CONSIDERATIONS ON THE PRINCIPLES OF DEVELOPMENT AND MANUFACTURING QUALITIES OF CHALLENGE AGENTS FOR USE IN HUMAN INFECTION MODELS

January 2022

Contents

EXECUTIVE SUMMARY

Human infection studies (also known as controlled human infection studies/models or challenge studies) have been an important tool in biomedical research for a number of decades. Infectious challenge agents used in these studies are pathogens to which a small number of volunteers are exposed under carefully controlled conditions to further understanding of different diseases. They have also been used as a platform to evaluate prophylactic and therapeutic drugs and for the testing, development and licensure of vaccines [for example the FDA approval of live oral cholera vaccine in 2016. With the increased use and scientific and regulatory acceptance of challenge models in an increasing number of countries, there is a growing need for establishing a set of common principles and/or practices for challenge agents that can be referenced, particularly in those regions where there is little regulatory guidance.

Human infection studies are always subject to ethical review and approval before they can be initiated regardless of where they are conducted. However, considerations relating to challenge agent manufacturing vary between countries due to differences in regulatory oversight, the categorisation of challenge agents and incorporation into medicinal/vaccine development processes.

A consortium of international experts with experience in the production of challenge agents, performance of challenge studies, and/or good manufacturing practices (GMP) was established to draft a considerations document, intended to discuss fundamental principles of selection, characterisation, manufacture, quality control and storage of challenge agents for international reference. In the absence of clear international guidance on this topic, the principles outlined in this document should be considered for implementation with the context of the pathogen and setting taken into consideration and for the purposes of improving volunteer safety, model reliability, and for interactions with regulatory agencies or other bodies which oversee human challenge studies. This document can be utilised across high-, middle- and low-income countries and can be applied whether the agent is manufactured in a certified GMP facility or in an academic laboratory by trained personnel with sufficient facilities, appropriate quality control measures and other best practices.

In summary, researchers and challenge agent developers should consider the following:

- Infectious challenge agent development
    - Agent selection and characterisation:
        - Use of the infectious challenge agent and the implications of its use (including route of inoculation, safety and the severity of illness and availability of therapies e.g., rescue treatments).
        - Applicable quality principles (e.g., identity, purity, and potency for challenge agent selection/design and characterisation).
    - Manufacturing preparation/manufacturing process: Ensuring suitably designed and controlled production processes are in place and monitored prior to manufacturing of the clinical batches.
        - Conception/design and qualification of the intended manufacturing process

        - Quality control strategies covering the whole process (e.g., testing for contaminating agents, challenge agent activity before release, stability profile, etc.)

- Manufacturing / routine manufacturing:
    - Manufacturing considerations, including: facilities, personnel, equipment and reagents, transfer of processes, quality checks, vendors.

1. Abbreviations

BSE	Bovine Spongiform Encephalopathy
BSL	Biosafety Level
CDMO	Contract Development and Manufacturing Organisation
CMC	Chemistry, Manufacturing and Controls
CQA	Critical Quality Attributes
Ph.Eur	European Pharmacopoeia
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HRA	Health Research Authority
IABS	International Alliance for Biological Standardization
IATA	International Air Transport Association
ICH	International Council of Harmonisation
IMPD	Investigational Medicinal Products Dossier
IND	Investigational New Drug
ISO	International Organization for Standardization
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time of Flight
MRG	Manufacturing Research Group
N/A	Not Applicable
NIMPD	Non-Investigational Medicinal Product Dossier
NRA	National Regulatory Agency
PFU	Plaque Forming Units
PPE	Personal Protective Equipment
R&D	Research and Development
RNA	Ribonucleic Acid
SOP	Standard Operating Procedures
TSE	Transmissible Spongiform Encephalopathy
USP	United States Pharmacopeia
WGS	Whole Genome Sequencing
WHO	World Health Organization

2. Introduction

Controlled human infection studies are studies in which human volunteers are deliberately inoculated with an infectious organism, called for this purpose “the challenge agent”, under carefully controlled conditions. Challenge agents may be wild type (i.e., retaining key pathogenicity characteristics found in community isolates), adapted and/or attenuated (reduction in pathogenicity compared to community isolates), or genetically modified (to have additional or fewer characteristics). Human infection studies were established over two hundred years ago and have become valuable tools in advancing our understanding of infectious diseases and evaluating interventions as part of proof of concept clinical trials [for example, as reviewed by Balasingam and Wilder-Smith (2016); Roestenberg et al. (2018); and Metzger et al. (2019). A wide variety of infectious agents have been utilised including parasites, viruses, and bacteria, each having their own considerations in the way they are developed and manufactured for use in human infection studies.

The intentional infection of healthy volunteers is an ethically sensitive subject; the complexities of which are discussed elsewhere [Jamrozik and Selgelid (2020); Selgelid and Jamrozik (2018) Bambery et al. (2016), Hope and McMillan (2004)] and beyond the scope of this document. The core ethical principles widely accepted by the research community and ethicists include the accurate characterization and minimization of risk to volunteers, coupled with clear and transparent communication of these risks to support volunteers providing informed consent to take part. Meeting these criteria necessitate thorough characterization of the identity, purity and potency of the challenge agent.

Human infection studies must be subject to ethical review and approval before they can be initiated regardless of where they are conducted globally. In many countries, independent ethical oversight is performed by review boards at the institutional level (e.g., Institutional review boards) and/or at the national level by Research Ethics Committees (e.g., Health Research Authority (HRA) in the UK). However, the oversight of the infectious challenge agent quality, its manufacturing and usage in Human infection studies varies greatly between countries. One outcome of the differing regulatory oversight of human infection studies globally is that challenge agent manufacturing considerations (including development, qualification, production and quality control strategy upon release) vary, largely because of the different ways challenge agents are categorized. In many cases the challenge agent does not fall within the mandate/writ of the National Regulatory Agency (NRA): as the challenge agent is not a medical agent or device intended for prevention/treatment of the disease, it is out of the regulator’s jurisdiction (i.e. the challenge agent is not included within the laws and/or regulations which dictate what products the NRA is able to regulate).

Due to the challenges of performing phase III efficacy studies either for rare infectious diseases, infections that occur in outbreaks or sporadically, or for those with widely used vaccines, there is increasing interest in the use of human infection studies in the development pathway of new vaccines, prophylactics, and treatments. This necessitates continued focus on the ethical, regulatory and biosafety considerations concerning the set-up, design and implementation of these studies. Regardless of how challenge agents are overseen in different regions, of primary concern is developing a challenge agent that generates clinically relevant results and is safe to use in humans. To this end, the principles laid out in this document may provide additional considerations that can impact safety when reviewing the manufacture and quality control aspects within dossiers submitted for human infection studies.

In 2017, the World Health Organization (WHO) published new guidance which outlined regulatory considerations and expectations for the use of human infection studies in vaccine development [World Health Organization Annex 10 (2017)]. However, that document does not provide guidance on the manufacture or quality control of the challenge stock, stating only that its quality should be "… comparable to a candidate vaccine at the same clinical trial phase." More recently, the need to agree and implement quality principles involved in the development, qualification and manufacture of challenge agents across the world has been highlighted by the UK The Academy of Medical Sciences Workshop Summary (2018), and by stakeholders (scientists, regulators, clinical staff, volunteers) involved in human infection studies [Bekeredjian-Ding et al. (2020), World Health Organization R&D Blueprint (2020) ,Levine et al. (2021)].

The human infection studies research community has extensively discussed the degree to which GMP guidelines apply to production of challenge agents, with respect to balancing the need for high quality manufacture against the consideration that challenge agents are not developed as marketed health products (i.e., therapeutic drugs or vaccines). Indeed, it is important to discuss "GMP" without overlooking the importance of wider considerations, including the development and characterisation of infectious challenge agents ahead of manufacturing, as has been done previously [Bekeredjian-Ding et al. (2020)]. Even if manufacturing is performed under strict GMP requirements the resulting batches may not necessarily be consistent or reliable if the process was not developed with due consideration.

In this document the considerations for the development and manufacture of infectious challenge agents are outlined, promoting trial participant safety whilst maximizing access to these agents and models in low-, middle- and high-income countries. This document has been developed independently of the manufacturing guidelines utilised for manufacturing medicinal products, although in this process we have taken advantage of previous constructive experience.

Considering the broad range of pathogens employed in human infection studies, and any potential future pathogens to be discovered, characterised and developed for use in these studies, it is unrealistic to provide specific details for every type of pathogen, this document therefore suggests an overarching approach to development and characterization of infectious challenge agents and their preparation.

3. INFECTIOUS CHALLENGE DEVELOPMENT CONSIDERATIONS

A. STEP 1: CHALLENGE AGENT SELECTION AND CHARACTERISATION

The manufacturing of a new challenge agent starts with the selection of the appropriate strain: defining the characteristics of the new challenge agent to be produced is recommended. In defining the characteristics the researcher/developer should also consider the intended usage of the challenge agent and assess the implications of usage (e.g., route of administration, safety, ability to characterise infection and the severity of illness and availability of anti-microbial treatment).

