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WHO Expert Committee on Biological Standardization

Seventy-ninth report

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.
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WHO Expert Committee on Biological Standardization
Seventy-ninth meeting held virtually 11 to 14 March 2024

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Abbreviations

ADCC antibody-dependent cellular cytotoxicity
AG-BRAS Advisory Group on Blood Regulation, Availability and Safety
BET bacterial endotoxin test
CEA carcinoembryonic antigen
CEACAM carcinoembryonic antigen-related cell adhesion molecule
CEPI Coalition for Epidemic Preparedness Innovations
COVID-19 coronavirus disease 2019
DNA deoxyribonucleic acid
EDQM European Directorate for the Quality of Medicines & HealthCare
ELISA enzyme-linked immunosorbent assay
GAS group A streptococcus
GBS group B streptococcus
GMP good manufacturing practice(s)
HIV human immunodeficiency virus
HPLC high-performance liquid chromatography
HTS high-throughput sequencing
IFU instructions for use
IL-2 interleukin-2
IU International Unit(s)
JUNV Junin virus
LASV Lassa virus
Lf limit of flocculation
mAb monoclonal antibody
MAT monocyte activation test
NAT nucleic acid amplification technique
NC3Rs National Centre for the Replacement, Reduction and Refinement of Animals in Research
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NCL</td>
<td>national control laboratory</td>
</tr>
<tr>
<td>NRA</td>
<td>national regulatory authority</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PDVAC</td>
<td>Product Development for Vaccines Advisory Committee</td>
</tr>
<tr>
<td>rDNA</td>
<td>recombinant deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RPT</td>
<td>rabbit pyrogen test</td>
</tr>
<tr>
<td>RSV</td>
<td>respiratory syncytial virus</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>severe acute respiratory syndrome coronavirus 2</td>
</tr>
<tr>
<td>TDM</td>
<td>therapeutic drug monitoring</td>
</tr>
<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
</tr>
<tr>
<td>TTP</td>
<td>thrombotic thrombocytopenic purpura</td>
</tr>
<tr>
<td>VACV</td>
<td>vaccinia virus</td>
</tr>
<tr>
<td>VLP</td>
<td>virus-like particle</td>
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1. Introduction

The seventy-ninth meeting of the WHO Expert Committee on Biological Standardization was held virtually from 11 to 14 March 2024. The meeting was opened on behalf of the Director-General of WHO by Dr Yukiko Nakatani, Assistant Director-General, Access to Medicines and Health Products. Dr Nakatani began by welcoming participants and thanking them fordevoting their time and expertise to the work of the Committee.

Dr Nakatani noted that the 18 members of the Committee represented a good balance of expertise, regional representation and gender, and went on to explain the ongoing efforts being made to broaden even further the expertise and global representativeness of the WHO Expert Advisory Panel on Biological Standardization from which Committee members were drawn.

Dr Nakatani then provided a brief update on WHO activities to ensure broader access to medicines and health products, highlighting the WHO support being provided in 120 countries to advance the goal of universal health coverage. This support included the WHO prequalification of vaccines, diagnostics and other biological products to promote more equitable access to such products worldwide. In addition, several medicines had now been added to the WHO Model List of Essential Medicines (EML), including new products for treating multiple sclerosis, cancer and cardiovascular conditions. With ongoing support from the WHO regulatory systems strengthening programme, a number of key improvements had also been made to the national regulatory systems in several countries.

Dr Nakatani went on to note a statement that had been made on behalf of the WHO African Region by Senegal at the recent Executive Board meeting (see section 2.3.1 below) and highlighted the significance of this to the work of the Committee. In addition, following the adoption of resolution WHA67.21 in 2014 on access to biotherapeutic products, WHO had continued to develop important standards and tools to facilitate access to biosimilar products of assured quality, safety and efficacy.

Dr Nakatani concluded by commenting on the ambitious agenda of the current meeting, noting in particular the proposed addendum to the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases. This addendum would provide guidance specifically on the evaluation of monoclonal antibodies (mAbs) for use against coronavirus disease 2019 (COVID-19). Dr Nakatani also noted the proposed updating of the 2011 WHO guidelines on good manufacturing practices for blood establishments. In these and other important areas, the expertise of the Committee will be key to informing the provision of WHO support to countries and strengthening the global health leadership provided by WHO.
Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Nakatani for her opening remarks. Dr Knezevic went on to note that as the directing and coordinating authority on international health within the United Nations system, WHO adheres to its organizational values of integrity, professionalism and respect for diversity, as set out in its Constitution. Driven by its mission to promote health worldwide, WHO, through its activities at headquarters, regional office and country office level, provides leadership on global health matters, shapes the health research agenda, sets norms and standards, articulates evidence-based policy options and provides technical support to countries. The setting of norms and standards for biological products, and promoting their implementation, is therefore a core function of WHO.

Dr Knezevic then reminded meeting participants that the decision-making body of WHO is the annual World Health Assembly attended by delegations from all WHO Member States. The agenda of the World Health Assembly is set in advance each year by the Executive Board, which consists of technical experts elected for 3-year terms. All of the reports and recommendations of the current Committee are submitted to the Executive Board for its consideration.

Dr Knezevic then outlined the procedures and working arrangements of the present meeting. An open information-sharing session involving all participants, including non-state actors, would be held on Monday 11 March 2024. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would then participate in the main meeting from Monday 11 March to Wednesday 13 March 2024. All final decisions and recommendations on the adoption of WHO written standards and the establishment of WHO measurement standards would be made in a closed session held on Thursday 14 March attended only by Committee members and WHO staff. Dr Knezevic concluded by expressing her thanks to WHO staff working in this area, colleagues from WHO collaborating centres and custodian laboratories, and all of the individual experts present.

Following the conclusion of the open information-sharing session, Dr Knezevic presented the declarations of interests that had been completed by Committee members, WHO temporary advisers and other participants. After evaluation, WHO had concluded that none of the interests declared constituted a significant conflict of interest, and that the individuals concerned would be allowed to participate fully in the meeting. In the absence of dissent, Professor Klaus Cichutek and Dr Silvano Wendel were elected as Co-chairs, and Dr Ian Feavers and Dr Diana Teo as Rapporteur and Co-rapporteur respectively.

The Committee then adopted the proposed agenda (WHO/BS/2024.2473).
2. General

2.1 Strategic directions in biological standardization

2.1.1 Update on recent and planned WHO written standards for biological products

Dr Knezevic summarized the WHO written standards for biological products recently adopted on the advice of the Committee, and provided an overview of the plans for new and revised such documents from 2024 onwards. The adoption of the following four documents between 2020 and 2023 had been of particular relevance during the COVID-19 pandemic: (a) the WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines; (b) Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations; (c) the WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use; and (d) the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases. Subject to the advice of the Committee, the latter document will be supplemented by a series of addenda covering considerations specific to mAbs against COVID-19, respiratory syncytial virus (RSV) disease, rabies, malaria and human immunodeficiency virus (HIV).

Following the 2022 adoption of the WHO manual for the preparation of reference standards for use as secondary standards in antibody testing, with its focus on antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an implementation workshop had been held in November 2023. The report of the workshop had been published on the WHO website and provided to the Committee. In addition, the WHO Guidelines on regulatory preparedness for the oversight of pandemic or other emergency use vaccines in importing countries had been adopted in 2023.

With regard to upcoming WHO written standards, Dr Knezevic indicated that revised WHO Guidelines on rotavirus vaccines would be proposed for adoption at the next meeting of the Committee in October 2024, with an update on progress made to date scheduled for the current meeting (see section 3.4.1 below). It was further anticipated that revised WHO Guidelines on post-approval changes for vaccines would be presented to the Committee in October 2025, and revised WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards in 2025 or 2026. The revision of several other WHO written standards for vaccines was expected to commence in 2026, and would include written standards on yellow fever virus, dengue, measles, mumps and rubella, bacille Calmette-Guérin and malaria vaccines, as well as more general WHO Guidelines on vaccine lot release. In addition, and guided by the advice of the WHO Product Development
for Vaccines Advisory Committee (PDVAC) (see section 2.2.1 below) and by stakeholder requests, the development of new WHO written standards on chikungunya, paratyphoid, shigella and group B streptococcus (GBS) vaccines was anticipated, along with WHO guidance on the evaluation of vaccines and other biological products using high-throughput sequencing (HTS) technologies.

Dr Knezevic concluded by noting the completion in 2023 of the independent, systematic review of WHO written standards by the National Centre for the Replacement, Reduction and Refinement of Animals in Research (NC3Rs) in the United Kingdom. During discussion of the full report of this review, the Committee had recommended the establishment of a working group to build on its findings and to help develop science-based guidance encouraging the replacement of animal testing with non-animal testing for the quality control of vaccines and biotherapeutic products. The recently formed working group was expected to present such guidance for consideration by the Committee by 2026.

2.2 Cross-cutting activities of other WHO committees and groups

2.2.1 Report from the WHO Product Development for Vaccines Advisory Committee

Dr Erin Sparrow summarized the outcomes of the most recent PDVAC meeting held in December 2023 at which a range of cross-cutting and vaccine-specific topics had been discussed. Dr Sparrow highlighted the provision of PDVAC support to the Gavi 2024 Vaccine Investment Strategy process, noting that new vaccines against tuberculosis, GBS, shigella and dengue had been shortlisted for Gavi investment. A number of such vaccines, along with a range of other novel vaccines and mAbs against infectious diseases, could be approved in the next 5–10 years.

Dr Sparrow went on to discuss the current tuberculosis vaccine pipeline in detail, which mainly comprises vaccines intended to boost waning immune responses to bacille Calmette-Guérin in adults and adolescents. Several different types of candidate vaccines were now at an advanced stage of clinical development, with PDVAC recommending that a WHO roadmap be developed setting out a pathway for their introduction. PDVAC further advised that such novel vaccines would require new written and measurement standards to support their WHO prequalification.

Dr Sparrow then noted the potential application of controlled human infection models in the development of vaccines against diarrhoeal and enteric pathogens, with several such models now existing or in development. Noting that the first bivalent typhoid conjugate vaccine and paratyphoid A vaccine might be approved on the basis of data obtained using such a model, Dr Sparrow highlighted the need for a regulatory consultation on the approval pathway of such vaccines. Once again, specific WHO guidance in this area may be required
to support vaccine licensure and WHO prequalification. In addition, the recent entry of several shigella candidate vaccines into the enteric vaccine pipeline would necessitate the development of an antiserum reference standard to harmonize the measurement of antibody responses and help identify correlates of protection.

Dr Sparrow further noted that GBS vaccines were also at an advanced stage of clinical development. Such vaccines were unlikely to be approved on the basis of traditional Phase III clinical studies as these were costly and logistically challenging to perform. A licensure pathway based on immune correlates of protection was therefore being explored with regulators and stakeholders to ensure the ongoing commitment of vaccine developers. An approach based on immune correlates of protection had already been used to approve the first chikungunya vaccine. PDVAC recommendations in this area had included the conducting of modelling, feasibility and other studies for a range of different scenarios to inform chikungunya vaccine advocacy at national and regional level.

Noting the recent publication of the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases, and noting the disease-specific addenda to this document due to be discussed at the current meeting, Dr Sparrow concluded by summarizing the developmental status of a number of such products. Related WHO activities included the development of general preferred product characteristics for mAbs used within routine immunization programmes, and of policy requirements for broadly neutralizing antibodies used to prevent the vertical transmission of HIV to infants.

The Committee thanked Dr Sparrow for her review and enquired whether there had been any progress towards the development of HIV-1 vaccines. Dr Sparrow noted that despite more than 40 years of research, there were currently no promising candidates in the pipeline. However, several promising mAb products were in development, with particular interest in their potential to prevent vertical transmission. The Committee also asked whether the recent success of COVID-19 messenger RNA vaccines had led to the development of other vaccines based on this technology. Dr Sparrow indicated that although several such products were in development, their reactogenicity profile and cold chain requirements meant that they were unlikely to replace existing vaccines based on older technologies. Enquiring about the current status of Nipah virus vaccine development, the Committee was informed that as such outbreak vaccines were on the agendas of the WHO R&D Blueprint and the Coalition for Epidemic Preparedness Innovations (CEPI) they did not come under the remit of PDVAC.

Responding to further questions from the Committee, Dr Sparrow emphasized the importance of post-marketing surveillance to confirm the efficacy of vaccines first licensed based on correlates of protection. Reflecting on the lessons learned from the licensure of the chikungunya vaccine, the Committee noted that although this was a pragmatic solution when efficacy studies were
impractical, it did rely on having a well-defined correlate of protection, ideally defined in International Units (IU). In this regard and more generally, the Committee encouraged the use of multinational randomized controlled studies across endemic areas to expedite the approval of new vaccines.

2.3 Other matters

2.3.1 Statement made during the 154th session of the Executive Board regarding the work of WHO expert committees

At its 154th session held in January 2024, the Executive Board was presented with the main outcomes and recommendations of the 77th meeting of the Committee held in March 2023, together with those of other WHO expert committees. At that occasion, the following statement was made:

Chairman, Ministers, Distinguished Delegates

SENEGAL has the honour of delivering this statement on behalf of the 47 Member States of the WHO African Region. The African Region would first of all like to congratulate the expert committees on the meetings they held during the biennium 2022–2023 from which came important recommendations on biological standardization, the selection and use of essential medicines and the evaluation of certain food additives.

These recommendations are of crucial importance for public policy and the programmes of the Organization and therefore we believe that:

1. The preparation of Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases is to be promoted. These would make it possible to provide specific updated guidance to national regulatory authorities and the manufacturers of biological products in order to guarantee their safety and efficacy.

2. The preparation of a regulatory framework for human cells and tissue and for innovative medical treatments that would favour regulatory harmonization of these products would also be useful.

3. We support the making available of WHO standards recognized internationally to make it possible to extend universal health coverage and the updating of the WHO Model List of Essential Medicines, taking into account needs but also efficacy and efficiency criteria so as to guarantee accessibility to all...

We urge the Executive Board to note the present report.
3. International Recommendations, Guidelines and other matters related to the manufacture, quality control and evaluation of biological products

3.1 General

3.1.1 Discontinuation of innocuity test

At its sixty-ninth meeting in 2018, the Committee had recommended the immediate cessation of any requirement to perform the innocuity test (also known as the abnormal toxicity or general safety test) in WHO written standards for biological products. It had further recommended that any mention of the test in WHO documents published before 2018 should be disregarded, acknowledging that the requirement could only be removed from such documents when they were revised or replaced. Despite these recommendations, a recent review commissioned by WHO and carried out by the National Centre for the Replacement, Reduction and Refinement of Animals in Research (NC3Rs) in the United Kingdom had indicated continued widespread use of the innocuity test.

To further highlight the recommendations of the Committee in this regard, it was agreed that an “important note” and a table listing all current WHO written standards on biological products published prior to 2018 that still mentioned the unneeded innocuity test would be added to Annex 1 of all reports of the Committee from October 2023 onwards. There was a consensus among Committee members that this would be an important addition that should continue to be made until all the relevant documents had been updated or withdrawn. Recognizing that many users of WHO written standards now accessed WHO documents directly from the WHO website, the Committee further suggested that the statement be reproduced on the relevant WHO webpages, along with other web-based approaches, to inform users that they should disregard any historic requirement to perform innocuity testing.

3.2 Biotherapeutics other than blood products

3.2.1 Nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of COVID-19

COVID-19 continues to pose a significant public health threat worldwide, with SARS-CoV-2 causing severe disease and, in some cases, long COVID, including among highly vulnerable groups. It is anticipated that as COVID-19 becomes endemic, it will continue to cause severe illness, hospitalizations and deaths as new variants emerge. In March 2023, the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases were adopted on the advice of the Committee. This document provides guidance on the evaluation of mAbs
and related products regardless of the target pathogen or toxin. During the adoption of these Guidelines, the Committee had recognized the need to also develop a number of addenda on disease-specific regulatory considerations.

