability of the new manufacturing process to the established process. If the process change affects the in vivo test, the need for revalidation should be considered and clinical data may be required for the approval by the NRA.

The in vitro assay that has been found most suitable for measuring the antigen content is the D-antigen ELISA. Although this assay is widely used, particular attention is required for its standardization. Some NRAs accept the use of polyclonal antisera, whereas others accept the use of monoclonal antibodies in the test. The use of different antibodies may give different results. The D-antigen specificity of the antibodies should be demonstrated. Whichever types of antisera are used, the validation studies should show that the assay can determine consistency of production. For a D-antigen ELISA to be valid, it should comply with specified criteria of linearity and parallelism. The effect of a change in the method of calculation of the D-antigen content on registered specifications should also be taken into account.

Other validated tests such as the multiplex antibody test or plasmon resonance technology (53, 54) may be used subject to the approval of the NRA.

If the use of an adjuvant in the final bulk interferes with the assay, a desorption or treatment step may be necessary before performing the D-antigen ELISA.

If the final bulk is formulated with poliovirus trivalent bulk and with other antigens into a combination vaccine, the suitability of performing the D-antigen ELISA on the final bulk will have to be determined. If the D-antigen ELISA is not suitable for a particular combination, an in vivo assay should be used.

The potency of the final bulk for each virus type should be approved by the NRA.

A.4.7.3 Preservative content

If preservative is added, its content in the final bulk should be determined by a method approved by the NRA. The preservative used and content permitted should be approved by the NRA.

As the use of thiomersal can result in loss of D-antigen content, 2-Phenoxyethanol has been the only preservative used by IPV manufacturers during the past 50 years.

A.4.7.4 Adjuvant (if applicable)

Each final vaccine bulk should be assayed for adjuvant content. This test may be omitted if it is performed on the final lot. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.
A.5  **Filling and containers**

The requirements concerning filling and containers given in Good manufacturing practices for biological products (44) should apply to vaccine filled in the final form. Single- and multiple-dose containers may be used.

A.6  **Control tests on the final lot**

Samples should be taken from each final lot for the tests described in the following sections. The following tests should be performed on each final lot of vaccine (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. The permissible limits for the different parameters listed under this section, unless otherwise specified, should be approved by the NRA.

Differences in the results of tests on novel IPVs – that is, deviations/excursions from the permissible limits – may be acceptable if justified and approved by the NRA.

A.6.1  **Inspection of final containers**

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded and recorded for each relevant abnormality. A limit should be established for the percentage of rejection.

A.6.1.1  **Appearance**

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

A.6.2  **Identity test**

An identity test should be performed on at least one labelled container from each final lot by an appropriate method. The potency test described in section A.6.5 may serve as the identity test.

A.6.3  **Sterility test for bacteria and fungi**

Each final lot should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.6.4  **General safety test (innocuity)**

The need to test the final lots of the vaccine for unexpected toxicity (also known as abnormal toxicity) should be agreed with the NRA. This test may be omitted.
from routine lot release once the consistency of production has been established to the satisfaction of the NRA, and when reliable good manufacturing practices are in place.

A.6.5 **Potency test**

The D-antigen content of each final lot should be determined using a validated immunochemical method (see sections A.4.5.4 and A.4.7.2) and calculated using a reference vaccine calibrated against the WHO International Standard (see section A.1.3).

In some countries, this test is omitted provided that the determination of the D-antigen content has been carried out with satisfactory results on the final bulk product and provided that a validation has been performed to demonstrate that there is no loss of potency between the final bulk product and the final lot, subject to approval by the NRA.

If the use of an adjuvant in the final bulk interferes with the assay, a desorption or treatment step may be necessary before performing the D-antigen ELISA. If treatment/desorption is not possible, the interference of the adjuvant should be documented and an in vivo assay should be performed (see section A.4.7.2 and Appendix 2).

In general, wIPVs formulated to contain 40, 8 and 32 D-antigen units or more per human dose for types 1, 2 and 3, respectively, are effective (55). Vaccines with lower D-antigen contents may be acceptable if supported by clinical data. Vaccines in which adjuvants are used, or vaccines produced from other seed viruses (for example, Sabin viruses), may also be licensed with a different antigenic composition if supported by clinical data.

If the final bulk is formulated from a trivalent bulk and other antigens into a combination vaccine, the suitability of performing the D-antigen ELISA on the final lot will have to be determined. If the D-antigen ELISA is not suitable for a particular combination, an in vivo assay such as that described in Appendix 2 should be used.

The potency of the vaccines for each virus type should be approved by the NRA.

A.6.6 **Protein content**

Poliomyelitis vaccine (inactivated) should not contain more than 10 µg of protein per human dose. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

If animal serum is used for the growth of cell cultures, the serum protein concentration (bovine serum albumin) in the final lot should be no more than 50 ng per human dose. The test for bovine serum albumin may be omitted if performed on the trivalent or final bulk, subject to approval by the NRA.
A.6.7 **Preservative content**
Where appropriate, the preservative content of each final lot should be determined by a method approved by the NRA. The method used and content permitted should be approved by the NRA. This test may be omitted if conducted on the final bulk.

A.6.8 **Endotoxin content**
The endotoxin content of each final lot should be determined by a method approved by the NRA. Levels should be consistent with levels found to be acceptable in vaccine lots used in pre-licensure clinical trials and approved by the NRA.

A.6.9 **Test for residual formaldehyde**
The content of free residual formaldehyde in each final lot should be determined by a method approved by the NRA. The limit should be approved by the NRA. This test may be omitted if performed on the trivalent bulk or on the final bulk.

A.6.10 **Test for pH**
The pH of each final lot should be determined and should be within limits approved by the NRA.

A.6.11 **Adjuvant and degree of adsorption (if applicable)**
If an adjuvant is used in the formulation, each final lot should be assayed for adjuvant content. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose. This test may be omitted on the final lot if performed on the final bulk.

The degree of adsorption of the antigen to the aluminium compounds (aluminium hydroxide or hydrated aluminium phosphate) in each final lot should be assessed.

A.6.12 **Residual antibiotics (if applicable)**
If any antibiotics are added during vaccine production, the residual antibiotic content should be determined and should be within limits approved by the NRA. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

A.7 **Records**
The requirements given in Good manufacturing practices for biological products (44) should apply.
A.8 **Retained samples**
The requirements given in Good manufacturing practices for biological products (44) should apply.

A.9 **Labelling**
The requirements given in Good manufacturing practices for biological products (44) should apply, and additionally the label on the container or package should include the following information:

- the designation(s) of the strain(s) of poliovirus contained in the vaccine
- the cell substrate used for the preparation of vaccine
- the D-antigen content of each poliovirus type
- the method and inactivating agent used to inactivate the virus
- the nature and amount of any stabilizer and preservative present in the vaccine
- the nature and amount of adjuvant, if applicable.

It is desirable for the label to carry the names of both the producer and the source of the bulk material if the producer of the final vaccine did not prepare it. The nature and amount of antibiotics present in the vaccine, if any, may be included.

A.10 **Distribution and shipping**
The requirements given in Good manufacturing practices for biological products (44) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (56).

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

A.11.1 **Stability testing**

Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (57). Stability testing should be performed at different stages of production when intermediate product is stored, namely on single harvests, inactivated purified monovalent pool, trivalent bulk, final bulk and final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. During vaccine production a shelf-life should be assigned to all in-process materials – particularly intermediates such as single harvests, inactivated purified monovalent pool, trivalent bulk and final bulk.
The stability of the vaccine in its final containers, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA. As a guide, containers from at least three consecutive final lots, and derived from different monovalent pools and different trivalent bulks, may be tested.

Where manufacturing involves only formulation of the final bulk from trivalent bulks supplied by another manufacturing establishment and the filling of final containers, stability data on the trivalent bulks should be generated if the storage container or storage conditions are changed, and the shelf-life until use should be established by the manufacturer performing the final fill.

The formulation of the vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (57). Data should be provided to the NRA in accordance with local regulatory requirements.

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

A.11.2 Storage conditions

Poliomyelitis vaccine (inactivated) should be stored at all times at a temperature between 2 °C and 8 °C. For novel vaccines, appropriate storage conditions should be validated and approved by the NRA.

A.11.3 Expiry date

The expiry date should be based on the shelf-life, and should be supported by stability studies and approved by the NRA. The expiry date should be based on the date of blending of the final bulk, the date of filling or the date of the first valid potency test on the final lot, which should be performed in an assay as described in Appendix 2.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals were inoculated with the final bulk.

Part B. Nonclinical evaluation of poliomyelitis vaccines (inactivated)

The nonclinical evaluation of candidate IPV vaccines should be based on the WHO guidelines on nonclinical evaluation of vaccines (8). The following issues relate specifically to new IPV candidates and should also be referred to when a significant change is made in the manufacturing process or vaccine formulation of a licensed IPV.
B.1 Characterization of poliovirus seed lots derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology)

The virus master and working seed lots derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology) that are used to manufacture a candidate IPV should be extensively characterized, as described in section A.3.1. Ideally, the characterization studies should be performed on seed lots used to prepare the vaccine batches tested in preclinical and clinical studies.

When attenuated poliovirus strains derived by recombinant DNA technology are used to prepare a candidate IPV, the mutations responsible for attenuation should be identified along with the mutations that can revert to a partial or full virulence phenotype. The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology with in vivo neurovirulence tests should be considered, and the decision whether to test or not should be scientifically justified and approved by the NRA. In addition, the genetic stability of the strains derived by recombinant DNA technology should be confirmed at the passage level (or beyond) used to prepare the vaccine. Efforts should also be made to develop an in vitro test to detect reversion to a partially or fully virulent virus.

B.2 Antigenic profile

The available evidence suggests that there may be significant differences in the antigenic composition of various IPV products developed independently (58, 59), particularly when comparing sIPV to wIPV. It is likely that antigenic profiles of IPV are influenced by the virus strains, cell substrates and process parameters used in manufacture. The antigenic structure of a candidate IPV should ideally be established using monoclonal antibodies (59, 60) of known specificity at the early stage of product development and should be used as a characterization tool for investigating vaccine stability and demonstrating manufacturing consistency during product development.

B.3 D-antigen content of IPV derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology)

The type-specific antigen content of current licensed wIPV is measured using various ELISA procedures (60) and is reported as D-antigen units relative to a reference preparation traceable to the International Standard. When attenuated strains (for example, Sabin strains and strains derived by recombinant DNA
technology) are used to prepare the candidate IPV, an in-house ELISA should be developed and implemented to determine type-specific D-antigen content. An in-house reference standard should be established at the early stage of product development and should be calibrated against the International Standard. A monitoring programme should be put in place to ensure the stability of the in-house reference standard and the comparability of its subsequent replacement. In addition, the ratio between virus titre (per millilitre) and D-antigen content (per millilitre) of purified monovalent pools prior to inactivation should also be established for each poliovirus type during product development and should be monitored during commercial production. This provides further assurance that the D-antigen content of commercial lots, throughout the product life-cycle, is comparable to lots shown to be safe and immunogenic in clinical studies.

Most licensed wIPV products have been formulated to contain 40, 8 and 32 D-antigen units per human dose. However, the D-antigen unit is not well defined with respect to various poliovirus strains used in manufacture and is known to be influenced by the specificity of the antibodies used as ELISA reagents. Therefore, it is not possible to directly compare the D-antigen content of various IPVs (for example, sIPV versus wIPV) measured using different monoclonal or polyclonal antibody-based ELISA procedures (61). It is recognized that IPV derived from attenuated strains or adjuvanted IPV may require different D-antigen content to induce adequate immune responses in humans.

B.4 Evaluation of immunogenicity in animal models

Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in suitable animal models (for example, rats). Proof-of-concept nonclinical studies should include the comparison of immunogenicity between a candidate IPV and a licensed IPV based on type-specific serum neutralizing antibody titres against both Sabin and wild-type strains. These studies may also assist in the selection of D-antigen content to be tested in the dose-finding studies in humans. However, it is important to note that immunogenicity data in animals do not reliably predict the antigen content that might be appropriate for inclusion as a single human dose in the final vaccine formulation. An assay using transgenic mice may be performed to compare the immune response and protection against virulent challenge induced by a candidate IPV to that induced by a licensed IPV (31, 32). In vivo tests are also important tools to be used as characterization tests to demonstrate comparable manufacturing processes when major changes are introduced.

When an adjuvant is included in the formulation, manufacturers should provide a rationale and immunogenicity data to support the use of an adjuvant in the vaccine (62).
B.5 Nonclinical safety studies

Decisions concerning the need for nonclinical safety studies, types of study and study design should be based on the general guidance provided, and special considerations addressed, in the WHO guidelines on nonclinical evaluation of vaccines (8) – for example, use of a delivery device or alternative administration routes such as intradermal. If a candidate IPV is formulated with a novel adjuvant or excipient (for example, stabilizer) then nonclinical safety studies should be conducted following the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (62).

Part C. Clinical evaluation of poliomyelitis vaccines (inactivated)

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (63) and Guidelines on clinical evaluation of vaccines: regulatory expectations (9).

A number of issues specific to the clinical evaluation of IPV are discussed in the following sections, which are applicable to IPV derived from wild-type strains as well as attenuated strains (for example, Sabin strains and strains derived by recombinant DNA technology).

C.1 General considerations

The establishment of the GPEI following the 1998 World Health Assembly resolution led to a dramatic decrease in poliomyelitis cases globally (11). Consequently, clinical efficacy studies to support the licensure of a candidate IPV are no longer feasible, and clinical evaluation should be based upon comparative assessment of the safety and immunogenicity of a candidate vaccine against an already licensed vaccine (comparator vaccine). The assessment of seroconversion should be based on the elicitation of serum neutralizing antibodies, which have been established as the basis of protection (64). The licensure of a candidate IPV should be based on a clear demonstration of non-inferiority in terms of immunogenicity when compared to a comparator vaccine.

C.2 Immunogenicity studies

C.2.1 Assessment of the immune response

A serum neutralizing antibody titre of ≥ 8 is considered to be a marker of clinical protection against poliomyelitis (65). The demonstration of an immune response to IPV vaccination should be based upon the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for polio antigen is defined as:
- for subjects seronegative at the pre-vaccination time point, post-vaccination antibody titres of ≥ 8;
- for subjects seropositive at the pre-vaccination time point, a ≥ 4-fold rise in antibody titres post-vaccination. In the event that the pre-vaccination titre is due to maternal antibodies, a 4-fold rise above the expected titre of maternal antibodies based on the pre-vaccination titre declining with a half-life of 28 days indicates seroconversion.

It is recommended that the assay used to assess serum neutralizing antibodies should be standardized, as described in the WHO Manual for the virological investigation of polio (66), particularly with respect to the use of appropriate cell lines, International Standards of anti-poliovirus sera and other important reagents. The level of neutralizing antibody present in a serum sample is expressed as a titre, which is the reciprocal of the highest serum dilution that inhibits the viral cytopathic effect in 50% of cell cultures.

For the evaluation of IPV derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology), serum neutralizing antibody titres against both Sabin and wild-type poliovirus should be determined in order to ensure that the conclusions of clinical studies are applicable to both types of strains. In view of the antigenic differences between the wild-type poliovirus strains, it may be useful to assess the neutralizing antibody titres using both recent wild-type isolates and the conventional strains in a subset of study subjects, if relevant.

The presence of neutralizing antibody against polioviruses is considered a reliable correlate of protection against poliomyelitis. However, immunity induced by one serotype does not provide protection against the other two serotypes.

C.2.2 Comparative immunogenicity studies
A candidate IPV should be directly compared with at least one well established and licensed IPV in prospective controlled studies.

In the event that no IPV products can be used in the country where the clinical studies are conducted due to country-specific regulations, the use of OPV as a comparator may be acceptable to the NRA provided that a high seroconversion rate of the comparator OPV has been well established in the study region. However, the use of OPV in clinical studies should be in compliance with the Polio Eradication and Endgame Strategic Plan 2013–2018 (15), and it must be noted that the use of tOPV is time limited as bOPV will be introduced and will be followed by the complete cessation of all OPV vaccination.

Non-inferiority studies to evaluate immunogenicity after completion of the primary vaccination series in the target population (for example, naive infants)
are required for regulatory approval of a candidate IPV. Persistence of the serum neutralizing antibodies after the primary series should also be investigated to recommend whether and when a booster dose is required. However, data concerning long-term antibody persistence might not be available prior to regulatory approval. The waning of antibodies over time is inevitable and should not be interpreted as indicating the need for a booster dose per se, as available data suggest that persistent immune memory may be sufficient to protect against poliomyelitis (67, 68).

C.2.3 Study population and region
In general, the first clinical study (Phase I) of a candidate IPV should be performed in healthy adults to assess vaccine safety. Due to the widespread use of IPV and OPV, the immunogenicity of a candidate IPV can be reliably evaluated only in a naive target population, such as infants.

Exposure of study subjects to circulating wild-type or OPV-derived poliovirus may enhance the immune response induced by IPV and, in turn, may affect study conclusions. Therefore, clinical trials to evaluate the immunogenicity of a candidate IPV should ideally be performed in regions where IPV is used exclusively. In the event that a clinical trial is conducted in areas where OPV is used routinely, special measures should be taken to minimize the potential exposure of study participants to live poliovirus (for example, undertaking clinical studies on a new IPV in a region where OPV is used on a campaign basis).

C.2.4 Endpoints and analyses
The primary study analysis should be based on the rate of seroconversion (as described in section C.2.1) against both Sabin and wild-type strains measured at approximately 4 weeks following completion of the primary infant immunization. The primary study objectives should be based on the demonstration of the non-inferiority of the seroconversion rates achieved with the candidate IPV versus the comparator vaccine.

The predefined clinical margins of non-inferiority should be justified, and the calculations of the proposed sample size required should be clearly explained in the study protocol. Further guidance on demonstrating non-inferiority trials is described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (9).

Comparison of geometric mean titres (GMTs) and reverse cumulative distributions of individual titres against both Sabin and wild-type poliovirus at 4 weeks post-primary should also be performed as a secondary analysis. While it may be that the GMT(s) for one or more poliovirus types induced by the candidate IPV derived from attenuated strains is lower than that induced by the comparator, it is not clear if a lower GMT at 4 weeks post-primary affects long-
term antibody persistence. Consequently, any significant differences in observed GMT (for example, not meeting pre-specified criteria) should be carefully considered by the NRA and a decision should be supported by additional studies of antibody persistence (as described in section C.2.2) and by a commitment to post-marketing studies (described in section C.5).

The minimum D-antigen content required for the candidate vaccine at the end of its shelf-life should be based on the D-antigen content of clinical lots shown to induce acceptable immune responses in clinical studies (for example, lots used in the dose-finding study).

**C.2.5 Immunization schedule**

Different immunization schedules are used for licensed wIPV in various regions and countries. It is common for IPV to be administered according to the same schedule as diphtheria, tetanus and pertussis (DTP) vaccines in order to achieve a high compliance rate. Clinical trial data have shown that the immune response induced by licensed wIPV varies according to the immunization schedule used. In general, longer intervals in the primary immunization series (for example, 2, 4 and 6 months) induce higher neutralizing antibody titres and a higher seroconversion rate \(^{69,70}\). An immunization schedule should be defined for the targeted country or region, wherever possible, and dose-finding and non-inferiority studies for a candidate IPV conducted according to this immunization schedule. However, it is not feasible to study a candidate vaccine using every possible schedule in all target regions. Manufacturers should justify the relevance of the clinical data provided to each country in which approval is sought and should discuss the basis for extrapolation of the findings. For example, satisfactory immune responses using a schedule with a short interval between immunizations (for example, 2, 3 and 4 months) would support an expectation that satisfactory immune responses would also be observed using a schedule with a longer interval (for example, 2, 4 and 6 months). However, the local and systemic reactogenicity associated with a candidate IPV may also differ between schedules within a specific population so there is still a need to collect safety data, prior to regulatory approval, for the proposed schedules (for example, 2, 4 and 6 months).