This section discusses the identification and assessment of Quality Considerations for a new challenge agent and its related Quality Attributes that should be considered prior to developing the manufacturing campaign. In this section, we recommend a list of applicable Quality Considerations and related Quality Attributes to consider. This list is not exhaustive and specificities for each type of challenge agent should be considered.

i. Definition of infectious challenge agent characteristics and intended usage

Summarising the known characteristics of a challenge agent in a table are recommended, as exemplified in Table 1, the summary characteristics table is intended to capture information regarding the challenge agent and its Quality Attributes throughout the development process. The information contained within this table will likely evolve as additional knowledge and experience of working with the challenge agent is gained. The generation of this table for any challenge agent should aim to be as dynamic as possible (and not considered as a static process) to allow for the incorporation of any new material generated on the characteristics and life of the use of the agent across different contexts e.g., translation of model across different populations (naïve vs previously exposed) might result in phenotype and/or genotype differences to be observed.

This table is intended to assist in the selection of a challenge strain or variant that meets the research study needs. Depending on the strain and/or variant chosen, there may be substantial implications for the clinical study as variations in virulence, transmissibility, and genetic stability could alter the virulence profile and thus the risks to volunteers. Any such variations would also impact the relevance of the data collected from the challenge study. Thus, it is important at an early stage to select a strain considering the balance between:

- The representativeness of the challenge agent to current isolates/variants to maximise the relevance of the model, where possible
- the need for virulence/attack rate
- ensuring the model is fit for its intended purpose (e.g. exploring pathogenicity, host-pathogen interactions, immunological profiles induced by infection, correlates of protection, testing efficacy of drugs, biologics and vaccines)
- a manageable symptom profile and a rapid and effective response to available treatment

Furthermore, the provenance of the strain (e.g., its origin, clinical isolate, storage condition and passage history) may need to be considered for designing the most appropriate manufacturing process, as well as regulatory aspects and the need for biosafety containment.

In Table 1, we propose a list of characteristics broadly applicable to challenge agents. Details provided are not exhaustive and more characteristics on the challenge agent can be captured on a case-by-case approach based on relevant justifications.

Table 1: Determination of an appropriate challenge strain

Development aspect	Characteristics	Description
Pre-Clinical	Identity and genome stability	Once the source of pathogen has been identified, the identity of the strain should be confirmed by whole-genome sequencing (WGS), or an appropriate pathogen-related assay suitable to confirm its identity. If relevant and appropriate, where the genome of the challenge agent can mutate during manufacturing process, its stability should be assessed. Where applicable, the clonality of infection should be confirmed with the genetic diversity clearly identified.
	Purity	Assess the likelihood of contamination with other pathogens and other non-pathogenic organisms derived from the originating sample (e.g., faecal parasites or bacteria present in faecal samples). WGS, metagenomic sequencing, selective cultures and other assays may be used to confirm the purity of isolate.
	Mechanism of actions and potency[1]	Assess need to confirm and validate virulence factor(s) with appropriate assays, when available, that may cause generalisable asymptomatic (infection/carriage) or symptomatic disease. Prior knowledge of the pathogenicity should help to select the final strain. If mechanism of actions leading to severe and/or irreversible symptoms are known, the virulence factor(s) responsible should be avoided as appropriate (e.g. use of Enterotoxigenic *Escherichia coli* [Harro et al. (2011)] instead of Shiga toxin-producing *Escherichia coli*). If appropriate to the study, the challenge agent can be modified for use, for example the development of the toxin-deleted El Tor strain JBK70 used in cholera challenge studies, Levine et al. (1988). For human infection studies involving the testing of vaccines, target antigen(s) from the vaccines need to be present in the challenge strain(s) to enable characterisation of the immune response and evaluate potential correlates of protection.
	Sensitivity to therapy, benefit and risks	Evaluate needs, risks and benefits of including a therapy (e.g., pre-emptive or rescue therapy) in the human infection study populations. Where applicable, known and possible drug resistance genes/markers should be identified and characterized, and periodically tested for their development. Confirm *in-vitro (and/or* in-vivo *pre-clinical models, if such exist)* sensitivity of a strain candidate to proven therapies and define a risk assessment strategy.
Clinical	Dose regimen	A dose-escalation/range finding study to determine optimal dose regimen in the intended populations e.g., suitable infection/disease that is safe for volunteers. Dose ranging studies can be determined for different contexts (e.g., naïve vs exposed population) to make the challenge agent profile applicable to different settings. Where possible and relevant, evidence of the dose level administered to each subject should be documented e.g., either by assessing fresh preparations before dose, using back titrations of residuals from the administered dose, or control of inoculum titres by ongoing infectious stability data from pre-prepared single use inoculum vials. Other considerations may be required for challenge agents delivered via biological vectors.
	Administration route	Intravenous, intranasal, oral, inhalation, subcutaneous, intradermal, mosquito/vector bite, transdermal, urethral catheter, vaginal, topical. The route of administration can impact the extent of the manufacturing process and quality control

[1] There may be limited preclinical data available for a given challenge agent, and while animal models may be available, the presentation in animal models may not necessarily translate well to human disease. Studies conducted with the same strain, including published journal papers, also provide information to assign the impact scoring for a challenge agent.

		requirements for the challenge agent; however, a challenge agent manufactured under conditions which are suitable to one route of administration may not be sufficient for another route of administration.
	Efficacy/potency	The strain should create as close to a natural infection or clinically measurable endpoint as possible. There should be appropriate methods available to confirm acquisition of infection/disease and, when used for vaccine/therapy efficacy studies, methods for measuring the response.
	Safety	The adverse event profile (e.g., minor, transient adverse effects), risks of developing a secondary infection, and transmissibility should be understood. Risks to the health of contact persons, including those manufacturing the agent and health care staff in the study facility should also be considered. It should be possible to terminate the infection before serious disease occurs, if needed. If pathogen persistence is probable after resolution of clinical symptoms, further appropriate risk assessment should be performed.
	Population	Populations included with reduced/minimal risk. If to be used in an at-risk population then the risks need to be effectively mitigated (e.g., through staged approach, early treatment, and/or additional measures used to ensure immunocompetence of participant such as complete blood count, immune function tests).
Regulatory	Registration	Depending on the locality and regulatory jurisdiction of the clinical study sight, the challenge agent may, or may not, require registration with the appropriate regulatory authority. Consideration may also be provided by the regulatory authority in the country where the challenge agent is manufactured if the NRA or ethical review board in the country in which the clinical study is being conducted requires. If registration of the challenge agent is required, it may be classified differently within different regulatory jurisdictions (e.g. IND, IMPD, NIMPD/Auxiliary Medicinal Product). Engagement with appropriate regulatory authorities may be required for genetically modified challenge agents.
	Biosafety containment	BSL2, BSL3 and GMO as per requirement, and environmental risk assessment should be considered.
CMC (Chemistry, Manufacturing, Controls)	Presentation	For example, fresh, lyophilised, cryopreserved, mono- or multi-dose presentation, as appropriate to the challenge agent.
	Formulation	Single or multi-use dose. Vial size, concentration/strength (bioactivity, and identification of tolerated dose ranges if known), fill volume. Use of appropriate diluent/media that is safe for humans. Diluent must be chemically-defined and free from animal products, or from an approved source, to prevent volunteer exposure to Bovine Spongiform Encephalopathy (BSE) and Transmissible Spongiform Encephalopathy (TSE).
	Production	Number of doses required over what time, ability to scale-up/out, high/low volume supply, affordability.
	Stability and storage	Determination of storage and stability (shelf life) by periodic testing of the agent at the provider site. Availability of appropriate storage conditions at trial site and when “in use” (i.e. after reconstitution). Retainment of used agent/dose vials or safe disposal of agent at the trial site. Appropriate stability assays may incorporate, where applicable, virulence/potency assays e.g., maintenance of invasion or inhibitory characteristics over time.

ii. Definition of quality considerations for an infectious challenge agent and related Critical Quality Attributes (CQAs).

Quality considerations for a challenge agent

Producing a document that can form a prospective summary of the desired quality characteristics of a challenge agent will help to identify the quality characteristics which should be considered during the design of the manufacturing process and the quality control strategy. Such a document will help to set the critical development goals of the manufacturing process such as establishing purity of the seed banks, finalization of the manufacturing processes, and development of a safe, potent, high quality wild type working challenge agent bank. An example of translating challenge agent characteristics into quality considerations is shown in Table 2.

Table 2: Example of translation of a Challenge Agent characteristic into a Quality Consideration

	Characteristics	**Quality considerations**
	Potency	Challenge agent quality attributes
Description	Need to confirm virulence factors (if information available) causing generalisable asymptomatic (infection/carriage) or symptomatic disease	Biological activity/potency: The potency of the challenge agent should be tested, as appropriate. The potency determination is the recommended way to monitor the dose received by the volunteers and establish the dose relationship. In some cases, and where an animal model exists, testing doses of the pathogen in animals may give confidence in whether the inoculum will behave as expected. Where needed and applicable, reference materials could be developed to ensure consistency in testing different challenge agent batches. The outcomes of human infections can also then inform and update the potency details in the challenge agent characteristics.

The list of quality considerations should provide an understanding of what will ensure the quality, safety and potency of the challenge agent and is the starting point for identifying the Critical Quality Attributes (CQAs) of the agent as well as the type of manufacturing process to be developed.

Table 3 provides a list of quality considerations and associated Critical Quality Attributes that could be broadly applicable to most challenge agents.