The Committee was provided with an overview of the development and structure of a proposed addendum setting out a number of supplementary considerations when evaluating the safety and efficacy of mAb products specifically intended for the prevention or treatment of COVID-19. Following two rounds of public consultation and subsequent revisions, the proposed addendum now consisted of seven sections ranging from general considerations to more detailed guidance on nonclinical and clinical evaluation. The Committee was reminded that separate detailed guidance on the production and quality control of mAbs was provided in the previously adopted WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use.

The Committee sought clarification on a number of issues, including on the guidance provided on the degree to which nonclinical and clinical data obtained for specific mAb formats or for specific variants of concern could be applied to other comparable mAb products. The Committee indicated that any new mAb format or mAb targeting a newly emerging variant of concern should be assessed as a new product, including in terms of their ability to neutralize the target variant. Assurance was provided to the Committee that clear guidance on these and related issues was provided in the parent Guidelines and the addendum when these were read in conjunction.

The Committee also highlighted two issues with the proposed section on international reference materials. While noting that such a section might helpfully be included in all relevant WHO Recommendations, Guidelines and guidance documents to promote the use of WHO measurement standards, the Committee was concerned that the section would become out of date once the cited standards were replaced or new variants emerged. Furthermore, the international reference materials in question were based on antisera, and although likely to be of utility, there was currently no evidence to support their use in evaluating mAbs. In light of these comments, it was agreed that the text would be modified to ensure that users check for the most up-to-date reference materials, and that more cautionary language would be used regarding the use of current international reference materials to characterize SARS-CoV-2 mAbs.

Despite a current lack of licensed products intended to be inhaled or administered intranasally, the Committee noted that prospective such mAb products against respiratory pathogens may be developed. The Committee agreed that such products would require a number of specific additional pharmacokinetic and pharmacodynamic considerations compared to parenterally administered mAbs, including considerations arising from the potential use of a delivery device.
Appropriate text was therefore added to the nonclinical and clinical sections of the addendum to address this issue. The Committee also suggested that although all currently approved neutralizing mAbs target the SARS-CoV-2 spike protein, the text be broadened to include mAbs targeting other antigens that may be in development. In addition, the Committee suggested some clarification of the guidance be provided on the use of available safety data to support the inclusion of pregnant women in clinical studies.

After due consideration of these and other minor modifications made to the text, the Committee recommended that document WHO/BS/2024.2466 be adopted and annexed to its report (Annex 2).

3.2.2 Development of WHO guidance on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention of respiratory syncytial virus disease

Worldwide, RSV is a leading cause of respiratory disease in all age groups, frequently causing severe morbidity and mortality in infants, young children and older adults. It is the second most common cause of infant mortality, with more than 99% of childhood deaths due to RSV occurring in low- and middle-income countries which typically lack safe and effective prevention or treatment options. Two prophylactic mAb products for use against RSV disease have recently been approved, with several others now in clinical development. Targeting neutralization-sensitive epitopes on the virus, these mAbs provide immediate protection against severe disease, especially in infants at particularly high risk.

The Committee was updated on progress made towards the development of an addendum to the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases that would address specific issues in the evaluation of mAbs used to prevent RSV disease. The addendum would be based on a format similar to that used for the addendum on COVID-19 mAbs recommended for adoption at the current meeting (see section 3.2.1 above). Its purpose would be to provide supplementary considerations in the evaluation of the safety and efficacy of prophylactic mAbs directed specifically against RSV, and would apply primarily to parenterally administered mAb products. A general considerations section will provide background on the pathology and epidemiology of RSV disease, as well as on the mechanism of action of RSV mAbs and their effectiveness. A section on nonclinical evaluation will provide guidance on the use of in vitro and in vivo studies to assess the pharmacodynamics and biological activities of candidate mAbs, with a section on clinical evaluation focusing on assessment of their prophylactic effect.

A drafting group had now been established and the proposed addendum was currently in preparation. Following two rounds of public consultation, it
was anticipated that the document would be submitted to the Committee for its review at its next meeting in October 2024.

While reflecting on the potential advantages of vaccines over mAbs for the prevention of RSV disease, the Committee also felt that access to both types of products would be desirable at present, and that the addendum would be a very important document in this context. The Committee looked forward to seeing the resulting document in due course.

3.3 Blood products and related substances

3.3.1 Revision of the WHO guidelines on good manufacturing practices for blood establishments

One of the strategic objectives of the WHO Action Framework to advance universal access to safe, effective and quality-assured blood products 2020–2023 is the establishment of well-functioning and efficiently managed blood services, with a functioning quality system as one of the specific outcomes. However, a lack of knowledge in implementing and maintaining good manufacturing practices (GMP) in blood establishments has been identified as a common barrier to achieving this objective. To help address this issue, efforts were now under way to update the 2011 WHO guidelines on good manufacturing practices for blood establishments as part of the 2023–2024 workplan of the WHO Advisory Group on Blood Regulation, Availability and Safety (AG-BRAS). As one of the planned activities of the WHO Action Framework, it was intended that the revised document would reflect new developments in the field and provide countries with practical guidance on how to establish reliable quality assurance systems for the whole chain of blood collection, testing, processing and distribution of blood components in blood establishments and hospital blood banks.

Following an overview of the WHO procedures for developing guidance documents, the Committee was provided with a summary of the objectives of the revised document, and of the rationale and justification for its development. The proposed revised document would set out the international principles and standards of GMP for blood establishments and hospital blood banks that should be implemented and enforced by the national regulatory authority (NRA) to ensure the quality and safety of blood components for transfusion and as starting material for further industrial manufacturing. The revised document would also be aligned with existing international documents in the field to facilitate harmonization in the global manufacturing and use of plasma products.

The Committee was informed that an expert working group comprising AG-BRAS members had been established and was being supported by the WHO Blood and Other Products of Human Origin team, with input from regional advisers. Following a detailed summary of the structure and contents of the proposed revised document, an update was provided on the progress made to
It was intended that a complete draft would be available in the first half of 2024 and that following a process of expert comment and public consultation, the final version would be published in late 2024. Explanation was given that although WHO planning clearance had been obtained based on developing a normative and standard-setting publication, the proposal was also being submitted to the Committee for its consideration as the 2011 WHO Guidelines had been developed with its involvement and subsequently jointly published by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The Committee welcomed the efforts made by the working group and AG-BRAS in revising this important document as this would directly contribute to the consistent production of safe and quality-assured blood, blood components and plasma products. Observing that the risks and challenges for GMP implementation had also changed over the years, the Committee noted that quality standards in hospital blood banks were often suboptimal, with unsatisfactory clinical transfusion practices in many resource-limited countries. Reflecting on how GMP and quality management concepts could best be implemented in these areas, the Committee noted that GMP would only apply to that which is necessary to maintain safety and quality during blood collection, testing, processing, release, and distribution to, and storage in, hospitals. This would not cover the key areas of clinical blood transfusion practices, which are instead part of the activities of the hospital quality system. It was acknowledged that the proposed document would thus cover quality system elements in blood establishments that also applied to hospital blood banks rather than the full scope of GMP in the latter setting. The Committee also suggested a number of additional topics that might usefully be considered in the document, such as donor data security/confidentiality when data is shared between blood establishments. It was also recognized that the relationship between the blood establishments and manufacturers of blood products was an important focus, as it was envisaged that blood establishments compliant with the revised WHO Guidelines would achieve a level of quality that would make plasma suitable for further manufacturing, with manufacturers of plasma-derive medicinal products looking to only source plasma from such GMP-compliant establishments.

The Committee concluded by discussing the proposed next steps in developing and publishing the revised document. Given the significant similarities between the WHO process for developing normative and standard-setting publications and the Committee process, the parallel publication of the proposed document appeared to be feasible. Under this parallel approach, the completed draft would undergo public consultation, followed by presentation of the final document to the Committee at its next meeting in October 2024. The final document would also be published on the WHO website to expedite user access and then independently published in the Technical Report Series, subject to the recommendations of the Committee.
Recognizing the important and pressing need for WHO guidance to countries in this area, the Committee expressed its support for the proposed revision and parallel publication of the WHO guidelines on good manufacturing practices for blood establishments.

3.4 **Vaccines and related substances**

3.4.1 **Revision of the WHO Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral)**

Rotavirus is a highly infectious cause of severe dehydrating gastroenteritis in children under the age of 5 years, and in the absence of antiviral treatment is best prevented by vaccination. Universal rotavirus vaccination of infants has been recommended by WHO for more than a decade and there is now considerable experience of using the available live attenuated vaccines. Since the adoption of the WHO Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral) in 2005 there have been numerous developments. These include the approval of the first two live attenuated rotavirus vaccines in Europe and the USA and then in numerous countries, and their WHO prequalification. Other live attenuated rotavirus vaccines have been licensed in India and prequalified by WHO, while live attenuated rotavirus vaccines have also been licensed nationally in China and Viet Nam. In addition, other candidate vaccines were currently being developed, including non-replicating rotavirus vaccines. Over the same period, several new and revised WHO guidance documents with broad applicability to vaccine standardization have been published that reflect technological advances in vaccine production, quality control and clinical evaluation.

In light of such technological advances and other developments, it had previously been proposed that the 2005 WHO Guidelines be updated, and the Committee was updated on the progress made to date. Following consultations with experts and stakeholders worldwide, a drafting group consisting of regulatory experts from several countries had prepared a series of draft revised texts. Once finalized, the revised document is intended for use by NRAs, national control laboratories (NCLs) and vaccine manufacturers, and will primarily address issues regarding live attenuated rotavirus vaccines for the prevention of disease. However, the sections on the nonclinical and clinical evaluation of candidate vaccines are intended to be applicable to all types of rotavirus vaccines.

The Committee was then provided with a detailed overview of the various sections of the current draft document which follows a format similar to recently adopted WHO vaccine Guidelines and Recommendations. Key changes in the document reflect current practice in the production and control of live attenuated rotavirus vaccines, provide guidance on the pharmacological evaluation of candidate vaccines developed on different platforms, elaborate on
toxicological testing (including the risk of intussusception) and provide guidance on the design of future trials (including in the context of available licensed rotavirus vaccines, and for different types of such vaccines).

Comments received following a second round of public consultation on the draft document had primarily focused on the requirement for sterility and sterile filtration. A small number of respondents had expressed concerns with regard to meeting sterile filtration requirements, and had advocated instead for confirmation of low bioburden as an end-point. In response, it was noted that whereas the original Guidelines required sterility at all production stages, the revised document was already more flexible as it introduces the possibility of using a bioburden test at an intermediate stage of production. However, such an approach requires that a sterilizing filtration be performed later in the production process. Conversely, and in line with long-established practice, such a filtration step is not mandatory or required if the entire manufacturing process is aseptic, and controlled by sterility testing and validated. This approach is consistent with the regulatory requirements of other entities, including the European Pharmacopoeia.

During discussion, a consensus was reached by the Committee that orally administered vaccines such as oral poliomyelitis vaccines and oral rotavirus vaccines should be sterile products given the target population and the implications of microbial contamination for storage. The Committee supported the revision of the wording in some places to provide more flexibility during production processes but strongly emphasized that whichever approach was used, the final product must in all cases be sterile.

Having agreed a further revised wording on sterility and sterile filtration, and having suggested several other amendments to the current text, the Committee recommended that the draft document be submitted for a third round of public consultation. The Committee looked forward to reviewing the final draft in more detail at its next meeting in October 2024, with a view to its potential adoption.
4. International reference materials – biotherapeutics other than blood products

4.1 WHO international reference standards for biotherapeutics other than blood products

4.1.1 First WHO International Standard for golimumab

Golimumab is a human therapeutic mAb originally isolated from a hybridoma clone generated from transgenic mice immunized with human tumour necrosis factor (TNF). It is structurally well characterized and binds with high affinity and specificity to both soluble and transmembrane forms of TNF thereby neutralizing its biological activity. Golimumab is used in adults as a TNF antagonist to treat moderate-to-severe rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, non-radiographic axial spondyloarthritis and moderate-to-severe ulcerative colitis. It is also used to treat polyarticular juvenile idiopathic arthritis in children. Variable efficacy among anti-TNF products has highlighted a potential need for therapeutic drug monitoring (TDM), which measures the levels of the therapeutic and of anti-drug antibodies. However, implementation of this approach has been hampered by a lack of standardization of the analytical methods used.

In order to develop a prospective reference standard for use in measuring both the in vitro biological activity of golimumab and golimumab concentrations in samples from treated patients, two international collaborative studies had been conducted using a donated drug preparation. This preparation was formulated similarly to WHO international standards for other TNF mAbs, and filled and lyophilized in accordance with WHO guidelines.

In the first study, 16 laboratories in 11 countries had assessed the suitability of the candidate material (NIBSC code 22/116) to standardize cell-based bioassays and TNF binding assays. Results indicated that the candidate material typically reduced the variability of potency estimates for tested preparations compared with estimates expressed relative to in-house reference standards. In the second study, using a panel of sera spiked with different amounts of candidate material 22/116, eight laboratories had assessed the ability of the candidate material to standardize the measurement of serum golimumab content in clinical laboratories. Results indicated that use of the candidate material reduced inter-laboratory variability thus harmonizing the clinical monitoring tests used for TDM. Accelerated thermal degradation studies indicated that the candidate material was stable during long-term storage at −20 °C, with additional studies indicating that the reconstituted candidate material was stable for at least 1 week at 4 °C or at room temperature. Freeze-thaw stability testing indicated that the potency of the candidate material was retained for at least four freeze-thawing cycles.
Noting the importance of this class of mAb product, the Committee considered this to have been an excellent study. However, concerns were expressed that assigning both IU and SI unitages to the same material might lead some users to erroneously relate activity to the mass of mAb. Reassurance was given that the two distinct groups of users would either be measuring bioactivity (in IU) or TDM (in µg/mL) but not both. In addition, the instructions for use (IFU) would clearly caution against the potential misuse of the reference material.

Following further minor clarifications, the Committee considered the report of the study (WHO/BS/2024.2467) and recommended that candidate material 22/116 be established as the First WHO International Standard for golimumab with assigned unitages of 500 IU/ampoule TNF neutralizing activity, 500 IU/ampoule TNF binding activity, 500 IU/ampoule FcγRIII binding activity and 500 IU/ampoule antibody-dependent cellular cytotoxicity (ADCC) activity. Noting that this material was intended to define IU of bioactivity for the purposes of assay harmonization, the Committee emphasized that it was not intended to define the specific activity of golimumab products or to serve as a reference product for biosimilarity determination or dosing requirements. The Committee further recommended that a unitage of 50 µg/ampoule also be assigned to the First WHO International Standard for golimumab for the purpose of TDM.

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

4.2.1 Proposed Third WHO International Standard for interleukin-2 (human, rDNA derived)

Interleukin-2 (IL-2) is approved for the treatment of metastatic renal cell carcinoma and melanoma. Clinical studies are also ongoing on the therapeutic use of IL-2 either on its own or as part of combination therapy for cancer immunotherapy, autoimmune disease and potentially for amyotrophic lateral sclerosis. The current WHO international standard (NIBSC code 86/500) had been established in 2013 and is used in the potency labelling of IL-2-based clinical products, and for calibrating commercial IL-2 reagents and immunoassay kits.

Compared to a rate of use of approximately 130 ampoules per year from 1986 to 2013 for the first WHO international standard (NIBSC code 86/504), the current international standard is being used at a rate of approximately 300 ampoules per year. Despite close monitoring and imposition of restrictions on distribution since late 2023, it was anticipated that stocks would likely be exhausted in around 2 years, and a replacement reference standard was now required.