The use of an IPV that is prepared from attenuated poliovirus strains or that contains a fractional antigen dose in a sequential IPV-OPV immunization schedule should be supported by clinical studies to ensure adequate levels of serum neutralizing antibodies against both wild-type and Sabin poliovirus.

**C.3 Concomitant administration with other vaccines**

IPV is commonly co-administered with other infant and toddler vaccines. Consequently, it is essential to evaluate the immune responses to a candidate IPV as well as to all other antigens co-administered in all the co-administration
situations claimed. Due to the large number of licensed vaccines that may need to be co-administered with IPV in infants and toddlers using a variety of schedules, it is not feasible for manufacturers to study every possible combination. The data on the effects of co-administration that are available at the time of initial licensure may be limited and should be expanded in post-approval studies. If study results indicate that immune responses are lower on co-administration with other vaccine(s), the NRA will need to consider the potential clinical consequences on a case-by-case basis.

C.4 Pre-licensure safety data

The general approach to safety assessment of a candidate IPV during pre-licensure clinical studies should be in accordance with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (9). The safety profile of a candidate IPV derived from attenuated strains is expected to be very similar to that of current licensed wIPV, which is very well tolerated. The NRA may decide that large safety studies are not required. However, if a candidate IPV includes novel adjuvants and/or excipients, or is administered using an alternative route and/or delivery device, a safety database similar in size to that requested for any new vaccine entity might be needed. This should be discussed and approved by the NRA on a case-by-case basis. In addition, it is likely that adverse events at the injection site are more frequent if a candidate IPV contains an adjuvant. This may be acceptable if the incidence of adverse events is comparable to that observed for other licensed adjuvanted vaccines and the benefit clearly outweighs the risks.

An appropriate pharmacovigilance plan should be developed and approved by the NRA prior to licensure.

C.5 Post-marketing studies and surveillance

Post-marketing surveillance should be undertaken during the initial post-approval years in collaboration with the NRA. Manufacturers and health authorities should work in collaboration with the global polio surveillance laboratory network to monitor new vaccines once they are introduced in immunization programmes. Enhanced safety surveillance is particularly important for vaccines which include novel adjuvants and/or excipients. Due to the possibility that the sIPV may induce a lower GMT for one or more poliovirus types, the persistence of antibody and the need for a booster dose should be studied in the post-marketing period.

The total duration of enhanced surveillance should be regularly reviewed by the NRA. If particular issues arise during pre-licensure studies or during
post-licensure safety surveillance, it may be necessary to conduct specific post-licensure safety studies.

**Part D. Recommendations for NRAs**

**D.1 General recommendations**

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (71) and Guidelines for independent lot release of vaccines by regulatory authorities (72) should apply. These recommendations specify that no new biological substance should be released until consistency in batch manufacturing and quality has been demonstrated.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of poliomyelitis vaccine (inactivated), should be discussed with and approved by the NRA.

For control purposes, the relevant International Standards currently in force should be obtained for the purpose of calibrating national/regional/working standards (73). The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release until international/national standard preparation is established.

Consistency of production has been recognized as an essential component in the quality assurance of poliomyelitis vaccine (inactivated). In particular, 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.

**D.2 Official release and certification**

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (72).

A protocol for the manufacturing and control of poliomyelitis vaccines (inactivated), based on the model protocol provided in Appendix 3 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request to release vaccine for use.

A lot release certificate signed by the appropriate NRA official should then be provided to the manufacturing establishment, and should certify that the lot of vaccine in question meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot. 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 in Appendix 4.
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References


Appendix 1

Overview of virus seeds used in IPV production

This appendix gives an overview of the history of virus seeds that are either currently used in production or may be used in the future. These include the wild strains used in current IPV production and the attenuated Sabin strains which are considered to pose a lower risk and are being developed as alternative seeds. Novel strains intended to be safer for use in production are also in development.

1. IPV made from virulent strains

Both classic IPV, which was developed by Jonas Salk and others and licensed in 1955, and the enhanced-potency IPV introduced in the late 1980s, are prepared from wild (virulent) polioviruses of three serotypes. The strains selected by Salk were Mahoney, MEF-1 and Saukett, representing types 1, 2 and 3, respectively. The Mahoney strain was isolated in 1941 by Drs Thomas Francis and Walter Mack from the pooled faeces of three healthy children in Cleveland, Ohio, USA (1). It was subsequently passaged by Salk, including 14 times in living monkeys and twice in monkey testicular cultures (2). The MEF-1 strain was isolated by the inoculation of monkeys in Egypt in 1940 (3) during a polio outbreak among allied troops of the Mediterranean Expeditionary Force (hence the name MEF). It was adapted by Schlesinger and Olitsky to growth in mice (4), and then transferred by Salk from the spinal cord of a paralysed mouse to tissue culture (2). The original Saukett strain was isolated by Salk in 1950 by direct inoculation of tissue culture with a faecal specimen from a paralysed patient (2). Seed stocks of the viruses were provided by Salk to most manufacturers and were used to establish their virus master seeds. An alternative strain of type-1 poliovirus (Brunhilde) is used by the Statens Serum Institute (SSI) in Denmark. This strain was isolated in 1939 by David Bodian from a pool of stool specimens from seven patients in Maryland, USA (5). The strain was provided to the laboratory of Dr John Enders at Harvard Medical School in Boston, Massachusetts, USA, and from there to Dr Arne Svedmyr’s laboratory in Stockholm, Sweden. Dr Svedmyr supplied SSI with the virus. Table 1 summarizes the history of isolation and early passaging of these viruses.
### Table 1

History of isolation and early passaging of wild polioviruses (WPVs) used in the production of IPV

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Source of isolation</th>
<th>Location</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahoney</td>
<td>Stool of 3 healthy children</td>
<td>Cleveland, Ohio, USA</td>
<td>1941</td>
<td>Francis &amp; Mack. See Sabin AB, Boulger LR, 1973 (1)</td>
</tr>
<tr>
<td>MEF-1</td>
<td>CNS of a paralysed patient</td>
<td>Egypt</td>
<td>1941</td>
<td>van Rooyen CE, Morgan AD, 1943 (3)</td>
</tr>
<tr>
<td>Saukett</td>
<td>Stool of a paralysed patient</td>
<td>USA</td>
<td>1950</td>
<td>Salk JE, 1953 (2)</td>
</tr>
<tr>
<td>Brunhilde</td>
<td>Stool of 7 patients</td>
<td>Massachusetts, USA</td>
<td>1939</td>
<td>Howe HA, Bodian D, 1941 (5)</td>
</tr>
</tbody>
</table>

Subsequent studies raised questions regarding these strains. The nucleotide sequence of MEF-1 was found to be very close to the sequence of another type-2 strain (Lansing; isolated in 1937 in Michigan, USA) with only 17 nucleotide and 2 amino acid differences (6). Since the strains were isolated 4 years apart in the Middle East and the USA, it is unlikely that the similarity represents a natural relatedness. In 1943, MEF-1 from the spinal cords of monkeys was adapted to growth in mice (4) and was found to be indistinguishable in terms of pathogenicity and immunological properties from Lansing, which was also adapted to growth in mice. A plausible explanation is that the Lansing strain used as a reference strain in Schlesinger and Olitsky’s laboratory was inadvertently substituted for MEF-1, and that all subsequent stocks of MEF-1 are derivatives of the Lansing strain. In addition, two common variants of MEF-1, differing by a small number of nucleotides, are in use in different laboratories and production facilities.

Saukett strains obtained from different laboratories and manufacturers differ significantly (7, 8) with the degree of diversity (~10% nucleotide substitutions), demonstrating that they are different strains. Some of the differences were observed in antigenic sites and could affect immunogenicity, suggesting that better characterization of vaccines in the future may need to include determination of the exact nucleotide sequences of virus master seed lots used by manufacturers.

The flow diagrams below (Figs. 1–3) show the history of each of the seed viruses used to prepare, according to IPV manufacturers, the master seed lots used to produce type-1, type-2 and type-3 IPV, respectively. These
diagrams provide only an overview of the use of different seeds based upon a written survey conducted in 2012 by WHO among vaccine manufacturers, and upon information obtained from subsequent consultations. The presentation of these diagrams should not be taken as an indication of any form of WHO qualification or approval of the strains or of the vaccines in the context of these WHO Recommendations.

The names of the manufacturers and agencies shown in the diagrams are given in full in Table 2.

Table 2
Full names of manufacturers and agencies shown in Figs. 1–3

<table>
<thead>
<tr>
<th>Manufacturer/Agency</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBio</td>
<td>Bilthoven Biologicals B.V. (formerly NVI), Bilthoven, Netherlands</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline Vaccines, Wavre, Belgium</td>
</tr>
<tr>
<td>RIVM</td>
<td>Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment), Bilthoven, Netherlands</td>
</tr>
<tr>
<td>Sanofi Pasteur (Canada)</td>
<td>Sanofi Pasteur, Canada</td>
</tr>
<tr>
<td>Sanofi Pasteur (France)</td>
<td>Sanofi Pasteur, Marcy L’Etoile, France</td>
</tr>
<tr>
<td>SSI</td>
<td>Statens Serum Institute, Copenhagen, Denmark</td>
</tr>
</tbody>
</table>

Fig. 1
History of seed virus used to produce type-1 IPV
Fig. 2
History of seed virus used to produce type-2 IPV

Fig. 3
History of seed virus used to produce type-3 IPV
2. IPV made from attenuated strains (Sabin)

Once the circulation of wild-type polio viruses has been ended, IPV manufacturing establishments will become the most likely potential source of virulent viruses. Such viruses will therefore need to be stringently contained to prevent their reintroduction into the environment. The Sabin vaccine strains used to manufacture OPV are less virulent than the wild strains and have been proposed as less hazardous seeds for IPV production in order to mitigate the risks of potential inadvertent release from production facilities. Sabin strains are known to be genetically unstable in infected humans and, to some extent, in production. To retain the attenuated phenotype the Sabin strains must be propagated under defined and well controlled conditions. In addition, during the manufacture of OPV each harvest is tested to monitor molecular consistency – for example, by MAPREC – and each monovalent bulk is tested for neurovirulence to be sure that the attenuated phenotype is retained. As the IPV product is inactivated, full characterization on every batch is not necessary but some assurance of consistent production is required. Sabin strains are considered less transmissible than the wild type so that, should they escape from the production facility and start to circulate within communities, they would pose a lesser risk. However, they can revert and may give rise to circulating vaccine-derived strains that are both transmissible and capable of causing outbreaks. Therefore, the use of Sabin strains in the manufacture of IPV may reduce biosecurity concerns compared to manufacture from virulent wild strains but does not eliminate such concerns entirely. Some testing or process validation will be required to show that the product is consistent and that the attenuated phenotype is retained in order to justify the level of containment used (see also General considerations and Part A of this document). Two sIPV-containing combination products based on attenuated Sabin strains have been licensed in Japan, and other sIPV vaccines are undergoing clinical evaluation in some countries. The derivation of Sabin strains has been described in the literature (1) and the detailed origin of seed viruses made from them can be found in Appendix 1 of the revised WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (9).

3. Other strains in development

Alternative attenuated strains of poliovirus, such as strains derived from recombinant DNA technology, are under development. They are intended to be both attenuated and genetically stable while possessing low or no infectivity for humans and thus be of negligible transmissibility. Such strains should pose a lower risk of inadvertent release from production facilities or of infecting production workers. They may include strains in which known attenuation
determinants are stabilized by targeted genetic changes, strains with alterations in codon usage in order to introduce multiple mutations to reduce virus replication efficiency, or viruses produced by other strategies. The phenotypes and stability of these strains will require confirmation.

References
Appendix 2

In vivo potency assay of IPV

Tests for evaluating the potency of IPVs include an in vivo assay for immune response. An appropriate WHO International Standard should be used to validate the assay. However, because of the diversity in the reactivity of different vaccines it is unlikely that an International Standard will be suitable for the standardization of in vivo assays of vaccines from all manufacturers. If this is shown to be the case, manufacturers should establish a product-specific reference preparation which is traceable to a lot of vaccine shown to be efficacious in clinical trials. The NRA should approve the reference preparation used and should agree with the potency limits applied. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and it should be replaced when necessary.

In recent investigations the in vivo potency assay in rats has been standardized (1) and shown to have advantages over previously described in vivo tests for IPV (2).

A suitable in vivo assay method consists of intramuscular injection into the hind limb(s) of rats of four dilutions of the vaccine to be examined and a reference vaccine, using for each dilution a group of not fewer than 10 rats of a suitable strain and which are free of specific pathogen. The number of animals used should be sufficient to allow for the calculation of potency to be made with 95% confidence limits within the 25–400% range. The number of dilutions and the number of animals used per dilution may differ from that specified here, provided that any alternative scheme gives at least the same sensitivity in the test. For each dilution, the weight of individual animals should not vary by more than 20% from the group mean. An inoculum of 0.5 mL is used per rat. The dose range is chosen so that a dose response to all three poliovirus types is obtained. The animals are bled after 20–22 days. Neutralizing titres against all three poliovirus types are measured separately using 100 CCID₅₀ of the Sabin strains as challenge viruses, Vero or Hep-2C as indicator cells and neutralization conditions of 3 h at 35–37 °C followed by 18 h at 2–8 °C. Results should be read after fixation and staining after 7 days of incubation at 35 °C. For the antibody assay to be valid, the titre of each challenge virus must be shown to be within the range of 30–300 CCID₅₀ and the neutralizing antibody titre of a control serum must be within two 2-fold dilutions of its geometric mean titre. The potency is calculated by comparing the proportions of animals defined as responders to the test vaccine and to the reference vaccine by the probit method. To define an animal as a responder, it is necessary to establish a cut-off neutralizing antibody titre.
for each poliovirus type. Owing to inter-laboratory variation, it is not possible to define cut-off values that could be applied by all laboratories. Instead, the cut-off values should be determined by each laboratory on the basis of a minimum series of three tests with the reference vaccine. The mid-point on a log₂ scale of the minimum and maximum geometric mean titres of the series of three or more tests is used as the cut-off value. For each of the three poliovirus types, the potency of the vaccine should not be statistically significantly less than that of the reference preparation. The test is not valid unless:

- the median effective dose (ED₅₀) for both the test and reference vaccines lies between the smallest and the largest doses given to the animals;
- the statistical analysis shows no significant deviation from linearity or parallelism;
- the confidence limits of the estimated relative potency fall between 25% and 400% of the estimated potency.

Laboratories that have established the parallel line method of analysis of antibody titres for the rat test may use it instead of converting titres to proportions of responders as in the probit method of analysis.

Laboratories are encouraged to validate alternative methods for the assay of neutralizing antibody to reduce the use of live polioviruses in laboratories. If IPV is formulated with other antigens into a combination vaccine, then the suitability of performing the rat immunogenicity test will have to be determined. If the immunogenicity test is performed, the potency of the final bulk for each virus type should be approved by the NRA.

The development of transgenic mice that express the human poliovirus receptor (TgPVR mice) (3–5) has led to the development of an immunization/challenge model that may be useful for assessment of vaccine efficacy. This test is not proposed for lot release. Any work with transgenic mice should comply with WHO guidelines (6).

**References**


Appendix 3

Model protocol for the manufacturing and control of poliomyelitis vaccines (inactivated)

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

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

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that will accompany the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate from the NRA or from the NCL of the country where the vaccine was produced or released stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on finished product (final lot)

International name: ______________________________
Trade name/commercial name: ______________________________
Product licence (marketing authorization) number: ______________________________
Country: ______________________________
Name and address of manufacturer: ______________________________
Name and address of licence holder if different: ______________________________
Virus strain: ______________________________
Origin and short history: ______________________________

Finished product (final lot)

Batch number: ______________________________
Final bulk: ______________________________
Type of container: ______________________________
Number of doses per container: ______________________________
Number of filled containers in this final lot: ______________________________
Volume of single human dose: ______________________________
Composition (D-antigen unit) of a single human dose:

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
</table>

Bulk numbers of monovalent pool suspensions:

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
</table>

Site of manufacture of each monovalent pool: ____________________________

Date of manufacture of each monovalent pool: ____________________________

Date of manufacture of trivalent bulk (blending): ____________________________

Date of manufacture of final bulk: ____________________________

Date on which last determination of potency was started, or date of start of period of validity: ____________________________

Shelf-life approved (months): ____________________________

Expiry date: ____________________________

Storage conditions: ____________________________

Nature and concentration of stabilizer: ____________________________

Nature of any antibiotics present in vaccine and amount per human dose: ____________________________

Release date: ____________________________

Starting materials

The information requested below is to be presented on each submission. Full details on master and working seed lots should be provided upon first submission only and whenever a change has been introduced.

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

If any cell lot or virus harvest intended for production was rejected during the control testing, this should also be recorded either in the following sections or on a separate sheet.