Table 3: Determination of quality considerations for a challenge agent

Quality Considerations	Critical Quality Attributes	Justification
Challenge agent quality attributes	Identification	To confirm challenge agent identity e.g., Targeted PCR, MALDI-TOF, WGS, and other assays to confirm use of the correct strain. Confirmation of treatment susceptibility of the strain, as appropriate.
	Purity/impurity profile Degradation products	Assessing impact of the presence of dead organisms and/or degradation products which may impact on challenge agents being fit for purpose, such as potency (e.g., defective interfering virus particles), safety to volunteers (e.g., bacterial toxins). It is important to note that in some challenge strains like enterotoxigenic *E. coli* (ETEC) and Cholera, evidence of toxin retention is needed. Purity also includes characterisation for adventitious agents and the animal/human-derived products issue
	Biological activity/Potency/Viability	The potency of the challenge agent should be evaluated, as well as stability of the agent during the anticipated storage time. Where needed and applicable, reference materials may be developed to ensure consistency in testing different challenge agent batches where needed. The development or acquisition of reference standard materials may be problematic for certain kinds of challenge product, such as the *Schistosoma mansoni* (Sm) cercariae due to their short viability span. Viability tests (e.g., colony forming units, plaque forming units) help determine the dose.
	Physical attributes	Depending on what is important for the challenge agent, elements to consider include: pathogen structure, size, stability against pH, temperature, UV light, and susceptibility to chemical agents including solvents and detergents. It is acknowledged that some of these may not be relevant for specific pathogens. However, knowledge of key physicochemical properties of a pathogen can be important for developing quality control methods.
	Assays	Use of fit-for-purpose, qualified assays (e.g., *in-vitro* functional assays) for characterisation and quality control testing. To support the development of the challenge agent, the analytical strategy should include appropriate assays for monitoring potency, identity, purity, product and process-related impurities, stability, and pathogen titre. Where relevant, reference standard materials may need to be developed to ensure consistency in testing different challenge agent batches.
	Excipients	Assess removal/impact of any carry-over antifungals/antibiotics from isolation and manufacturing steps. Where diluents are used to prepare inoculum for clinical studies or used in storage of the challenge agent, their use should be justified and should be chosen carefully (e.g., biological activity of diluents, allergens, animal-free).
	Challenge agent release	Label volume and quantity/concentration of organisms. Consistency of potency within storage containers or vial fills. It is acknowledged that in some cases this may be less relevant e.g., bacterial challenge material that is then regrown and freshly harvested with dose preparation prior to challenge.
	Microbial limits/Adventitious agent testing	Challenge agents should be free from other pathogens of concern to humans (animal/human viruses, bacteria, fungi, endotoxin, mycoplasma). Avoid the use of animal and/or human derived products in manufacturing as much

		as possible to minimise the risk of contaminating pathogens (e.g., Transmissible Spongiform Encephalopathies (TSEs)). The use of documentation regarding microbial safety should be referred to (for comparison please also see Section 3.2.A CTD).
Dosage form	Fresh, lyophilised or cryopreserved	The way a challenge agent is stored and transported at the end of the manufacturing process can have an impact on its potency. The dosage form should be adapted to the route of inoculation and practicability at the clinical site.
Route of administration	Oral, intravenous, insect bite, transdermal, intradermal, subcutaneous inhalation, intranasal, topical, urethral catheter, vaginal. This is a non-exhaustive list.	The usage of vectors can have an impact on the transmission of other vector-borne diseases as well as manufacturing process (e.g., maintaining an insectarium feeding and infecting the vectors) and potency. Non-injectable routes of administration (e.g., oral, intranasal, or topical) may not require the same level of purity as an intravenous delivered agent.
Stability	Stability during manufacturing process, storage over time and during freezing/thawing steps + Real-time stability studies and in-use stability	There are potentially two types of stability to consider: 1) Phenotypic and genetic stability of the agent due to possible mutations during the process: For some pathogens, number of passages can have an impact on genome stability. Viruses and bacteria can be subject to mutations or emergence of variants during the production process. Pathogenicity, viability, motility and morphology should be also considered during manufacturing. 2) Stability profile in terms of degradation, viability and potency: Stability studies should cover real-time stability to determine the shelf-life and also in-use stability to determine the instructions at the clinical trial site (for example, for containers with more than one dose, the following information should be provided: storage duration and conditions once the container is open).
Container Closure System	Suitable container closure system to achieve the target shelf life and to ensure challenge agent integrity during shipping	Single-dose vials/containers can be advantageous in certain situations (such as performing preparation of the inoculum at different sites with varying level of sterility conditions) and sometimes this is either not possible or provides no advantage. An evaluation of what is most appropriate should be performed depending on the pathogen and intended usage. Container characteristics and appropriate labelling to be described. Secondary packaging, needed for shipping, should be described and may be adapted to the study site or clinical sponsor. Temperature of shipping and maintenance of temperature during transit should be monitored using log tag devices.

Critical Quality Attributes (CQA)

As per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, a Critical Quality Attribute is "a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality" (ICH Q8 – R2).

Potential CQAs should be selected based on prior knowledge and current understanding of the challenge agent and the associated disease (Table 3). The list of potential CQAs can be adapted when the final form of the challenge agent and the manufacturing process are selected and as challenge agent knowledge and process understanding increase.

Challenge agent CQAs will typically include properties or characteristics that affect:

- identity: confirmation of identity of the pathogen must be performed (e.g., assessing genotype, serotype, subtype or variant). Sufficiently high coverage and long read sequencing to allow an assembled genome should be performed, where possible and relevant to the challenge agent. In many cases targeted PCR may be sufficient, for example where the genome is not subject to significant change. Which assays used for a given pathogen should be risk assessed on a case-by-case basis.
- impurities: considerations of contaminants, including all relevant and potentially important adventitiously introduced materials not intended to be part of the manufacturing process (e.g., adventitious viral, bacterial, fungi, endotoxin, mosquito salivary gland components, or mycoplasma contamination).
- biological activity/potency/viability: where possible, direct and indirect assessment of potency should be considered (using appropriate assays) to allow proper assessment of the dose(s) given.
- genetic stability: the challenge agent genome and protein expression should be considered in a risk assessment and, if deemed relevant for the challenge agent, their stability should be assessed at more than one stage of the manufacturing process to determine any changes in virulence, susceptibility and viability.

Quality risk management can be used to prioritise the list of potential CQAs for subsequent evaluation. The level of severity varies according to the impact score (level of impact on pathogenicity and safety) and the likelihood score (likelihood of risk becoming an issue). Thus, the criticality of CQAs can be determined as follows: Severity = Impact x Likelihood.

As the goal is to link challenge agent attributes either directly or indirectly to pathogenicity and safety, the impact score is restricted to those characteristics that have the potential to impact this, as assessed by non-clinical and clinical studies, as well as knowledge of disease in the community. Prior knowledge and current data on the challenge agent should help to determine the severity of the CQAs.

Below, we propose a non-exhaustive list of CQAs to be considered and some specificities for different types of challenge agent.

<u>CQA 1: Pathogen strain</u>

Relevance and justification of the strain: the distribution of pathogen strains/variants can vary significantly across geographical regions. When choosing a strain, factors that may be considered include:

- Vaccine and drug requirements – strain relevance for the intended purpose of human infection studies is important to consider (e.g., alignment with the strains within the vaccines to be tested [homologous or heterologous], geographical representation, presence of resistance genes, and virulence factors [e.g., Tuberculosis challenge model using BCG as the challenge agent would not be suitable to evaluate vaccine candidates containing RD1 antigens, which are absent from BCG]).

- Location of the manufacturing site (research laboratory or subcontractor/Contract Development and Manufacturing Organisation (CDMO)) and clinical study site(s) – implications of the geographical location(s) and immunological background of the population(s). Factors include assessing the risk of accidental release into the community and the impact of the strain/variant in the study site local population (e.g., Dengue challenge or *Plasmodium* infection considerations in naïve or endemic countries, certain strains of *Leishmania* are geographically restricted, use of native snails for *Schistosoma mansoni* depending on country of residence). If a human infection study is performed in an endemic region, information on the strain/variant may be crucial to optimise screening and recruitment of suitable participants (e.g., recruiting those who are immunologically naïve or with low level immunity (e.g., malaria, enteric pathogens) for the challenge strain to achieve desired attack rates and disease severity). There may be a need to investigate different doses of the challenge agent within an endemic setting and/or previously exposed population, depending on the nature of the human infection study requirements.

- Variants, virulence, and mutations – pathogen virulence, stability of key virulence factor expression, availability, and sensitivity to treatments, and transmission potential may be important when selecting an infectious challenge agent strain/variant. It is recommended to assess and validate if mutations in key areas of the pathogen genome may impact these factors. Demonstration of efficacy of vaccines and antimicrobials against specific variants may be required (e.g., potential resistant mutants), and this may be considered in the selection of the challenge agent. Data from scientific literature and applying modelling and simulation techniques may support this evaluation. As relevant to the pathogen and as determined by risk assessment, targeted PCR or WGS should be used to assess relevant mutations at key sites in the isolate as well as genetic changes that occur due to manufacturing process in order to assess potential impacts: drug susceptibility of the material or strain, virulence drift/shift, as well as environmental impact, could be included as applicable.