It was proposed that a candidate replacement material would be prepared using full human sequence IL-2 derived from recombinant DNA (HEK293 cells) formulated in 1% human serum albumin, 0.5% trehalose and PBS. With
the exception of the buffer used for the second WHO international standard, this was essentially the formulation that had been used for both of the previous two WHO international standards, and had proven to be stable. Although the IL-2 in the preparation would be glycosylated, it had previously been shown that glycosylation would not affect bioactivity in the assays used if the complete human sequence was present. The Committee was informed that the source material was now on site and that trial fills had been completed with a definitive fill scheduled. To meet the increasing rate of demand, it was proposed that a batch size of 8000 ampoules would be prepared – double that of the previous two batches. An international multi-centre collaborative study would be conducted in 2024–2025 to evaluate the performance of the candidate material and to assign a unitage relative to the second WHO international standard. It was anticipated that the results of the collaborative study would be submitted for consideration by the Committee in 2026.

Noting the importance of the WHO international standard for IL-2 in the development and production of IL-2-based products, and for the investigation of novel therapeutic approaches, and the short time available for establishing a replacement international standard, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a Third WHO International Standard for interleukin-2 (human, rDNA derived).

4.2.2 Proposed Fourth WHO International Standard for endotoxin
Testing parenteral medicinal products for pyrogens is essential to prevent serious adverse events caused by contamination or inconsistent production, with bacterial endotoxin being the most common pyrogen contaminating biological products. The WHO international standard for endotoxin has been widely used to calibrate critical reference materials used in three compendial methods: the rabbit pyrogen test (RPT); the bacterial endotoxin test (BET); and the monocyte activation test (MAT). Users of these international standards include pharmacopoeias, regulatory agency laboratories, pharmaceutical companies and pyrogen testing reagent manufacturers worldwide.

The current Third WHO International Standard for endotoxin (NIBSC code 10/178) was established in 2012. To ensure a harmonized approach to endotoxin standardization, a portion of this material had also been used to establish EDQM and US Pharmacopoeia reference materials, with an understanding that this harmonized approach would be continued. With the impending removal of the RPT from the European Pharmacopoeia in 2026, and increasing use of recombinant BET reagents as part of the move towards animal-free testing, it is anticipated that the level of demand will increase. Therefore, although current stocks were expected to last for a further 5–6 years, it was being proposed that development of the replacement standard should begin immediately.
It was proposed that a donated lyophilized bulk endotoxin material derived from *Escherichia coli* (Braude strain) would be used to develop a candidate material. This bacterial endotoxin was the same source material that had been used for the first and second WHO international standards, and for previous lots of the companion reference materials. However, as stocks of the source material itself were also running low, and would only be sufficient for the development of one or two future replacement standards, it was proposed that the replacement international standard be formulated at a lower concentration (5000 IU/vial) to conserve stocks of the source material and ensure its efficient use. Current methods for endotoxin testing were now very sensitive, and users had been consulted on the proposed reduction. No responses or objections had been received regarding this change and broader consultation was planned. The source material was already on site and would be formulated in same way as the previous WHO international standards, with a definitive fill in late 2024. An international multi-centre collaborative study would then be conducted in 2025 to evaluate the performance of the candidate replacement material and to assign potency. It was anticipated that the results of the collaborative study would be submitted for consideration by the Committee by no later than October 2026.

Noting that only the BET would be included in the collaborative study and that the RPT would no longer be included due to the move towards animal-free pyrogen testing, the Committee reflected on the role and possible future inclusion of the MAT. In response it was noted that, in general, the MAT was considered to be more useful for detecting non-endotoxin pyrogenic substances and not as suitable for endotoxin. Having also been assured that no complaints had been received in response to the proposed reduction in potency, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a Fourth WHO International Standard for endotoxin.

### 4.3 Proposed discontinuations and updates – biotherapeutics other than blood products

#### 4.3.1 Proposed discontinuation of the First WHO International Standard for calcitonin ASU 1–7 eel calcitonin analogue (elcatonin)

Elcatonin, an eel analogue of calcitonin, inhibits the absorption and autolysis of bone thereby lowering blood calcium levels. It is primarily used to reduce pain associated with osteoporosis. The First WHO International Standard for calcitonin, ASU 1–7 eel calcitonin analogue (elcatonin) (NIBSC code 84/614) was developed in the 1980s for the calibration of therapeutic elcatonin preparations using in vivo bioassays. Stocks of this international standard were expected to be exhausted within 2–3 years. However, in light of its highly restricted geographical use and associated low level of demand, any continuing need for such a global international standard was likely to be minimal, with the regional
use of elcatonin potentially supported through available regional or national standards. In addition, and in line with the increasing application of the 3Rs principles (Replacement, Refinement, Reduction) regarding the use of animals in research, there should now be a transition away from the assignment of IU to elcatonin products based on the use of in vivo rat bioassays towards the dosing of such products in mass units using animal-free methods. It was noted that other calcitonin analogues were now being controlled using physicochemical methods in both the European Pharmacopoeia and US Pharmacopoeia.

The Committee was therefore informed of the decision of the custodian laboratory not to prepare a replacement international standard, and was presented with two options going forward. The first option was to simply discontinue the WHO international standard upon exhaustion of the current stocks. The second option would be for another custodian laboratory to develop a replacement WHO international standard.

During discussion, the potential implications of discontinuation of the WHO international standard for users in China and Japan were considered, and clarification sought on the availability of regional and national standards. The monograph for elcatonin in the Japanese Pharmacopoeia includes an in vivo bioassay supported by a national standard traceable to the WHO international standard, and it was opined that the current batch of this national standard would likely last for some time, with any future replacements calibrated against it. The Committee was further informed that in China, elcatonin was marketed as a chemical drug and was controlled by national standards based on in vivo bioassay methods in Chinese Pharmacopoeia monographs.

Exploring the feasibility of using alternative assays such as high-performance liquid chromatography (HPLC) to characterize the international standard, the Committee was advised that although theoretically possible due to the relatively small size and lack of complexity of the elcatonin peptide, the presence of albumin in the preparation might be problematic, with issues encountered in developing such physicochemical alternatives in Japan. One possible long-term solution would be to calibrate and establish a reference material using HPLC based on widely available EDQM and US Pharmacopoeia standards for calcitonin, but this would depend on the acceptability of this approach, as in some countries this would require changes to the way in which products were controlled.

Reflecting on the importance of customer outreach efforts, the Committee was informed that sales restrictions had been put in place to minimize the risk of a large one-off order depleting the stock, with letters now being sent out to notify customers that the future of the standard was uncertain and would be clarified. Noting that customers requesting the material had not yet been informed of the intention to discontinue supply, the Committee recommended that further
steps be taken to notify users and to ensure that any concerns are taken into consideration. It was agreed that specific mention of this issue in the Executive summary of the current meeting would be a useful first step in the broader dissemination of the current intentions.

After due consideration, the Committee agreed that a decision on the proposed discontinuation of the First WHO International Standard for calcitonin, ASU 1–7 eel calcitonin analogue (elcatonin) would be taken at its next meeting in October 2024 to provide sufficient time and opportunity for the information to be disseminated and feedback obtained. The Committee also recommended that any interest from other WHO collaborating centres or institutions in establishing a replacement standard, including existing institutions in China and Japan responsible for maintaining national standards, should be explored. Sufficient material should be curated from current stocks to support the establishment of such a replacement standard by another institution or to support any potential regional standards that might be developed.
5. International reference materials – blood products and related substances

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

5.1.1 Proposed WHO International Reference Reagent for autoantibodies to ADAMTS13 (human, plasma)

Thrombotic thrombocytopenic purpura (TTP) is a rare disorder caused by ADAMTS13 deficiency, with a reported annual incidence of around 6 per million population. ADAMTS13 is a plasma protease enzyme responsible for the cleavage of von Willebrand factor, which is involved in blood clotting. Prompt and accurate diagnosis is essential, as the onset of clinical symptoms can be rapid with 90% of cases fatal if left untreated. TTP may either be congenital due to an inherited deficiency (cTTP) or immune mediated due to autoantibodies against ADAMTS13 (iTTP). Differential diagnosis is based on Bethesda-type activity assays or enzyme-linked immunosorbent assays (ELISA), and determines the treatment modalities employed. Once recovered, patients must also be monitored every 6–12 months in order to detect any onset of recurrence.

Several different activity assays and one ELISA-based binding assay are currently available commercially, with significant discrepancies observed between the different methods, and no common units of measurement exist between the functional inhibitor and binding antibody methods. The Bethesda assay is also inherently variable due to the use of different plasma pools by laboratories. The standardization of assays and quantification of inhibitory activity will therefore be important both for harmonization and for ensuring correct patient diagnosis, monitoring and treatment.

It was proposed that a WHO international reference reagent for ADAMTS13 autoantibodies for use by assay manufacturers and clinical laboratories in hospitals managing TTP patients be developed using pools of plasma collected from iTTP patients. An international collaborative study involving 10–20 laboratories would be conducted to evaluate the suitability of a candidate material using functional Bethesda-type assays to measure reduction in ADAMTS13 activity or ELISA-based assays to detect anti-ADAMTS13 antibodies. As the project was still in its early stages, the source material had not yet been acquired but a network of clinicians had indicated their willingness to provide the source material. Based on the current indicative timeline, it was anticipated that the results of the collaborative study would be submitted for consideration by the Committee in 2027. It was noted that the high cost of assays might limit study participation and thus geographical representation.

The Committee observed that in absolute terms, TTP was not that uncommon, with several recent publications on case numbers. Access to source
material would not be a problem as large volumes of plasma were usually collected from iTTP patients during plasma exchange and discarded as waste material. Having been assured of the support of the clinician network in sourcing material for this project, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a WHO International Reference Reagent for autoantibodies to ADAMTS13 (human, plasma).

6.1 WHO international reference standards for in vitro diagnostics

6.1.1 First WHO International Reference Panel for Lassa virus RNA for NAT-based assays

The Committee was reminded that at its seventy-fifth meeting in April 2022, a twin proposal had been made to establish both a WHO international standard and WHO international reference panel for Lassa virus (LASV) RNA for use in NAT-based assays. The Committee had subsequently recommended the establishment of the First WHO International Standard for Lassa virus RNA for NAT-based assays (NIBSC code 21/112). However, in the case of the international reference panel, the Committee had recommended that additional panel member characterization and performance studies be conducted prior to its establishment. The Committee had further recommended that due to insufficient data at 3 months, along with a positive microbiological result obtained for two panel members, additional stability testing be conducted for both the international standard and international reference panel.

The Committee was informed that additional in-house characterization testing had now been conducted on the international reference panel with the data indicating that previously observed variability in potency estimates for two panel members was not due to sample commutability issues but was assay dependent. This further demonstrated the utility and fitness for purpose of all reference panel members in evaluating NAT-based assay performance across different LASV lineages.

The Committee was further informed that the recommended additional stability testing had also been completed for both the international standard and international reference panel for up to 1-year timepoints. In the case of the international standard, minimal loss was demonstrated across all timepoints up to 37 °C and only a slight loss of potency observed at 45 °C at 6 months and 1 year. Based on Arrhenius model calculations, only a negligible loss in potency per year was predicted when stored at the recommended temperature of −20 °C, and no change was necessary in the original recommendation to ship the international standard at ambient temperature. For the international reference panel, stability assessments completed at timepoints up to 1 year also indicated minimal loss of potency in all samples for up to 3 months at temperatures up to 37 °C, and up to 1 year at 4 °C and 20 °C. However, some loss of potency after 1 year was observed at 37 °C and 45 °C. No clear differences were observed in the stability of panel members found to have a higher residual moisture content, or that had previously returned a positive microbiological test result. The data again supported the recommendation for long-term storage at −20 °C and shipment at ambient temperature. As it was not possible to predict long-term stability using the Arrhenius model, periodic stability testing of the panel would be undertaken.
Noting that LASV lineages I and VI were not represented in the international reference panel, the Committee was reassured that this was due to their lower level of circulation, and that samples could be incorporated into the panel if required. On the issue of preferred gene target, the Committee was informed that the L-gene is not LASV specific and cross-reacts with other arenaviruses. Most assay kits would therefore include both L-gene and GPC-gene targets with a positive result for either target gene sufficient to suggest LASV infection. Commenting that materials shipped at ambient temperature might be affected by rising global temperatures, particularly in hot climates, the Committee was assured that this would be guided by the results obtained at 37 °C and 45 °C during stability testing, and that samples were also monitored during transit.

Having considered the above matters and the addendum (WHO/BS/2024.2468) to the original report of the study, the Committee recommended that the following four candidate materials be established without assigned unitage as the First WHO International Reference Panel for Lassa virus RNA for NAT-based assays (available under NIBSC code 22/108):

- Candidate material 21/102 – LASV Lineage II
- Candidate material 21/106 – LASV Lineage III
- Candidate material 21/108 – LASV Lineage V
- Candidate material 21/104 – LASV Lineage VII.

The Committee noted that the IFU would include a statement indicating that the panel had been formulated to the same target potency based on quantification of the common lentiviral gene, and that LASV-specific NAT-based assays have detected discrepancies when targeting the S-segment that may be assay dependent.

6.1.2 First WHO International Standard for HIV-1 p24 antigen

The core HIV-1 capsid protein p24 is present in abundance during the early stages of HIV-1 infection and detectable during days 15–50 post infection, thus making it a suitable target for the diagnosis of early-stage infection. Sensitive fourth-generation serological tests utilize the detection of both antibodies and HIV-1 p24, thereby narrowing the window period for diagnosis compared to antibody-only testing.

The WHO International Reference Reagent for HIV-1 p24 antigen (NIBSC code 90/636) was established in 1992 to support the development and standardization of p24 assays. In 2009, the revised European Union common technical specifications for in vitro diagnostic medical devices required HIV-1 p24 assays to have a limit of detection of 2 IU/mL based on calibration against the WHO international reference reagent. In light of dwindling stocks of the
WHO international reference reagent, a proposal to develop a First WHO International Standard for HIV-1 p24 antigen had been endorsed by the Committee in October 2022.

Prior to this proposal, and due to the scarcity of high-titre HIV-1 stock materials, a pilot study had been conducted to evaluate both a commercially sourced recombinant p24 protein and an in-house preparation of virus-like particles (VLPs) as alternative source materials. Although the recombinant p24 protein had been found to be suitable for most assay systems, it was of an unknown subtype and exhibited some discrepancy on Abbot platforms. The VLPs had produced more consistent results in the study, appeared to be very stable and would be easy to replace. The Committee had therefore agreed that the VLP material would be suitable for further evaluation as a candidate material. It had also been decided to formulate the proposed WHO international standard at a lower concentration as the international reference reagent needed to be highly diluted to evaluate current assays within their dynamic range.

An international collaborative study involving 15 laboratories in seven countries had been conducted to evaluate the suitability of the candidate material (NIBSC code 22/230) and to calibrate it against the current WHO international reference reagent using a range of commercial qualitative HIV-1/2 fourth-generation immunoassay systems. The candidate material was suitably diluted in pooled negative plasma and lyophilized, with post-fill testing indicating that oxygen content and residual moisture levels were within acceptable ranges. Stability testing had been conducted at four timepoints (1, 3, 6 and 12 months), with calculations based on the Arrhenius equation predicting a loss of 0.083% per year when stored at −20°C. Using a wide range of different methods (including 3 prototype assays), the candidate material had been assessed as part of a blinded panel of study samples which had also included the international reference reagent, a liquid preparation of the candidate material, a recombinant p24 protein preparation prepared from the same material used in the pilot study, a clinical HIV-1 antigen- and antibody-positive sample identified as subtype B, and two clinical HIV-1 antigen-positive window period samples identified as subtype C. All samples were tested in duplicate on three occasions.