Control of source materials (section A.3)

Virus seed (every submission) (section A.3.1)

Vaccine virus strain(s) and serotype(s): ____________________________

Substrates used for preparing seed lots: ____________________________

Origin and short history: ____________________________

Authority that approved the virus strains: ____________________________

Date of approval: ____________________________
Information on seed lot preparation (every submission) (section A.3.1.3)

Virus master seed (VMS) and virus working seed (VWS) (to be provided upon first submission only and whenever a change has been introduced)

Strain used: ____________________________________________
Source of VMS: _________________________________________
VMS and VWS lot number: _________________________________
Name and address of manufacturer: _________________________
VWS passage level from VMS: _____________________________
Dates of inoculation: _________________________________
Dates of harvest: ________________________________
Number of containers: _________________________________
Conditions of storage: _________________________________
Dates of preparation: _________________________________
Maximum passage levels authorized: ____________________

Tests on VMS and VWS (first submission only)

Tests for bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: __________________________________________
Number of vials tested: _________________________________
Volume of inoculum per vial: _____________________________
Volume of medium per vial: ______________________________
Observation period (specification): ______________________

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date of start of test</th>
<th>Date of end of test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25 °C</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>30–36 °C</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Test for mycoplasmas

Method used: __________________________________________
Volume tested: _________________________________
Media used: ______________________________________
Temperature of incubation: __________________________
Observation period (specification): __________________
Positive controls (list of species used and results): ____________
<table>
<thead>
<tr>
<th>Subcultures at day 3</th>
<th>Date of start of test</th>
<th>Date of end of test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcultures at day 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcultures at day 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcultures at day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Indicator cell-culture method (if applicable)
- **Cell substrate used:**
- **Inoculum:**
- **Date of test:**
- **Passage number:**
- **Negative control:**
- **Positive controls:**
- **Date of staining:**
- **Results:**

### Virus titration
- **Date of test:**
- **Reference batch number:**
- **Date of test:**
- **Result:**

### Identity test
- **Method used:**
- **Date of start of test:**
- **Date of end of test:**
- **Result:**

### Test in rabbit kidney cell cultures
- **Number of cell cultures:**
- **Total volume inoculated:**
- **Period of observation:**
- **Result:**

### Test for adventitious agents
- **Date(s) of satisfactory test(s) for freedom from adventitious agent:**
- **Volume of virus seed samples for neutralization and testing:**
Batch number of antisera used for neutralization virus seed:  
Method used:  
Date of start of test:  
Date of end of test:  
Result:  

Absence of SV40 
Method used:  
Date of start of test:  
Date of end of test:  
Results:  

Tests for neurovirulence (if applicable) 
In vitro tests: MAPREC test for attenuated strains (if applicable) 
MAPREC 
Date of test:  
Type 1 
Ratio of % of the sum of both mutations 480-A and 525-C of bulk sample to the International Standard, or level of mutations:  
Result of test of consistency of production:  
Result of test of comparison with the International Standard:  

Type 2 
Ratio of % of 481-G of bulk sample to the International Standard, or level of mutations:  
Result of test of consistency of production:  
Result of test of comparison with the International Standard:  

Type 3 
Ratio of % of 472-C of bulk sample to the International Standard, or level of mutations:  
Result of test of consistency of production:  
Result of test of comparison with the International Standard:  


In vivo tests for neurovirulence (if applicable)

Neurovirulence test in monkeys: ________________________________
Result of blood serum test in monkeys prior to inoculation: ________________________________
Number and species of monkeys inoculated: ________________________________

Quantity (CCID\textsubscript{50}) inoculated in each test monkey: ________________________________
Number of “valid” monkeys inoculated with test sample: ________________________________
Number of positive monkeys observed inoculated with test sample or with reference: ________________________________
Reference preparation: ________________________________
Number of “valid” monkeys inoculated with reference: ________________________________
Number of positive monkeys observed: ________________________________
Mean lesion score of test sample: ________________________________
Mean lesion score of reference: (see also attached forms giving details of histological observations and assessment): ________________________________
C\textsubscript{1} constant value: ________________________________

Neurovirulence test in transgenic mice for attenuated strains (if applicable)

Strain of mice inoculated: ________________________________
For each dose of the seed sample: ________________________________
Number of mice inoculated: ________________________________
Number of mice excluded from evaluation: ________________________________
Number of mice paralysed: ________________________________
Results of validity tests for each dose of the reference virus: ________________________________
Number of mice inoculated: ________________________________
Number of mice excluded from evaluation: ________________________________
Number of mice paralysed: ________________________________

Virus assay results for each dose inoculated (residual inoculums)

Paralysis rates for test vaccine at each dose: ________________________________
Paralysis rates for reference virus at each dose: ________________________________
Results: ________________________________
Log odds ratio: ________________________________
L1 and L2 values: ________________________________
Pass/fail decision: ________________________________
Cell banks (every submission) (section A.3.2)

Information on cell banking system

- Name and identification of substrate: ____________________________
- Origin and short history: ____________________________
- Authority that approved the cell bank: ____________________________
- Master cell bank (MCB) and working cell bank (WCB)
  lot numbers and date of preparation: ____________________________
- Date MCB and WCB were established: ____________________________
- Date of approval by NRA: ____________________________
- Total number of ampoules stored: ____________________________
- Passage level (or number of population doublings) of cell bank: ____________________________
- Maximum passage approved: ____________________________
- Storage conditions: ____________________________
- Method of preparation of cell bank in terms of number of freezes and efforts made to ensure that a homogeneous population is dispersed into the ampoules: ____________________________

Tests on MCB and WCB (first submission only) (section A.3.2)

- Percentage of total cell-bank ampoules tested: ____________________________

Identity test

- Method: ____________________________
- Specification: ____________________________
- Date of test: ____________________________
- Result: ____________________________
- Growth characteristics: ____________________________
- Morphological characteristics: ____________________________
- Immunological marker: ____________________________
- Cytogenetic data: ____________________________
- Biochemical data: ____________________________
- Results of other identity tests: ____________________________

Tests for adventitious agents

- Method used: ____________________________
- Number of vials tested: ____________________________
- Volume of inoculum per vial: ____________________________
- Date of start of test: ____________________________
- Date of end of test: ____________________________
- Result: ____________________________
### Tests for bacteria, fungi and mycoplasmas

#### Tests for bacteria and fungi

**Method used:**

- Number of vials tested:
- Volume of inoculum per vial:
- Volume of medium per vial:
- Observation period (specification):

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date of start of test</th>
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</thead>
<tbody>
<tr>
<td>20–25 °C</td>
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</tr>
<tr>
<td>30–36 °C</td>
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</tr>
<tr>
<td>Negative control</td>
<td></td>
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</tr>
</tbody>
</table>

#### Test for mycoplasmas

**Method used:**

- Volume tested:
- Media used:
- Temperature of incubation:
- Observation period (specification):
- Positive controls (list of species used and results):

<table>
<thead>
<tr>
<th>Subcultures at day 3</th>
<th>Date of start of test</th>
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<tr>
<td>Subcultures at day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Indicator cell culture method (if applicable)**

- Cell substrate used:
- Inoculum:
- Date of test:
- Passage number:
- Negative control:
- Positive controls:
- Date of staining:
- Results:
Control of vaccine production (section A.4)

Control cell cultures (section A.4.1)

Virus type (1, 2 or 3) *(a separate protocol should be completed for each type)*

Control of production cell cultures
Lot number of MCB: ________________________________
Lot number of WCB: ________________________________
Date of thawing of ampoule of WCB: ________________________________
Passage number of production cells: ________________________________
Date of preparation of control cell cultures: ________________________________
Results of microscopic observation: ________________________________

*Tests on control cell cultures (provide information on control cells corresponding to each single harvest)*

- Ratio of control to production cell cultures: ________________________________
- Incubation conditions: ________________________________
- Period of observation of cultures: ________________________________
- Dates observation started/ended: ________________________________
- Ratio or proportion of cultures discarded for nonspecific reasons: ________________________________
- Results of observation: ________________________________

*Tests for haemadsorbing viruses*

- Quantity of cell tested: ________________________________
- Method used: ________________________________
- Date of start of test: ________________________________
- Date of end of test: ________________________________
- Results: ________________________________

*Tests for adventitious agents on supernatant culture fluids*

- Method used: ________________________________
- Date of start of test: ________________________________
- Date of end of test: ________________________________
- Result: ________________________________

*Identity test*

- Method used: ________________________________
- Date of start of test: ________________________________
- Date of end of test: ________________________________
- Result: ________________________________
Control of cell cultures for vaccine production (section A.4.2)

Observation of cultures for adventitious agents on day of inoculation

Results of microscopic observation: ____________________________________________

Control of single harvests (section A.4.3)

Batch number(s) and virus type: ____________________________________________

Date of inoculation: ______________________________________________________

Date(s) of harvest: ______________________________________________________

Volume(s), storage temperature, storage time and approved storage period: ______

Freedom from bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: __________________________________________________________

Number of vials tested: _________________________________________________

Volume of inoculum per vial: ____________________________________________

Volume of medium per vial: _____________________________________________

Observation period (specification): _______________________________________

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
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</tr>
<tr>
<td>Negative control</td>
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</tr>
</tbody>
</table>

Test for mycoplasmas

Method used: __________________________________________________________

Volume tested: ________________________________________________________

Media used: __________________________________________________________

Temperature of incubation: _____________________________________________

Observation period (specification): ______________________________________

Positive controls (list of species used and results): ______________________

<table>
<thead>
<tr>
<th></th>
<th>Date of start of test</th>
<th>Date of end of test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
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<td>Subcultures at day 3</td>
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<tr>
<td>Subcultures at day 7</td>
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</tbody>
</table>
Table continued

<table>
<thead>
<tr>
<th>Subcultures at day 14</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcultures at day 21</td>
<td></td>
</tr>
</tbody>
</table>

**Indicator cell culture method (if applicable)**
- Cell substrate used: ________________________________
- Inoculum: ________________________________
- Date of test: ________________________________
- Passage number: ________________________________
- Negative control: ________________________________
- Positive controls: ________________________________
- Date of staining: ________________________________
- Results: ________________________________________

**Virus titration**
- Date of test: ________________________________
- Reference batch number: ________________________________
- Date of test: ________________________________
- Result: ________________________________________

**Identity test**
- Method used: ________________________________
- Date of start of test: ________________________________
- Date of end of test: ________________________________
- Result: ________________________________________

**Purified monovalent pools before inactivation (section A.4.4)**
- Batch number(s) and virus type: ________________________________
- Date of inoculation: ________________________________
- Date(s) of harvest: ________________________________
- Volume(s), storage temperature, storage time and approved storage period: ________________________________

**Test for residual cellular DNA**
- Method used: ________________________________
- Date of start of test: ________________________________
- Date of end of test: ________________________________

**Virus titration**
- Date of test: ________________________________
- Reference batch number: ________________________________
Date of test: __________________________
Result: __________________________

Identity test
Method used: __________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: __________________________

D-antigen content
Reference used: __________________________
Method used: __________________________
Date of test: __________________________
Result: __________________________

Protein content
Method used: __________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: __________________________
Details of filtration and/or clarification and/or
purification (if applied): __________________________

Date: __________________________
Additional tests on monovalent pools produced from Sabin vaccine seeds or
from seeds derived by recombinant DNA technology: for example, in vitro
tests (such as MAPREC) for attenuated strains, or in vivo neurovirulence
test in transgenic mice for attenuated strains (if applicable)
(See above in tests on virus seeds) __________________________

Inactivation of monovalent product (section A.4.5)
Agent(s) and concentration at the beginning and
end of inactivation: __________________________
Temperature of inactivation: __________________________
Date of start of inactivation: __________________________
D-antigen units at start of inactivation: __________________________
Date of taking first sample: __________________________
Date of completion of inactivation: __________________________
D-antigen units at end of inactivation: __________________________
Test for effective inactivation (after removal/neutralization of inactivating agent)

Sample size tested: ________________________________
Date of first sample: ________________________________
Date of second sample: ________________________________
Details of testing procedure: ________________________________
Period of observation of cell cultures: ________________________________
Period of observation of subcultures: ________________________________
Result: ________________________________
Result of challenge of used culture with live virus: ________________________________

Tests for bacteria and fungi

Method used: ________________________________
Number of vials tested: ________________________________
Volume of inoculum per vial: ________________________________
Volume of medium per vial: ________________________________
Observation period (specification): ________________________________

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<tr>
<th>Incubation</th>
<th>Media used</th>
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<td>Negative control</td>
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</table>

D-antigen content

Method used: ________________________________
Reference used: ________________________________
Date of test: ________________________________
Result: ________________________________

Trivalent bulk product (monovalent pools incorporated)
Date of preparation: ________________________________
Preservative (if added, type and concentration): ________________________________

Tests on trivalent bulk (section A.4.6)

Test for absence of infective poliovirus: ________________________________
Sample size tested: ________________________________
Details of testing procedure: ________________________________
Period of observation of cell cultures: ________________________________
Period of observation of subcultures: ________________________________
Result: ________________________________
Tests for bacteria and fungi
Method used: ____________________________
Number of vials tested: ____________________________
Volume of inoculum per vial: ____________________________
Volume of medium per vial: ____________________________
Observation period (specification): ____________________________

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<td>Negative control</td>
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Residual formaldehyde
Method used: ____________________________
Result: ____________________________

D-antigen content
Method used and acceptance limits for test results: ____________________________
Reference used: ____________________________
Date of test: ____________________________
Result: ____________________________

Control of final bulk (section A.4.7)
Tests for bacteria and fungi
Method used: ____________________________
Number of vials tested: ____________________________
Volume of inoculum per vial: ____________________________
Volume of medium per vial: ____________________________
Observation period (specification): ____________________________

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<td>Negative control</td>
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Potency test(s)

D- antigen test
Method used and acceptance limits for test results: ______________________
Reference used: ______________________
Date of test: ______________________
Result: ______________________
Results (and date) of in vivo tests (in rats)
(if performed): ______________________

Preservative content (if applicable)
Date of test: ______________________
Method used: ______________________
Result: ______________________

Adjuvant (if applicable)
Date of test: ______________________
Method used: ______________________
Result: ______________________

Filling and containers (section A.5)
Final lot number: ______________________
Total volume for final filling: ______________________
Date of filling: ______________________
Number of vials after inspection: ______________________
Number of vials filled: ______________________

Control tests on final lot (section A.6)

Inspection of final containers
Date of test: ______________________
Results: ______________________

Appearance
Date of test: ______________________
Results: ______________________

Identity test
Method used: ______________________
Date of start of test: ______________________
Date of end of test: ______________________
Result: ______________________
Tests for bacteria and fungi

Method used: ____________________________

Number of vials tested: ________________________

Volume of inoculum per vial: ________________________

Volume of medium per vial: ________________________

Observation period (specification): ________________________

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</table>

General safety test (if applicable)

Date of start of test: ________________________

Date of end of test: ________________________

Result: ________________________

Potency test(s)

D-antigen test

Method used and acceptance limits for test results: ________________________

Reference used: ________________________

Date of test: ________________________

Result: ________________________

Results (and date) of in vivo tests (in rats)

(if performed): ________________________

Protein content

Content of protein in mg per human dose: ________________________

Serum protein tests (if applicable)

Result: ________________________

Preservative content (if applicable)

Date of test: ________________________

Method used: ________________________

Result: ________________________
Endotoxin content
Date of test: ________________________________________
Method used: ________________________________________
Result: _____________________________________________

Test for residual formaldehyde
Date of test: ________________________________________
Method used: ________________________________________
Result: _____________________________________________

pH
Date of test: ________________________________________
Result: _____________________________________________

Adjuvant (if applicable)
Date of test: ________________________________________
Method used: ________________________________________
Result: _____________________________________________

Residual antibiotics (if applicable)
Date of test: ________________________________________
Method used: ________________________________________
Result: _____________________________________________

Certification by the manufacturer
Name of head of quality control (typed) __________________________

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

I certify that lot no. __________________ of trivalent poliomyelitis vaccine (inactivated), whose number appears on the label of the final containers, meets all national requirements and/or satisfies Part A¹ of the 2014 WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated).²

Signature ____________________________________________
Name (typed) __________________________________________
Date __________________________________________________

¹ With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
Certification by the NRA
If the vaccine is to be exported, attach the NRA Lot Release Certificate (as shown in Appendix 4), a label from a final container and an instruction leaflet for users.
Appendix 4

Model NRA Lot Release Certificate for poliomyelitis vaccines (inactivated)

Certificate no. ____________________

The following lot(s) of poliomyelitis vaccine (inactivated) produced by ______ ___________________________ 1 in __________________________, 2 whose numbers appear on the labels of the final containers, meet all national requirements 3 and Part A 4 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (2015) 5 and complies with WHO good manufacturing practices for pharmaceutical products: main principles; 6 Good manufacturing practices for biological products; 7 and Guidelines for independent lot release of vaccines by regulatory authorities. 8 The release decision is based on ____________________________ 9

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container used;

1 Name of manufacturer.
2 Country of origin.
3 If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.
4 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
9 Evaluation of the summary protocol, independent laboratory testing and/or procedures specified in a defined document etc., as appropriate.
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other appropriate authority)

Name (typed) ____________________________________________
Signature ________________________________________________
Date ____________________________________________________
Annex 4

Guidelines on procedures and data requirements for changes to approved vaccines

1. Introduction
2. Scope
3. General considerations
4. Terminology
5. Reporting categories for quality changes
   5.1 Major quality changes
   5.2 Moderate quality changes
   5.3 Minor quality changes
6. Reporting categories for safety, efficacy and/or product labelling information changes
   6.1 Safety and efficacy changes
   6.2 Product labelling information changes
   6.3 Urgent product labelling information changes
   6.4 Administrative product labelling information changes
7. Procedures
   7.1 Procedures for prior approval supplements
   7.2 Procedures for minor quality changes
   7.3 Procedures for urgent product labelling information changes
   7.4 Procedures for administrative product labelling information changes
8. Special considerations
   8.1 Adjuvants
   8.2 Influenza vaccines
   8.3 Bridging studies
9. Authors and acknowledgements
10. References
    Appendix 1 Reporting categories and suggested review timelines
    Appendix 2 Changes to the antigen
    Appendix 3 Changes to the final product
    Appendix 4 Safety, efficacy and product labelling information changes
Guidelines published by 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.
1. Introduction

Changes to the vaccine manufacturing process or product labelling information often need to be implemented after a new vaccine has been approved (that is, licensed or marketing authorization (MA) received). Changes may be made for a variety of reasons, such as to maintain the routine production of vaccines (for example, replenishment of cell banks, seed lots and reference standards), to improve the quality attributes of the vaccine or the efficiency of manufacture (for example, changes in the manufacturing process, equipment or facility) or to update product labelling information (for example, to add a new indication and/or improve the management of risk by adding a warning, limiting the target population, changing the dosage regimen and adding information on co-administration with other vaccines or medicines).

National regulatory authorities (NRAs) and MA holders should recognize that:

- any change to a vaccine may impact upon the quality, safety and efficacy of that vaccine;
- any change to the information associated with the vaccine (that is, product labelling information) may impact on the safe and effective use of that vaccine.

The regulation of changes to approved vaccines is one of the most important elements in ensuring that vaccines of consistent quality, safety and efficacy are distributed after they receive authorization or licensure. WHO provides support to its Member States through the provision of written standards and guidelines (1–3). However, the NRAs of Member States requested further guidance on the data needed to support changes to approved vaccines to ensure the comparability – with respect to quality, safety and efficacy – of vaccines manufactured with the change. Although it is difficult to provide guidance that applies 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. The categories of such 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, the contents of 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 of the principles and technical specifications set out in this document be made only
on condition that they ensure a level of vaccine quality, safety and efficacy at least equivalent to that which would be achieved by following the guidance provided here (that is, ensure that the risks of introducing vaccines for use in public health programmes are no greater than those that are outlined in this document).

2. Scope

This document provides guidance for NRAs and MA holders on the regulation of changes to the original MA dossier or product licence for an approved vaccine in terms of: (a) procedures and criteria for the appropriate categorization and reporting of changes; and (b) the data required to enable NRAs to evaluate the impact of the change on the quality, safety and efficacy of the vaccine. Additionally, the purpose of these WHO Guidelines is to assist NRAs in establishing regulatory procedures for post-approval changes to vaccines.

The guidance given below applies to the manufacture and use of approved prophylactic vaccines for humans. However, the general principles set out in this document may also apply to other biological products.

3. General considerations

For each change to the original MA dossier or product licence the MA holder should decide if the information in the original MA or product licence needs to be supplemented (that is, requires the official submission of a supplement or a change application dossier to the NRA) based on the guidance provided in this document. Prior to implementing the change, the MA holder should assess the effects of the change and demonstrate through appropriate studies (analytical testing, functional assays, and/or clinical or nonclinical studies) the absence of any negative effect of the change on the quality, safety and efficacy of the vaccine. A supplement requiring approval prior to implementation of a change is referred to as a prior approval supplement (PAS). In general, no change should be implemented without the approval of the NRA unless otherwise indicated in this document (for example, minor quality changes).

Changes to approved vaccines are categorized on the basis of a risk analysis. When a change affects the manufacturing process, this assessment should include evaluation of the effect of the change on the quality (that is, identity, strength, purity and potency) of the final product as it may relate to the safety and/or efficacy of the vaccine. When a change affects the clinical use or product labelling information, this assessment should include evaluation of the effect of the change on the safety and efficacy of the vaccine. Changes that may potentially have a major or moderate impact require submission of a PAS to the NRA. For each change, the supplement should contain information developed
by the MA holder to allow the NRA to assess the effects of the change. When changes may potentially have a minimal impact or no impact on product quality, safety and efficacy, they should be recorded and retained by the manufacturer or MA holder.

Assessment of the extent to which the quality change (also referred to as manufacturing change) affects the quality attributes (that is identity, strength, purity and potency) of the vaccine is generally accomplished by comparing manufacturing steps and test results from in-process and release testing of pre-change and post-change processes, and determining if the test results are comparable (that is, the antigen, intermediate or final product made after the change should be shown to be comparable to and/or to meet the acceptance criteria of the final product made before the change). However, additional supporting data may be required, as noted in Appendices 2–4 below.

An MA holder making a change to an approved vaccine should also conform to other applicable laws and regulations, including good manufacturing practice (GMP), good laboratory practice (GLP) and good clinical practice (GCP). MA holders should 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 of equipment used in the manufacturing process generally require installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ). 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 may occur routinely, may be required before submission of a supplement for a major manufacturing change such as a move to a new facility, or may be triggered by a major manufacturing change such as a change in production capacity or filtration or purification systems.