<u>CQA 2: Identity</u>

To produce an infectious challenge agent, the identity of the strain must be confirmed (e.g., genotype, serotype, subtype). It is recommended to make use of well-established, recognised and qualified methods that are applicable to the challenge agent. Targeted PCR, WGS and MALDI-TOF should also be considered, in risk assessments, as assays to confirm identity and genetic changes over time. Where appropriate to the pathogen, and feasible, the challenge strain should undergo targeted PCR or whole genome sequencing (WGS) at the start and end of manufacturing to detect relevant genetic changes. It should be noted that with some pathogens such as RNA viruses, viral quasispecies may be present in batches. For RNA viruses, on average there is one or more mutations per viral particle, and

sequencing often will yield more than one nucleotide per position. The sequence is therefore a consensus sequence, and some "mutations" (apparent sequence changes from start to end of manufacturing) are expected and can be acceptable.

For parasitic challenge agents that will be used in the manufacturing process and given to volunteers, the Manufacturing Research Group (MRG) should identify the parasite development stage and, if appropriate, sex, as these characteristics may have a direct impact on study design and volunteers' safety.

Consideration should be given to submitting the full genome sequence of the strain to a repository that is accessible to the scientific community. With any meaningful updates on the genome over time submitted periodically when this is applicable e.g., any mutations that arise, resistance markers, virulence factors, and/or phenotypes identified.

CQA 3 Purity / impurities

A critical aspect of challenge agent manufacturing is to control, minimise, or eliminate levels of contaminating agents (viruses, mycoplasma, bacteria, fungi) in the inoculum. The contamination could originate from the clinical isolate, starting and/or raw materials, or be introduced during the manufacturing process.

The potential contaminating organisms to be tested and their acceptable bioburden levels highly depend on the pathogen being manufactured, the type of isolate from which it was derived (e.g. blood, faeces, respiratory exudate), the source of the isolate (e.g., recombinant viruses, paediatric or adult origin, geographic origin, medical history of host), the manufacturing facility and process used (e.g., reagents, vector facilities and materials used for growth and maintenance of vectors), the permissiveness of any substrates used to grow the pathogen (e.g., eggs, mammalian cells, bacterial growing mediums, vectors), and inoculation delivery route (e.g., oral, intravenous, intranasal, vector-borne, epidermal, topical).

A risk assessment should be performed to evaluate the possibility of reactivation of cryptic (integrated, quiescent) forms of adventitious agents. A thorough testing for the presence or absence of relevant and potentially important contaminating bacteria, fungi, viruses, and mycoplasma should be performed at the level of finished product at a minimum, although their control and testing earlier in the manufacturing process is highly advisable as their presence in a final product may require that the entire batch to be discarded. Endotoxin and/or non-endotoxin pyrogen testing might also be considered; however, the challenge agent may interfere with the assay(s) and the acceptable levels of endotoxins and non-endotoxin pyrogens are highly dependent on the route of administration. Where applicable, consideration should be given to assess the need for any additional testing during the maintenance of the stock or batch of the challenge agent.

It may be beneficial to discuss the adventitious agent testing program upfront with the applicable regulators and/or subject matter experts as appropriate.

These tests should be performed with current methods as methods described in the European, US or other pharmacopoeia. Where the situation is warranted, alternative/newer qualified testing methods may be acceptable (e.g., Targeted PCR, WGS, where high coverage sequencing can be used to identify sequences not from the challenge agent), if justified (ICH Topic Q 5 A (R1) Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin; World Health Organization Expert Committee on Biological Standardization Sixty-fifth Report - Technical Report Series No. 993 Annex 2 (2015)).

CQA 4: Potency

Potency should be evaluated using described and appropriate *in-vitro* assays as much as possible. If required, *in-vivo* potency assays could be performed but should be justified. When appropriate, the techniques used should demonstrate optimum growth of the challenge agent and assist in the identification of the pathogen infectious doses (alongside historical knowledge of pathogenicity in humans) that are taken forward to be used for clinical characterisation / dose optimisation. It is recognised that there may be challenge agents that do not have suitable *in-vitro* assays to assess potency. In those cases, appropriate indirect assessments of the potency should be considered to allow proper assessment of the dose(s) given, such as establishing the PCR copy number and relate to the e human infectious dose of the challenge agent in humans (e.g., Norovirus), enumeration of the number of vector bites that a volunteer receives and the resulting attack rate (e.g., *Plasmodium vivax and Leishmania*).

Viability and infectivity of the strain should be assessed during the manufacturing process and for different storage conditions, where possible. The manufacturing process should seek to limit the presence of non-viable pathogens in the inoculum and the implications of presence of non-viable and viable pathogen should be considered. Presence of non-viable organisms can potentially result in various undesirable inoculum traits: higher doses needed to achieve the desired infectivity or attack rates (e.g., malaria infected erythrocytes which could contain a percentage of uninfected versus infected cells, defective interfering particles competing with viable viruses), induction of immune responses by non-viable pathogens, presence of undesirable toxins etc. In some cases, the proportions of viable organisms that are pathogenic or non-pathogenic may also need to be considered (e.g., *Shigella* challenge agent).

CQA 5: Raw materials

Raw materials should be of the quality that are appropriate for the route of administration. For example, an agent that is administered orally only needs to be manufactured using food-grade (or better) materials.

Production of an infectious challenge agent whether by isolation, cultivation or using a recombinant technology might require materials such as: culture media, buffers, water, serum, trypsin or other enzymes, amino acids, antibiotics, and medicinal/drug interactions which might be added in the formulation buffers for storage. Stringent sourcing requirements and acceptance criteria for all materials derived from human and/or animal origin should be adequately defined according to their intended use. When alternatives are available, it is recommended to avoid where possible the use of animal-derived products to mitigate the risks of relevant and potentially important adventitious agents being present in final batches (e.g., TSEs) or employ certified TSE-free sources. As an example, bacteria should ideally be grown using media with no materials of animal origin (e.g., GMO-free soya peptone or 'Veggietones', (Osowicki et al. (2019). All raw materials should be defined, and their use should be justified. Reagents used in challenge agent manufacturing should be traceable throughout the production process, including certificate of analysis, or equivalent, and specifications sheets relevant to batch production.

When the raw materials, reagents and/or excipients are mentioned in a Pharmacopoeia, appropriate references should be given. If not mentioned in a Pharmacopoeia, a justification should be given that they are fit for purpose.

CQA 6: Starting materials (source of pathogens and seed banks)

A challenge agent seed bank system (usually a two-tiered system with a Master seed and working seed) should be established derived from an original isolate of the challenge agent (from a previously challenged volunteer, or from a community case of disease or asymptomatic infection, or produced using recombinant technology [e.g., development of a rhinovirus inoculum using reverse genetics Gern et al. (2019)].

To establish seed banks, intended for use in the manufacturing of the challenge agent, the following information need to be documented:

- seed banking system
- characterisation of the seed through quality control testing at the various passage levels
- passage history and derivation history of seeds, including documented donor screening, testing and medical history, as available (ICH Topic Q 5 D Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products), and chain of custody from isolation of the pathogen from the original host.

In the case that recombinant technology is applied to obtain a strain, the full genome sequence, phylogeny analysis, source, genetic stability, and phenotypic characteristics should be documented.

When the developed challenge agent is considered a genetically modified organism (GMO), local applicable regulations will need to be followed, including the relevant biosafety institutions. Where appropriate, techniques, such as CRISPR editing, could be used to ensure genetically modified challenge agents are marker (e.g., antibiotic resistance gene) free.

CQA 7: Cell substrates for production

Where production of the challenge agent relies on use of cell culture it is recommended to apply a similar approach as used for generation of pathogen seed, master and working banks to generate cell banks, where applicable. Cell lines have the potential to influence consistency of the manufacturing process and quality of the challenge agent. Adequate characterisation of the cell substrates is necessary to monitor and control the manufacturing process. It is advised to use well-characterised and, where possible, validated cell lines.

CQA 8: Hosts or vectors for manufacturing

The manufacturing of a challenge agent may require the use of an intermediate host. For example, the production of some parasites may require the use of a vector: *Plasmodium falciparum* with the use of mosquitos (*Anopheles*) and *Schistosoma mansoni* with the use of snails (*Biomphalaria glabrata)*.

In some cases, these vectors may be used during the clinical phase where they serve as a biological system to allow optimal parasite development and for administering the challenge agent into humans- e.g., *Leishmania major* with the use of sandflies.

In both situations, like the recommendations provided above for pathogen banks and cell banks, vectors need to be characterised and the growth and maintenance of these vectors documented. Risk assessments should include documentation of environmental impact of vector release (in particular if the vector is not native to the country), and bioburden concerns. For the latter, it is recommended to use sterile raw materials as much as possible and where not possible, risk analyses and risk mitigation measures should be considered.

For example, production of aseptic *Anopheles stephensi* mosquitoes (Lyke et al. (2010): eggs from mosquitoes are disinfected and placed in a custom medium for growth to pupae. Adult female mosquitoes are fed with *Plasmodium falciparum* gametocytes in transfusion-qualified human erythrocytes and serum. A proportion of the eggs, pupae, blood meal, and mosquitoes are cultured to assess for microbial growth. In a view of preventing microbial contamination of the insects and the parasite production, a production chamber can be designed for this purpose. All solutions and materials introduced in this chamber should be sterile (water, larva growth broth, feeding solutions).

B. STEP 2: DESIGN OF THE MANUFACTURING PROCESS - PROCESS DESCRIPTION AND QUALITY CONTROL STRATEGY

Once the new challenge agent is selected and characterised, a reliable manufacturing process (at lab scale) should be developed. Establishing a Quality Control Strategy while designing and developing the manufacturing process, is recommended. This includes a set of processes and testing to be put in place, to ensure the manufacturing process is followed and documented and the challenge agent meets the predefined quality criteria for final release of each routine production batch.