A total of 35 datasets were produced, with low intra-laboratory variability indicating good precision for all of the assay types used. Potency estimates for the candidate material were calculated relative to the international reference reagent. Following exclusion of a number of outlier results and of the results obtained using the not yet established prototype assays, a potency of 44.1 IU/ampoule was established for the candidate material using a robust mean rather than geometric mean to remove bias, and with results from laboratories using the same assay combined to remove any over-representation of specific assay systems. Assessment of the effect of lyophilization on the candidate material was assessed by evaluating
its relative potency against the liquid preparation, with currently unexplained variations in estimated potency observed. Although a significant degree of non-commutability was observed for the candidate material, the level observed was equivalent to that of the current international reference reagent. The implications of the revised common technical specifications for in vitro diagnostic medical devices for manufacturers were also investigated by calculating the approximate limit of detection for all laboratories and methods. Assuming a value of 44 IU/ampoule for the candidate material, all assays produced values ranging from 0.14 IU to 1.99 IU with the exception of the Abbott Determine HIV Early Detect assay.

The Committee expressed concern regarding the performance of the Abbott assay and noted that although not marketed within the European Union, it would still be marketed in other countries, and hence should be considered a problem. Further noting that only subtypes B and C had been used for the study, and that other subtypes might be more prevalent in some parts of the world, the Committee was informed that this was because the WHO international reference reagent was subtype B, while the inclusion of subtype C (the circulating variant) had previously been recommended. It was also clarified that panels of p24 clades have been produced and can be used to assess the performance of assays against different subtypes. Recognizing the significant challenges involved in obtaining window period samples for this project, the Committee offered its support with regard to the sourcing of such donations in future projects.

Having considered the report of the study (WHO/BS/2024.2470), the Committee recommended that candidate material 22/230 be established as the First WHO International Standard for HIV-1 p24 antigen with an assigned unitage of 44 IU/ampoule.

6.2 Proposed new projects and updates – in vitro diagnostics

6.2.1 Proposed First WHO International Standard for carcinoembryonic antigen

Carcinoembryonic antigen (CEA) is a glycoprotein found at low levels (< 5 ng/mL) in the blood of healthy adults. CEA levels are raised in some cancers (including bowel, lung, breast and pancreatic cancer), as well as during liver disease and inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis. Raised CEA levels may also be predictive of metastatic disease in some cancers, such as breast and colon cancers. CEA levels are measured using serum/plasma-based immunoassays which are widely used as a diagnostic tool, and for monitoring disease progression and response to treatment.

A WHO international reference preparation (NIBSC code 73/601) had been prepared in 1976 using an extract of native CEA purified from liver cancer tissue and formulated in 0.5% lactose, and was used predominantly to calibrate test kits measuring CEA in patient samples. As stocks of this preparation were
now running low, and are expected to be exhausted by 2025, it was proposed that a WHO international standard be developed to replace the current international reference preparation. The proposed international standard would be prepared using a native material and formulation similar to that used for the current international reference preparation.

As CEA contains a mixture of different carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), proteomic analysis of both the current international reference preparation and trial-filled candidate materials had been conducted to determine any impact of variable CEACAM distribution on continuity. The analysis had demonstrated CEACAM5 to be present in highest abundance, with some diversity noted in other less abundant CEACAMs. As CEACAM5 is known to be the most prevalent and clinically relevant protein targeted by commercial assays, it was concluded that any slight difference in CEACAM distribution would not affect continuity. It was also proposed, following discussion with kit manufacturers, to formulate the international standard using 2 µg of material (compared to the 10 µg used in the current international reference preparation) as this would be sufficient to cover the dynamic ranges of current assays and would reduce waste. Furthermore, by using isotope dilution mass spectrometry it may now be possible to assign SI units to the international standard. This would help to address an observed issue of uptake of the international reference preparation (calibrated in IU) since most CEA immunoassays report results in ng/mL.

An international collaborative study involving 10–12 test kit manufacturers and clinical laboratories performing CEA assays would be conducted to evaluate the suitability of the candidate material. It was anticipated that the results of the collaborative study would be submitted for consideration by the Committee in 2025.

Noting that the current international reference preparation had remained in use for 48 years, the Committee reflected that this had been due to a combination of a large initial batch size and lower uptake rather than the use of competing standards. The issue of low adoption would hopefully be addressed by the use of SI units in the proposed international standard. The Committee also considered the risk of discontinuity between the existing international reference preparation (expressed in IU/mL) and the proposed international standard (expressed in ng/mL). This was acknowledged to be an important issue that would be taken into consideration during the proposed collaborative study. The Committee was further reassured that a collaborative project currently under way with the International Federation of Clinical Chemists on tumour marker harmonization (including CEA) would provide an opportunity to discuss any potential impact of this shift with clinicians and assay manufacturers.

Reflecting on the potential impact of variable CEACAM distribution, the Committee opined that the candidate material should ideally be obtained from the same type of tissue and be relevant to the diagnostic target of the immunoassays.
Clarification was given that the international reference preparation had been derived from a liver biopsy of a metastatic tumour caused by colon cancer, and that efforts would be made to ensure that a similar type of material was sourced. While acknowledging that further detailed CEACAM analysis would not be feasible or materially change the overall concept, the Committee recommended that proposals for such future standards should underscore the need to obtain source material from similar types of tissue to ensure continuity and relevance.

The Committee endorsed the proposal (WHO/BS/2024.2472) to develop a First WHO International Standard for carcinoembryonic antigen.

6.2.2 Proposed First WHO International Standard for antibodies to Junin virus

Junin virus (JUNV) is the etiological agent of Argentine haemorrhagic fever, which causes significant morbidity and has a mortality rate of 15–30%. The virus is spread through a rodent reservoir that is so far confined to Argentina. Although only 10–15 cases have been reported in recent years, approximately 5 million people are considered to be at risk and live within the endemic area in which the rodent reservoir is present. Post-exposure treatment options are limited to the transfusion of immune plasma and the off-label use of ribavirin and favipiravir. A live attenuated vaccine (Candid#1) has been licensed in Argentina since 1992 but its use is limited to adult populations at risk due to concerns regarding the stability of the attenuated phenotype. Several alternative vaccine platforms are now in preclinical development.

JUNV is a member of the family Arenaviridae – which is divided into New World viruses (such as JUNV) and Old World viruses (such as LASV). Global pandemic preparedness efforts are moving towards an approach based on identifying prototype pathogens that could represent viruses within a whole taxonomic group, rather than focusing on individual priority pathogens. In this regard, LASV has been identified as a prototype pathogen for Old World arenaviruses, with JUNV a likely suitable prototype pathogen for New World arenaviruses.

Given the expected development of JUNV vaccines, therapeutics and diagnostics, assay standardization and calibration will be vital for the accurate evaluation and regulatory approval of such products. In-house ELISA, immunofluorescence assays and neutralization assays are currently used to detect antibodies to JUNV. The availability of a WHO international standard for use by JUNV vaccine manufacturers, NCLs and other public health laboratories, therapeutic antibody producers, assay kit manufacturers and research laboratories would support the further development and standardization of such assays, align with the prototype pathogen approach and complement the First WHO International Standard for anti-Lassa virus immunoglobulin G established on the advice of the Committee in 2021.
The candidate material would be prepared from human plasma or serum obtained from convalescent individuals and Candid#1 vaccine recipients. Following screening for JUNV and other bloodborne viruses, the material would undergo virus inactivation treatment in accordance with protocols developed by the Advisory Committee on Dangerous Pathogens to ensure safety during broader distribution. The Committee was informed that financial and practical support in sourcing and developing the candidate material would be provided by CEPI, with support in securing and characterizing source materials also being provided by the National Institute of Human Viral Diseases in Argentina.

An international collaborative study involving 15–20 laboratories would then be conducted to evaluate the performance of the candidate material, assign unitage and assess commutability. Participants would include NCLs, vaccine manufacturers, and clinical and academic laboratories performing a range of serological assays for the detection of JUNV antibodies. Potential difficulties were anticipated in identifying such laboratories across all WHO regions given the highly geographically restricted circulation of JUNV. It was provisionally anticipated that the proposed collaborative study would be conducted in early 2025, with the results submitted for consideration by the Committee in October 2025.

Recognizing that little was known regarding serological cross-reactivity between Old World and New World arenaviruses, the Committee encouraged further investigation into this aspect. In response to a query regarding the use of pseudovirus or wild-type virus during the project, the Committee was informed that only pseudovirus systems could be used for neutralization tests in the custodian laboratory but that National Institute of Human Viral Diseases laboratories were using a test based on wild-type virus, thus allowing for the use of both systems during screening and characterization of the candidate material.

Acknowledging the need for this reference material, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a First WHO International Standard for antibodies to Junin virus.

7.1 WHO international reference standards for use in high-throughput sequencing technologies

7.1.1 First WHO International Reference Panel for adventitious virus detection in biological products by high-throughput sequencing

HTS technologies have the ability to detect both known and novel adventitious viruses in biological products. As an alternative to conventional adventitious virus detection, which relies on in vivo animal testing and in vitro cell culture assays or on polymerase chain reaction (PCR) assays, HTS has the potential to expand the breadth of virus detection while also significantly shortening the time required for the quality control testing of biological products. Importantly, the increasing implementation of HTS for this purpose aligns with, and provides support for, the shift towards reducing the use of animals in such testing. Encouraged by regulatory guidance worldwide, the need for international viral standards for HTS process qualification and validation to support the application of HTS to biological product virus safety testing has long been recognized.

An international collaborative study involving nine laboratories in six countries had therefore been conducted to evaluate the suitability of seven viruses representing diverse virus families for use as a potential WHO international reference panel for adventitious virus detection in biological products by HTS. The following viruses had been selected based on their distinct physicochemical and genome properties:

- human betacoronavirus OC43 (hCoV)
- porcine circovirus type 1 (PCV1)
- mammalian orthoreovirus type 1 (REO)
- feline leukemia virus (FeLV)
- Epstein-Barr virus (EBV)
- human respiratory syncytial virus (RSV)
- minute virus of mice (MVM).

The viruses were spiked together at different spike levels but all laboratories included testing at $10^4$ genome copies/mL of each virus into $10^5$ genome copies/mL of adenovirus 5 to evaluate the breadth of virus detection using HTS in a high-titre virus background mimicking a low-complexity biological sample with reduced host cellular content – for example, a viral vaccine seed or virus vector preparation. Participating laboratories followed a common protocol for preparing the spiked samples and then used their own HTS workflow protocols.
All laboratories returning data detected the seven viruses between $10^4$ and $10^5$ genome copies/mL. Differences observed in virus detection between laboratories reflected the different protocols used in the HTS workflow.

The Committee commended this excellent study and noted its timeliness in the context of ongoing work to develop WHO guidance on the implementation of 3Rs principles when testing biological products – which included the recent establishment of a WHO working group on the implementation of 3Rs principles. Further commenting on the related need for written guidance and training on the application of HTS in ensuring the quality and safety of such products, the Committee noted that several conferences on the application of HTS technologies in biological product testing had been held by the International Alliance for Biological Standardization.

Noting the urgent need for this reference panel and its relevance in ensuring the safety of cell and gene therapy products, the Committee enquired as to the anticipated demand and the likely effects of this on supplies. The Committee was assured that the 1000 panels prepared should be sufficient for about 5 years, with the expectation that panel titres would be sufficiently high to last an individual organization for at least 1 year.

Enquiring about the potential impact of endogenous viruses in the cell lines used to prepare the virus stocks, the Committee was informed that although two retroviruses had been detected this did not affect the performance of the panel. Similarly, contamination of the panel with residual host cell DNA did not contribute significantly to the spike levels given the high titre of panel members. However, the level of contamination with host cell DNA would be recorded in the certificate of analysis.

Recognizing the importance of such a panel in helping to understand the impact of bioinformatics on the sensitivity of HTS for detecting extraneous agents, the Committee was informed that while the data to support establishment of the panel had been based on targeted bioinformatics, collaborative study participants had also returned data using non-targeted bioinformatics to reflect the real-life situation, and these data would be published separately in the scientific press. Reflecting on the prospect of HTS replacing in vivo and other in vitro tests for the detection of adventitious viruses, the Committee concluded that although it was impossible to generalize for all viruses, HTS was typically proving to be more sensitive than animal-based methods and was not associated with the drawbacks of PCR-based testing, such as mismatched primers. However, even though The European Pharmacopoeia indicates that HTS may be used as a replacement method, it would ultimately be the responsibility of the NRA to consider the risks associated with specific products.

Having considered the report of the study (WHO/BS/2024.2471), the Committee recommended that the panel of seven viruses be established as the
First WHO International Reference Panel for adventitious virus detection in biological products by high-throughput sequencing, with the following assigned unitages:

- CBER-FSCUST-90 (hCoV) \(2.6 \times 10^{10}\) genome copies/mL
- CBER-FSCUST-91 (PCV1) \(8.1 \times 10^{9}\) genome copies/mL
- CBER-FSCUST-92 (REO) \(1.5 \times 10^{10}\) genome copies/mL
- CBER-FSCUST-93 (FeLV) \(4.0 \times 10^{10}\) genome copies/mL
- CBER-FSCUST-94 (EBV) \(2.8 \times 10^{7}\) genome copies/mL
- CBER-FSCUST-95 (RSV) \(5.5 \times 10^{10}\) genome copies/mL
- CBER-FSCUST-96 (MVM) \(1.2 \times 10^{10}\) genome copies/mL.

The Committee further recommended that a set of five individual virus preparations established in 2020 as WHO international reference reagents for adventitious virus detection in biological products by HTS now be discontinued in line with the proposed succession plan. These valuable resources should now be solely used as CBER research reagents to support HTS development by new users, with a clear indication that these reagents no longer have the status of WHO international reference standards.
8. International reference materials – vaccines and related substances

8.1 WHO international reference standards for vaccines and related substances

8.1.1 WHO International Reference Reagent for diphtheria antitoxin for use in flocculation test (equine)

Diphtheria toxoid is typically administered in combination with tetanus and pertussis as DTP vaccines, and is also a carrier protein in several glycoconjugate vaccines. Reliable evaluation of the concentration and quality of diphtheria toxoid in combination vaccines depends upon the accuracy and robustness of the routinely used flocculation test which requires the use of hyperimmune equine diphtheria antitoxin as a critical reagent. The equine diphtheria antitoxin used in the flocculation test is produced from hyperimmune horse serum, with a lyophilized preparation of hyperimmune equine antitoxin (NIBSC code 63/007) previously available as a non-WHO reference material. However, this reagent had been widely used and stocks were now completely depleted. In 2023, the Committee had therefore endorsed a proposal to develop a First WHO International Reference Reagent for diphtheria antitoxin for use in flocculation test (equine).

The Committee was informed that a purified diphtheria equine immunoglobulin preparation produced from a pool of equine serum had been filled and lyophilized in ampoules to produce the candidate material (NIBSC code 23/102). The candidate material had an estimated diphtheria antitoxin potency of 1032 IU/ampoule as assessed by toxin neutralization assay. As the proposed international reference material would be used as a reagent (not a calibrant) and as only a single method was involved, characterization of the candidate material was undertaken in a small collaborative study involving seven laboratories in five countries. Study participants calibrated the candidate material against the Third WHO International Standard for diphtheria toxoid for use in flocculation test (NIBSC code 13/212) using the Ramon flocculation method.

Study results (expressed in Lf-eq/mL against 13/212) gave an overall geometric mean value of 956 Lf-eq/mL for the candidate material (range 890–1032 Lf-eq/mL), with low intra- and inter-laboratory geometric coefficients of variation observed. Initial data from an accelerated thermal degradation study indicated that the candidate material would have good long-term stability, with further testing to be performed. The 1831 ampoules produced would likely be sufficient for at least 6–7 years.

Discussing the differences in results that had been noted between different laboratories, the Committee was informed that this was most likely attributable to the subjectivity of the flocculation test and difficulty in seeing the end-point. The Committee also noted that sourcing sufficient material for
this type of standard was challenging and advised that sufficient time be allowed when sourcing future replacements.