Certain major changes, such as changes in the vaccine antigen composition (for example, addition of virus or bacterial types), use of new cell substrates (for example, use of cells unrelated to the established master cell bank (MCB) or pre-MCB material) or changes in the composition of vaccine adjuvants are generally considered to be a new product and as such require the submission of a product licence application for a new MA. In addition, in some countries a change in the quantity of antigen per dose of vaccine also requires a product licence application for a new MA (see section 8.2 for changes to the seasonal influenza virus vaccine composition; and Appendix 2 (changes 9.a and 10.a) for information on changes to the cell banks and seed lots, respectively).

Administrative changes related to acquisitions and mergers, company names or contact information should be submitted directly to the NRA as general correspondence to the MA or product licence. When these changes affect the product labelling information, the revised labelling items should be submitted to the NRA, as described in this document (see section 6.4).
The implementation of new regulations should not affect vaccine supply and access by the public to vaccines. NRAs are therefore strongly encouraged to establish requirements that are commensurate with public health priorities and with their own regulatory capacity and resources. NRAs of vaccine-procuring countries should strongly consider establishing alternative procedures for the expedited approval of changes on the basis of previous expert review and approval of the same changes by the NRAs of countries in which the vaccines are produced and/or licensed, or on the basis of decisions made by 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 to support the change, as outlined in this document. Nevertheless, responsibility for the final regulatory decision on the approval of the change will still lie with the receiving NRA (see section 7 and Appendix 1).

To ensure vaccine supply and encourage adequate reporting of changes by manufacturers, NRAs should also consider establishing procedures for the concurrent (that is, parallel) review of changes to each product. Vaccine production requires the replenishment of biological starting materials such as cell banks, seed lots and reference standards, which are considered routine changes beyond the control of manufacturers. Consequently, these changes often need to be reviewed concurrently with other manufacturing or safety and efficacy changes. Similarly, clinical safety and efficacy changes, such as the addition of a new indication for a vaccine or a new age group for use of a vaccine, require considerable supporting data and review time and should not preclude or impede the review of unrelated manufacturing changes or the immediate implementation of urgent changes to product labelling information. However, multiple related changes may be submitted in the same supplement (see section 7).

The establishment of regional NRA associations or networks that can serve as forums for sharing information and exchanging experience on technical issues and regulatory decisions is highly encouraged. The development of such networks would expand the capacity of individual NRAs through work-sharing and recognition of the decisions of other NRAs in the network, thus avoiding unnecessary repetition of evaluations of the same change by multiple members of the network. NRA associations should establish work-sharing procedures that ensure the protection of confidential proprietary information with the engagement of MA holders and experts on the proprietary laws of each country. Any regional association or network of NRAs should, at a minimum, ensure the confidential nature of the technical information in the MA or licence application, especially information on product quality.

Establishing networks would be part of capacity-building activities for countries in each region. A fully functional regional network would be a
long-term goal, but cooperation can begin in the short term with the sharing of scientific information and experience regarding regulatory decisions on the evaluation of changes to approved products. Meetings should be organized periodically to promote transparency and mutual confidence between the NRAs. Effective regional networks could serve as the foundations for achieving full mutual recognition among NRAs.

In these WHO Guidelines, descriptions of the reporting categories are provided for both quality changes (section 5) and for safety, efficacy and product labelling information changes (section 6). Proposed recommendations on the regulatory procedures for the reporting of changes to NRAs are described in section 7. 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 antigen and intermediates) and Appendix 3 (for the final product). Examples of changes that affect clinical use and product labelling information (safety, efficacy, dosage, administration, vaccine components and expiry date) are provided in Appendix 4.

4. Terminology

The definitions given below apply to the terms as used in these WHO Guidelines. They may have different meanings in other contexts, including the compendial references and regulations or guidelines issued by NRAs and by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

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

**Antigen:** the following definitions apply in this document:

- The active ingredient in a vaccine against which the immune response is induced. Antigens may be: (a) live attenuated or inactivated preparations of bacteria, viruses or parasites; (b) crude cellular fractions or purified antigens, including recombinant proteins (that is, those derived from recombinant DNA expressed in a host cell); (c) polysaccharides and conjugates formed by covalent linkage of polysaccharides to components such as mutated or inactivated proteins and/or toxoids; (d) synthetic antigens; (e) polynucleotides (such as plasmid DNA vaccines); or (f) living vectored cells expressing specific heterologous antigens. Also referred to as “immunogen” in other documents.
Also used to describe (a) a component that may undergo chemical change or processing before it becomes the antigen or active ingredient used to formulate the final product (also referred to as an “intermediate” in other documents); or (b) an active ingredient present in an unmodified form in the final product (also referred to as “drug substance” or “active substance” in other documents). For example, in this document the term “antigen” applies, in the case of a polysaccharide conjugated vaccine, to the polysaccharide intermediate as well as to the conjugated polysaccharide that will not undergo further modification prior to formulation.

Cell bank: a collection of vials of cells of uniform composition (though not necessarily clonal) derived from a single tissue or cell, and used for the production of a vaccine directly or via a cell bank system. The following terms are used in these Guidelines – master cell bank (MCB): a bank of a cell substrate from which all subsequent cell banks used for vaccine production will be derived. The MCB represents a well characterized collection of cells derived from a single tissue or cell; and working cell bank (WCB): a cell bank derived by propagation of cells from an MCB under defined conditions and used to initiate production of cell cultures on a lot-by-lot basis. Also referred to as “manufacturer’s working cell bank” in other documents.

Change: refers to a change that includes, but is not limited to, the product composition, manufacturing process, quality controls, equipment, facilities or product labelling information made to an approved MA or licence by the MA holder. Also referred to as “variation” in other documents.

Comparability study: the activities, including study design, conducting of studies and data evaluation that are designed to investigate whether the pre- and post-change products are comparable. In addition to routine analysis performed during production and control of the antigen or final product, these evaluations typically include a comparison of manufacturing process steps and parameters impacted by the change, characterization studies and an evaluation of product stability following the change. In some cases, nonclinical or clinical data might contribute to the conclusion reached.

Comparability protocol: establishes the tests to be done and acceptable limits to be achieved to demonstrate the lack of a negative effect of specific manufacturing changes on the safety or effectiveness of the product. A comparability protocol is a highly specific, well defined plan for the future implementation of a quality (that is, manufacturing) change. Also referred to as “post-approval change management protocol” in other documents.

Container closure system: refers to the following components: (a) a primary container closure system is a packaging component (for example, a vial or pre-filled syringe) that is in, or may come into, direct contact with the final
product dosage form, or components that contribute to the container/closure integrity of the primary packaging material for a sterile product; and (b) a secondary container closure system is a packaging component (for example, a carton or tray) that is not, and will not be, in direct contact with the dosage form.

**Dosage form:** in this document “dosage form” refers to 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.

**Excipient:** any component of the final product other than the active component/antigen and the packaging material. Also referred to as “inactive ingredient” in other documents. In the context of this document, adjuvants are not considered to be excipients.

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

**Final product:** a finished dosage form (for example, suspension or lyophilized cake) that contains an active ingredient, generally but not necessarily in association with inactive ingredients (excipients) or adjuvants. Also referred to as “finished product” or “drug product” in other documents.

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

**Intermediate:** a material produced during steps in the manufacture of a vaccine that undergoes further processing before it becomes the final product. See the definition for **Antigen** above.

**Manufacturer:** any person or legal entity engaged in the manufacture of a product subject to MA or licensure. In other documents, “manufacturer” may also refer to any person or legal entity that is an applicant or a holder of a MA or product licence where the applicant assumes responsibility for compliance with the applicable product and establishment standards. See the definition for **Marketing authorization holder** below.

**Marketing authorization (MA):** a formal authorization for a medicine to be marketed. Once an NRA approves an MA application for a new medicine, the medicine may be marketed and may be available for physicians to prescribe. Also referred to as “product licence” or “licence” in this and other documents.

**Marketing authorization application (MA application):** a formal application to the NRA for approval to market a new medicine. The purpose of the MA application is to determine whether the medicine meets the statutory standards for safety, effectiveness, product labelling information and manufacturing. Also referred to as “licence application” in other documents.
Marketing authorization holder (MA holder): any person or legal entity that has received MA or licensure to manufacture and/or distribute a medicine. It also refers to a person or legal entity allowed to apply for a change to the MA or licence. Also referred to as the “manufacturer” or “applicant” in this and other documents.

Product labelling information: printed materials that accompany a prescription medicine and all labelling items, namely: (a) prescribing information (an instruction circular that provides product information on indication, dosage and administration, safety and efficacy, contraindications and warnings, along with a description of the product for health care providers (also referred to as “summary of product characteristics” or “package insert” in various countries); (b) patient labelling or consumer information; (c) inner label or container label; and (d) outer label or carton.

Quality attribute: a physical, chemical, biological or microbiological property or characteristic. A critical quality attribute refers to a characteristic or property that should be within an appropriate limit, range or distribution to ensure the desired product quality.

Quality change: in the context of this document, quality change refers to 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 reagents or solvents intended for use in the production of starting materials, intermediates or final products.

Seed lot: a preparation of live cells (prokaryotic or eukaryotic) or viruses constituting the starting material for the vaccine antigen. A seed lot is of uniform composition (although not necessarily clonal), is derived from a single culture process and is aliquoted into appropriate storage containers, from which all future vaccine production will be derived either directly or via a seed lot system. The following derived terms are used in these Guidelines – master seed lot (MSL): a lot or bank of cells or viruses from which all future vaccine production will be derived. The MSL represents a well characterized collection of cells or viruses of uniform composition. Also referred to as “master virus seed” for virus seeds, “master seed bank” or “master seed antigen” in other documents; and working seed lot (WSL): a cell or viral seed lot derived by propagation from the MSL under defined conditions and used to initiate production of vaccines on a lot-by-lot basis. Also referred to as “working virus seed” for virus seeds, “working seed bank” or “working seed antigen” in other documents.

Specification: the quality standard (that is, tests, analytical procedures and acceptance criteria) provided in an approved application to confirm the quality of antigens (drug substances), final products (drug products), intermediates, raw materials, reagents, components, in-process materials, container closure systems
and other materials used in the production of the antigen (drug substance) or final product (drug product). For the purpose of this definition, acceptance criteria mean numerical limits, ranges or qualitative criteria for the applied tests.

**Starting material:** any material used at the beginning of the manufacturing process, as described in an MA or product licence. Generally, the term refers to a substance of defined chemical properties and structure that contributes an important and/or significant structural element (or elements) to the active substance (for example in the case of vaccines, synthetic peptides, synthetic glycans and starting materials for adjuvants). The starting material for an antigen (drug substance) obtained from a biological source is considered to consist of: (a) cells; (b) microorganisms; (c) plants, plant parts, macroscopic fungi or algae; or (d) animal tissues, organs or body fluid from which the antigen (drug substance) is derived.

**Supplement:** written request submitted to the NRA to approve a change in the original application for MA (or product licence) or any other notification to add to (that is, supplement) the information in the original MA 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.

**Vaccine:** a preparation containing antigens capable of inducing an active immune response for the prevention, amelioration or treatment of infectious diseases.

**Vaccine efficacy:** the relative reduction in disease incidence or severity in vaccinated individuals compared to unvaccinated individuals measured in a randomized, placebo-controlled clinical trial. In the context of these Guidelines, vaccine efficacy has a broad meaning and relates to all clinical data obtained to ensure vaccine efficacy, immunogenicity or field effectiveness.

### 5. Reporting categories for quality changes

Based on the potential effect of the quality change (for example, manufacturing change) on the quality attributes (that is, identity, strength, purity and potency) of the vaccine, and the potential impact of this on the safety or efficacy of the vaccine, a change should be categorized and identified as:

- a major quality change
- a moderate quality change, or
- a minor quality change.

The implementation of changes in the major or moderate categories requires reporting to the NRA in order to supplement the information in the
original MA or product licence. The major and moderate quality changes should be reviewed and approved by the NRA prior to implementation of the change.

Minor quality changes that are expected to have a potential minimal effect or no effect on the quality, safety or efficacy of the vaccine do not require submission of a supplement. The changes included in this category may be implemented by the MA holder without prior review and approval by the NRA. However, a list of minor changes should be made available by the MA holder upon request by the NRA.

Further information on each category is given below. In addition, Appendices 2 and 3 provide a comprehensive list of major, moderate and minor quality changes, and the information required to support each change. Appendix 2 includes changes to the antigen or intermediates and Appendix 3 includes changes to the final product. 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 or efficacy of the vaccine, 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 based on a change-specific risk assessment using the principles and examples provided in this document. The NRA should consider establishing a mechanism that allows for the updating of its guidelines to address technological changes that require new regulatory category classifications.

5.1 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 vaccine. The MA holder should submit a PAS and receive a notification of approval from the NRA before implementing the change. For a change in this category, the supplement should specify the products concerned and should include a detailed description of the proposed change. Additional supporting information is needed, as noted in Appendix 2 for the antigen and in Appendix 3 for the final product, and should include information on: (a) 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; (d) updated product labelling information; and (e) summaries of relevant standard operating procedures (SOPs) or a list referencing previously approved relevant SOPs. In some cases, major quality changes may also require nonclinical and/or clinical data. The recommendations given in WHO guidelines on nonclinical evaluation of vaccines (4), Guidelines on clinical evaluation of vaccines: regulatory
expectations (5), Guidelines on stability evaluation of vaccines (6), other related
WHO guidance (7–12), and recommendations for specific products and adjuvants
should apply.

5.2 **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 vaccine. The MA holder
should submit a supplement and receive a notification of approval from the NRA
before implementing the change. The requirements for the supplement content
of the moderate quality changes are the same as for the major quality changes
(see section 5.1 above). However, the amount of supporting data required will
generally be less than for major changes and the review time should be shorter.

5.3 **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 vaccine. The changes
included in this category may be implemented by the MA holder without prior
review by the NRA (that is, such changes do not need to be reported to and
approved by the NRA). However, these changes must be retained as part of the
product’s record by the manufacturer or MA holder, must comply with GMP
requirements and must be available for review during GMP inspections.

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 vaccine lot
release protocol, the MA holder should inform the institution responsible for
reviewing the release of vaccine lots (see introductory sections in Appendices 2
and 3).

For each approved product, the MA holder or manufacturer should
maintain a comprehensive chronological list of all quality changes, including
minor quality changes that occur in all production areas. Additionally, this list
should include a description of the manufacturing and quality control changes,
including the manufacturing site(s) or area(s) involved, the date each change was
made, and the references of relevant validations and SOPs. The data to support
minor quality changes, as listed in Appendices 2 and 3, should be available to the
NRA upon request or during inspections.

When minor quality changes are related to a major or moderate change,
they should be described in the supplement for the major or moderate quality
change (see section 7.2).
6. Reporting categories for safety, efficacy and/or product labelling information changes

After assessing the effect of a change related to clinical use or to product labelling information on the safe and effective use of a vaccine, MA holders should classify this change as belonging to one of the following categories:

- 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 before implementation is needed).

The product labelling information includes prescribing information (or package insert) for health care providers or patients, outer label (carton), and inner label (container label). After approval, the MA holder should promptly revise all promotional and advertising items relating to the vaccine to make them consistent with implementation of the product labelling information change.

Further information on each category is provided in the following sections, with examples of efficacy, safety and product labelling information changes considered to be appropriate for each category provided in Appendix 4.

6.1 Safety and efficacy changes

Safety and efficacy changes are changes that have an impact on the clinical use of the vaccine in relation to safety, efficacy, dosage and administration, and that require data from clinical studies to support the change. Safety and efficacy changes require supplement submission and approval prior to implementation.

Generally, safety and efficacy changes affect the product labelling information and have the potential to increase or decrease the exposure levels of the vaccine, either by expanding the population that is exposed or by changing dosage or dosing. These changes may relate to the clinical use of the vaccine, for example:

- 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 Some NRAs consider that changes in the route of administration or strength may require a new MA. Furthermore, in some cases, changes involving the subcutaneous and intramuscular administration routes may not require a new application while others, such as changes from intramuscular to intranasal administration routes, may require a new application.
- change in the recommended dose and/or dosing schedule, including the addition of a booster dose;
- co-administration with other vaccines or medicines;
- deletion or reduction of existing risk-management measures (such as contraindications, adverse events, warnings or cautionary text/statements in the product labelling information).

The type and scope of the required supporting 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 vaccine attributes and the disease that the vaccine is designed to prevent. Other considerations include:

- robustness of the immune response elicited by the vaccine and availability of a correlate of protection (that is, data establishing a threshold level of antibody needed to protect against the development of disease following exposure);
- availability of animal models;
- vaccine attributes (for example, live as opposed to inactivated vaccines).

MA holders are encouraged to consult with NRAs on the adequacy of the clinical 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. NRAs may also be consulted on the data required to support such changes.

For nonclinical and clinical studies, the recommendations given in WHO guidelines on nonclinical evaluation of vaccines (4), Guidelines on clinical evaluation of vaccines: regulatory expectations (5) and other related WHO guidance (7–12) should apply.

For a change under this category, the MA holder should submit a supplement to the NRA that may include the following:

- detailed description and rationale of the proposed change;
- summary of the methods used and studies performed to evaluate the effect of the change on the vaccine’s safety or efficacy;
- amended product labelling information;
- clinical studies (protocol, statistical analysis plan and clinical study report);
- clinical assay methods (including SOPs) and validations;
- the pharmacovigilance plan.
6.2 **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 currently approved for use of the vaccine through:

- identification or characterization of any adverse event following immunization (AEFI) resulting in the addition or strengthening of risk-management measures for an adverse event identified to be consistent with a causal association to immunization with the vaccine concerned;
- identification of subgroups for which the benefit-to-risk profile of the vaccine has the potential to be less favourable;
- addition or strengthening of risk-management measures, including instructions on dosing or any other conditions of use.

Product labelling information changes require supplement submission and approval prior to distribution of the product. Supplements for product labelling information changes related to clinical use often require data from pharmacovigilance reports (“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 MA holder should submit a supplement to the NRA that may include the following:

- detailed description and rationale of the proposed change
- pharmacovigilance reports and statistical analysis of results
- amended product labelling information.

6.3 **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 currently approved for use of the vaccine. MA holders should consult with the NRA and agree on the supporting documentation required prior to supplement submission.

6.4 **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 vaccine. 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, as follows:
Examples of product labelling information changes that require approval by the NRA prior to implementation are changes in the name of the MA holder that are due to a merger, or changes in the proper name or trade name of the vaccine. The 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 changes to a distributor’s address or minor changes in format. These changes should be reported to the NRA as part of subsequent supplements for safety and efficacy changes or product labelling information changes when updated product labelling information is included.

7. Procedures

Establishing procedures and criteria for the adequate oversight of changes is the responsibility of the regulators. Therefore, NRAs should establish written instructions regarding the submission procedures and timelines with action dates, to be consulted by MA holders when they prepare to submit a supplement for a change. 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 reviews of major quality changes that do not require clinical data, compared to safety and efficacy changes that do require clinical data. Examples of regulatory categories and review timelines are provided in Appendix 1 below.

MA holders may contact the NRA to determine the appropriate category of a supplement prior to submission of the information in support of a change, especially if the change is not included in Appendices 2–4 of this document. Similarly, MA holders may also consult NRAs for major changes (such as the introduction of new equipment, change in process step or facility expansion) that require the inclusion of a GMP certificate and may trigger a pre-submission inspection, or that may require clinical data to support a change in safety and efficacy or in product labelling information. MA holders should generally be encouraged to contact the NRA regarding plans for future changes and proposed filing dates for changes to existing products in order to aid NRAs in planning the allocation of review resources. NRAs should establish procedures for the conducting and recording of communications between themselves and MA holders.