Key points to consider are:

- Quality control strategy: acceptance criteria for quality control (QC) release
- Manufacturing process description (flow diagram) and characterisation
- Control Tests
- Final formulation and storage conditions
- Stability
- Labelling

An integrated approach to a control strategy for a challenge agent includes elements which impact both the process and the product. This should include establishing the QC release strategy at the different steps of production (linked to CQA), as well as the control strategy during production.

i. Manufacturing process description and characterisation

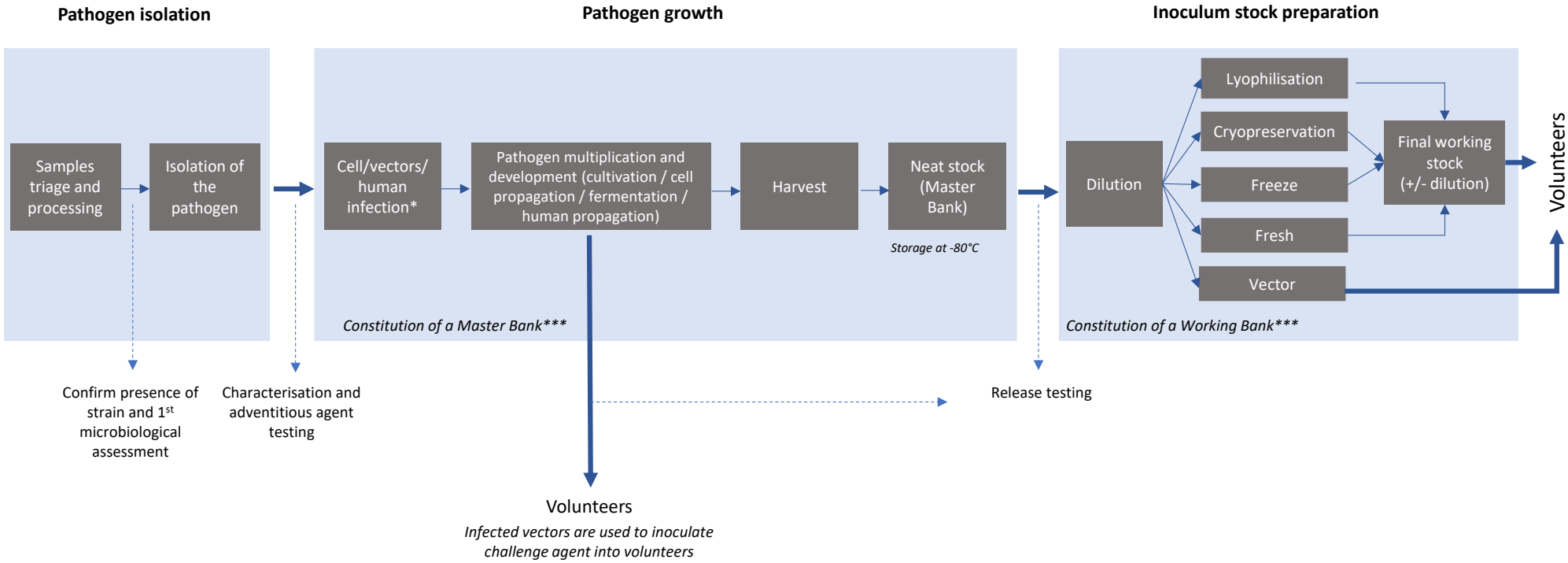
The development of an infectious challenge agent should include a summarised description of the manufacturing process specifically designed for producing the desired challenge agents, including manufacturing steps and quality attributes of the challenge agent.

Flowchart:

A flowchart is recommended for this purpose: the process of manufacturing of a challenge agent can be conceptualised as shown in Figure 1.

The flowchart of the entire process should be prepared, including from the starting material and, where applicable, from cell and/or seed banks. The flowchart should capture the critical steps and intermediate products used in the process (e.g., use of vectors to deliver parasites, like mosquito, or seed virus stocks).

The process may include activities performed post manufacturing like management and preparation of the challenge agent at the clinical site. Information on procedures used to transport material during the manufacturing process of the intermediates or final product, including transportation and storage conditions (e.g., thermo-loggers should be used for temperature monitoring during transport from manufacturing site to clinical site) and holding times, should be provided. A detailed process for destruction of the challenge agent should be provided.



* To obtain infected mosquitoes, Master and Working banks are firstly manufactured and in a second time challenge agent is used to infect mosquitoes.
** Ideally, the seed stock would already be a purified human clinical isolate
*** Not applicable for hookworms and schistosomes

Figure 1: Schematic of overall challenge agent process

Manufacturing area:

If possible, the manufacturing area should be physically separated from the procurement area/storage area. No un-authorised personnel should enter the production area/dedicated space. If different pathogens or strains are processed and stored in the same manufacturing area there is an increased risk of cross-contamination during each step of the procedure (e.g., via processing equipment or in storage containers such a liquid nitrogen tanks), therefore adequate control measures to prevent cross-contamination should be put in place. A suggested best practice would be to use a cleaning protocol shown to be effective at removing any agents which may have previously been grown in the facility.

Testing at critical steps of manufacturing and acceptance criteria:

The manufacturing process should be controlled at the level of critical steps or intermediates. For example, after constitution of seed virus stock, tests should be performed (e.g., contaminants and characterisation testing) prior to, or in parallel to, amplification of the virus in a cell substrate. Testing of the seed stock for contaminating agents can be more focused than testing further downstream in production or in the final product. For example, for viruses and certain parasites (e.g, preparation of *Plasmodium vivax* infection red blood cell stock) obtained from humans, contaminating agent testing could be directed to detection of other relevant and potentially important human pathogens. Similarly, testing for bovine and porcine viruses is advisable on animal-derived products (before being used in the manufacturing process) and should also be considered at the end of the manufacturing process.

If multiple batches are manufactured and required to be directly comparable, reproducibility of the process and consistency of the final batch of challenge agent should be demonstrated. This is particularly important when there is a risk of drift in the strain or specificity of the pathogen. Verification of the robustness of the process under development is required to ensure that, at the time of routine production, there is inter-batch reproducibility and consistency. However, there are many instances where this is either not possible or direct comparability is not required. In either case, the reproducibility of the process and the risks and implications on the pathogen batches being fit-for-purpose should be considered.

Where applicable, the storage conditions should be assessed and determined (e.g., time, temperature). Attention should be paid to storage information post manufacturing, especially at the clinical site.

Upon receipt at the site, the challenge agent should be accompanied with its certificate of analysis (or equivalent), and clear storage information:

- The storage information should include, but is not limited to:
  - Storage conditions, such as:
    - Any temperature-specific instructions (i.e., store at room temperature, below 25°C, frozen -70^{C}, lyophilized, etc.)
    - Other environmental considerations (i.e., protect from sunlight, etc.)
  - storage instructions following any reconstitution that may be performed as well as direction on the steps to be taken/liability if storage considerations have not been adhered to.
  - Re-test dates, as appropriate

ii. Final formulation (dosage form)

Current human infection models use challenge agents in different forms: fresh, lyophilised or cryopreserved. The lyophilised and cryopreserved forms are increasingly preferred as they may provide more reproducible challenge agent potency and dosing characteristics.

The MRG should consider assessing impact of lyophilisation and cryopreservation methods on challenge agent viability, infectivity and stability. A viability quality check after freeze-thaw can be used to assist in accounting for potential loss when thawed for challenge. Once the final formulation is selected, the MRG should provide all documentation regarding lyophilisation or cryopreservation process development. If the MRG decides to proceed with a lyophilised form, the other parameters that need to be considered include primary and secondary drying, ramp rate, and chamber pressure during the lyophilisation run. The freezing step is a critical process parameter impacting both process performance and quality attributes (e.g., potency). For example, it is known that the different methods of freezing can significantly impact the overall crystal structure (e.g., faster freezing – LN2 blast freezing) and can lead to differences in ice structure with an impact on the overall drying properties of the challenge agent.

For the final formulation given to the volunteers, challenge agents may need to be diluted or processed (e.g., introduced into a vector) at the clinical site prior to inoculating. Diluents should be manufactured to the same principles and quality as would be required for the challenge agent and route of administration, ensuring that relevant and potentially important contaminating agents are not introduced, and that it does not adversely affect the performance of the challenge agent. Consideration should be given to the premises, equipment, cleaning, trained staff, and consumables used for the dilution process to ensure minimizing risks.

iii. Analytical tests

As part of a comprehensive approach to control and verification that the manufacturing process can produce a challenge agent that meets the assigned CQAs, a testing strategy should be employed. The control strategy for a challenge agent should include:

- Identity testing
- Purity testing
- Specifications (final batch testing)

Identity (characterisation): testing of certain attributes outside of the batch release testing for the purposes of demonstration of consistency and where necessary comparability. A specific testing plan may be developed based on the risk to product quality (e.g., confirm genome stability after one or several passages using targeted PCR or WGS techniques (acknowledging expected inherent instability in some pathogens, as seen with quasispecies in RNA viruses); confirm potency and check no attenuation of the strain).

Purity and impurities: while contaminating agent testing should be part of release testing, the presence of undesired pathogens should be tested at critical process steps. For example, the use of animal-based raw material can represent a risk of introduction of a potentially important adventitious

agent and tests, where applicable, should be included early in the process to mitigate this risk. All cell substates and cell/seed banks should be tested for contaminating agents prior to their use in the manufacturing process.