Having considered the report of the study (WHO/BS/2024.2469), the Committee recommended that candidate material 23/102 be established as the WHO International Reference Reagent for diphtheria antitoxin for use in flocculation test (equine) with no assigned unitage.

8.2 Proposed new projects and updates – vaccines and related substances

8.2.1 Proposed Fifth WHO International Standard for diphtheria toxoid (adsorbed)

Diphtheria toxoid-containing vaccines are among the most commonly used and successful human vaccines, with more than 30 different products approved worldwide. As well as being an essential part of the primary immunization programme for children, these vaccines are also used to boost immunity in adolescents and adults. Ensuring the continuing supply of effective vaccines depends on confirmation of their potency against a reference standard calibrated in IU.

Following their establishment in 2009, both the Fourth WHO International Standard for diphtheria toxoid (adsorbed) and European Pharmacopoeia biological reference preparation (BRP batch 4) for diphtheria toxoid have been widely used for potency testing by vaccine manufacturers and control laboratories. As stocks of both materials were now low, replacement materials were required. It was therefore proposed that an international collaborative study involving laboratories in several WHO regions be conducted to calibrate replacement WHO and European Pharmacopoeia standards in IU. Two candidate materials would be prepared from adsorbed diphtheria toxoids provided by vaccine manufacturers and evaluated in a study similar to that used to calibrate the existing standards. It was anticipated that the proposed collaborative study would be conducted in early 2025 and its results submitted for consideration by the Committee in October 2025.

The Committee agreed that this was a straightforward and well-designed project to replace the existing standards and endorsed the proposal (WHO/BS/2024.2472) to develop a Fifth WHO International Standard for diphtheria toxoid (adsorbed).

8.2.2 Proposed Second WHO International Standard for Vi polysaccharide of S. Typhi

Typhoid fever is caused by infection with Salmonella enterica subspecies enterica serovar Typhi (S. Typhi) expressing a Vi polysaccharide capsule, which is both a virulence factor and protective antigen. As immunization is the most cost-effective
preventative strategy for controlling typhoid, especially in areas where multidrug-resistant strains are endemic, licensed polysaccharide and glycoconjugate vaccines are widely used in at-risk populations. The quality, safety and potency of such vaccines are principally assessed using physicochemical methods to ensure batch-to-batch consistency, with the WHO international standard being used to calibrate secondary reference materials used in the different methods to quantify the Vi polysaccharide content of bulk intermediates and final formulations. The First WHO International Standard for Vi polysaccharide of S. Typhi (NIBSC code 16/126) had been established in 2017 and due to low stocks a replacement will be needed within 3 years.

A working freeze-dried material (previously filled under NIBSC code 17/260) had been identified as a suitable replacement, with sufficient ampoules available to last for more than 10 years at the current rate of use. The candidate material would be assessed for its suitability in an international collaborative study involving approximately 20 laboratories. Based on the study results, polysaccharide content would be assigned in milligrams using quantitative nuclear magnetic resonance spectroscopy as an accepted primary method for quantifying organic compounds in mass units. However, other assays would also be included to assess the suitability of the candidate material across a range of test methods. It was anticipated that the proposed collaborative study would be conducted in 2024 and its results submitted for consideration by the Committee in 2025.

Having been assured of the stability of the candidate Vi polysaccharide preparation, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a Second WHO International Standard for Vi polysaccharide of S. Typhi.

8.2.3 Proposed First WHO International Standard for antibodies to group A streptococcus antigens (human serum)

Infection with group A streptococcus (GAS) is associated with a range of diseases from mild pharyngitis to severe necrotizing fasciitis, as well as autoimmune conditions such as rheumatic heart disease. Despite a high burden of disease worldwide, GAS vaccine development has historically been hampered by a number of scientific, regulatory and commercial challenges. Following concerted global efforts to prioritize GAS vaccine development, particularly to reduce the global burden of rheumatic heart disease, a number of candidate vaccines based on M proteins and non-M protein antigens were now in preclinical or clinical development.

The development of an international reference standard for antibodies to GAS antigens will be an important step in standardizing the assays used to compare antibody responses to prospective GAS vaccines, developing correlates of protection and supporting the epidemiological surveillance studies that will
underpin vaccine implementation programmes. Such a standard may also be used to standardize assays developed for the serodiagnosis of GAS infection. A multi-centre international collaborative study was therefore being proposed to develop a WHO international standard for antibodies to group A streptococcus antigens using pooled serum obtained from either vaccinees or convalescent individuals. A multiplex immunoassay measuring antibodies against target GAS antigens would be used to assess the suitability and stability of the candidate materials. It was anticipated that the proposed collaborative study would be conducted in late 2025 and its results submitted for consideration by the Committee in 2026.

Recognizing the challenge of obtaining serum from convalescent or vaccinated subjects with reactivity to the relevant antigens, the Committee was assured that this could be achieved by screening individual donations and pooling only those exhibiting appropriate reactivities. Given the expected timing of clinical trials, the project would likely begin by screening convalescent serum donations. The Committee went on to query the current status of functional assays and the potential impact of this on the development of the proposed reference standard. Clarification was given that most advanced such assays were currently based on antigen-binding platforms, which would therefore guide the evaluation of the prospective standard.

Acknowledging the timeliness of this project, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a First WHO International Standard for antibodies to group A streptococcus antigens (human serum).

8.2.4 Proposed First WHO International Standard for antibodies to vaccinia virus

Vaccinia virus (VACV) is a member of the Orthopoxvirus genus that includes variola, cowpox and Mpox viruses. Antigenic similarity between these viruses confers cross-reactivity and preclinical studies have shown that VACV vaccines also provide a level of immunity against Mpox and are efficacious in reducing Mpox disease severity. This led to the use of VACV-based vaccines for the control of Mpox during the public health emergency of international concern declared in 2022. Although this emergency had now been declared over, Mpox remains a significant burden in endemic countries, with three licensed VACV vaccines currently available and others under development.

Recognizing the need for serological assays to measure vaccine-induced immune responses, evaluate new treatments and monitor disease epidemiology, a proposal to develop a WHO international standard for antibodies to Mpox virus to support assay development and harmonization had been endorsed by the Committee in March 2023. During preparations for the collaborative study to evaluate the candidate materials, it was recognized that several prospective participants had developed assays capable of distinguishing between the antibody
responses to Mpox virus and VACV. Differentiating these responses in both vaccinated and infected individuals would be important in understanding the immune responses to vaccination versus infection, interpreting seroprevalence studies and conducting clinical trials for new vaccines.

The Committee was informed that a candidate material (NIBSC code 05/124) had been prepared in 2005 from pooled defibrinated human plasma obtained from volunteers vaccinated with the New York City Board of Health strain of VACV, and then freeze-dried and stored. This material had subsequently been evaluated in a 2010 collaborative study and proposed for establishment as a WHO international standard to replace the previous standard for smallpox virus antibodies. However, due to the limited number of assays used in the study, and the differential performance of the candidate material compared with the anti-smallpox serum, its establishment had not been supported. The committee was informed that approximately 2600 ampoules were still available and although originally intended for inclusion as one of the samples in the collaborative study for the Mpox virus antibody standard, it was now proposed to instead evaluate candidate material 05/124 in parallel for its suitability as a separate WHO international standard for antibodies to VACV given the ability of some participant laboratories to distinguish antibody responses to the two viruses.

The Committee was further informed that 16 collaborative study participants had now been recruited and would analyse the candidate materials using both neutralization and binding antibody detection methods. Although the possibility of cross-reactivity between the different orthopoxviruses may complicate the interpretation of the collaborative study results, it was nevertheless anticipated that the study outcomes would be submitted for consideration by the Committee in early 2025.

After due consideration, the Committee endorsed the proposal (WHO/BS/2024.2472) to develop a First WHO International Standard for antibodies to vaccinia virus.

8.3 Proposed discontinuations and updates – vaccines and related substances

8.3.1 Proposal not to proceed with the development of a First WHO International Standard for antibodies to influenza A(H7N9) virus

In 2014, a proposal to develop a First WHO International Standard for antibodies to influenza A(H7N9) virus was endorsed by the Committee at a time when this virus was causing severe respiratory disease in humans in China. Since 2018, changes in zoonotic influenza epidemiology have meant that pandemic preparedness efforts were now focused on other influenza subtypes and the Committee was informed that it would not now be possible to source the serum-
positive samples needed to prepare the candidate material. In addition, given the
dramatic decline in the number of human infections, and successful control of the
disease in poultry through immunization efforts, the need for this international
standard no longer existed.

While noting the potential value of such a reference material for
pre-pandemic preparedness for the re-emergence of influenza A(H7N9), the
Committee was nevertheless confident that in the event of such re-emergence
that a reference standard could be prepared quickly as there would then be
a ready supply of convalescent serum. Recognizing the challenge in sourcing
material at the current time, the Committee agreed that a final decision on
whether or not to proceed with the development of a First WHO International
Standard for antibodies to influenza A(H7N9) virus would be taken at its
meeting in October 2024.
Annex 1

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

WHO Recommendations, Guidelines and other documents in the field of biological product development and standardization are intended to be scientific and advisory in nature. Each of these documents provides guidance for national regulatory authorities (NRAs), developers and manufacturers of biological products, and others who may have to decide upon appropriate methods for ensuring that such products are safe, reliable and potent. In the case of WHO Recommendations and Guidelines, the guidance provided may, if an NRA so desires, be adopted as definitive national requirements or used as the basis of such requirements.

WHO Recommendations, Guidelines and other guidance documents for biological products are formulated by international groups of experts, and are adopted on the recommendation of the WHO Expert Committee on Biological Standardization. Following adoption, the documents are published in the WHO Technical Report Series⁴ as part of the respective full report of the Committee, and as listed in this annex. The full reports of the Committee are freely available for download at https://iris.who.int/. Hard copies of the reports can also be purchased from:

WHO Press
World Health Organization
20 avenue Appia, 1211 Geneva 27
Switzerland
Email: bookorders@who.int
Website: www.who.int/bookorders

In all cases in which a previous version of a WHO Recommendations, Guidelines or other guidance document has been revised and superseded by an updated document, it is of paramount importance that only the latest version of the document is used. All documents listed in this annex are current, with no previous versions shown. The annex has also been arranged alphabetically either by product type or regulatory topic to facilitate easy identification of the most up-to-date document.

⁴ Abbreviated in the following pages to “TRS”.
Important note on the immediate discontinuation of the innocuity test

At its sixty-ninth meeting in 2018, the Committee recommended the immediate discontinuation of any requirement to perform the innocuity test (also known as the abnormal toxicity test or general safety test) in all future WHO Recommendations, Guidelines and other guidance documents for biological products published in the Technical Report Series. The Committee further recommended that any mention of this test in any still current WHO document published prior to 2018 be disregarded (Table 1).

These recommendations represent a significant step towards increasingly science-based regulation and regulatory convergence at the global level, while also promoting the application of the 3Rs principles (Replacement, Refinement, Reduction) by reducing the use of animals in biological product quality control and lot release testing.

Table 1
List of current WHO documents for biological products published prior to 2018 in which any mention of the innocuity test (also known as the abnormal toxicity test or general safety test) should be disregarded

<table>
<thead>
<tr>
<th>Product</th>
<th>WHO document</th>
<th>Name of test as it appears in document</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue fever vaccines (live, attenuated)</td>
<td>Annex 2: TRS 979</td>
<td>General safety</td>
</tr>
<tr>
<td>Diphtheria vaccines (adsorbed)</td>
<td>Annex 4: TRS 980</td>
<td>Innocuity</td>
</tr>
<tr>
<td>DT-based combined vaccines</td>
<td>Annex 6: TRS 980</td>
<td>[Control of final product]</td>
</tr>
<tr>
<td>Ebola vaccines</td>
<td>Annex 2: TRS 1011</td>
<td>General safety (innocuity)</td>
</tr>
<tr>
<td>Hepatitis A vaccines (inactivated)</td>
<td>Annex 2: TRS 858</td>
<td>General safety</td>
</tr>
<tr>
<td>Hepatitis B vaccines (recombinant)</td>
<td>Annex 4: TRS 978</td>
<td>General safety (innocuity)</td>
</tr>
<tr>
<td>HFRS vaccines (inactivated)</td>
<td>Annex 2: TRS 848</td>
<td>General safety</td>
</tr>
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<td><em>Haemophilus influenzae</em> type b conjugate vaccines</td>
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<tr>
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<td>Vaccines (stability evaluation of)</td>
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<tr>
<td>Varicella vaccine (live)</td>
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<tr>
<td>Yellow fever vaccines (live, attenuated)</td>
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DT = diphtheria and tetanus; HFRS = haemorrhagic fever with renal syndrome; VLP = virus-like particle; MMR = measles, mumps and rubella.
List of all current WHO documents for biological products

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<td>Revised 2018, TRS 1016 (2019); Amendment 2020, TRS 1028 (2021)</td>
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<td>Tetanus vaccines (adsorbed)</td>
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<td>Vaccines: changes to approved vaccines; procedures and data requirements</td>
<td>Adopted 2014, TRS 993 (2015)</td>
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# Annex 2

Nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of COVID-19

Addendum to Annex 2 of WHO Technical Report Series, No.1048

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

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<td>ACE2</td>
<td>angiotensin-converting enzyme 2 (receptor)</td>
</tr>
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<td>ADA</td>
<td>anti-drug antibody</td>
</tr>
<tr>
<td>ADE</td>
<td>antibody-dependent enhancement (of disease)</td>
</tr>
<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>AESI</td>
<td>adverse event of special interest</td>
</tr>
<tr>
<td>COVID-19</td>
<td>coronavirus disease 2019</td>
</tr>
<tr>
<td>Fc</td>
<td>fragment crystallizable (region)</td>
</tr>
<tr>
<td>FcγR</td>
<td>Fc gamma receptor</td>
</tr>
<tr>
<td>GMT</td>
<td>geometric mean titre</td>
</tr>
<tr>
<td>IMP</td>
<td>investigational medicinal product</td>
</tr>
<tr>
<td>MAAE</td>
<td>medically attended adverse event</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>PD</td>
<td>pharmacodynamics</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetics</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
</tr>
<tr>
<td>RBD</td>
<td>receptor binding domain</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription-polymerase chain reaction</td>
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<tr>
<td>S</td>
<td>spike (glycoprotein)</td>
</tr>
<tr>
<td>SAE</td>
<td>serious adverse event</td>
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<tr>
<td>SARS-CoV-2</td>
<td>severe acute respiratory syndrome coronavirus 2</td>
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1. Introduction

Evaluating the safety and efficacy of monoclonal antibodies (mAbs) and related products intended for the prevention or treatment of infectious diseases requires different considerations than mAb products that target endogenous proteins, such as those intended for the treatment of noncommunicable diseases. To help address such differences, the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases (1) was adopted in 2023 on the recommendation of the WHO Expert Committee on Biological Standardization. These Guidelines outline the general principles applicable to the evaluation of mAbs for use against infectious diseases. However, although the document provides guidance on evaluating the safety and efficacy of mAb products regardless of the targeted pathogen, it was recognized that pathogen-specific considerations would potentially affect the interpretation and application of the guidance provided.

2. Purpose and scope

The current addendum is intended to provide supplementary considerations when evaluating the safety and efficacy of mAb products directed specifically against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens. This includes mAb products intended for pre- and post-exposure prophylaxis as well as for the treatment of coronavirus disease 2019 (COVID-19). These considerations are applicable to mAbs and related products, including single and co-formulated mAbs against SARS-CoV-2. However, some mAb products (for example, bispecific mAbs or those with a different mechanism of action) may require additional nonclinical studies and the NRA should be consulted on the need for such studies. Unless otherwise indicated, the guidance applies to products that are administered parenterally.