To aid in the acceptance of submissions for review, the covering letter accompanying a supplement for a quality change should specify that the change is being reported in 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 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 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 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. The list should be part of the covering letter. If the submission has been inappropriately classified, the MA holder should be notified. Minor quality changes that are related to a moderate or major quality change should be included in the PAS if they were implemented after the submission of a previous supplement for a moderate or major quality change. For example, a minor change such as the narrowing of a specification should be included in a supplement for a moderate or major change which includes updated quality control release information.

Regulation of post-approval changes is part of the whole regulatory framework which incorporates elements such as MA, GMP inspection, lot release and post-marketing surveillance (PMS). These activities are often performed by different branches of the NRA. It is essential that these different branches – particularly the MA (or regulatory affairs), GMP inspection and lot release branches – interact and exchange information effectively and that the roles and responsibilities of each branch are clearly defined, especially when they operate as separate entities. When multiple branches 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 are pivotal.

**Expedited review procedures**

NRAs of vaccine-procuring countries that decide to recognize 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 vaccines are produced and/or licensed (see Appendix 1). On the basis of regulatory and regional considerations, regulatory procedures for recognizing the decision of other NRAs on the approval of changes could include:

- The NRA recognizes the decision of other regulatory authorities and does not perform a review of supporting data, but is informed of the change. The submission consists of a covering letter from the MA holder informing the procuring NRA of the change and including as an attachment a copy of the approval letter issued by the NRA of the producing and/or licensing country.
- The NRA performs an assessment of the decision of the NRA from the producing and/or licensing country to determine if recognition of that NRA’s decision is appropriate. The submission consists of: (a) the covering letter from the MA holder informing the procuring NRA of the change; (b) a copy of the approval letter issued by the NRA of the producing and/or licensing country; (c) assessment reports and relevant correspondence from the NRA of the producing and/or licensing country (if made available by the NRA); and (d) a detailed description of the change with no supporting data.
- The NRA performs a partial review and evaluation of a complete package of supporting data, as originally submitted in the vaccine-producing and/or -licensing country and/or as recommended in these WHO Guidelines.

Similarly, recognition of inspection activities conducted by the authorities in the place where a vaccine is produced may also be considered 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 7.4 and Appendix 1).
In special or urgent circumstances, an MA holder may ask the NRA to expedite the review of a supplement for public health reasons (for example, a vaccine shortage, or during an epidemic or pandemic) or if a delay in making the change would impose extraordinary hardship on the MA holder or manufacturer.

Multiple changes

Multiple related changes, involving various combinations of individual changes, may be submitted in the same supplement. For example, a site change may also involve changes to the equipment and manufacturing process, or a vaccine component change may necessitate a change in a specification. For submissions that include multiple changes, the MA holder should clearly specify which data support each change.

Multiple major or moderate quality changes for the same vaccine 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 to a moderate or major quality change should be included in the supplement for the moderate or major quality change. If the changes are related, the MA holder should indicate the association between the proposed changes. Such changes could affect both the antigen and the final 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 MA holder to divide the changes into separate submissions and to re-submit 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 vaccine. This reclassification should be communicated to the MA holder at the start of the assessment.

7.1 Procedures for prior approval supplements

The procedures in this section apply to all changes requiring approval prior to implementation: that is, 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: (a) the type of submission (for example, major quality change, moderate quality change, safety and efficacy change); (b) a list of the change(s) and a rationale for the change(s) with sufficient detail to allow for processing and reviewer assignments by NRAs; (c) an indication of the general type of supporting data; and (d) cross-referenced information if applicable (including product name, MA holder’s name, submission type control number and date of submission/approval);

- Completed documents or forms based on NRA requirements, such as a medicines submission application form, signed and dated;
- The anticipated date for implementation of the change;
- GMP document information, as applicable;
- A rationale for the change and a justification for the selected reporting category;
- When relevant, a side-by-side comparison showing the differences between the approved manufacturing process (including quality control tests) and the proposed ones (see section 5);
- When relevant, clinical study reports, pharmacovigilance reports, and annotated and clean drafts of product labelling information (see section 6).

In addition to the above common information items, the specific information required to support the various quality changes is outlined in Appendices 2 and 3. It should be noted that the common information items listed above are not included under each of the various changes outlined in these appendices. All data recommended to support a change should be provided with the submission along with all appropriate common information items. When recommended supporting data cannot be submitted, a detailed rationale should be provided.

If the same change is applicable to multiple products, a separate submission is generally required for each product but the data may be cross-referenced. When cross-references are made to information that has been submitted previously, the details of the cross-referenced information should be indicated in the covering letter (for example, brand name of the product, name of manufacturer and/or MA holder, submission type, control number and date approved).

Submissions filed in electronic or paper format should be based upon 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 there are two possible outcomes:

- If the NRA determines that the information in a supplement indicates no adverse impact on the quality, safety or efficacy of the product manufactured with the change, the NRA will issue a written approval notification by which 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 demonstrate 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 MA holder. If the identified deficiencies are minor, they may be addressed without stopping the review clock. If the deficiencies are major or are not resolved during the allotted review time frame, the NRA may decide to issue a written notification of noncompliance by means of which the review clock is stopped, the change may not be implemented and the product manufactured with the change may not be distributed.

In the case of a noncompliance notification being issued, the following outcomes are possible:

- If the information in the MA holder’s response document to the noncompliance notification is adequate and all identified deficiencies are resolved in a satisfactory manner, the NRA will issue a written notification of approval by which the change can be implemented and the product manufactured with the change can be distributed.

- If the information in the MA holder’s response document to the noncompliance notification is not adequate and not all identified deficiencies are resolved in a satisfactory manner, the NRA will issue a written notification of rejection by means of which 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 the MA holder’s responses to the notification of noncompliance in cases where the review is stopped. Documentation subsequent to the original supplement submission (in response to information requests or noncompliance notifications) should be submitted and filed as amendments to the original supplement, and communications with MA holders should be properly recorded.
Appeal procedures should be established for resolving disagreements and disputes between the NRA and the MA holder. Such procedures should allow the MA holder to request a re-evaluation of the submitted application in cases where the application is rejected by the NRA.

In some cases, following approval, the distribution of a vaccine made with a change may be delayed to allow for depletion of the previously approved vaccine or to allow for global approval. Therefore, the MA holders should provide the anticipated date for implementation of the change. If deemed necessary, any issues related to the implementation dates and distribution of product with the approved manufacturing changes should be communicated to the NRA.

NRAs may consider the following approaches when an MA holder is submitting changes.

**Comparability protocol**

A comparability protocol (also referred to as a “post-approval change management protocol” in other documents) establishes a framework for a well defined and highly specific plan for the future implementation of a quality change, including the tests to be done and acceptable limits to be achieved to demonstrate the lack of negative effects caused by specific manufacturing changes on the quality, safety or efficacy of a vaccine. For some changes, the routine quality tests performed to release the antigen or final product are not considered adequate for assessing the impact of the change, and additional in-process tests and characterization tests may be needed (for example, addition of bioburden and endotoxin tests to support the removal of preservatives from the manufacturing process). 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 allow for a more expedient distribution of a product by permitting the MA 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. This concept is not discussed in further detail in these Guidelines as the use of a comparability protocol is not currently harmonized among NRAs. It is the decision of the NRA whether or not to include the review and approval of comparability protocols in its approach to regulating changes to approved vaccines. For NRAs currently taking this approach, 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 the comparability protocol in relation to the comparability data should be established by the NRA at the time the comparability protocol is approved.
Production documents

Production documents (that is, executed lot records) are not required to support changes to the MA dossier or product licence. However, such documents may be requested during review and should be available to the NRA upon request or during inspections.

7.2 Procedures for minor quality changes

Minor quality changes do not require notification to, or prior approval from, the NRA for their implementation. However, any minor changes that have been implemented should be noted in the affected documents (for example, SOPs and batch records). As recommended in Appendices 2 and 3 of this document, minor quality changes should be recorded or compiled with related supporting data in a document or file dedicated to minor changes. The documents or files for all minor quality changes should be available to the NRA upon request or during inspections.

Minors quality changes that have previously been implemented and are related 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. In general, changes to SOPs which are not mentioned in Appendices 2 and 3 do not need to be submitted to the NRA for approval.

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 the change or the supporting data are not considered to be acceptable, the MA holder may be requested to file a major or moderate quality change supplement.

For changes that are not reported, if the NRA determines (during an inspection or review of related changes) that the information relating to the change fails to demonstrate the continued safety or efficacy of the product manufactured using the changes, the NRA will try to resolve the problem with the MA 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 MA 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.

7.3 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, the addition of a contraindication or warning) NRAs should establish a specific
mechanism to allow for the immediate or speedy approval and implementation of such changes on a case-by-case basis after previous agreement between NRAs and MA holders.

Since product labelling safety updates invariably need to be implemented and are generally approved, NRAs should establish a mechanism by which urgent product labelling changes that have been approved in the country where the vaccines are produced and/or licensed may be implemented immediately upon receipt of the supplement by the NRAs of countries procuring the vaccines. Such accelerated procedures would contribute to the dissemination of the most current information to health care providers, and would also help to mitigate the effects of discrepancies between labelling information in different countries and between the information posted on different web sites.

7.4 Procedures for administrative product labelling information changes

Administrative product labelling information changes may require approval prior to implementation depending on the scope of the change. For example, changes in the name of the MA holder require approval before implementation while minor formatting changes do not (see Section 6.4).

For an administrative product labelling information change that requires approval prior to implementation, the MA 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 subsequent supplements 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.

8. Special considerations

8.1 Adjuvants

Because adjuvants are considered to be components of vaccines, each new adjuvanted vaccine is considered to be a new entity that will require appropriate physicochemical characterization and nonclinical and clinical evaluation. It is the specific antigen-adjuvant formulation (as a whole) that is tested in nonclinical and clinical trials and which receives MA or licensure on the basis of demonstration of safety and efficacy.
There is substantial diversity among vaccine adjuvants, antigens and the diseases they are designed to prevent. Therefore, the supporting information needed for adjuvant-related changes will depend upon product-specific features, the clinical indications and the impact of the change. The recommendations in WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (12) should be followed.

8.2 Influenza vaccines

To ensure that influenza vaccines are effective against circulating influenza viruses, WHO reviews global virological and epidemiological data twice a year, and if necessary recommends new vaccine strain(s) in accordance with the available evidence for the northern and southern hemispheres (13, 14). WHO and NRAs recommend the use of certain vaccine virus strains on the basis of their antigenic characteristics. Influenza vaccine viruses are usually derived from isolates obtained from laboratories in the WHO Global Influenza Surveillance and Response System.

For seasonal influenza vaccines, annual changes in the vaccine strain composition are considered to be moderate quality changes because of extensive experience with such changes and in order to maximize the flexibility and brevity of the review process. MA holders of approved seasonal vaccines are expected to submit a supplement for a moderate quality change to support annual changes in the influenza strain composition. To allow for the timely distribution of vaccines, NRAs should review the supplement as part of a streamlined and prompt process. The supporting quality information generally consists of: (a) information on the source of the seed viruses; (b) passage history until establishment of working seeds; (c) results of quality release tests performed on working virus seeds (including identity confirmation); and (d) specific validation data (including inactivation kinetics). Generally, stability data for antigen bulks or final drug product produced in the previous influenza season are expected to be submitted to continuously support the approved shelf-life. In addition, updated product labelling information items (package insert and inner and outer labels with relevant strain composition and formula year) should be provided (13).

Changes to the manufacturing processes, posology and product labelling information of influenza vaccines that are not related to the annual update should follow the normal categorization process, as described in Appendices 2–4, and should not be included in the strain change supplements to avoid delays in the approval process. Due to time constraints related to the seasonality of influenza vaccines, changes that are not related to vaccine strain composition should be timed such that approval will allow for vaccines manufactured with the change to be distributed prior to the start of the influenza season.
8.3 Bridging studies

Clinical bridging studies are trials in which a parameter of interest (such as manufacturing process, formulation or dosing schedule) is directly compared with a changed version of that parameter with respect to the effect of the change on the product’s clinical performance. The comparison of immune responses and safety outcomes (for example, rates of common and serious AEFIs) is often the primary objective. If the immune response and safety profiles are similar, the safety and efficacy of the vaccine can be inferred.

In some cases, safety and efficacy data comparing the approved vaccine to the vaccine produced with the change may be required by NRAs. The following are examples of manufacturing changes that may require clinical bridging studies:

- use of a new or re-derived antigen (that is, re-derived virus seed or bacterial cell bank) or host cell line (that is, re-derived MCB);
- new agents used for inactivation or splitting of the antigen;
- a new dosage form;
- a new formulation (for example, amount of ingredients, adjuvants, preservatives or reactogenic residual components from the manufacturing process).

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The second draft was then prepared by the same principal authors following a consultation held in Geneva, 16–17 April 2013 and attended by: Mr P. Akut, Serum Institute of India Ltd., India; Dr M-C. Annequin, Agence Nationale de Sécurité du Médicament et des Produits du Santé, France; Ms S. Boucher, Health Canada, Canada; Mr S. Callewaert, GlaxoSmithKline Biologicals, Belgium; Dr M. Eisenhawer, World Health Organization, Regional Office for South-East Asia, India; Dr S. Fakhrzadeh, Food and Drug Organization, the Islamic Republic of Iran; Dr I. Feavers, National Institute for Biological Standards and Control, England; Dr M.C. Flores, Representante de la Dirección General Adjunta de Control y Aseguramiento de la Calidad, Mexico; Dr S. Gagneten, United States Food and Drug Administration Center for Biologics Evaluation and Research, USA; Dr K. Hara, Pharmaceuticals and Medical Devices Agency, Japan; Dr I. Hura, Central Drugs Standard Control Organisation, India; Mrs T. Irviyanti, PT Bio Farma, Indonesia; Mrs T. Jivapaiparnpong, Institute of Biological Products, Thailand; Dr I. Knezevic, World Health Organization, Switzerland; Dr H. Langar, World Health Organization, Regional Office for the Eastern Mediterranean, Egypt; Ms Y. Lee, National Institute of Food and Drug Safety Evaluation, Republic of Korea; Dr D. Lei, World Health Organization, Switzerland; Dr M. Leroux-Lepage, Sanofi Pasteur, France; Dr J. Luo, Center for Drug Evaluation, China; Mr M. McGoldrick, Merck Research Laboratories, USA; Dr A. Meek, World Health Organization, Switzerland; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr R. Morales, World Health Organization, Switzerland; Mr M. Moreira, Brazilian Health Surveillance Agency, Brazil; Ms S. Munnangi, Biological E Limited, India; Ms N. Nguyen, Crucell, Switzerland; Dr Y. Nunez, Centro para el Control Estatal de la Calidad de los Medicamentos, Cuba; Mrs C. Olaayan, National Agency for Food and Drug Administration and Control, Nigeria; Dr K. Omori, Pharmaceuticals and Medical Devices Agency, Japan; Ms S. Padayachee, National Department of Health, South Africa; Dr D. Pfeifer, World Health Organization, Regional Office for Europe, Denmark; Dr L.V. Phung, National Institute for Control of Vaccine and Biologicals, Viet Nam; Dr C. Saillez, GlaxoSmithKline Biologicals, Belgium; Dr J. Southern (Developing Country Vaccine Regulators’ Network representative), South Africa; Mrs P. Thanaphollert, Food and Drug Administration, Thailand; Dr A.R.T. Utami, National Agency of Drug and Food Control, Indonesia; Mr M. Vyas, Novartis Vaccines & Diagnostics, USA; Dr J. Wang, National Institutes for Food and Drug Control, China; Mr M. Welin, Medical Product Agency, Sweden; Dr D.J. Wood, World Health Organization, Switzerland.

The document WHO/BS2014.2238 was prepared by the same principal authors, taking into account comments received from national regulators and
vaccine manufacturers during a round of public consultation on the WHO Biologicals website in 2014. Further changes were then made by the WHO Expert Committee on Biological Standardization.

10. References


Appendix 1

Reporting categories and suggested review timelines

It is recommended that NRAs establish review timelines to allow MA holders or applicants to plan the implementation of changes. The review times established will depend upon the capability of the NRA, the impact of the change and the amount of data required to support the change. As a result, the review time frames for major changes should be longer than those for moderate changes. The review times suggested in Table 1 below are shown as examples, based upon 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 MA holder, either by the issuance of an approval notification or a noncompliance notification with a list of comments and deficiencies. In the case of the latter, the MA 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 MA holder. If minor deficiencies are identified during the initial review cycle, the NRA may communicate these to the MA holder without stopping the clock to try to finalize the assessment within the established timeline (see section 7.1).

For product labelling information changes which address urgent safety issues, procedures should be in place to allow for the expedited implementation of such changes (see section 7.4).

For annual updates of influenza virus strain composition, the review timeline of moderate quality change supplements should be as short as possible (around 30 days). This may be achieved by reducing the amount of supporting information required and by clearly describing to MA holders the required content and format of the information to be submitted (see section 8.2).

Table 1

Examples of review timelines for a prior approval supplement (PAS)

<table>
<thead>
<tr>
<th>Category</th>
<th>Supplement</th>
<th>Maximum review period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major quality changes</td>
<td>PAS</td>
<td>6 months</td>
</tr>
<tr>
<td>Moderate quality changes</td>
<td>PAS</td>
<td>3 months</td>
</tr>
</tbody>
</table>
Table 1 continued

<table>
<thead>
<tr>
<th>Category</th>
<th>Supplement</th>
<th>Maximum review period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quality changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor quality changes</td>
<td>Do not require notification to the NRA(^a)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Safety, efficacy and product labelling information changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety and efficacy changes</td>
<td>PAS</td>
<td>10 months</td>
</tr>
<tr>
<td>Product labelling information changes</td>
<td>PAS</td>
<td>5 months</td>
</tr>
<tr>
<td>Urgent product labelling information changes (^b)</td>
<td>PAS for urgent safety restrictions</td>
<td>Immediate implementation on receipt of supplement by the NRA</td>
</tr>
<tr>
<td>Administrative product labelling information changes</td>
<td>PAS</td>
<td>30 days</td>
</tr>
<tr>
<td><strong>Administrative product labelling information changes</strong></td>
<td></td>
<td>Do not require approval prior to implementation(^c) N/A</td>
</tr>
</tbody>
</table>

N/A: not applicable.

\(^a\) Minor quality changes that are related to a moderate or major quality change should be included in the PAS if they have been implemented after the submission of a previous supplement for a moderate or major quality change (for example, a minor change such as the narrowing of a specification should be included in a supplement for a moderate or major change which includes updated quality control release information).

\(^b\) 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 MA holders.

\(^c\) 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 supplements for safety and efficacy changes or product labelling information changes.

NRAs of countries that procure vaccines from countries where the vaccines are produced and/or licensed are encouraged to establish alternative regulatory procedures for the expedited approval of changes that have previously been approved by the licensing NRAs. As described in section 7 above, expedited regulatory approval procedures that could be established include:

- 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 producing and/or licensing country to determine if recognition of the latter’s decision is appropriate. In this case, NRAs could establish abbreviated review timelines, such as 2 months for major quality changes, 4 months for safety and efficacy changes, and immediate implementation upon 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 package of supporting data, as originally submitted in the vaccine producing and/or licensing country and/or as recommended in these WHO Guidelines. In this case, timelines could range from those shown in Table 1 or could be abbreviated as described in the preceding bullet point.
Appendix 2

Changes to the antigen

The examples presented in this appendix are intended to assist with the classification of changes made to the quality information for a vaccine antigen. The information summarized in the antigen table below provides recommendations 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 the next higher level of change – 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 MA holder (if any of the supporting data outlined for a given change are not provided, are different or are not considered applicable then adequate scientific justification should be provided);

- the *reporting category* (that is, major, moderate or minor quality change).

It is important to note that the NRA reserves the right to request additional information or material, as deemed appropriate, or to define conditions not specifically described in this document in order to allow for adequate assessment of the quality, safety and efficacy of a vaccine. In addition, MA holders should contact the NRA if a change not included in the antigen table below has the potential to impact upon vaccine quality.