Specifications (final batch testing): tests with associated acceptance criteria conducted either during the manufacturing process on key intermediates (the so-called in-process testing) and at the final batch release stage on a set of quality attributes to confirm quality of the strain for forward processing and challenge agent for use in clinical setting and distribution to other sites.

The following tests should be considered in the final batch testing package and the results should be documented in a certificate of analysis:

- Identification – confirmation of identity
- Purity/Impurity profile
- Biological activity/potency/viability

  If biological activity testing cannot be performed for QC release due to *in-vitro* tests not being available or appropriate, assessments of biological activity may be confirmed in humans, for example in a characterisation study or retrospectively (e.g., quantification of parasites delivered by mosquito vectors). This information should be captured as part of the challenge agent documentation for each released batch.

- Quantity - quantity may provide different but relevant information compared to biological activity/potency/viability, as this also includes non-infectious particles. For example, in the case of respiratory challenge viruses, PCR $\log_{10}$copy number/mL provides useful quantitative information additional to potency, as may be measured by a tissue culture assay (e.g., $TCID_{50}$/mL or pfu/mL).
- Microbiological quality testing, including relevant and potentially important adventitious agent testing (more details provided in adventitious agent testing section). When relevant, additional tests might need to be added such as: osmolality, pH, residual moisture content.

iv. Filling and containers

Care should be taken to ensure that the materials from which the container (if possible and relevant, the use of Ph.Eur/USP or equivalent is recommended) and, if applicable, the closures made do not adversely affect the quality of the challenge agent under the recommended conditions of storage.

v. Stability

A stability plan should be generated that assesses the stability profile of the challenge agent during production and storage. A stability protocol or Standard Operating Procedures, including setting aside a proportion of the challenge agent vials for stability testing should be developed, where appropriate. When relevant, attention should be paid to genome and phenotypic stability during the manufacturing process and during storage, and the other critical quality attributes should also be considered (e.g., viability, motility, morphology). Stability tests should be justified and qualified, with appropriate replicates, and assay controls.

If possible, the stability protocol could include real-time studies, accelerated/stress studies/forced degradation and in-use studies (e.g., WGS or specific targeted PCR to assess genomic stability).

For real-time studies, the frequency of testing applied may benefit from consideration of ICHQ5C and ICHQ1 frequency guidelines. For example, if storing the pathogen for >1 year, consider frequent testing in the first year (e.g., every 3 months), less during the second year (e.g., every six months), and annually thereafter. The frequency applicable will depend on the type of challenge agent and prior knowledge on the stability properties. For example, influenza and rhinovirus are known to be relatively stable viruses when stored appropriately at -70℃. In this case the challenge agents may be tested annually or before a new study is initiated. However, if a batch is actively being used in clinical studies, then more frequent testing (e.g., every six months) may be considered. Care should be given to pathogens that are particularly sensitive to external conditions or environmental factors that could affect potency, purity and quality of the challenge agent, such as temperature, light, shearing, and clumping. In some cases, only inoculation of humans is possible to confirm the potency of the challenge agent. For example, some strains of Norovirus, while stable and difficult to inactivate, cannot easily be assayed for potency due to poor growth on cell lines. In this case, potency can be confirmed when propagating a new batch in humans.

Stability testing should be conducted on the dosage formulation that is packaged in the container closure system intended for use in the clinical studies. The frequency of stability testing would depend on challenge agent and dosage formulation.

vi. Labelling

Where appropriate and relevant to the challenge agent, the labelling recommendations provided in the WHO Good Manufacturing Practices for biological products should be followed. This would need to also reflect in-country or regulatory specific requirements in the event that some more parameters are required.

The label of the carton enclosing one or more final containers, or the leaflet accompanying the container, should include:

- the name of the challenge agent.
- Concentration and volume.
- Quantity and/or number of doses, if the product is issued in a multiple dose container (this may not be possible to include in some instances e.g., for bacterial challenge material that is then regrown and freshly harvested prior to challenge).
- if applicable, a statement indicating the volume and nature of diluent to be added to reconstitute the challenge agent, specifying the diluent to be used and a statement to the effect that after the challenge agent has been reconstituted it should be used without delay or, if not used immediately, stored for a maximum of what time under conditions and temperature formally shown not to affect stability for a maximum period defined by stability studies.
- the temperature recommended during storage and transport.

- the expiry date (if known and appropriate), or in the case the expiry date is not known or it is not appropriate, then the label may refer to or rely on the supporting material (i.e. stability plan). Some examples for consideration to include on the label (not limited to) using "N/A" (not applicable) or the first retest date (taken from the stability plan). In any case, the associated documentation should clarify the expiry or retesting strategy, and where expiry date cannot be assessed with confidence, the labeling should encourage the end-user to prepare the final product in the shortest period of time prior to administration in order to minimize the risk of denaturation/inactivation of the agent during undocumented storage and/or transportation up to the administration site, prior to use of the challenge agent.

vii. Challenge agent dossier for clinical site usage

Where available, data should be included within the challenge agent documentation to inform, as guidance, the clinical usage of the challenge agent. This may include, but is not limited to:

- Protocol for inoculum preparation including list of ingredients/adjuvants/excipients, handling instructions, equipment/PPE requirements, containment level, concentrations/dosage if applicable, administration methods etc.
- Contraindications
    - Known effects on subjects with specific conditions/biology i.e. anaphylaxis, etc. Care should be given to not include populations that are at risk of enhanced disease in the contraindication section, as in some instances challenge agents may be applicable to be used in at risk populations when managed in the correct way e.g., as has been previously carefully done with rhinovirus for inducing exacerbations in patients with asthma and COPD.
- Warnings and Precautions
    - A list of possible effects of challenge agent via the planned route of administration
    - Precautions for clinical staff to take in the handling and administration of the challenge agent, including minimizing the time period from reconstitution/preparation of the dose up to its administration.
    - Pre-existing diseases/conditions that may be adversely affected by the challenge agent in question based on its pathogenicity
    - Additional risks/mitigation strategies for challenge agents requiring maintenance within animal hosts local to clinical sites and the potential risk to the population if a community outbreak was to occur
- Adverse reactions
    - Expected and reported unexpected reactions to the challenge agent, if known
- Medicinal/drug interactions
    - Known treatments/drugs which both can affect and be affected by the challenge agent due to its pathogenicity
    - If appropriate and known, sensitivity of the challenge agent to therapies can be identified e.g., those routine therapies that may be used in the challenge studies. Depending on the type of challenge study and challenge agent given, therapies may be planned to halt disease progress / prevent progression to severe disease and/or treat/resolve infection.

- Use in specific populations
    - Any known data/publications of the challenge agents use in specific populations e.g., pregnancy, paediatric, geriatric, immunosuppressed etc.
- Instructions for waste management including disinfection of laboratory benches /surfaces, and where risk assessments require it, testing thereafter for viability of the agent and for molecular remnants using PCR methods.

4. MANUFACTURING CONSIDERATIONS

A. Manufacturing of infectious agent and post-manufacturing activities: facility, premises, equipment, and personnel

Once the challenge agent is fully characterised, and the manufacturing process is established and deemed ready to be transferred for routine production: for volunteer safety and model reliability, there are few additional considerations that the MRG should take into account when manufacturing batches of the challenge agent for clinical use.

The following considerations for challenge agent manufacturing are recommended in relation to:

- Technology Transfer
- Selection and qualification of subcontractors
- Quality management system
- Personnel
- Facility, premises, equipment
- Documentation
- Distribution and transport

Additional regulatory requirements for challenge agent manufacturing may be needed depending on the region. It should be noted that the above recommendations are also formalized in GMP, ICH or ISO. A list of resources that may assist the MRG are referenced in Annex 1.

Technology transfer

For a variety of reasons, the challenge strain may be manufactured by a third party or by a group different from the one that originally designed, characterised and developed the challenge agent.

In this case, it is recommended that the developer/sponsor prepares a Product Development Report (PDR), and technology transfer packages including key elements regarding process performance including characterisation, manufacturing process up until preparation of the inoculum at the clinical site.

The technology transfer can be completed with appropriate documented training.

Selection and approval of subcontractors

The manufacturing of the challenge agent can be outsourced to a subcontractor that could be a Contract Development and Manufacturing Organisation (CDMO) and some tests (e.g., adventitious agent testing or sequencing) could be outsourced to another research group or subcontractor. Whoever is selected as subcontractor, they should be qualified for their experience in related fields (infectious disease, vector manufacturing, sequencing, adventitious agent testing), production of pathogen (e.g., for the development of vaccine), cell bank production and maintenance. The manufacturer should have a quality system in place for the intended subcontracted activities. In case

of several subcontractors intervening for the manufacture and quality control a single convention should be established.

Quality management system

According to the ISO organisation "A Quality Management System, often called a QMS, is a set of internal rules that are defined by a collection of policies, processes, documented procedures and records. This system defines how a company will achieve the creation and delivery of the product or service they provide to their customers".

It is recommended that the MRG put in place a quality management system that documents the selection, characterisation and manufacturing steps, with a view to help control the manufacturing campaign appropriately. For example, Standard Operating Procedures (SOP) should be implemented appropriately. Part of the quality management system put in place by the MRG (the sponsor of the Challenge agent project) should encompass an audit system of the subcontractor(s) selected and the quality management system in place.