It should be noted that mAbs and related products that target endogenous human antigens (for example, those which block the angiotensin-converting enzyme 2 (ACE2) receptor or cytokines) are not within the scope of this addendum as these require different considerations for evaluating their safety and efficacy.

Separate and detailed guidance on the production and quality control of mAbs is provided in the WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use (2).
3. Terminology

The following definitions apply to the terms as used in this addendum. These terms may have different meanings in other contexts. It should be noted that additional terms relevant to this addendum are defined in the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases (1).

**COVID-19**: the disease caused by infection with SARS-CoV-2.

**Long COVID**: health problems that persist or develop after infection with SARS-CoV-2, and which may last from several weeks to years.

**SARS-CoV-2**: the virus that causes COVID-19.

**Variant**: a virus that possesses mutations which may confer altered transmissibility, receptor binding affinity, virulence, morbidity or mortality. A number of SARS-CoV-2 variants have been labelled as “variants of interest” (VOI), “variants of concern” (VOC) or variants under monitoring (VUM) depending on their emerging dominance among actively circulating strains.

4. General considerations

SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus belonging to the genus *Betacoronavirus*. The virus first emerged in Wuhan, China in 2019, with sustained human-to-human transmission confirmed shortly afterwards, followed by its rapid spread worldwide. Evidence of sustained global transmission led WHO to declare COVID-19 a pandemic in March 2020. COVID-19 was subsequently officially declared to no longer be a public health emergency of international concern by WHO on 5 May 2023. Nevertheless, the disease remains a major threat, with SARS-CoV-2 still in circulation in most regions of the world.

SARS-CoV-2 is transmitted primarily by the respiratory route producing a mucosal infection after a short incubation period that results in a range of disease symptoms and outcomes – from asymptomatic to severe disease leading to hospitalization and death, as well as long COVID in some cases (3–7). Moreover, there is a possibility that SARS-CoV-2 will become endemic and will continue to cause substantial levels of hospitalization and death due to the emergence of new variants. This is of particular concern among vulnerable groups such as the immunocompromised and those with underlying comorbidities (8, 9).

Monoclonal antibodies, vaccines and other therapeutics against SARS-CoV-2 were developed rapidly and authorized for use, initially under emergency procedures. These early mAbs (10–12) and other products were all based on the genetic sequence of the ancestral Wuhan strain and provided protection against severe disease, hospitalization and death. However, to date, no correlates of protection or threshold of protection have been established and
challenges remain in directly comparing the neutralizing antibody activity of different products (13). It is clear that some of these early products now exhibit reduced virus neutralization activity against SARS-CoV-2 variants, and a number have been withdrawn from use, especially in regions with high levels of Omicron circulation (10, 11, 14, 15). Although most early authorized mAbs have to varying degrees lost their ability to neutralize variant strains, this finding is primarily based on in vitro testing and does not necessarily reflect clinical experience (10, 13). Protection against COVID-19 was initially provided both through SARS-CoV-2 neutralizing antibodies and through the induction of broader cellular immunity following infection and/or vaccination (16). To date, although SARS-CoV-2 variants have evolved to evade neutralizing antibodies, with consequences for infection, virus shedding and transmission, these have not significantly affected the longer lasting and broader cellular immune responses (10, 11, 16).

Nevertheless, it is clear that the emergence of variant strains of SARS-CoV-2 poses a major challenge to product development and evaluation of efficacy in clinical use (9, 17–19). In response to this challenge, considerable efforts are now under way to develop SARS-CoV-2 mAbs and vaccines that are escape resistant (10, 11, 15, 20). For example, progress in B-cell technologies has accelerated the identification and rapid isolation of candidate antibodies which can overcome variants arising through antigenic shift (10, 11, 21).

The principal target of both mAb and vaccine development has been the virus trimeric transmembrane spike (S) glycoprotein which protrudes from the virus surface and mediates its entry into host cells by binding to the ACE2 receptor (10, 22). Entry requires cleavage of the S transmembrane glycoprotein generating S1 and S2 subunits to initiate fusion of the viral and cell membranes upon entry. The SARS-CoV-2 S glycoprotein contains a furin-like cleavage site for host cell proteases (23). Neutralizing mAbs targeting the receptor binding domain (RBD) of the S glycoprotein, the S2 subunit or the S1/S2 proteolytic cleavage site have been variously affected by the emergence of SARS-CoV-2 variants (10, 11, 14, 20, 24, 25). To expedite the development of new mAbs, consideration is being given by some but not all regulatory authorities to immunobridging studies in support of licensure (see section 7.4.2 below). However, it is important that such an approach be discussed directly with the relevant NRA.

Clearly, the ongoing evolution of SARS-CoV-2 requires continuous monitoring for significant changes in local circulating variant strains which might impact the performance of mAbs, both in clinical studies and in use (26). Similarly, careful attention needs to be given to the virus strains used in nonclinical and clinical evaluation studies to ensure that the virus preparation used is well characterized and standardized with respect to variant strains (27).

Although the emergence of resistant variants of SARS-CoV-2 is an issue of concern with regard to efficacy, no major safety signals have been identified regarding the use of mAbs to prevent or treat COVID-19. However, the potential
for antibody-dependent enhancement (ADE) of disease is always a possibility, and is an important aspect to consider as part of nonclinical and clinical evaluation programmes (28, 29). The complexities of assessing and predicting mAb-induced clinical ADE of disease in humans, including the poor predictability of both in vitro systems and animal models, are discussed in detail elsewhere (28). Particular attention should be given to the effects of any engineered modifications of the fragment crystallizable (Fc)-mediated effector functions of mAbs that may, for example, have been made to increase the half-life of the antibody (10, 11).

5. International reference materials

WHO international reference standards are the primary reference materials used worldwide and such standards are available for SARS-CoV-2 antibodies to support the development of serological assays and to increase the comparability of results obtained by different laboratories. The WHO international reference standards related to SARS-CoV-2 antibodies available at the time of publication of the current document are:

- Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin (30);
- First WHO International Standard for antibodies to SARS-CoV-2 variants of concern (31); and
- First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern (32), and subsequent panel expansion to include Gamma and Omicron variants (33).

Although the above standards are likely to be useful for laboratories characterizing SARS-CoV-2 mAbs, further studies are needed to determine whether these polyclonal plasma standards can effectively harmonize the measurement of mAb neutralizing activity between laboratories.

Furthermore, it should be noted that when stocks become exhausted or new variants emerge, new or replacement standards are established on the recommendation of the WHO Expert Committee on Biological Standardization. Users should take steps to ensure use of the most recent and appropriate WHO international standard, international reference panel or other international reference standard.

6. Nonclinical evaluation

There are several important factors to consider when designing nonclinical studies for mAbs intended to prevent or treat SARS-CoV-2 infection. Such studies should characterize the targeted SARS-CoV-2 binding site/epitope and
the ability of the mAb to neutralize virus variants. The primary pharmacological effector functions of the mAb should be considered, especially if the Fc region of the mAb has been engineered. Any potential risk of unwanted or unexpected cross-reactivity with human cells or tissues, ADE or viral resistance should also be explored.

For the assessment of inhaled or intranasally administered mAbs, the selection of an animal model should take into consideration the differences in the anatomy and physiology of human and animal respiratory systems. The animal model selected should be justified when designing proof-of-concept studies for demonstrating mAb antiviral activity. If a delivery device (for example, nebulizer or dry powder inhaler) is required, its mechanism of delivery should be similar to the device intended for clinical use in humans. In some cases, additional studies may be required to ensure optimal conditions for mAb delivery in the animal model to be used.

6.1 Pharmacodynamics studies
The pharmacodynamics (PD) of the mAb should be characterized using in vitro assays.

6.1.1 Target antigen or epitope
To date, all neutralizing mAbs against COVID-19 target the SARS-CoV-2 S protein and exhibit both antigen binding and neutralizing activity. The ability of such a mAb to recognize the S protein should be demonstrated and its binding affinity measured. In addition, inhibition of binding of the S protein RBD to the human target ACE2 receptor should be demonstrated.

The epitope on the S protein targeted by the mAb should be identified for single-formulated mAbs. In the case of co-formulated mAbs (where there are two or more mAbs within a final product) or bispecific mAbs that target two binding epitopes, each targeted binding epitope should be identified. This is to ensure that the co-formulated or bispecific mAbs do not compete for the same epitope or have overlapping epitopes that could lead to antagonism.

In future, mAbs that target relevant SARS-CoV-2 antigens other than the S protein may be developed. In such cases, the ability of the mAb to recognize the targeted virus epitope should be demonstrated. If the mAb is intended to inhibit virus binding to a human cell then its ability to prevent virus binding and subsequent infection should be demonstrated.

6.1.2 Virus neutralization assays
The primary antiviral mechanism of mAbs is direct virus neutralization. The in vitro virus neutralization activity of the mAb should be assessed against historical,
currently dominant and emerging variants. Virus neutralization activity can be demonstrated using a live virus assay (for example, focus-reduction neutralization test, plaque-reduction neutralization test or surrogate neutralization assay) and/or a pseudovirus neutralization assay (34, 35).

For co-formulated mAbs, the virus neutralization activity of each constituent mAb should be tested and any synergistic activity reported. For bispecific mAbs, the virus neutralization activity of each independent targeted epitope should be tested and reported.

6.1.3 Effector function assays

The need for effector function assays should be justified and is predominately important in the assessment of immunoglobulin G1 products. The secondary antiviral mechanism of mAbs is the effector functions driven by Fc gamma receptor (FcγR) interactions. The effector properties of the mAb, such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC), should be assessed.

If the Fc region of the mAb has been engineered, the engineered pharmacological effects, such as extending mAb half-life or attenuation of Fc binding activity to Fc receptors, should be assessed and reported.

6.1.4 Assessment of antibody-dependent enhancement of disease

Clinical evidence of ADE of COVID-19 is limited and this has not been observed with currently approved mAb therapies or COVID-19 vaccines in humans. However, mAbs can mediate ADE via FcγR interaction or complement component C1q (36, 37), and so the potential risk of ADE cannot be ruled out. Therefore, evaluation of the potential for ADE of disease should be carried out and the results reported. The selection of an in vitro assay(s) for assessing ADE should take into consideration current understanding of SARS-CoV-2 infection and ADE, and the reliability of the assay(s) in predicting mAb-induced clinical ADE of disease in humans. Although in vitro assays have limitations, they may provide useful information relevant to the potential risk of ADE (for example, information on virus neutralization, virus uptake and infectivity, or cytokine production) (28).

6.1.5 Virus resistance assessment

The effectiveness of mAbs has been threatened by the emergence of resistant SARS-CoV-2 variants, with reductions in the magnitude of neutralization of such variants using existing treatments reported (38). Therefore, the neutralization activity of the mAb against these variants should be evaluated in
virus neutralization assays using emerging variants from clinical surveillance, experimentally derived viral escape mutants and/or modelled predicted escape mutants. In addition, the risk of emergence of resistant viruses to the investigational mAb under treatment due to the use of suboptimal doses should be investigated in vitro before initiation of clinical studies. Where resistance is observed, genotyping, phenotyping and cross-resistance analyses of the potential escape mutants should be conducted.

6.2 In vivo studies

For animal proof-of-concept studies demonstrating antiviral activity, preference should be given to animal models in which the SARS-CoV-2 infection is reflective of the human infection and of the anticipated mechanism of action of the mAb. The similarity of SARS-CoV-2 infection in the chosen animal model to human infection and disease should be described and justified. With the ongoing detection of SARS-CoV-2 variants, consideration should also be given to assessing the similarity of infection across different variants in the animal model and in humans.

Several animal models for COVID-19 have been developed for the testing of mAbs and vaccines. Each of the following models are able to reproduce some aspects of the clinical and pathological features of COVID-19 in humans (39, 40).

- Syrian hamsters have been established as an animal model for COVID-19 due to the similarity of hamster and human ACE2. Viral replication is observed in the respiratory and gastrointestinal tracts following infection. Laboured breathing and weight loss are observed clinical symptoms. Severe interstitial pneumonia with inflammation is observed more in aged or male hamsters compared to young or female hamsters. The induction of serum neutralization antibodies has been observed following infection. In addition, hamsters have been shown to transmit SARS-CoV-2 by both close contact and non-contact routes.

- Normal mice are not a relevant model for COVID-19 as SARS-CoV-2 does not bind effectively to mouse ACE2. However, transgenic mice expressing human ACE2 or the use of mouse-adapted SARS-CoV-2 strains have made the mouse a useful model. Mice display a range of clinical symptoms (such as weight loss) and pathological disease symptoms (such as lung inflammation).

Any animal species used for in vivo studies should be chosen carefully and thoroughly justified. For scientific and ethical reasons, it is desirable to apply the 3Rs principles of “Replace, Reduce, Refine”.
that vary in severity. The use of mouse-adapted virus models or human ACE2 transgenic mice can also be a useful tool for studying infection with SARS-CoV-2 variants.

- Ferrets have long been a model for studying human respiratory viruses such as influenza and respiratory syncytial virus, and are now being used to investigate SARS-CoV-2 transmission and COVID-19. Ferrets display mild clinical disease that includes fever, wheezing and nasal discharge. Virus replication is observed in the respiratory and gastrointestinal tracts. Histopathological studies have shown pneumonia with lung inflammation. Transmission studies have demonstrated virus transmission by both close contact and non-contact routes, suggesting that airborne transmission of SARS-CoV-2 among ferrets is possible, making them a useful model for such studies.

- Non-human primates have been infected with SARS-CoV-2 variants and studies have shown high levels of viral replication in both the upper and lower respiratory tract. Non-human primates display mild clinical disease but with notable histopathology findings of pneumonia. Severe disease has been observed in aged non-human primates. The induction of natural protective immunity through innate, humoral and cellular immune responses following infection has also been observed. Non-human primates should only be considered as a last resort option, and the selection of this model should be extensively justified.

Based on the above differences in clinical and pathological aspects, the selection of animal models for characterizing the potential clinical use of the mAb (for prophylaxis or treatment, or both) should be justified. Furthermore, the design of the proof-of-concept study should also reflect the intended clinical use(s) of the mAb (that is, for pre-exposure prophylaxis and/or post-exposure prophylaxis, and/or for treatment).