Supporting data should be provided according to the submission format accepted by the NRA. For example, for NRAs that accept the ICH common technical document (CTD) and/or ICH eCTD formatted submissions, the supporting data should be provided in the appropriate sections of the CTD modules and not in separate documents. For the placement of data in the appropriate section of the CTD please see the ICH guidelines (1, 2).

For additional information on data requirements to support quality changes, WHO guidelines on GMP requirements and stability evaluation of vaccines (3, 4) should be consulted, together with relevant ICH guidelines.
Quality changes to comply with updated compendia and/or pharmacopoeia

NRAs should make a list of the recognized compendia and/or pharmacopoeia available to MA holders. Manufacturers are expected to comply with the current versions of compendia and/or pharmacopoeia as referenced in the approved MA. Changes in the compendial and/or pharmacopoeial methods or specifications referenced by a particular NRA do not need to be submitted for review, but information on such changes should be available for inspection.

In some cases, changes to comply with recognized compendia and/or pharmacopoeia may require approval by the NRA prior to implementation regardless of the timing of the change with respect to the date the 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, tests for potency), for changes which have an impact on any items of the product labelling information, and for changes which may potentially affect the quality, safety or efficacy of the product.

Quality changes affecting lot release

Where post-approval changes to the antigen affect the lot release protocol (for example, changes to test procedures, reference standards or laboratory sites) or sample testing requirements for lot release, the MA holder should inform the institution responsible for reviewing the release of vaccine 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 done in accordance with an approved protocol and specification. Nevertheless, these changes must be reported to the NRA or NCL as appropriate.

General information

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Change in the name of the antigen</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

*Note: This change generally applies only to influenza vaccines (see section 8.2).*

Conditions

None
### Supporting data

1. Revised product labelling information (all labelling items).
2. Information on the proposed nomenclature of the antigen and evidence that the proposed name for the antigen is recognized (for example, proof of acceptance by WHO).

### Manufacture

#### Description of change

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Change to an antigen manufacturing facility:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. replacement or addition of a manufacturing facility for the antigen bulk, or any intermediate of the antigen</td>
<td>None</td>
<td>1–4, 6–8</td>
<td>Major</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>2, 4–8</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. deletion of a manufacturing facility or manufacturer of an antigen intermediate, or antigen bulk</td>
<td>5, 6</td>
<td>None</td>
<td>Minor</td>
</tr>
</tbody>
</table>

#### Conditions

1. The new manufacturing facility/suite is an approved antigen manufacturing site.
2. Any changes to the manufacturing process and/or controls are considered either moderate or minor.
3. The new facility/suite is under the same quality assurance/quality control (QA/QC) 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 (such as recurrent deviations, recurrent out-of-specification events, environmental monitoring failures and so on).

#### Supporting data

1. Evidence that the facility is GMP compliant.
2. Name, address and responsibility of the proposed facility.
3. Process validation study reports.


Table continued

Supporting data

4. Comparability of the pre- and post-change antigen 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 vaccine, existing relevant nonclinical and clinical data, and aspects of vaccine 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 and release testing results as quantitative data, in a comparative tabular format, for at least three (3) consecutive commercial-scale batches of the pre- and post-change antigen. 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, and/or the use of fewer than 3 batches may be acceptable where justified and agreed by the NRA.

7. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale antigen batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months of testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the antigen 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

8. Updated post-approval stability protocol.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Change to the antigen fermentation, viral propagation or cellular propagation process:</td>
<td>None</td>
<td>1–7, 9, 11</td>
<td>Major</td>
</tr>
<tr>
<td>a. a critical change (a change with high potential to have an impact on the quality of the antigen or final product) (for example, incorporation of disposable bioreactor technology)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Description of change</td>
<td>Conditions to be fulfilled</td>
<td>Supporting data</td>
<td>Reporting category</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------------</td>
<td>-----------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>b. a change with moderate potential to have an impact on the quality of the antigen or final product (for example, extension of the in vitro cell age beyond validated parameters)</td>
<td>2, 4</td>
<td>1−6, 8, 10</td>
<td>Moderate</td>
</tr>
<tr>
<td>c. a noncritical change with minimal potential to have an impact on the quality of the antigen or final product (for example, 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; or duplication of a fermentation train)</td>
<td>1−6, 9−11</td>
<td>1−4</td>
<td>Minor</td>
</tr>
</tbody>
</table>

4. Change to the antigen purification process involving:

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. a critical change (a change with high potential to have an impact on the quality of the antigen or final 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 antigen)</td>
<td>None</td>
<td>1, 2, 5−7, 9, 11, 12</td>
<td>Major</td>
</tr>
<tr>
<td>b. a change with moderate potential to have an impact on the quality of the antigen or final product (for example, a change in the chemical separation method, such as from ion-exchange HPLC to reverse-phase HPLC)</td>
<td>2, 4</td>
<td>1, 2, 5−7, 10, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>Description of change</td>
<td>Conditions to be fulfilled</td>
<td>Supporting data</td>
<td>Reporting category</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------------------</td>
<td>----------------------------</td>
<td>-----------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>c. a noncritical change with minimal potential to have an impact on the quality of the antigen or final product (for example, addition of an in-line filtration step equivalent to the approved filtration step)</td>
<td>1−5</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
</tbody>
</table>

5. Change in scale of the manufacturing process:

a. at the fermentation, viral propagation or cellular propagation stage

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>3−6, 11−13</td>
<td>2, 3, 5−7, 9, 11</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

b. at the purification stage

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 3, 5, 7</td>
<td>2, 5−7, 9, 11</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

6. Change in supplier of raw materials of biological origin (for example, fetal calf serum, human serum albumin, trypsin)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4, 8, 12, 13</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

7. Change in source of raw materials of biological origin

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4, 7, 12, 13</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

8. Introduction of reprocessing steps

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>8, 10, 11, 14</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Conditions

1. No change in the principle of the sterilization procedures of the antigen.
2. The change does not have an impact on the viral clearance data or the chemical nature of an inactivating agent.
3. No change in the antigen specification outside the approved limits.
4. No change in the impurity profile of the antigen outside the approved limits.
5. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
6. The change does not affect the purification process.
7. The change in scale is linear with respect to the proportionality of production parameters and materials.
8. The change is for compendial raw materials of biological origin (excluding human plasma-derived materials).
9. The new fermentation train is identical to the approved fermentation train(s).
10. No change in the approved in vitro cell age.
11. The change is not expected to have an impact on the quality, safety or efficacy of the final product.
Table continued

**Conditions**

12. No change in the proportionality of the raw materials (that is, the change in scale is linear).
13. The change in scale involves the use of the same bioreactor (that is, it does not involve the use of a larger bioreactor).
14. The need for reprocessing is not due to recurrent deviations from the validated process and the root cause triggering reprocessing is identified.

**Supporting data**

1. Justification for the classification of the change(s) as critical, moderate or noncritical as this relates to the impact on the quality of the antigen.
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 antigen for non-recombinant product.
4. For antigens 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, and use and previous acceptance of the material) (5).
5. Process validation study reports.
6. Comparability of the pre- and post-change antigen 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 vaccine, existing relevant nonclinical and clinical data, and aspects of vaccine 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 (3) consecutive commercial-scale batches of the pre- and post-change antigen. 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, and/or the use of fewer than 3 batches may be acceptable where justified and agreed by the NRA.
Supporting data

8. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for one (1) commercial-scale batch of the pre- and post-change antigen. 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 MA holder if outside the specification (with proposed action). The use of a smaller-scale batch may be acceptable where justified and agreed by the NRA.

9. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale antigen batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months of testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the antigen 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

10. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least one (1) commercial-scale antigen 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months of testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the antigen 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 and agreed by the NRA.

11. Updated post-approval stability protocol and stability commitment to place the first commercial-scale batch of the final product manufactured using the post-change antigen 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) (5).

13. Information demonstrating comparability of the raw materials/reagents of both sources.

14. Data describing the root cause triggering the reprocessing, as well as validation data (for example, extended hold-times and resistance to additional mechanical stress) to help prevent the reprocessing from having an impact on the antigen.
<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>9. Change to the cell banks:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note: New cell substrates that are unrelated to the licensed master cell bank (MCB) or pre-MCB material generally require a new application for MA or licence application.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. generation of a new MCB</td>
<td>1</td>
<td>1, 2, 5, 7−9</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. generation of a new working cell bank (WCB)</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>2−4</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>c. change in cell bank storage site</td>
<td>7</td>
<td>10</td>
<td>Minor</td>
</tr>
<tr>
<td><strong>10. Change to the seed lots:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note: New viral or bacterial seeds that are unrelated to the master seed lot (MSL) or pre-MSL material generally require a new application for MA or licence application.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. generation of a new MSL</td>
<td>1</td>
<td>1, 5−9, 11</td>
<td>Major</td>
</tr>
<tr>
<td>b. generation of a new working seed lot (WSL)</td>
<td>2, 3</td>
<td>5−9, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>2−4</td>
<td>5−6</td>
<td>Minor</td>
</tr>
<tr>
<td>c. generation of a new WSL by extending the passage level of an existing WSL beyond an approved level</td>
<td>None</td>
<td>5−7, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>d. change in seed lot storage site</td>
<td>7</td>
<td>10</td>
<td>Minor</td>
</tr>
<tr>
<td><strong>11. Change in cell bank/seed lot testing/storage site</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5, 7</td>
<td>10</td>
<td>Minor</td>
<td></td>
</tr>
<tr>
<td><strong>12. Change in cell bank/seed lot qualification protocol</strong></td>
<td>None</td>
<td>3, 4</td>
<td>Moderate</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>Minor</td>
<td></td>
</tr>
</tbody>
</table>

**Conditions**

1. The new MCB is generated from a pre-approved MCB or WCB or the new MSL is generated from a pre-approved MSL or WSL.
2. The new cell bank/seed lot is generated from a pre-approved MCB/MSL.
3. The new cell bank/seed lot is at the pre-approved passage level.
4. The new cell bank/seed lot 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/seed lot.
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/seed lot and the transport conditions of the cell bank/seed lot has been validated.
Table continued

Supporting data
1. Qualification of the cell bank or seed lot 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/seed lot qualification protocol.
4. Updated cell bank/seed lot qualification protocol.
5. Comparability of the pre- and post-change antigen 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 vaccine, existing relevant nonclinical and clinical data, and aspects of vaccine use.
6. Quality control test results as quantitative data in tabular format for the new seed lot.
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 (3) consecutive commercial-scale batches of the antigen derived from the new cell bank/seed lot. Matrixing, bracketing, the use of smaller-scale batches, and/or the use of fewer than 3 batches may be acceptable where justified and agreed by the NRA.
8. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale antigen batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the antigen 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.
10. Evidence that the new company/facility is GMP compliant.
11. Revised information on the quality and controls of critical starting materials (for example, specific pathogen-free eggs and chickens) used in the generation of the new WSL, where applicable.
### Description of change

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>13. Change in equipment used in the antigen manufacturing process, such as:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. introduction of new equipment with different operating principles and different product contact material</td>
<td>None</td>
<td>1–6</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. introduction of new equipment with the same operating principles but different product contact material</td>
<td>None</td>
<td>1, 3–6</td>
<td>Moderate</td>
</tr>
<tr>
<td>c. introduction of new equipment with different operating principles but the same product contact material</td>
<td>None</td>
<td>1–3, 5, 6</td>
<td>Moderate</td>
</tr>
<tr>
<td>d. replacement of equipment with equivalent equipment (including filter)</td>
<td>None</td>
<td>1, 5–7</td>
<td>Minor</td>
</tr>
</tbody>
</table>

### Conditions

None

### 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 (1) commercial-scale batch of the antigen 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 MA holder if outside specification (with proposed action).
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 demonstrating requalification of the equipment or requalification of the change.
7. Rationale for regarding the equipment as similar/comparable, as applicable.
### Description of change

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14. Change in specification for the materials, involving:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. raw materials/intermediates: widening of the approved specification limits for starting materials/intermediates, which may have a significant effect on the overall quality of the antigen and/or final product and are not changes to the cell banks or seed lots</td>
<td>None</td>
<td>1, 3–6, 8, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. raw materials/intermediates: narrowing of the approved specification limits for starting materials/intermediates</td>
<td>1–4</td>
<td>1, 3–7</td>
<td>Minor</td>
</tr>
<tr>
<td><strong>15. Change to in-process tests and/or acceptance criteria applied during manufacture of the antigen, involving:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. narrowing of in-process limits</td>
<td>3, 5, 8, 9</td>
<td>2, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>b. addition of new in-process test and limits</td>
<td>4, 5, 10, 11</td>
<td>2–6, 8, 10</td>
<td>Minor</td>
</tr>
<tr>
<td>c. deletion of a non-significant in-process test</td>
<td>4–6</td>
<td>2, 6, 9</td>
<td>Minor</td>
</tr>
<tr>
<td>d. widening of the approved in-process limits</td>
<td>None</td>
<td>2–6, 8, 10, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>e. deletion of an in-process test which may have a significant effect on the overall quality of the antigen</td>
<td>None</td>
<td>2, 6, 8, 10</td>
<td>Moderate</td>
</tr>
<tr>
<td>f. addition or replacement of an in-process test as a result of a safety or quality issue</td>
<td>None</td>
<td>2–6, 8, 10</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>16. Change in in-process controls testing site</strong></td>
<td>3–5, 7, 8</td>
<td>12</td>
<td>Minor</td>
</tr>
</tbody>
</table>

### 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. No change in the antigen specification outside the approved limits.
4. No change in the impurity profile of the antigen outside the approved limits.
### Conditions

5. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.

6. The test does not concern a critical attribute (for example, content, impurity, any critical physical characteristics or microbial purity).

7. The replaced analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity, if applicable.

8. No change in the in-process controls outside the approved limits.

9. The test procedure remains the same, or changes in the test procedure are minor.

10. Any new test method does not concern a novel non-standard technique or a standard technique used in a novel way.

11. The new test method is not a biological/immunological/immunochemical or physicochemical method or a method using a biological reagent (does not include standard pharmacopoeial microbiological methods).

### 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 antigen.

2. Revised information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed antigen.

3. Updated antigen specification, if changed.

4. Copies or summaries of analytical procedures, if new analytical procedures are used.

5. Validation study reports, if new analytical procedures are used.

6. Comparative table or description, where applicable, of pre- and post-change in-process tests/limits.

7. Description of the batches and summary of in-process and release testing results as quantitative data, in a comparative tabular format, for one (1) commercial-scale batch of the pre- and post-change antigen. 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 MA holder if outside specification (with proposed action). The use of a smaller-scale batch may be acceptable where justified and agreed by the NRA.

8. 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 (3) consecutive commercial-scale batches of the pre- and post-change antigen. 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 and/or the use of fewer than 3 batches may be acceptable where justified and agreed by the NRA.

9. Justification/risk assessment showing that the attribute is non-significant.
Table continued

Supporting data


11. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

12. Evidence that the new company/facility is GMP compliant.

Control of the antigen

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>17. Change affecting the quality control (QC) (release and stability) testing of the antigen, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. transfer of the QC testing activities for a non-pharmacopoeial assay to a new company not approved in the current MA or licence</td>
<td>1−3 1, 2</td>
<td></td>
<td>Minor</td>
</tr>
<tr>
<td>b. transfer of the QC testing activities for a pharmacopoeial assay to a new company not approved in the current MA or licence</td>
<td>1 1, 2</td>
<td></td>
<td>Minor</td>
</tr>
</tbody>
</table>

Conditions

1. The transferred QC test is not a potency assay (for example, the test may be a bioassay such as an endotoxin assay or sterility assay).

2. No changes to the test method.

3. Transfer within a site approved in the current MA for the performance of other tests.

Supporting data

1. Information demonstrating technology transfer qualification.

2. Evidence that the new company/facility is GMP compliant.
<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>18. Change in the specification used to release the antigen, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. deletion of a test</td>
<td>None</td>
<td>1, 5, 8</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. addition of a test</td>
<td>1–3</td>
<td>1–3, 5</td>
<td>Minor</td>
</tr>
<tr>
<td>c. replacement of an analytical procedure</td>
<td>None</td>
<td>1–5</td>
<td>Moderate</td>
</tr>
<tr>
<td>d. change in animal species/strains for a test (for example, new species/strains, animals of different age, new supplier where genotype of the animal cannot be confirmed)</td>
<td>None</td>
<td>6, 7</td>
<td>Moderate</td>
</tr>
<tr>
<td>e. minor changes to an approved analytical procedure</td>
<td>4–7</td>
<td>1, 4, 5</td>
<td>Minor</td>
</tr>
<tr>
<td>f. change from an in-house analytical procedure to a recognized compendial/pharmacopoeial analytical procedure</td>
<td>4, 7</td>
<td>1–3</td>
<td>Minor</td>
</tr>
<tr>
<td>g. widening of an acceptance criterion</td>
<td>None</td>
<td>1, 5, 8</td>
<td>Moderate</td>
</tr>
<tr>
<td>h. narrowing of an acceptance criterion</td>
<td>1, 8, 9</td>
<td>1</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**Conditions**

1. The change does not result from unexpected events arising during manufacture (for example, new unqualified impurity or change in total impurity limits).
2. No change in the limits/acceptance criteria outside the approved limits for the approved assays.
3. The addition of the test is not intended to monitor new impurity species.
4. No change in the acceptance criteria outside the approved limits.
5. The method of analysis is the same and is based on the same analytical technique or principle (for example, a change in column length or temperature, but not a different type of column or method) and no new impurities are detected.
6. The modified analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity.
7. The change does not concern potency testing.
8. Acceptance criteria for residuals are within recognized or approved acceptance limits (for example, within ICH limits for a Class 3 residual solvent, or pharmacopoeial requirements).
9. The analytical procedure remains the same, or changes to the analytical procedure are minor.
Supporting data
1. Updated antigen specification.
2. Copies or summaries of analytical procedures, if new analytical procedures are used.
3. Validation reports, if new analytical procedures are used.
4. Comparative results demonstrating that the approved and proposed analytical procedures are equivalent.
5. Justification for deletion of the test or for the proposed antigen specification (for example, tests, acceptance criteria or analytical procedures).
6. Data demonstrating that the change in animals/strains give results comparable to those obtained using the approved animals/strains.
7. Copies of relevant certificate of fitness for use (for example, veterinary certificate).
8. Declaration/evidence that consistency of quality and of the production process is maintained.

Reference standards or materials

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>19. Qualification of a new reference standard against a new primary international standard</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>20. Change in the reference standard from in-house (no relationship with international standard) to pharmacopoeial or international standard</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>21. Qualification of a new lot of reference standard against the approved reference standard (including qualification of a new lot of a secondary reference standard against the approved primary standard)</td>
<td>1</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>22. Change to reference standard qualification protocol</td>
<td>None</td>
<td>3, 4</td>
<td>Moderate</td>
</tr>
<tr>
<td>23. Extension of reference standard shelf-life</td>
<td>2</td>
<td>5</td>
<td>Minor</td>
</tr>
</tbody>
</table>
### Table continued

**Conditions**
1. Qualification of the new reference standard is according to an approved protocol.
2. The extension of the shelf-life is according to an approved protocol.

**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 and 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.

### Container closure system

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>24. Change in the primary container closure system(s) for the storage and shipment of the antigen</td>
<td>None</td>
<td>1, 2, 4, 5</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1, 3, 5</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**Conditions**
1. The proposed container closure system is at least equivalent to the approved container closure system with respect to its relevant properties.