Personnel

- Training, hygiene, and expertise

All personnel employed in areas where the challenge agents are manufactured should receive appropriate training specific to the pathogen and to their work. Personnel should be given relevant information and training in safe working and microbiology (e.g., use of PPE). The Personnel working in areas where contamination is a hazard, such as clean areas or areas where highly active, toxic, and infectious or sensitising materials are handled, should be given specific appropriate training based on risk assessments.

Personnel for manufacturing and quality control should have an adequate background in relevant scientific disciplines with sufficient practical experience to enable them to exercise their management function for the process concerned.

In some circumstances the immunological status of personnel may need to be taken into consideration for their safety. Attention should be paid to personnel health; if relevant, staff vaccination status should be checked and regular health checks performed, as appropriate to the setting.

In general, visitors should be excluded from production areas. When an audit of the production areas is planned, the auditors should have access to the complete area. The auditor should fulfil all the access procedures.

In the case a Biosafety Risk Group pathogen is manufactured, it is recommended to the MRG to identify and involve the person responsible for Health and Safety.

- Authorised person

Attention should be paid to the authorised/qualified person responsible for batch certification and release procedures. Detailed description of their qualification (education and work experience) should be provided. This person can be an external or internal resource.

Facility, equipment, and related processes

- Qualification and modification of operations

Equipment operating ranges (defined in the user requirement specifications) should be capable of being maintained during routine production, as necessary.

Equipment and premises used for manufacturing should be suitable and qualified for aseptic production (where required). It is recommended that dedicated, agent-specific or single-use equipment are used in the production, whenever possible.

Any modifications made to equipment, utility systems, materials, manufacturing processes, or procedures during production campaign, may change the parameters or affect the expected outcomes. Changes made during production campaign would need to be strictly controlled by seeking approval before implementing it, including the evaluation of possible effects and associated risks.

In cases where routine production requires inter-batch reproducibility, consistency and comparability verification of the established processes is needed, once the transfer has taken place.

- Premises and equipment

It is recommended to use a dedicated area for the duration of manufacturing of the considered challenge agent.

Thorough cleaning should be performed before initiation of challenge agent preparation. Cleaning and sanitisation should consider the fact that processes often include the use of growth media and other growth promoting agents in addition to the pathogen itself.

The MRG should explain, for the considered challenge agent, how to ensure the effectiveness of cleaning, sanitisation, and disinfection, including elimination of residues of used agents. Environmental and personal safety precautions should be taken during cleaning and sanitisation processes. The use of cleaning and sanitising agents should not pose any major risk to the performance of the equipment. The use of closed systems such as a positive pressure isolator or Class 2 safety cabinet to improve asepsis and containment should be considered where practicable.

Control measures should be taken to prevent contamination, mix-up, and cross contamination.

A small proportion of the approved starting material (e.g., the working cell bank) may be kept in the production area for the period of the batch manufacture or campaign, in case contamination occurs in the production area during production. Appropriate storage conditions and controls should be maintained during this temporary storage. These materials should not return to the general stock.

In the manufacturing facilities, the mix-up of entry and exit of personnel should be avoided using separate changing rooms or through procedural controls, especially if Biosafety Risk Group 3 organisms are handled (WHO, Laboratory Safety Manual, 3rd edition).

In regard to production of a challenge agent that necessitates the use of a live host (e.g., human, mouse, snail, mosquito, sand fly), it is recommended to perform a risk assessment of the propagation in the host, identifying what additional risks are possible due to their inclusion, identify the likelihood of them occurring, and explore mitigation strategies to minimise the probability of those risks becoming issues, and/or to reduce the impact if they do occur. For example, assessing the risks associated with the use of an insectarium to house vectors such as mosquitoes or sand flies. In this case, depending on where the insectarium is housed, risks could include accidental release of vectors into the environment (with or without the challenge agent) or accidental contamination of the insectarium with endemic vectors containing pathogens other than the intended challenge agent. In a similar vein, some vectors cannot be entirely “cleanly” produced, for example sandflies used to deliver *Leishmania* need to feed on faeces that naturally contain bacteria. A mitigating approach in

this instance may be to utilise antibiotics to treat bites, where appropriate. Likewise, when propagating *Schistosoma*, consideration should be given to both housing snails as well as rodents. Each species presents their own risks in regards i) cleanliness of animals (e.g., using Specific Pathogen Free animals where possible), and ii) infestations and accidental release. For challenge agents in which humans are used to propagate pathogens for subsequent human infection studies, consideration should be given both to i) potential adventitious agents introduced (similar to those considerations for obtaining pathogen isolates), and ii) transmission prevention measures (similar to those used for the main challenge experiments).

- Containment and Environmental Risk Assessment

Dedicated production areas should be used for the handling of Biosafety Risk Group 2 and 3 pathogens. These facilities should comply with local regulations for the appropriate biosafety level. Use of pathogens above Biosafety Risk Group 3 may be permitted by the NRA according to the biohazard classification of the organisation, the risk assessment of the challenge agent and its emergency demand.

Airborne dissemination of live microorganisms and viruses used for the manufacturing process, including those from personnel, should be avoided.

Adequate precautions should be taken to avoid contamination of the drainage system with dangerous effluents. When appropriate, to minimise the risk of cross-contamination, drainage systems should be designed in a way effluent can be neutralised or decontaminated. Specific and validated decontamination systems may be needed.

Air-handling systems should be designed, constructed, and maintained to minimise the risk of cross-contamination between different manufacturing areas. The need for a dedicated air-handling system should be based on Quality Risk Management (QRM) principles, considering the biohazard classification and containment requirements of the relevant pathogen and process/equipment risks. In the case of Biosafety Risk Group 3 pathogens, air should not recirculate to any other areas of the manufacturing facility and should be extracted through High Efficiency Particulate Air (HEPA) filters that are regularly checked for performance.

Primary containment equipment should be designed and initially qualified for integrity to ensure that escape of the pathogens into immediate working area and outside environment is prevented. It is recommended that tests should be performed to ensure equipment is in proper working condition.

We recommend that decontamination measures should be available for each pathogen. Where different strains of a single bacteria species or very similar viruses are involved, the decontamination process should be appropriate for one representative strain, unless the strains vary significantly in their resistance to the decontamination agents used. Measures may include PCR testing of representative samples taken from the lab.

If GMO strains are manufactured, the MRG should consider safety management and should follow national guidelines related to the manufacture, storage, use and disposal of GMOs.

- Clean rooms

As part of the control strategy, the degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the manufacture of challenge agent and to the production step, considering the potential level of contamination of the starting materials and the risks to the final batch of the challenge agent. The MRG should use cleaning methods that have been proven to be capable of eliminating the previous products followed by testing the risk of cross contamination.

It is recommended that the MRG refer to the WHO guidance document [World Health Organization Report (2012)] to develop the environmental classification requirements for the challenge agent manufacturing process.

Documentation

Where multiple challenge agents are manufactured in the same facility it is important to consider the processes in place for minimizing the risk of cross contamination as well as ensuring robust documentation (see section 3B "manufacturing area").

- Batch processing records

It is recommended to prepare a batch processing record and a summary of the protocol for each batch for the purpose of batch release.

The processing records of regular production batches should provide a complete account of the manufacturing activities of each batch of challenge agent (e.g., list of equipment, details of ancillary consumables, reagent batches; aliquots numbers), showing that it has been produced, tested, and dispensed into containers in accordance with the approved procedures.

Manufacturing batch records should be retained for an undetermined time and should be ready for inspection by the NRA, as appropriate.

- Documentation for batch release

A form should be filled and signed by an authorised person (as described in Section 4A "Personnel") from the MRG for each critical step during the manufacturing campaign.

Starting materials may also require additional documentation on source, origin, supply chain, methods of manufacture and controls applied, to ensure an appropriate level of control (including microbiological quality) where applicable.

It is recommended that a Certificate of Analysis, or equivalent document, signed-off by an authorised person should be provided for each starting material.

It is recommended that a Certificate of Analysis or equivalent should be released for each new batch produced. This document will inform if the challenge agent has passed critical tests (identity, viability, microbiology quality testing) and should be signed to assure that each batch has been checked for compliance with the agreed specification.

- Batch definition

The purpose of the batch definition is to ensure consistency and traceability. A clear definition of a production batch from sourcing to labelling of final container should be provided.

Attention should be paid to shipment consideration where the following information should be provided:

- Name, manufacturing batch number and expiry date
- Place of origin of challenge agent manufacture and contact details
- Destination of shipment
- Identification of permits/documentation required specific to different countries including high, medium and low-income countries

- Specific shipment packing considerations, including reference to the storage and stability data of the challenge agent.

Distribution and transport

To maintain the viability of the challenge agent and adherence to biosafety containment, shipments should meet IATA standards and be correctly packaged, maintained within specified temperature ranges and packages should contain cold-chain monitors (where needed). Consideration should be given to the different aspects of the shipping process. For international shipments, potential delays at customs should be anticipated.

Consideration should be given to assessing the risks to challenge agent integrity when transporting between sites as well as in storage. As an example, risk mitigation measures may include splitting shipments of the challenge agent over time in case of batch transport failures or splitting storage of vials across different freezers or sites in case of freezer failures.

B. Challenge agent documentation in a view of potential discussion with regulatory authorities and use in clinical trials.