The characteristics of SARS-CoV-2 infection and COVID-19 outcomes in the above animal models are summarized in Table 1. This summary table is provided here for information only and the more detailed information available in the scientific literature on the use of selected animal models for SARS-CoV-2 and COVID-19 (39–41) should be taken into consideration when designing proof-of-concept studies.
<table>
<thead>
<tr>
<th>Relevant animal models</th>
<th>Infection characteristics and disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rodent</strong></td>
<td></td>
</tr>
<tr>
<td>Syrian hamster</td>
<td>• Susceptible to SARS-CoV-2 as hamster ACE2 is similar to human ACE2</td>
</tr>
<tr>
<td></td>
<td>• Main clinical disease symptom is weight loss</td>
</tr>
<tr>
<td></td>
<td>• High levels of viral replication in lungs at early stage after infection followed by rapid decline in virus</td>
</tr>
<tr>
<td></td>
<td>levels, and no virus detected 1 week after challenge</td>
</tr>
<tr>
<td></td>
<td>• Lung histopathological changes observed (for example, pneumonia, inflammatory cell infiltration)</td>
</tr>
<tr>
<td></td>
<td>• Severe clinical disease observed more often in males than in females and aged hamsters</td>
</tr>
<tr>
<td></td>
<td>• Naturally clear infection</td>
</tr>
<tr>
<td>Transgenic mouse</td>
<td>• Expressed human ACE2 (hACE2) allows SARS-CoV-2 infection</td>
</tr>
<tr>
<td></td>
<td>• Main clinical disease symptom is weight loss but some models may also show respiratory distress</td>
</tr>
<tr>
<td></td>
<td>• Respiratory tract infection following virus challenge but other organs (for example, brain, heart) may be</td>
</tr>
<tr>
<td></td>
<td>infected due to secondary infection or expression of hACE2</td>
</tr>
<tr>
<td></td>
<td>• Lung histopathological changes observed (for example, pneumonia, diffuse alveolar damage, inflammatory cell</td>
</tr>
<tr>
<td></td>
<td>infiltration)</td>
</tr>
<tr>
<td></td>
<td>• Severe disease observed when challenged with higher viral load</td>
</tr>
<tr>
<td>Mouse-adapted SARS-CoV-2 mouse</td>
<td>• Mutations (N501Y or Q498T and P499Y) in RBD region of SARS-CoV-2 makes it adaptive to mouse ACE2</td>
</tr>
<tr>
<td></td>
<td>• Clinical signs typically limited to mild weight loss but loss of pulmonary function also observed with</td>
</tr>
<tr>
<td></td>
<td>mouse-adapted virus carrying Q498T and P499Y mutations</td>
</tr>
<tr>
<td></td>
<td>• Mild to moderate pneumonia observed with mouse-adapted virus carrying N501Y mutation</td>
</tr>
<tr>
<td></td>
<td>• Respiratory tract infection observed</td>
</tr>
</tbody>
</table>
### Table 1 continued

<table>
<thead>
<tr>
<th>Relevant animal models</th>
<th>Infection characteristics and disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Ferret</td>
<td>• Naturally susceptible to SARS-CoV-2</td>
</tr>
<tr>
<td></td>
<td>• Clinical symptoms similar to humans (fever and mild respiratory symptoms)</td>
</tr>
<tr>
<td></td>
<td>• Viral replication observed in respiratory tract (nasal wash, lungs)</td>
</tr>
<tr>
<td></td>
<td>• Interstitial pneumonia observed</td>
</tr>
<tr>
<td></td>
<td>• No deaths from infection observed</td>
</tr>
<tr>
<td><strong>Non-human primate</strong></td>
<td>– non-human primates should only be considered as a last resort option, and the selection of this model should be extensively justified</td>
</tr>
<tr>
<td>Rhesus macaque</td>
<td>• Mild clinical disease (for example, fever, weight loss)</td>
</tr>
<tr>
<td></td>
<td>• High levels of viral replication in respiratory tract (detected by nasal swab, bronchoalveolar lavage, lung tissue examination); lung histopathological changes (for example, pneumonia, pulmonary discoloration) similar to humans</td>
</tr>
<tr>
<td></td>
<td>• Severe disease not observed</td>
</tr>
<tr>
<td></td>
<td>• Naturally cleared infection</td>
</tr>
<tr>
<td>Cynomolgus macaque</td>
<td>• Mild clinical disease (for example, fever, weight loss)</td>
</tr>
<tr>
<td></td>
<td>• High levels of viral replication in respiratory tract (detected by nasal swab, bronchoalveolar lavage, lung tissue examination); lung histopathological changes (for example, diffuse alveolar damage, pulmonary discoloration) similar to humans</td>
</tr>
<tr>
<td></td>
<td>• Severe disease not observed</td>
</tr>
<tr>
<td></td>
<td>• Naturally cleared infection</td>
</tr>
<tr>
<td>African green monkey</td>
<td>• Clinical disease and histopathological changes similar to rhesus or cynomolgus macaques</td>
</tr>
<tr>
<td></td>
<td>• Severe disease (acute respiratory distress syndrome) observed in aged monkeys</td>
</tr>
</tbody>
</table>

Although the use of animal models has advanced the development of several COVID-19 therapeutics, there is no specific animal model optimized to mimic human SARS-CoV-2 infection and COVID-19. The selection of appropriate animal models for proof-of-concept studies should take into consideration the disease outcome of each animal model with regard to the intended study end-points.

The design of proof-of-concept studies should also ensure the use of a well-characterized virus challenge strain and acceptable route of inoculation.
The minimum anticipated biological effect level (MABEL) or biological effective dose (BED) should be used to select dose levels and to optimize the anticipated therapeutic effect.

7. Clinical evaluation

There are several factors to be considered in the clinical development programmes of anti-SARS-CoV-2 mAbs as they impact the clinical trial design and end-points to be used. One important consideration is whether the product is intended to be used as a prophylactic (for pre-exposure prophylaxis and/or post-exposure prophylaxis), as a therapeutic, or both.

For treatment indications, the timing of administration of the mAb is especially relevant. Current anti-SARS-CoV-2 mAbs were generally found to be more effective when administered early to patients with symptomatic COVID-19 and prior to hospitalization (42, 43). Some, but not all, studies suggest that such mAbs may be associated with worse outcomes for patients requiring high-flow oxygen or mechanical ventilation (44–46).

Inhaled or intranasally administered mAbs are currently under development and may provide some advantages due to more-localized administration and lower systemic exposure. Additional considerations may be required for these alternative routes of administration, such as compatibility of the delivery device with the mAb formulation, mAb distribution within the airways and potential for systemic exposure. In addition, robust pharmacokinetic and pharmacodynamic modelling should be performed. For efficacy evaluation, consideration may be given to measuring the prevention of infection. Sponsors should consult with the NRA to help ensure a comprehensive regulatory approach for such products.

Because of the functionality of the mAb, healthy volunteers may not be suitable candidates for therapeutic efficacy trials, but may be appropriate for prophylactic studies. Healthy volunteers may also provide useful data on product safety, preliminary pharmacokinetics (PK) and potential for anti-drug antibody (ADA) induction in Phase I studies. PK parameters may require confirmation in infected patients to highlight any differences compared to healthy volunteers. As repeated administration of the mAb may alter its safety and activity profiles, repeat-dosing studies should be conducted to support the use of additional administrations.

Clinical trial duration can vary depending on the biological half-life of the mAb. A number of anti-SARS-CoV-2 mAbs have been engineered for increased half-lives of approximately 6 months. The duration of follow-up for participants should be appropriate for the investigational product to provide information on its long-term efficacy and safety, and should be discussed with the NRA.
Participants in clinical trials should be representative of the population targeted for eventual product use. This population should include individuals who are unlikely to mount an adequate immune response to COVID-19 vaccination secondary to immunocompromised status, elderly people and/or subjects with comorbidities such as:

- obesity
- cardiovascular disease, including hypertension
- chronic lung disease, including asthma
- type 1 or type 2 diabetes mellitus
- chronic kidney disease, including those on dialysis
- chronic liver disease.

All of these have been identified as groups at high risk of severe COVID-19 and death (47). However, the immunocompromised population is also quite heterogeneous and the risk of progression to severe disease, even in those adequately vaccinated, can vary considerably between the different pathologies.

The COVAXID cohort study reported the 1-year follow-up immune response of 356 subjects after COVID-19 messenger RNA vaccination in a real-world setting. Subjects who had undergone solid organ transplant and who had been treated with mycophenolate mofetil, those with common variable immunodeficiency, with chronic lymphocytic leukaemia treated with ibrutinib, or with X-linked agammaglobulinemia exhibited lower vaccine responses compared to other groups of immunocompromised patients (48). Those with a higher risk of disease progression even after vaccination thus require alternative therapies. In this regard, several randomized trials and real-world studies have investigated the role of mAbs in reducing hospitalization and preventing progression from asymptomatic to symptomatic disease, and even death (49).

The complications that result from the inclusion of immunocompromised individuals in clinical studies include ethical concerns, for example with regard to the comparator used (that is, whether a placebo or active comparator is used). The extrapolation of efficacy data in low-risk patients, based on neutralizing antibody titres, may be reasonable, and should be discussed early in clinical development with the NRA and ethics committee. In addition, in immunocompromised patients, the virus can remain viable for a longer period of time which prolongs the duration of potential spreading. Studies have shown that mAbs can reduce the time needed to clear the replicating virus, which not only benefits infected immunocompromised individuals through the avoidance of longer isolation periods (50) but also reduces the risk of virus transmission to others.

Vaccines and mAb therapies are complementary approaches to prophylaxis and treatment of COVID-19. However, due to the specificity of
immune support provided by mAbs, certain variants of SARS-CoV-2 can evade the response. One related issue is that the early mAbs are no longer effective against more recently circulating VOC, reinforcing the belief that new mAbs with conserved efficacy across different VOC are needed. Therefore, the decision to administer mAbs should be based on factors such as the regional prevalence of resistant variants, and on individual patient health status (51, 52).

The epidemiological situation, including circulating variants, should be monitored and noted in the study report as any change in circulating virus variants may have a significant impact on the clinical efficacy of the mAb product. The viral strain of infected patients should thus also be determined and recorded during the clinical study. In addition, continued monitoring of emerging viral variants and of the neutralization activity of the mAb against them is vital.

Furthermore, the risk of development of viruses resistant to the investigational mAb should be evaluated in all clinical breakthrough cases, using phenotypic, genotypic and cross-resistance analysis.

7.1 Inclusion and exclusion criteria

For considerations specific to the inclusion or exclusion of pregnant or breastfeeding women see section 7.4.4 below.

7.1.1 Prophylaxis

Inclusion criteria

- Participants who can benefit from passive immunization with antibodies.
- Medically stable participants.
- Result from SARS-CoV-2 serology and RT-PCR testing at screening.
- Able to understand and comply with study requirements/procedures based on the assessment of the investigator.

Exclusion criteria

- Significant infection or other acute illness, including fever > 37.8 °C on the day prior to or day of randomization.
- Known history of allergy or reaction to any component of the study drug formulation.
- Previous known hypersensitivity, infusion-related reaction or severe adverse reaction following administration of a mAb.
- Bleeding disorder or prior history of significant bleeding or bruising following intramuscular injections or venepuncture.
- Any other significant disease, disorder or finding that may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to participate in the study or impair interpretation of the study data.
- Receipt of any investigational medicinal product (IMP) in a set period (as defined by the sponsor) immediately prior to the study, or expected receipt of an IMP during the period of study follow-up, or concurrent participation in another interventional study.

7.1.2 **Treatment**

**Inclusion criteria**

- Participant has a documented laboratory-confirmed SARS-CoV-2 infection.
- WHO Clinical Progression Scale score > 1 and < 4 (53).
- Participant must be dosed with the IMP within a set period (as defined by the sponsor) following self-reported onset of COVID-19 related symptoms (mild to moderate COVID-19).
- One or more of the signs/symptoms relevant to COVID-19 infection (for example, cough, sore throat, shortness of breath or difficulty breathing at rest or with activity, body pain or muscle pain/aches, fatigue, headache, chills, nasal obstruction or congestion, nasal discharge, nausea or vomiting, diarrhea, new loss of taste or smell).
- Oxygenation saturation of ≥ 92% obtained at rest by study staff within 24 hours prior to Day 1 (unless participant regularly receives chronic supplementary oxygen for an underlying lung condition).

**Exclusion criteria**

- Current hospitalization for severe COVID-19, requiring oxygen therapy or mechanical ventilation.
- Previous known hypersensitivity, infusion-related reaction or adverse reaction to any mAb, or known allergy to components of the IMP or placebo.
- Current requirement or anticipated impending need for mechanical ventilation.
- Any significant disease, disorder or finding that may increase risk to the participant and that might affect their ability to participate in the study.
- Participant must not participate in another clinical trial for the treatment of COVID-19 or SARS-CoV-2 during the study period until reaching hospitalization or 28 days after entry into the study (whichever is earliest).
- Receipt of systemic steroids or inhaled steroids prior to study entry, unless a stable dose is being used for a chronic condition.
- Receipt of any other IMP or expected receipt of an IMP during the study follow-up period, or concurrent participation in another interventional study.

7.2 Phase I studies

Phase I and first-in-human studies are conducted to determine the initial safety and tolerability of the IMP following completion of the essential nonclinical studies. Clinical experience has demonstrated that most COVID-19 mAb products are, in general, well tolerated.

The determination of starting dose, dose escalation steps and maximum exposure for first-in-human studies should take into consideration all available nonclinical information (for example, PD, PK, toxicokinetics and toxicological profiles, and dose or exposure/effect relationships) as well as safety and toxicity information derived from testing in a relevant animal model during nonclinical evaluation. For additional information on animal models of SARS-CoV-2 infection see section 6.2 above.

Products with the same antibody scaffolding and manufacturing process used for previously authorized anti-SARS-CoV-2 mAbs (that is, a product that only differs from the authorized product in the epitope binding site) may leverage the clinical development of the authorized product to expedite certain aspects of their clinical development. However, this should be discussed with the NRA, particularly if the mechanism of action has changed. Phase I trials may be conducted in healthy volunteers to determine the mAb safety profile, PK and potential physiological responses. If the product is intended to be administered in the elderly, in children or in other specific groups, then safety and tolerability data may be required for those specific groups.

7.3 Clinical pharmacology

7.3.1 Pharmacokinetics

Multiple-dose PK studies may not be required if the mAb is intended to be given only in a single dose. However, if the product is intended to be repeatedly administered, safety and tolerability data may be required to support the dosing regimen.
7.3.2 **Pharmacodynamics**

The PK, combined with nonclinical PD target levels, should guide the doses to be evaluated. Such studies may involve the ex vivo assessment of the neutralizing activity of the mAb in serum collected at different timepoints following administration.

7.4 **Phase II and III studies**

7.4.1 **Efficacy**

The clinical trial design of Phase II and III studies for efficacy determination will depend on whether the mAb is intended to be a prophylactic or therapeutic product.

The efficacy of a prophylactic mAb should be evaluated in terms of its ability to prevent the disease or progression to severe disease, but may also be assessed in terms of its ability to eliminate the pathogen, reduce the viral load or reduce virus shedding.

The efficacy of a therapeutic mAb should be evaluated in terms of its ability to prevent disease progression (that is, prevent deterioration in overall clinical status, hospitalization or death) and/or reduce clinically relevant endpoints, such as time to sustained alleviation of symptoms, following confirmation of infection. The efficacy of a therapeutic mAb may also include the ability to eliminate the pathogen, reduce the viral load or reduce virus shedding.

An emphasis should be placed on designing randomized controlled trials that take into account the intended target population, the selected clinical end-point(s) (Table 2) and case definitions (53).

The local epidemiology of circulating variants may also affect efficacy outcomes, particularly if the mAb has different binding affinities to such variants. For this reason, in vitro neutralizing assays against any identified new variant should be conducted to ensure that the mAb retains activity against this new variant and that the study can safely be continued.

The selection of an appropriate authorized product as a comparator for use in efficacy trials will also require careful consideration and may vary depending on intended use – that is, for prevention or treatment. A randomized controlled double-blind trial design should be used in efficacy studies intended to prevent or treat infections. A placebo control may be considered when there is no appropriate comparator, no known therapeutic agent is effective, or when the natural history of the untreated infectious disease is relatively benign or self-limiting (that is, of low risk to patients) and where switching to an approved treatment is ensured in case of progression to severe disease. Any other current standard of care practices for the prevention or treatment of the infection must be provided to all participants regardless of the treatment arm. It is recommended that in all cases, these issues are discussed in advance with the NRA and ethics committee.
7.4.2 Immunobridging

To accelerate initial approval of novel mAbs manufactured on the same platform technology as already approved mAbs, an immunobridging approach could be an acceptable pathway for mAbs intended for prevention (54). The immunobridging should be based on a cross-variant comparison in a non-inferiority study with an approved mAb with the same indication. The geometric mean titres (GMTs) of neutralizing antibodies at Day 28 of an already approved mAb product against a virus strain for which efficacy was shown (for example, Alpha) should be compared to the GMTs achieved at the same timepoint with the new investigational mAb product against circulating variants. The acceptable non-inferiority margin when using a comparison of GMT values should be discussed with the NRA. Following initial approval, post-marketing efficacy data (including data from the investigation of breakthrough cases) should be collected, neutralizing antibody concentrations monitored to determine the timing of antibody waning, and long-term efficacy and safety data generated for at least 6 months.