**Supporting data**
1. Information on the proposed container closure system (for example, description, composition, materials of construction of primary packaging components and specification).
2. Data demonstrating the suitability of the container closure system (for example, extractable/leachable testing).
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 interaction studies, and extractable/leachable studies).
Table continued

Supporting data

4. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale antigen batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the antigen 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

5. Comparative table of pre- and post-change specifications.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>25. Change in the specification of the primary container closure system for the antigen, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. deletion of a test</td>
<td>1, 2</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>b. addition of a test</td>
<td>3</td>
<td>1−3</td>
<td>Minor</td>
</tr>
<tr>
<td>c. replacement of an analytical procedure</td>
<td>6, 7</td>
<td>1−3</td>
<td>Minor</td>
</tr>
<tr>
<td>d. minor changes to an analytical procedure</td>
<td>4−7</td>
<td>1−3</td>
<td>Minor</td>
</tr>
<tr>
<td>e. widening of an acceptance criterion</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>f. narrowing of an acceptance criterion</td>
<td>8</td>
<td>1</td>
<td>Minor</td>
</tr>
</tbody>
</table>

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 nor result in a potential impact on the performance of the antigen.
3. The change is not necessitated by recurring events arising during manufacture or because of stability concerns.
Table continued

Conditions
4. There is no change in 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 tightens precision, accuracy, specificity and sensitivity.
8. The change is within the range of approved acceptance criteria or has been made to reflect a new pharmacopoeial monograph specification for the container closure component.

Supporting data
1. Updated copy of the proposed specification for the primary container closure system.
2. Rationale for the change in specification for a primary container closure system.
3. Description of the analytical procedure and, if applicable, validation data.

Stability

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>26. Change in the shelf-life/hold-time for the antigen or for a stored intermediate of the antigen, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. extension</td>
<td>None</td>
<td>1−5</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1−5</td>
<td>1, 2, 5</td>
</tr>
<tr>
<td>b. reduction</td>
<td>None</td>
<td>1−5</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>2−4</td>
</tr>
</tbody>
</table>

Conditions
1. No changes to the container closure system in direct contact with the antigen with the potential of impact on the antigen, or to the recommended storage conditions of the antigen.
2. The approved shelf-life is at least 24 months.
3. Full long-term stability data are available covering the proposed shelf-life and are based on stability data generated on at least three (3) commercial-scale batches.
4. Stability data were generated in accordance with the approved stability protocol.
5. Significant changes were not observed in the stability data.
6. 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.
Table continued

Supporting data
1. Summary of stability testing and results (for example, studies conducted, protocols used and results obtained).
2. Proposed storage conditions and shelf-life, as appropriate.
4. Justification of 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 at least three (3) commercial-scale batches). For intermediates, data to show that the extension of shelf-life has no negative impact on the quality of the antigen. Under special circumstances and with prior agreement of the NRA, interim stability testing results and a commitment to notify the NRA of any failures in the ongoing long-term stability studies may be provided.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>27. Change in the post-approval stability protocol of the antigen, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. significant 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</td>
<td>None</td>
<td>1–6</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 2, 4–6</td>
<td>Minor</td>
</tr>
<tr>
<td>b. addition of time point(s) into the post-approval stability protocol</td>
<td>None</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>c. addition of test(s) into the post-approval stability protocol</td>
<td>2</td>
<td>1, 2, 4, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>d. deletion of time point(s) from the post-approval stability protocol beyond the approved shelf-life</td>
<td>None</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>e. deletion of time point(s) from the post-approval stability protocol within the approved shelf-life</td>
<td>3</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
</tbody>
</table>

Conditions
1. For the replacement of an analytical procedure, the new analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity.
### Table continued

#### Conditions

2. The addition of test(s) is not due to stability concerns or to the identification of new impurities.

3. The approved antigen shelf-life is at least 24 months.

#### Supporting data

1. Copies or summaries of analytical procedures, if new analytical procedures are used.

2. Validation study reports, if new analytical procedures are used.

3. Proposed storage conditions and/or shelf-life, as appropriate.


5. If applicable, stability testing results to support the change to the post-approval stability protocol or stability commitment (for example, data showing greater reliability of the alternative test).

6. Justification for the change to the post-approval stability protocol.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>28. Change in the storage conditions for the antigen, involving:</td>
<td>None</td>
<td>1–4</td>
<td>Moderate</td>
</tr>
<tr>
<td>a. addition or change of storage condition for the antigen (for example, widening or narrowing of a temperature criterion)</td>
<td>1, 2</td>
<td>1–3</td>
<td>Minor</td>
</tr>
</tbody>
</table>

#### 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 labelled 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 at least three (3) commercial-scale batches).
References


Appendix 3

Changes to the final product

The examples presented in this appendix are intended to assist with the classification of changes made to the quality information of the final product. The information summarized in the final product table below provides recommendations 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 the next higher level of change – 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 MA holder (if any of the supporting data outlined for a given change are not provided, are different or are not considered applicable then adequate scientific justification should be provided);

- the *reporting category* (that is, major, moderate or minor quality change).

It is important to note that the NRA reserves the right to request additional information or material, as deemed appropriate, or to define conditions not specifically described in this document in order to allow for adequate assessment of the quality, safety and efficacy of a vaccine. In addition, MA holders should contact the NRA if a change not included in the final product table below has the potential to impact upon vaccine quality.

Supporting data should be provided according to the submission format accepted by the NRA. For example, for NRAs that accept the ICH common technical document (CTD) and/or ICH eCTD formatted submissions, the supporting data should be provided in the appropriate sections of the CTD modules and not in separate documents. For the placement of data in the appropriate section of the CTD please see the ICH guidelines (1, 2).

For additional information on data requirements to support quality changes, WHO guidelines on GMP requirements and stability evaluation of vaccines (3, 4) should be consulted, together with relevant ICH guidelines.
Quality changes to comply with updated compendia and/or pharmacopoeia

NRAs should make a list of the recognized compendia and/or pharmacopoeia available to MA holders. Manufacturers are expected to comply with the current versions of compendia and/or pharmacopoeia as referenced in the approved MA. Changes in the compendial and/or pharmacopoeial methods or specifications referenced by a particular NRA do not need to be submitted for review, but information on such changes should be available for inspection.

In some cases, changes to comply with recognized compendia and/or pharmacopoeia may require approval by the NRA prior to implementation regardless of the timing of the change with respect to the date the 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, tests for potency), for changes which have an impact on any items of the product labelling information, and for changes which may potentially affect the quality, safety or efficacy of the product.

Quality changes affecting lot release

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 MA holder should inform the institution responsible for reviewing the release of vaccine 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 done in accordance with an approved protocol and specification. Nevertheless, these changes must be reported to the NRA or NCL as appropriate.

Description and composition of the final product

Note: Changes in dosage form and/or presentation may, in some cases, necessitate the filing of a new application for MA or licensure. MA holders are encouraged to contact the NRA for further guidance.
### Description of change

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>29. Change in the description or composition of the final product, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. addition of a dosage form or change in the formulation (for example, lyophilized powder to liquid, change in the amount of excipient or new diluent for lyophilized product)</td>
<td>None</td>
<td>1−10</td>
<td>Major</td>
</tr>
</tbody>
</table>

*Note: Change in formulation does not include changes in antigen(s) or adjuvants. A change in antigen(s) or adjuvant(s) requires the filing of a new application for MA or licensure. MA holders are encouraged to contact the NRA for further guidance.*

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. change in fill volume (that is, same concentration, different volume)</td>
<td>None</td>
<td>1, 5, 7, 10</td>
<td>Major</td>
</tr>
<tr>
<td></td>
<td>1, 2</td>
<td>1, 5, 7</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>1–3</td>
<td>5, 7</td>
<td>Minor</td>
</tr>
</tbody>
</table>

### Conditions

1. No changes classified as major in the manufacturing process to accommodate the new fill volume.
2. No change in the dose recommended.
3. Narrowing of fill volume while maintaining the lower limit of extractable volume.

### Supporting data

1. Revised final product labelling information (as applicable).
2. Characterization data demonstrating that the conformation and immunogenicity of the antigen is comparable in 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 final product, as appropriate (for example, choice of excipients, compatibility of antigen and excipients, leachates or compatibility with new container closure system, as appropriate).
Table continued

Supporting data

5. Information on the batch formula, manufacturing process and process controls, control of critical steps and intermediates, and process validation study reports.

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 (3) 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- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

10. Supporting clinical data or a justification for why such studies are not needed.

Description and composition of the final product: change to an adjuvant

Note:

- Change in type/structure of a chemical adjuvant, in the type of a biological adjuvant or in a component of a biological adjuvant may necessitate the filing of a new application for MA or licensure. MA holders are encouraged to contact the NRA for further guidance.

- For additional guidance on the required supporting data for quality changes for chemical and biological adjuvants, see recommendations for other changes to the final product, such as changes to facilities, equipment, manufacturing process, quality control, shelf-life, and so on, as applicable.
<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>30. Change involving an approved chemical/synthetic adjuvant:</strong></td>
<td>None</td>
<td>4, 5, 10, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>a. change in supplier of a chemical/synthetic adjuvant</td>
<td></td>
<td>1−3</td>
<td>Minor</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>3−5, 10, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. change in manufacture of a chemical/synthetic adjuvant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>7−11</td>
<td>Moderate</td>
</tr>
<tr>
<td>c. change in specification of a chemical/synthetic adjuvant (including tests and/or the analytical procedures)</td>
<td></td>
<td>7−9</td>
<td>Minor</td>
</tr>
</tbody>
</table>

| **31. Change involving a biological adjuvant:** | None | 1−7, 10−13 | Major |
| a. change in supplier of a biological adjuvant | | | |
| | None | 1−7, 10−12 | Major |
| b. change in manufacture of a biological adjuvant | | | |
| | None | 6−10 | Moderate |
| c. change in specification of a biological adjuvant (including tests and/or the analytical procedures) | | 7−8 | Minor |

**Conditions**

1. The specification of the adjuvant is equal to or narrower than the approved limits (that is, narrowing of acceptance criterion).
2. The adjuvant is an aluminium salt.
3. The change in specification consists of the addition of a new test or of a minor change to an analytical procedure.
4. There is no change in the manufacturer and/or supplier of the adjuvant.

**Supporting data**

1. Information assessing the risk with respect to potential contamination with adventitious agents (for example, impact on the viral clearance studies, BSE/TSE risk) (5).
2. Information on the quality and controls of the materials (for example, raw materials, starting materials) used in the manufacture of the proposed adjuvant.
3. Flow diagram of the proposed manufacturing process(es), a brief narrative description of the proposed manufacturing process(es), and information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed adjuvant.
Table continued

Supporting data

4. Process validation study reports (for example, for manufacture of the adjuvant) unless otherwise justified.

5. Description of the general properties, including stability, characteristic features and characterization data of the adjuvant, as appropriate.

6. Comparability of the pre- and post-change adjuvant 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 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 adjuvant, existing relevant nonclinical and clinical data, and aspects of vaccine use.

7. Updated copy of the proposed specification for the adjuvant (and updated analytical procedures if applicable).

8. Copies or summaries of analytical procedures, if new analytical procedures are used.

9. Validation study reports, if new analytical procedures are used.

10. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for at least three (3) consecutive commercial-scale batches of the final product with the pre-change (approved) and post-change (proposed) adjuvant, as applicable. Comparative test results for the approved adjuvant do not need to be generated concurrently; relevant historical testing results are acceptable.

11. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

12. Supporting nonclinical and clinical data, if applicable.

13. Evidence that the facility is GMP compliant.
Description and composition of the final product: change to a diluent

*Note: Changes to diluents containing adjuvants and/or antigens are considered final products and as such the corresponding changes to final product (not diluent) should be applied.*

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>32. Change to the diluent, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. change in manufacturing process</td>
<td>None</td>
<td>1−5</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>1, 3</td>
<td>1−4</td>
<td>Minor</td>
</tr>
<tr>
<td>b. replacement of or addition to the source of a diluent</td>
<td>None</td>
<td>1−5</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>1−3</td>
<td>1−3</td>
<td>Minor</td>
</tr>
<tr>
<td>c. change in facility used to manufacture a diluent (same company)</td>
<td>1, 2</td>
<td>1, 3, 5</td>
<td>Minor</td>
</tr>
<tr>
<td>d. addition of a diluent filling line</td>
<td>1, 2, 4</td>
<td>1, 3, 5</td>
<td>Minor</td>
</tr>
<tr>
<td>e. addition of a diluent into an approved filling line</td>
<td>1, 2</td>
<td>1, 3, 5</td>
<td>Minor</td>
</tr>
<tr>
<td>f. deletion of a diluent</td>
<td>None</td>
<td>None</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**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 final product specification outside the approved limits.
3. The proposed diluent is commercially available in the NRA country/jurisdiction.
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 (3) 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.
Supporting data
4. Updated stability data on the product reconstituted with the new diluent.
5. Evidence that the facility is GMP compliant.

Manufacture

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>33. Change involving a final product manufacturer/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>manufacturing facility, such as:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. replacement or addition of a manufacturing facility for</td>
<td>None</td>
<td>1−7</td>
<td>Major</td>
</tr>
<tr>
<td>the final product (including formulation/filling and</td>
<td>1−5</td>
<td>1−3, 5−8</td>
<td>Moderate</td>
</tr>
<tr>
<td>primary packaging)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. replacement or addition of a secondary packaging facility,</td>
<td>2, 3</td>
<td>1−3</td>
<td>Minor</td>
</tr>
<tr>
<td>a labelling/storage facility or a distribution facility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. deletion of a final product</td>
<td>None</td>
<td>None</td>
<td>Minor</td>
</tr>
<tr>
<td>manufacturing facility</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conditions
1. The proposed facility is an approved formulation/filling facility (for the same company/MA holder).
2. There is no change in the composition, manufacturing process and final product specification.
3. There is no change in the container/closure system and storage conditions.
4. The same validated manufacturing process is used.
5. The newly introduced product is in the same family of product(s) or therapeutic classification as the products already approved at the site, and also uses the same filling process/equipment.

Supporting data
1. Name, address and responsibility of the proposed production facility involved in manufacturing and testing.
2. Evidence that the facility is GMP compliant.
3. Confirmation that the manufacturing process description of the final product has not changed as a result of the submission (other than the change in facility), or revised description of the manufacturing process.
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. Process validation study reports. The data should include transport between sites, if relevant.

6. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for at least three (3) consecutive commercial-scale batches of the pre- and post-change final 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.

7. Comparative pre- and post-change test results for the manufacturer's characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

8. Rationale for considering the proposed formulation/filling facility as equivalent.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>34. Change in the final product manufacturing process, such as:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. scale-up of the manufacturing process at the formulation/filling stage</td>
<td>1−4</td>
<td>1–6</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. addition or replacement of equipment (for example, formulation tank, filter housing, filling line and head, and lyophilizer); see change 13 above.</td>
<td>None</td>
<td>1–8</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>2, 7–9</td>
</tr>
<tr>
<td>c. addition of a new scale bracketed by the approved scales or scale-down of the manufacturing process</td>
<td>1–4</td>
<td>1, 4</td>
<td>Minor</td>
</tr>
</tbody>
</table>
Table continued

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>d. addition of a new step (for example, filtration)</td>
<td>3</td>
<td>1–6</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

**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 SOPs are utilized).
3. The change should not be a result of recurring events having arisen during manufacture or because of stability concerns.
4. No change in the principle of the sterilization procedures of the final 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).

**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 final product.
2. Information on the in-process control testing, as applicable.
3. Process validation study reports (for example, media fills), as appropriate.
4. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for at least three (3) consecutive commercial-scale batches of the pre- and post-change final 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.
5. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.
Table continued

Supporting data

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. Information demonstrating requalification of the equipment or requalification of the change.
9. Rationale for regarding the equipment as similar/comparable, as applicable.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>35. Change in the controls (in-process tests and/or acceptance criteria) applied during the manufacturing process or on intermediates, such as:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. narrowing of in-process limits</td>
<td>2, 3, 7</td>
<td>1, 5</td>
<td>Minor</td>
</tr>
<tr>
<td>b. addition of new in-process test and limits</td>
<td>2, 3, 8, 9</td>
<td>1−6, 8</td>
<td>Minor</td>
</tr>
<tr>
<td>c. deletion of a non-significant in-process test</td>
<td>2−4</td>
<td>1, 5, 7</td>
<td>Minor</td>
</tr>
<tr>
<td>d. widening of the approved in-process limits</td>
<td>None</td>
<td>1−6, 8, 9</td>
<td>Major</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1−3</td>
<td>Moderate</td>
</tr>
<tr>
<td>e. deletion of an in-process test which may have a significant effect on the overall quality of the final product</td>
<td>None</td>
<td>1, 5, 6, 8</td>
<td>Major</td>
</tr>
<tr>
<td>f. addition or replacement of an in-process test as a result of a safety or quality issue</td>
<td>None</td>
<td>1−6, 8</td>
<td>Moderate</td>
</tr>
<tr>
<td>36. Change in in-process controls testing site</td>
<td>1−3, 5, 6</td>
<td>10</td>
<td>Minor</td>
</tr>
</tbody>
</table>

Conditions

1. No change in final product specification outside the approved limits.
2. No change in the impurity profile of the final 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).
Table continued

**Conditions**

5. The replaced analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity, if applicable.
6. 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.
8. Any new test method does not concern a novel non-standard technique or a standard technique used in a novel way.
9. The new test method is not a biological/immunological/immunochemical or physicochemical method or a method using a biological reagent (does not include standard pharmacopoeial microbiological methods).