In a view of potential discussions with regulatory authorities, we suggest consideration of collating information about the challenge agent, covered in the section 3 and 4 of this considerations document, in a pathogen dossier. For the routine manufacture aspect (when applicable), we recommend preparing a manufacturing file where the roles and responsibilities of the various actors/subcontractors, quality control and batch release are well described, with the description of the overall quality system (plus the individual quality system from each subcontractor) put in place to allow monitoring of the various activities. The MRG may benefit from engaging with regulators and/or ethics committees before beginning manufacture of the challenge agent where appropriate.

ANNEX 1: RELEVANT RELATED LIST GUIDELINES

The following listed ISO, GMP, and ICH principles are provided for reference. They may assist the MRG in establishing relevant procedures., avoid missing important items related to the pathogen manufacture, as well as helping avoid reproducing what may already be in available that can be modified.

For consideration, current versions of the ICH guidelines can be found on the ICH website:

- ICHQ5a: Viral safety evaluation of Biotechnology products derived from cell lines of human or animal origin
- ICHQ5c: Quality of biotechnological products: Stability testing of biotechnological/biological products
- ICHQ5d: Derivation and characterisation of cell substrates used for production of biotechnological/biological products
- ICHQ 6b: Specifications: test procedures and acceptance criteria for biotechnological/biological products
- ICHQ 7: Good manufacturing practice
- ICHQ 8: Pharmaceutical development
- ICHQ 9: Quality risk management
- ICHQ 10: Pharmaceutical quality system
- ICHQ 11: Development and manufacture of drug substances

For consideration, current versions of the ISO guidelines can be found on the ISO website:

ISO 9001:2015 Quality management systems - requirements

ACKNOWLEDGEMENTS & KEY CONTRIBUTORS

The views expressed are those of the authors and not necessarily those of the institutions, organisations, or affiliations.

An international consortium was created on the funders' behalf (Wellcome Trust and HIC-Vac) and led by hVIVO Ltd to draft this document: Mr Alexander Mann, hVIVO, UK; Mrs Carine La, hVIVO, UK; Mr Alan Bell, hVIVO, UK; Dr Andrew Catchpole, hVIVO, UK; Mr Bruno Speder, hVIVO, UK; Dr Jean-Hugues Trouvin (Affiliate), IABS-EU, France; Dr Hilde Depraetere (Affiliate), European Vaccine Initiative - EVI, Germany; Dr Pieter Neels, IABS-EU, Belgium; Dr Kawsar R. Talaat, John Hopkins Bloomberg School of Public Health, USA; Dr Chad K. Porter* (Affiliate), Naval Medical Research Center, USA; Dr Akamol E. Suvarnapunya* (Affiliate), Naval Medical Research Center, USA; Dr Louis Bourgeois (Affiliate), PATH, USA; Professor Meta Roestenberg, Leiden University Medical Center, The Netherlands; Dr Angela Van Diepen, Leiden University Medical Center, The Netherlands; Professor Peter Kremsner, Tübingen University, Germany and Centre de Recherches Médicales de Lambarene, Gabon; Ms Evelyne Kestelyn, Oxford University Clinical Research Unit, Viet Nam; Dr Beno N. Yakubu, NAFDAC, Nigeria; Mrs Ade-Abolade Khadijah, NAFDAC, Nigeria; Dr Amrita Sekhar, Translational Health Science and Technology Institute, India; Professor Gagandeep Kang, The Wellcome Trust Research Laboratory Division of Gastrointestinal Sciences, Christian Medical College, India.

The core principles of the manufacturing document were created by Mr Alexander Mann, Mrs Carine La, Dr Hilde Depraetere, Dr Jean-Hugues Trouvin and Dr Pieter Neels in discussion and agreement with the consortium, Wellcome Trust (Ms Shobana Balasingam, Dr Cecilia Chui, Ms Sarah Meillon) and HIC-Vac (Dr Emma Smith). Individual meetings were held with various experts in regulatory and/or the human challenge field which served as an important source of information during the preparation of the document. These included Dr Joshua Osowicki, Murdoch Children's Research Institute, Australia; Ms Kristy I Azzopardi, Murdoch Children's Research Institute, Australia; Professor Daniela M Ferreira, Liverpool School of Tropical Medicine, UK; Dr Jay R. Laver, University of Southampton, UK; Professor Jeremy Brown, University College London, UK; Professor Jeffrey M. Bethony, George Washington University, USA; Ms Linda Schellhaas, Consultant to The George Washington University, Quality Reviews Inc, USA; Professor Jetsumon Sattabongkot Prachumsri, Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; Dr Wanlapa Roobsoong, Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; Professor James Gern, University of Wisconsin School of Medicine and Public Health, USA; Professor Robert C. Read, Faculty of Medicine and Institute for Life Sciences, University of Southampton, and NIHR Southampton Biomedical Research Centre, UK; Dr Andrew Gorringe, Public Health England, UK; Professor Paul Kaye, University of York, UK; Dr Richard Isbrucker, WHO, Switzerland; Dr Nicolas Noulin, hVIVO, UK; Mrs Fanny Nascimento Moura Viana, ANVISA, Brazil; Mrs Adriane Alves De Oliveira, ANVISA, Brazil; Professor Rodrigo Correa-Oliveira, Fiocruz, Brazil.

The first draft of this document was prepared by Mrs Carine La and Mr Alex Mann with input from Mr Alan Bell, Mr Bruno Speder, Dr Jean-Hugues Trouvin, Dr Hilde Depraetere, and Dr Pieter Neels. This draft took into account both the individual feedback from experts in the field as well as comments from the Strasbourg (2016), Rockville (2018), Langen (2019), Oxford (2020) meetings organised by IABS and from

the Academy of Medical Sciences workshop held on 6 February 2018 in the UK, as well as significant review input from the consortium and affiliates, Wellcome Trust and HIC-Vac.

For public consultation, the consortium approved the draft document that was presented and discussed at the IABS webinar held on 31 March 2021 and distributed in advance to the attendees to garner feedback that could be incorporated into the second draft. The authors would like to acknowledge the report of the conference organised by the International Alliance for Biological Standardization (IABS) published in 5 October 2021, the contributors to the presentations (Professor Jeffrey M. Bethony, Dr Pauline Meij and Dr Akamol E. Suvarnapunya) and panel discussion (Professor Isabelle Bekeredjian-Ding, Department of Medical Microbiology and Hygiene, University Hospital Heidelberg, Paul-Ehrlich Institute, Germany ; Dr Pieter Neels, IABS-EU, Belgium; Ms Winfred Badanga Nazziwa, UNCST Uganda; Dr Hilde Depraetere, EVI, Germany; Mr Eric Karikari-Boateng, FDA Ghana, Ghana; Mr Alex Mann, hVIVO, UK; Dr Pauline Meij, LUMC, the Netherlands; Professor Jetsumon Sattabongkot Prachumsri, Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; Dr Paula Salmikangas, NDA, Finland; Dr Dean Smith, Health Canada, Canada; Dr Scott Stibitz, FDA, USA; Dr Peter Stjärnkvist, MPA, Sweden ; Dr Jean-Hugues Trouvin, IABS-EU, France; Dr Wim Van Molle, Sciensano, Belgium), as well as the attendees' feedback.

While the document was originally produced as a White Paper, following discussions with the Wellcome Trust and HIC-Vac, it was confirmed that the document was to be adjusted into a Considerations Document to better align with the goals of the project.

Further drafts were prepared by Mrs Carine La and Mr Alex Mann, in consultation with Dr Jean-Hugues Trouvin and Dr Hilde Depraetere, that included input from rounds of consultation with the consortium and affiliates, Wellcome Trust and HIC-Vac as well as reviews by an external regulatory and challenge agent experts including Dr Joshua Osowicki, Murdoch Children's Research Institute, Australia; Ms Kristy I Azzopardi, Murdoch Children's Research Institute, Australia; Professor Andrew C Steer, Murdoch Children's Research Institute, Australia; Dr Ben Morton, Malawi-Liverpool-Wellcome Trust, Malawi & Liverpool School of Tropical Medicine, UK; Professor Daniela M Ferreira, Liverpool School of Tropical Medicine, UK; Professor Jeremy Brown, University College London, UK; Professor Jetsumon Sattabongkot Prachumsri, Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; Professor James Gern, University of Wisconsin School of Medicine and Public Health, USA; Dr Andrew Gorringe, Public Health England, UK; Professor Paul Kaye, University of York, UK; Dr Richard Isbrucker, WHO, Switzerland; Dr Melissa Kapulu, KEMRI-Wellcome Trust Research Programme, Kenya and University of Oxford, UK; Professor Stephen Gordon, Malawi-Liverpool-Wellcome Trust, Malawi & Liverpool School of Tropical Medicine, UK; Mrs Fanny Nascimento Moura Viana, ANVISA, Brazil; Mrs Adriane Alves De Oliveira; Professor Rodrigo Correa-Oliveira, Fiocruz, Brazil; Professor Sebastian Johnston, Imperial College London, UK; Professor Helen McShane, University of Oxford, UK; Dr Moses Egesa, MRC/UVRI and LSHTM Uganda Research Unit, Uganda; Ms Emmanuella Driciru, MRC/UVRI and LSHTM Uganda Research Unit, Uganda.

Considering comments from the expert panels, consortium and affiliates, Wellcome Trust, HIC-Vac, and the participants of the IABS meeting held on March 31st, 2021, the document was finalised.

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