Deciding upon the acceptability of an immunobridging approach, particularly for bispecific mAbs and mAbs with a different mechanism of action, will be in the remit of the NRA.

Presently, there are not sufficient data to derive a specific mAb concentration or neutralizing threshold to derive a correlate of protection for SARS-CoV-2.

7.4.3 Safety

The continual evaluation of mAb product safety is an important component within all phases of clinical studies. Although mAbs generally have a very good safety profile, each product is unique and should be considered independently.

Safety data should be obtained from a sufficient number of subjects during the clinical trials to characterize and quantify the product safety profile, which can include the type, frequency and severity of adverse drug reactions. In some cases, it may be possible to consider safety data from multiple clinical studies if both the products tested and the study conditions are sufficiently similar.

Evaluating the safety and tolerability of anti-SARS-CoV-2 mAbs should include the recording of all adverse events (AEs), serious adverse events (SAEs), medically attended adverse events (MAAEs) and adverse events of special interest (AESIs) over the duration of the study (Table 2).

Product reactogenicity should also be clearly characterized by monitoring immune responses to the mAb through ADA titres and immune system activity.
## Table 2
Objectives, estimands and clinical end-points

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Estimand description/end-point</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Estimate the efficacy of the mAb | An appropriate time frame for the assessment of efficacy should be provided to the NRA based on the end-point being assessed (for example, 6 months for prophylaxis, Day 28 for treatment)  
For pre-exposure prophylaxis, a binary response whereby a participant is defined as a COVID-19 case if a SARS-CoV-2 RT PCR-positive symptomatic illness occurs post dose(s) of the mAb and prior to the specified time frame.  
For post-exposure prophylaxis, a composite outcome of either hospitalization or progression of symptoms post dose(s) of the mAb during the specified time frame.  
For treatment, a composite outcome of medical attendance visits, hospitalization or death from any cause and/or time to sustained resolution of symptoms post dose(s) of the mAb during the specified time frame. |
<p>| Estimate the safety and tolerability of the mAb | AEs, SAEs, MAAEs and AESIs during the study period |
| <strong>Secondary</strong> |                                |
| Estimate the efficacy of the mAb in preventing severe or critical symptomatic COVID-19 | Incidence of SARS-CoV-2 RT-PCR-positive severe or critical symptomatic illness occurring after dosing with the mAb |
| Estimate the efficacy of the mAb in preventing COVID-19-related emergency department visits | The incidence of COVID-19-related emergency department visits occurring after dosing with the mAb |
| Assess the PK of the mAb following administration of an appropriate dose via an appropriate route | Serum concentrations |
| Evaluate ADA response to the mAb in serum | Incidence of ADA to the mAb in serum |</p>
<table>
<thead>
<tr>
<th>Objectives</th>
<th>Estimand description/end-point</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exploratory</strong></td>
<td></td>
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</tbody>
</table>
| Estimate the efficacy of the mAb over a longer time frame | An appropriate time frame for assessment of efficacy for pre-exposure prophylaxis should be provided to the NRA based on the end-point being assessed. (for example, 12 months for prophylaxis)
A binary response whereby a participant is defined as a COVID-19 case if a SARS-CoV-2 RT-PCR-positive symptomatic illness occurs post dose(s) of mAb and prior to the specified time frame. |
| Determine anti-SARS-CoV-2 mAb levels in serum following the administration of the mAb | Post-treatment GMT and geometric mean fold rise from baseline value through an extended time frame |
| Quantify SARS-CoV-2 viral loads in infected participants treated with the mAb | Viral genome copies in nasopharyngeal swabs at illness visits as determined by quantitative RT-PCR |
| Quantify the duration of viral shedding in participants with symptomatic COVID-19 treated with the mAb | Duration of SARS-CoV-2 shedding in saliva |
| Characterize the risk of development of resistance to the mAb in patients with virological failure | Genotypic analysis and biochemical and/or susceptibility analysis of SARS-CoV-2 variants |
| Assess additional immune responses following administration of the mAb | Other exploratory assays for humoral, mucosal and cellular immune responses may be performed based upon emerging safety, efficacy and PD data. |
| Estimate the efficacy of the mAb in preventing long COVID | The incidence of long COVID occurring after dosing with the mAb |

* RT-PCR-positive = reverse transcription-polymerase chain reaction-positive.

* As the rate of progression to severe COVID-19 has significantly decreased due to the increased levels of immunization and seropositivity in the population, as well as the lower progression rates seen with Omicron variants, the primary efficacy end-point of progression to severe disease or death may no longer be appropriate. The use of an alternative end-point (such as sustained resolution of symptoms or non-progression of the clinical status) should be considered. In all cases, consultation with the NRA is recommended during trial design and end-point selection.
7.4.4 **During pregnancy and breastfeeding**

Some studies have shown that COVID-19 infection during pregnancy was associated with a greater probability of maternal, fetal and neonatal complications, including pre eclampsia, increased risk of admission to an intensive care unit for the mother, preterm birth and neonatal mortality compared to non-infected pregnant women (55, 56). However, many studies related to SARS-CoV-2 infection in pregnancy were performed among hospitalized patients, which may have led to overestimation of the risk of severe outcomes as not all cases of SARS-CoV-2 infection in the pregnant population were included (57, 58). Moreover, pregnant women who were obese and women with comorbidities were more likely to develop severe disease or to present greater risk of complications related to COVID-19 than pregnant women without such conditions (57, 59, 60).

The extensive physiological changes associated with pregnancy may alter drug PK and PD, thus directly affecting the safety and efficacy of any drug administered during pregnancy through alterations in drug absorption, distribution, metabolism and excretion (61).

Currently, information on medicinal drug use in pregnancy and breastfeeding generally is collected in the post-marketing setting, using data from observational studies such as pregnancy exposure registries and other cohort studies, case control studies and surveillance methods. However, this approach commonly results in delayed access to new medicinal products for pregnant and breastfeeding women. There are multiple reasons for considering the inclusion of pregnant women in clinical trials, including:

- Women need safe and effective treatment during pregnancy.
- Failure to establish the dose/dosing regimen, safety and efficacy of treatments during pregnancy may compromise the health of women and/or the fetus.
- In some settings, the enrolment of pregnant women in clinical trials may offer the possibility of direct benefits to such women and/or their fetus that are unavailable outside the research setting.
- The development of accessible treatment options for pregnant women is a significant public health issue.

Systematic consideration should be given to the possible use of any new medicine by pregnant and breastfeeding women and, where warranted, to planning formal investigations in these populations. Such planning should take into account different variables, such as the benefit and risk perspective, as well as the need for systematic and timely study of medicines likely to be used in this population to support dosing, use rationale and other aspects (62). Data obtained to date, mainly from clinical settings, suggest that COVID-19 mAb products seem to be well tolerated and likely to be safe when used during pregnancy (63–68).
Sponsors should consult with the NRA early in the product development phase on the requirements for specific nonclinical studies, and on the potential inclusion of pregnant women in clinical studies to promote the health of pregnant women and their fetus, and to inform prescribing decisions during pregnancy. Proper follow-up of the mother-child pair should also be considered to fully determine the impact of product administration on maternal and newborn health (63–68).

### 7.4.5 Human challenge studies

Human challenge studies of SARS-CoV-2 infection have been conducted to better understand COVID-19, especially during the early stages of infection, and for the potential evaluation of candidate vaccines, antiviral drugs and antibodies (69, 70). The first reported study (70) paid careful attention to the preparation of the challenge stock and used highly characterized virus, including whole genome sequencing to confirm that the challenge virus was unaltered compared to the original isolate. Although not technically a clinical trial (no product was being investigated), regulatory oversight of the challenge strain was provided by the United Kingdom Medicines and Healthcare products Regulatory Authority, which confirmed that its manufacture would be suitable for use in future efficacy studies of an IMP. However, it may not be possible to undertake such studies in some jurisdictions and the relevant NRA should always be consulted directly. Such studies have not yet been used to assess the efficacy of mAbs but may be used in the future.

### 7.4.6 Paediatric considerations

In children, severe COVID-19 is uncommon. However, those with certain underlying conditions (such as cardiovascular, respiratory, neuromuscular or malignant disease, or immunocompromised individuals) are prone to unfavourable outcomes. Therefore, while most children infected with SARS-CoV-2 will recover without therapy, treatment of mild or moderate infection should be considered in paediatric patients at highest risk of progression to severe disease. This would be in alignment with the current indication for the use of mAbs against SARS-CoV-2 in adults.

None of the currently licensed mAbs against SARS-CoV-2 are authorized for use in children under 12 years of age. In addition, even for mAbs against SARS-CoV-2 that have already been commercialized, safety and efficacy data in paediatric patients are limited. Furthermore, the additional data available from observational studies are associated with limitations. With regard to post-authorization data, it is important to highlight that the generation of data in children has been greatly hampered by the loss of effectiveness of early mAbs against recently circulating VOC. Overall, the data generated so far do not suggest
an excessive risk of toxicity in children compared with adults, and mAbs seem to be well tolerated. However, the lack of a comparator group in studies makes clear estimation of the effectiveness of mAbs in preventing COVID-19 progression in children difficult. Therefore, further studies are needed to fully define the safety and efficacy of mAb therapy in the paediatric population (71–74).

The inclusion of children and adolescents in clinical trials should always be considered when planning a study to avoid knowledge gaps and to facilitate early access to new medicinal products (73–77). Sponsors are encouraged to discuss paediatric drug development with the NRA early in clinical development, including: (a) the potential for extrapolating efficacy data from studies in adults; (b) appropriate PK trials in paediatric subjects to support dose selection; (c) the recommended size of the pre-approval safety database in children; and (d) the targeted age group(s) (78–80).

### 7.4.7 Post-authorization studies

The potential risk of treatment failure due to the development of SARS-CoV-2 variants resistant to the mAb, along with the potential risks associated with any biological therapy (including mAbs) should continue to be assessed post-authorization.

Data monitoring (including systematic and proactive review of the emerging data) should be conducted using all available data sources, for example by evaluating:

- new and cumulative nonclinical data (antiviral activity and viral resistance);
- data on variants detected in clinical studies among patients who received mAbs;
- spontaneous reports related to lack of efficacy, including information for variant lineages; and
- literature reports or studies conducted by public health authorities.

The requirements for a risk-management plan, Phase IV studies and/or use of real-world evidence and data should be discussed with the NRA.

### Authors and acknowledgements

The first draft of this WHO addendum was prepared by Dr A. Chia (lead author for the Nonclinical evaluation section), Health Sciences Authority, Singapore; Dr E. Griffiths (lead author for the General considerations section), consultant, United Kingdom; Dr R. Isbrucker (lead author for the Introduction, and Purpose and scope sections) and Dr J. Lacroix (lead author for the Clinical evaluation section), Health
Canada, Canada. The draft document was then reviewed and revised by a drafting group comprising Dr A. Chia, Dr E. Griffiths, Dr R. Isbrucker, Dr J. Lacroix, and Dr S. Buchholz and Dr M. Gonzalez-Tome, European Medicines Agency, Netherlands (Kingdom of the); and Dr B. Klug, Paul-Ehrlich-Institut, Germany; and by Dr I. Knezevic and Dr E.K. Kim, World Health Organization, Switzerland.

The resulting draft document was posted on the WHO Biologicals website from 1 November to 4 December 2023 for a first round of public consultation. Comments were received from Dr S. Hufton and Dr G. Mattiuzzo, Medicines and Healthcare products Regulatory Agency, United Kingdom; Dr J. Wang, National Institutes for Food and Drug Control, China; Dr S. Tognarelli, Paul-Ehrlich-Institut, Germany; Dr T. Cohen, AstraZeneca, USA; the Nonclinical working party, 3Rs working party, and Pregnancy group, European Medicines Agency, Netherlands (Kingdom of the); Dr J. Holst, Holst PharmaWorks, Norway; and Dr R. Gupta, Vir Biotechnology, USA.

All comments received were collated and distributed to the drafting group members for their consideration, and revisions to the text made accordingly. The revised document (WHO/BS/2024.2466) was then posted on the WHO Biologicals website from 11 January to 15 February 2024 for a second round of public consultation. Comments were received from Dr S. Fakhrzadeh, Consultant, Iran (Islamic Republic of); Dr I. Feavers, United Kingdom; Bharat Biotech International Limited, India; Dr S. Silviera, Brazilian Health Regulatory Agency, Brazil; Dr J. Southern, South African Health Products Regulatory Authority, South Africa; European Medicines Agency, Netherlands (Kingdom of the); and Dr T. Cohen, AstraZeneca, USA. All comments received were taken into consideration and an updated document prepared.

Editorial review of the resulting document was then completed by Dr T. Waddell, United Kingdom in accordance with WHO requirements for all documents appearing in the WHO Technical Report Series.

Further changes were made to document WHO/BS/2024.2466 by the WHO Expert Committee on Biological Standardization.

References


Annex 3

New and replacement WHO international reference standards for biological products

The provision of global measurement standards is a core normative WHO activity. WHO international reference standards are widely used by manufacturers, regulatory authorities and academic researchers in the development and evaluation of biological products. The timely development of new reference standards is crucial in harnessing the benefits of scientific advances in new biologicals and in vitro diagnosis. At the same time, management of the existing inventory of WHO international reference standards requires an active and carefully planned programme of work to replace established materials before existing stocks are exhausted.

The considerations and guiding principles used to assign priorities and develop the programme of work in this area have previously been set out as WHO Recommendations. In order to facilitate and improve transparency in the priority-setting process, a simple tool was developed as Appendix 1 of these WHO Recommendations. This tool describes the key considerations taken into account when assigning priorities, and allows stakeholders to review and comment on any new proposals being considered for endorsement by the WHO Expert Committee on Biological Standardization.


At its meeting held via video conference on 11–14 March 2024, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list. Each of the WHO international reference standards shown in the table below should be used in accordance with its instructions for use (IFU).

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### Additions\(^\text{10}\)

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<tr>
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<td><strong>Standards for use in high-throughput sequencing technologies</strong></td>
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<tr>
<td>Adventitious virus detection in biological products by high-throughput sequencing(^\text{11})</td>
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\(^\text{10}\) Unless otherwise indicated, all materials are held and distributed by the Medicines and Healthcare products Regulatory Agency, Potters Bar, Herts, EN6 3QG, United Kingdom.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

WHO Expert Committee on Biological Standardization
Seventy-eighth report.
WHO Technical Report Series, No. 1054, 2024 (xvii + 95 pages)

WHO Expert Committee on Biological Standardization
Seventy-seventh report.
WHO Technical Report Series, 1048, 2023 (xiv + 137 pages)

WHO Expert Committee on Biological Standardization
Seventy-sixth report.
WHO Technical Report Series, 1045, 2023 (xvi + 330 pages)

WHO Expert Committee on Biological Standardization
Seventy-fifth report.
WHO Technical Report Series, 1043, 2022 (xii + 252 pages)

WHO Expert Committee on Biological Standardization
Seventy-fourth report.

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

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

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

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

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

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email: bookorders@who.int; order online: www.who.int/bookorders
This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological products used in medicine, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of issues brought to the attention of the Committee at its meeting held virtually in March 2024. Of particular relevance to manufacturers and national regulatory authorities are the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, the WHO document entitled Nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of COVID-19 was adopted.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: biotherapeutics other than blood products; blood products and related substances; in vitro diagnostics; standards for use in high-throughput sequencing technologies; and vaccines and related substances.

A series of annexes is then presented which includes an updated list of all WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products (Annex 1). The above WHO document adopted on the advice of the Committee is then presented as part of this report (Annex 2). Finally, all new and replacement WHO international reference standards for biological products established during the March 2024 meeting are summarized in Annex 3. The updated full online catalogue of WHO international reference standards is available at: https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/catalogue.