**Supporting data**

1. Revised information on the controls performed at critical steps of the manufacturing process and on intermediates of the proposed antigen.
2. Updated final product specification if changed.
3. Copies or summaries of analytical procedures, if new analytical procedures are used.
4. Validation study reports, if new analytical procedures are used.
5. Comparative table or description, where applicable, of current and proposed in-process tests.
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 (3) consecutive commercial-scale batches of the pre- and post-change final 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.
9. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.
10. Evidence that the new company/facility is GMP compliant.
### Description of change

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>37. Change in the specification used to release the excipient, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> This change excludes adjuvants. See adjuvant-specific changes above for details (changes 30 and 31).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. deletion of a test</td>
<td>5, 8</td>
<td>1, 3</td>
<td>Minor</td>
</tr>
<tr>
<td>b. addition of a test</td>
<td>4</td>
<td>1–3</td>
<td>Minor</td>
</tr>
<tr>
<td>c. replacement of an analytical procedure</td>
<td>1–3</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>d. minor changes to an approved analytical procedure</td>
<td>None</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>e. change from an in-house analytical procedure to a recognized compendial analytical procedure</td>
<td>None</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>f. widening of an acceptance criterion</td>
<td>None</td>
<td>1, 3</td>
<td>Moderate</td>
</tr>
<tr>
<td>g. narrowing of an acceptance criterion</td>
<td>3, 4, 6, 7</td>
<td>1</td>
<td>Minor</td>
</tr>
</tbody>
</table>

### 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 tightens 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.
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 or 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.
Table continued

**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 final product).

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>38. 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</td>
<td>None</td>
<td>2−7</td>
<td>Major</td>
</tr>
<tr>
<td>39. Change in the source of an excipient from a TSE risk (for example, animal) source to a vegetable or synthetic source</td>
<td>None</td>
<td>1, 3, 5, 6</td>
<td>Moderate</td>
</tr>
<tr>
<td>40. Replacement in the source of an excipient from a TSE risk source to a different TSE risk source</td>
<td>5, 6</td>
<td>2−7</td>
<td>Minor</td>
</tr>
<tr>
<td>41. Change in manufacture of a biological excipient</td>
<td>None</td>
<td>2−7</td>
<td>Major</td>
</tr>
<tr>
<td><strong>Note:</strong> This change excludes biological adjuvants; see adjuvant-specific changes above for details (changes 30 and 31).</td>
<td>2</td>
<td>2−7</td>
<td>Moderate</td>
</tr>
<tr>
<td>41. Change in manufacture of a biological excipient</td>
<td></td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>42. Change in supplier for a plasma-derived excipient (for example, human serum albumin)</td>
<td>None</td>
<td>3−8</td>
<td>Major</td>
</tr>
<tr>
<td>42. Change in supplier for a plasma-derived excipient (for example, human serum albumin)</td>
<td>3, 4</td>
<td>5, 6, 9</td>
<td>Moderate</td>
</tr>
<tr>
<td>43. Change in supplier for an excipient of non-biological origin or of biological origin (excluding plasma-derived excipient)</td>
<td>None</td>
<td>2, 3, 5−7</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Note:</strong> This change excludes adjuvants; see adjuvant-specific changes above for details (changes 30 and 31).</td>
<td>1, 5, 6</td>
<td>3</td>
<td>Minor</td>
</tr>
</tbody>
</table>
Table continued

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>44. Change in excipient testing site</td>
<td>1</td>
<td>10</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**Conditions**

1. No change in the specification of the excipient or final 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 (5).
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 (5).
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 (3) commercial-scale batches of the proposed excipient.
6. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.
Table continued

Supporting data

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 (5)) 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. 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 final product

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>45. Change affecting the QC testing of the final product (release and stability), involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note: Transfer of testing to a different facility within a GMP-approved site is not considered to be a reportable change but is treated as a minor GMP change and reviewed during inspections.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. transfer of the QC testing activities for a non-pharmacopoeial assay (in-house) to a new company or to a different site within the same company</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. transfer of the QC testing activities for a pharmacopoeial assay to a new company</td>
<td>1</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
</tbody>
</table>

Conditions

1. The transferred QC test is not a potency assay or a bioassay.

Supporting data

1. Information demonstrating technology transfer qualification.

2. Evidence that the new company/facility is GMP compliant.
<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>46. Change in the specification used to release the final product, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. for products or components subject to terminal sterilization by heat (for example, diluent for reconstitution of lyophilized vaccines), replacing the sterility test with process parametric release</td>
<td>None</td>
<td>1, 2, 6, 8, 10</td>
<td>Major</td>
</tr>
<tr>
<td>b. deletion of a test</td>
<td>None</td>
<td>2, 9, 10</td>
<td>Moderate</td>
</tr>
<tr>
<td>c. addition of a test</td>
<td>1, 2, 9</td>
<td>2–4, 8</td>
<td>Minor</td>
</tr>
<tr>
<td>d. change in animal species/strains for a test (for example, new species/strains, animals of different ages, and/or new supplier where genotype of the animal cannot be confirmed)</td>
<td>None</td>
<td>5, 11</td>
<td>Moderate</td>
</tr>
<tr>
<td>e. replacement of an analytical procedure</td>
<td>None</td>
<td>2–4, 7, 8</td>
<td>Moderate</td>
</tr>
<tr>
<td>f. minor changes to an approved analytical procedure</td>
<td>3–6</td>
<td>3, 8</td>
<td>Minor</td>
</tr>
<tr>
<td>g. change from an in-house analytical procedure to a recognized compendial analytical procedure</td>
<td>3, 6</td>
<td>2–4</td>
<td>Minor</td>
</tr>
<tr>
<td>h. widening of an acceptance criterion</td>
<td>None</td>
<td>2, 8, 10</td>
<td>Moderate</td>
</tr>
<tr>
<td>i. narrowing of an acceptance criterion</td>
<td>7–10</td>
<td>2</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**Conditions**

1. No change in the limits/acceptance criteria outside the approved limits for the approved assays.
2. The additional test is not intended to monitor new impurity species.
3. No change in the acceptance criteria outside the approved limits.
4. 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.
Table continued

Conditions
5. The modified analytical procedure maintains or tightens precision, accuracy, specificity and sensitivity.
6. The change does not concern potency testing.
7. The change is within the range of approved acceptance criteria.
8. 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).
9. The change does not result from unexpected events arising during manufacture (for example, new unqualified impurity, or impurity content outside of the approved limits).
10. The analytical procedure remains the same, or changes to the analytical procedure are minor.

Supporting data
1. Process validation study reports on the proposed final product.
2. Updated copy of the proposed final product specification.
3. Copies or summaries of analytical procedures, if new analytical procedures are used.
4. Validation study reports, if new analytical procedures are used.
5. Data demonstrating that the change in animals gives results comparable to those obtained using the approved animals.
6. Description of the batches and summary of results as quantitative data for a sufficient number of batches to support the process parametric release.
7. Description of the batches and summary of results as quantitative data, in a comparative tabular format, for at least three (3) commercial-scale batches of the final product.
8. Justification for the change to the analytical procedure (for example, demonstration of the suitability of the analytical procedure in monitoring the final product, including the degradation products) or for the change to the specification (for example, demonstration of the suitability of the revised acceptance criterion in controlling the final product).
9. Justification for the deletion of the test (for example, demonstration of the suitability of the revised specification in controlling the final product).
10. Declaration/evidence that consistency of quality and of the production process is maintained.
11. Copies of relevant certificates of fitness for use (for example, veterinary certificate).
# Reference standards or materials

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>47. Qualification of a reference standard against a new primary international standard</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>48. Change of the reference standard from in-house (no relationship with international standard) to pharmacopoeial or international standard</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>49. Qualification of a new lot of reference standard against the approved reference standard (including qualification of a new lot of a secondary reference standard against the approved primary standard)</td>
<td>1</td>
<td>2</td>
<td>Minor</td>
</tr>
<tr>
<td>50. Change to the reference standard qualification protocol</td>
<td>None</td>
<td>3, 4</td>
<td>Moderate</td>
</tr>
<tr>
<td>51. Extension of the shelf-life of the reference standard</td>
<td>2</td>
<td>5</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**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.

**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 and 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.
### Container closure system

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>52. Modification of a primary container closure system (for example, new coating, adhesive, stopper or type of glass)</td>
<td>None</td>
<td>1–7</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>1–3</td>
<td>3</td>
<td>Minor</td>
</tr>
</tbody>
</table>

*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 29.c above.*

| 53. 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 |
|                                                                                       |     |        |         |

| 54. Deletion of a container closure system                                           | None | 1      | Minor   |
|                                                                                      |     |        |         |

*Note: The NRA should be notified of the deletion of a container closure system, and product labelling information should be updated, as appropriate.*

### Conditions

1. No change in the type of container closure or materials of construction.
2. 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).

### Supporting data

1. Revised product labelling information, as appropriate.
2. For sterile products, process validation study reports, or providing equivalency rationale. For a secondary functional container closure system, validation testing report.
3. Information on the proposed container closure system, as appropriate (for example, description, materials of construction of primary/secondary packaging components, performance specification).
Table continued

**Supporting data**

4. Results demonstrating protection against leakage, no leaching of undesirable substance and compatibility with the product, and results from the toxicity and biological reactivity tests.

5. Summary of results as quantitative data, in a comparative tabular format, for at least three (3) consecutive commercial-scale batches of the pre- and post-change final 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- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.

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 transportation and/or interaction studies demonstrating the preservation of protein integrity and maintenance of sterility for sterile products; results of maintenance of sterility in multidose containers and results of user testing).

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>55. Change in the supplier for a primary container closure component, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. replacement or addition of a supplier</td>
<td>1, 2</td>
<td>4, 5</td>
<td>Minor</td>
</tr>
<tr>
<td><strong>Note:</strong> A change in container closure system involving new materials of construction, shape or dimensions would require supporting data such as is shown for change 52 above.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. deletion of a supplier</td>
<td>None</td>
<td>None</td>
<td>Minor</td>
</tr>
</tbody>
</table>
### Table continued

#### Conditions
1. 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. No change in the specification of the container closure component outside the approved limits.

#### Supporting data
1. Information on the supplier and make of the proposed container closure system (for example, certificate of analysis, description, materials of construction of primary packaging components, specification).
2. Data demonstrating the suitability of the container closure system (for example, extractable/leachable testing).
3. Comparative pre- and post-change test results for the manufacturer’s characterized key stability-indicating attributes for at least three (3) commercial-scale final product batches 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 lots on the stability programme are acceptable. The data should cover a minimum of 3 months testing unless otherwise justified. Additionally, the manufacturer should commit to undertake real-time stability studies to support the full shelf-life/hold-time of the final 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, the use of fewer than 3 batches and/or use of forced degradation or accelerated temperature conditions for stability testing may be acceptable where justified and agreed by the NRA.
5. Letter from the MA holder certifying that there are no changes to the container closure system.
6. Certificate of analysis for the container provided by the new supplier and comparison with the certificate of analysis for the approved container.

#### Description of change

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>56. Change in the specification used to release a primary container closure component or functional secondary container closure component, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. deletion of a test</td>
<td>1, 2</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>b. addition of a test</td>
<td>3</td>
<td>1, 2</td>
<td>Minor</td>
</tr>
<tr>
<td>c. replacement of an analytical procedure</td>
<td>6, 7</td>
<td>1–3</td>
<td>Minor</td>
</tr>
<tr>
<td>d. minor changes to an analytical procedure</td>
<td>4–7</td>
<td>1–3</td>
<td>Minor</td>
</tr>
<tr>
<td>Description of change</td>
<td>Conditions to be fulfilled</td>
<td>Supporting data</td>
<td>Reporting category</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>---------------------------</td>
<td>-----------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>e. widening of an acceptance criterion</td>
<td>None</td>
<td>1, 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>f. narrowing of an acceptance criterion</td>
<td>8</td>
<td>1</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**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 nor result in a potential impact on the performance of the final product.
3. The change is not necessitated by recurring events arising during manufacture 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.
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 and sensitivity.
8. The change is within the range of approved acceptance criteria or has been made to reflect new pharmacopoeial monograph specifications for the container closure component.

**Supporting data**

1. 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**

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>57. Change in the shelf-life of the final product, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. extension (includes extension of shelf-life of the final product as packaged for sale, and hold-time after opening and after dilution or reconstitution)</td>
<td>None</td>
<td>1−5</td>
<td>Moderate</td>
</tr>
<tr>
<td>Description of change</td>
<td>Conditions to be fulfilled</td>
<td>Supporting data</td>
<td>Reporting category</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------------------</td>
<td>----------------------------</td>
<td>-----------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>b. reduction (includes reduction as packaged for sale, after opening, and after dilution or reconstitution)</td>
<td>None</td>
<td>1–5</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

### Conditions

None

### Supporting data

1. Updated product labelling information, as appropriate.
2. Proposed storage conditions and shelf-life, as appropriate.
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 (3) commercial-scale batches.

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>58. Change in the post-approval stability protocol of the final product, involving:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. major 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</td>
<td>None</td>
<td>1–6</td>
<td>Moderate</td>
</tr>
<tr>
<td>b. addition of time point(s) into the post-approval stability protocol</td>
<td>None</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>c. addition of test(s) into the post-approval stability protocol</td>
<td>1</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>d. deletion of time point(s) from the post-approval stability protocol beyond the approved shelf-life</td>
<td>None</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
<tr>
<td>e. deletion of time point(s) from the post-approval stability protocol within the approved shelf-life</td>
<td>2</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
</tbody>
</table>
### Table continued

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>f. replacement of the sterility testing by the container/closure system integrity testing</td>
<td>None</td>
<td>1, 2, 4, 6</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4, 6</td>
<td>Minor</td>
</tr>
</tbody>
</table>

**Conditions**

1. The addition of the test(s) is not due to stability concerns or to the identification of new impurities.
2. The approved shelf-life of the final product is at least 24 months.
3. The method used to demonstrate the integrity of the container/closure system has already been approved as part of a previous application.

**Supporting data**

1. Copies or summaries of analytical procedures, if new analytical procedures are used.
2. Validation study reports, if new analytical procedures are used.
3. Proposed storage conditions and or shelf-life, as appropriate.
5. If applicable, stability testing results to support the change to the post-approval stability protocol or stability commitment (for example, data showing greater reliability of the alternative test).
6. Justification of the change to the post-approval stability protocol or stability commitment.

---

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>59. Change in the labelled storage conditions for the final product or the diluted or reconstituted vaccine, involving:</td>
<td>None</td>
<td>1–4, 6</td>
<td>Moderate</td>
</tr>
<tr>
<td>a. addition or change of storage condition(s) for the final product, or for diluted or reconstituted vaccine (for example, widening or narrowing of a temperature criterion, or addition of or change to controlled temperature chain conditions)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. addition of a cautionary statement (for example, “Do not freeze”)</td>
<td>None</td>
<td>1, 2, 4, 5</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
Table continued

<table>
<thead>
<tr>
<th>Description of change</th>
<th>Conditions to be fulfilled</th>
<th>Supporting data</th>
<th>Reporting category</th>
</tr>
</thead>
<tbody>
<tr>
<td>c. deletion of a cautionary statement (for example, “Do not freeze”)</td>
<td>None</td>
<td>1, 2, 4, 6</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

**Conditions**

None

**Supporting data**

1. Revised product labelling information, as applicable.
2. Proposed storage conditions and shelf-life.
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 (1) commercial-scale batch unless otherwise justified.
6. Results of stability testing under appropriate conditions covering the proposed shelf-life, generated on at least three (3) commercial-scale batches unless otherwise justified.

**References**

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 given 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 inner and outer vaccine labels.

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 (that is, 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 data required to support that change (as provided in Appendices 2 and 3 for quality changes). MA 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.

In some cases, safety and efficacy data comparing the approved clinical use (for example, indications or dosing regimens) of a vaccine with a new one may be required. Such studies, often referred to as clinical bridging studies, are trials in which a parameter of interest (such as formulation, dosing schedule or population group) is directly compared with a changed version of that parameter to assess the effect of the change on the product’s clinical performance. Comparisons of immune responses and safety outcomes (for example, rates of common and serious AEFIs) are often the primary objectives. If the immune response and safety profiles are non-inferior, then the efficacy and safety of the vaccine can be inferred.

Examples of safety and efficacy changes that require data from clinical studies, post-marketing observational studies or extensive post-marketing safety data include:

- change to the indication:
  (a) addition of a new indication (such as prevention of a previously unspecified disease);
(b) modification of an approved indication (such as 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:
  (a) addition of a new vaccination regimen (such as addition of accelerated vaccination regimens);
  (b) addition or modification of the existing vaccination regimen (such as addition of a booster dose or modification of the recommended time interval for booster vaccinations).

- Change to add information on shedding and transmission.

- Change to the use in specific at-risk groups (such as addition of information on use in pregnant women or immunocompromised patients).

- Change to add information on co-administration with other vaccines or medicines.

- Change to add a new route of administration.\(^1\)

- Change to add a new dosage form\(^1\) (such as replacement of a suspension for injection with a lyophilized cake).

- Change to add a new strength.\(^1\)

- Change to add a new delivery device.\(^1\) (such as adding a needle-free jet injector).

- 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 (such as use in pregnant women).

**Product labelling information changes**

Supplements on product labelling information change should be submitted for changes which do not require clinical efficacy data, safety data or extensive pharmacovigilance (safety surveillance) data. Product labelling information changes require approval prior to implementation of the change.

Examples of product labelling information changes associated with changes that have an impact on clinical use include:

- Addition of an adverse event identified as consistent with a causal association with immunization with the vaccine concerned.

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\(^1\) Some NRAs consider that these changes may require a new application for MA or licence.
- Change in the frequency of occurrence of a given adverse reaction.
- Addition of a contraindication or warning (such as 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 the provision of recommended risk-management actions (for example, required testing prior to vaccination, specific monitoring following vaccination and ensuring patient awareness of certain risks).
- Strengthening or clarification of product labelling information text 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 vaccine.

In some cases, the safety-related changes listed above may be urgent and may require rapid implementation (for example, the addition of a contraindication or warning). To allow for the rapid 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 MA holder (see section 7.3 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 on the safe and efficacious use of the vaccine. 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 approval prior to implementation by the MA holder include:

- Change in the name of the MA holder and/or manufacturer (such as change of name due to a merger).
- Change in the trade name of the vaccine.

Examples of changes which **do not** require approval by the NRA prior to implementation include:

- 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 MA holder’s contact information (for example, customer service number or web site addresses) or the distributor’s name.
- Update of the existing information for referenced literature without adding or removing references.
- Changes made to comply with an official compendium (such as change of common name).
- Minor changes to the text to add clarity in relation to maintaining consistency with common label phrase standards (for example, a change from “not recommended for children” to “not for use in children”).

These administrative product labelling information changes (that is, changes that have been implemented since the time of the last approved product labelling information not subject to prior approval) should be included when submitting subsequent supplements for safety and efficacy changes, or for product labelling information changes (see section 7.4).
# Annex 5

## Biological substances: WHO International Standards, Reference Reagents and Reference Panels

A list of WHO International Standards, Reference Reagents and Reference Panels for biological substances is available at: [http://www.who.int/biologicals](http://www.who.int/biologicals).

At its meeting in October 2014, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list.

### Additions

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotherapeutics other than blood products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteinizing hormone, human pituitary</td>
<td>33 IU/ampoule</td>
<td>Third WHO International Standard</td>
</tr>
<tr>
<td>Proinsulin, human</td>
<td>7.0 µg/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Blood products and related substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs 13 (ADAMTS13)</td>
<td>Function: 0.91 IU/ampoule Antigen: 0.92 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Activated blood coagulation factor XI</td>
<td>9.8 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>12/148 Lupus negative 12/150 Lupus moderate positive 12/152 Lupus strong positive</td>
<td>First WHO Reference Panel</td>
</tr>
</tbody>
</table>

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1 Unless otherwise indicated, all materials are held and distributed by the National Institute for Biological Standards and Control, Potters Bar, Herts, EN6 3QG, England. Antibiotic reference preparations identified by an * in the above list are held and distributed by the European Directorate for the Quality of Medicines & HealthCare, Council of Europe, 7 allée Kastner, CS 30026 F-67081, Strasbourg, France. Materials identified by an ** in the above list are held and distributed by the Paul-Ehrlich-Institut, 63225 Langen, Germany.
### Preparation Activity Status

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin</td>
<td>8.0 IU/ampoule</td>
<td>Fourth WHO International Standard</td>
</tr>
</tbody>
</table>

**In vitro diagnostic device reagents**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B virus surface antigen</td>
<td>47.3 IU/mL</td>
<td>Third WHO International Standard</td>
</tr>
<tr>
<td>Hepatitis C virus core antigen**</td>
<td>3200 IU/mL</td>
<td>First WHO International Standard</td>
</tr>
</tbody>
</table>

**Toxoplasma gondii** DNA for NAT-based assays

<table>
<thead>
<tr>
<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^6$ IU/mL</td>
<td>First WHO International Standard</td>
</tr>
</tbody>
</table>

**Vaccines and related substances**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Activity</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-malaria (<em>Plasmodium falciparum</em>) human serum</td>
<td>100 units/ampoule</td>
<td>First WHO Reference Reagent</td>
</tr>
<tr>
<td>Haemophilus influenzae type b capsular polysaccharide</td>
<td>$4.904 \pm 0.185$ mg/ampoule</td>
<td>Second WHO International Standard</td>
</tr>
</tbody>
</table>
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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

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

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

For further information, please contact: WHO Press, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (tel. +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int; order on line: www.who.int/bookorders).
This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological substances, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of general issues brought to the attention of the Committee. The next part of the report, of particular relevance to manufacturers and national regulatory authorities, outlines the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, a WHO guidance document on the Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine was adopted along with WHO Guidelines on procedures and data requirements for changes to approved vaccines and revised WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated).

Subsequent sections of the report provide information on the current status and proposed development of international reference materials in the areas of antibiotics; biotherapeutics other than blood products; blood products and related substances; in vitro diagnostic device reagents; 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) followed by the above three WHO documents adopted on the advice of the Committee (Annexes 2–4). All additions and discontinuations made during the 2014 meeting to the list of International Standards, Reference Reagents and Reference Panels for biological substances maintained by WHO are summarized in Annex 5. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/.