Registration of Human Vaccines Guideline

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Promoting the Quality of Medicines Plus (PQM+)

Contact Information

Promoting the Quality of Medicines Plus Program
United States Pharmacopeia
12601 Twinbrook Parkway
Rockville, MD 20852 USA
Tel: +1-301-816-8166
Fax: +1-301-816-8374
Email: PQMplus@USP.org

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About PQM+

The Promoting the Quality of Medicines Plus (PQM+) Program is a five-year cooperative agreement between USAID and USP to sustainably strengthen medical product quality assurance systems in low- and middle-income countries. The program works to improve medical product quality through cross-sectoral and systems strengthening approaches and the application of international quality assurance standards across the pharmaceutical system. By sharing scientific expertise and providing technical support and leadership, PQM+ helps create resilient and robust local health systems that address diseases such as HIV/AIDS, tuberculosis, malaria, and neglected tropical diseases, as well as improve maternal, newborn, and child health.

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PQM+ program team contributed for continuous effort with input and suggestion to develop this guideline.

1. Major General Mohammad Yousuf, Director General, DGDA
2. Md. Salahuddin, Director, Admin, DGDA
3. Dr. Syed Umar Khyyam, CoP, PQM+ Program, USP
4. Dr. Souly Phanouvong, Director Regulatory Strengthening, PQM+ Program, USP
5. Tanya Malpica Lianos, Program Manager, PQM+ Program, USP
6. Kristina Campbell, Editor, PQM+ Program, USP
7. Md. Shah Newaj, Consultant, PQM+ Program, USP
8. Dilruba Hossain, Technical Advisor-QMS, PQM+ Program, USP
9. Tabassum Munira, Snr Technical Officer and Technical Lead – QC, PQM Program, USP
10. Shaiful Islam Khan, Snr Technical Officer and Technical Lead-RSS, PQM Program, USP
11. Md. Mehedi Hasan, Technical Officer-RSS, PQM+ Program, USP

Acronyms

ADR	adverse drug reaction
AEFI	adverse event following immunization
API	active pharmaceutical ingredient
BET	bacterial endotoxins test
BP	British Pharmacopoeia
BSE	bovine spongiform encephalopathy
CTD	common technical document
DGDA	Directorate General of Drug Administration
DMF	drug master file
DNA	deoxyribonucleic acid
DP	differential pressure
ELISA	enzyme-linked immunoassay
EP	European Pharmacopoeia
EUA	emergency use authorization
ICH	International Council on Harmonization
IPV	inactivated poliovirus vaccine
MS	master seed
MCB	master cell bank
MPR	master production record
MWCB	manufacturer’s working cell bank
mRNA	messenger RNA
NCL	national control laboratory
PO	purchase order
PMS	post-marketing surveillance
PQM+	Promoting the Quality of Medicines Plus
QA	quality assurance
QC	quality control
RB	risk-based
RB-PMS	risk-based post-marketing surveillance
rDNA	recombinant DNA.
TRS	Technical Report Series
USAID	U.S. Agency for International Development
USP	United States Pharmacopeia
WCB	working cell bank
WHO	World Health Organization

Introduction

Registration guidelines for human vaccines are essential for ensuring the safety, Efficiency, and Quality of vaccines as well as for building public trust in the vaccination process.

Vaccines are immunogens; when introduced into the body, they stimulate the immune system to react to a specific pathogen, resulting in the development of antibodies to the targeted microorganism.

Vaccines for human use may contain:

- Inactivated organisms conveyed by chemical or physical means that maintain adequate immunogenic properties.
- Live organisms that are naturally virulent or have been treated to attenuate their virulence while retaining adequate immunogenic properties.
- Antigens extracted from or secreted by organisms or produced by recombinant DNA (rDNA) technology, vector-based vaccines, or nucleic acid based (e.g., mRNA).

To increase immunogenicity, antigens can be used in their native state or detoxified by chemical or physical means and may be aggregated, polymerized, or conjugated to a carrier. However, if any vaccine complies with product-specific World Health Organization Technical Report Series (WHO TRS) or British Pharmacopoeia/United States Pharmacopeia/ European Pharmacopoeia monographs and has satisfactory non-inferiority clinical study data with a marketed vaccine, it will also be considered a requirement for vaccine registration in Bangladesh. All applications should be submitted per the common technical document (CTD) dossier format. Any emergency authorization (EUA) vaccines, if applicable, should follow this guideline for full market authorization.

Part 1: Manufacturing and Control Requirements of Vaccines

I. Active Substance

Fermentation, cultivation, isolation, or synthesis generally starts with raw materials to produce active substances. Preparation, characterization, and purification of intermediates are the subsequent steps for the active substance. The purity and quality of the active substance depend not only on downstream testing, but also on proper control of the manufacturing and synthetic process.

Minimal levels of impurities depend on the below-listed controls:

- Ensure that starting materials for the preparation of active substances have the appropriate quality and purity, including the seed organisms and reagents;
- When manufacturing the active substance, put in place established in-process controls for intermediates;
- Use process validation for manufacturing active substances to confirm the consistency of the quality and purity of active substances; and
- Perform an independent quality control test, per designed specifications, before the final release of the active substances.

A. Description and Characterization [13]

Complete the steps outlined in this important section for each drug substance identified as present in the final drug product. This is applicable for single or combination vaccines.

A.1 Description [13]

The description contains transparent information about the drug substance or active ingredient. Provide the biological name (including clone designation and/or strain) or chemical name, (including any established name). The outlines should include the source of the cells, including microbes from which the drug substances were derived, the active components of the cell fractions or purified antigens, and the physical and chemical properties of the synthetic drug substance or active ingredient. Describe any chemical conjugation or alteration of the drug substance and list any inactive substances that may be present within the active substance or drug substance.

A.2 Biological Activity [2]

Characterization of vaccines may include the following:

- Specific identity testing (e.g., Western blot analysis or enzyme-linked immunoassay [ELISA]);
- Cytometric analysis;
- Neurovirulence testing, if appropriate;
- Serotyping;
- Inactivation studies;
- Electrophoretic typing;
- Neutralization assays;
- Testing B-cell and T-cell response; and
- Titrations.

Provide all relevant in vivo and in vitro biological testing (bioassays) performed on the manufacturer's reference standard lot or another relevant lot to demonstrate the potency and biological activity or activities of the active substance described, as well as the results. For market authorization, include a detailed description of the protocol or method used for each bioassay, the control standards used, the validation of the inherent variability of the test, and the established acceptance limits for each biological assay. Also include specific antibody properties used in immunochemical or serological assays.

B. Manufacturer [2]

B.1 Identification [2]

The application should include name(s), address(es), the Directorate General of Drug Administration (DGDA) registration number, and any other relevant organizational information for each manufacturer responsible for drug substances manufacturing procedure or testing operations. This could include independent contractors, other locations/sites owned or company subsidiaries

operated by the applicant. This section should incorporate a discussion of the operations performed by each party, including the responsibilities delegated to each party by the applicant.

C. Method of Manufacture[2]

Provide detailed descriptions of manufacturing and controls to demonstrate proper quality control and prevention of possible contamination and cross-contamination with adventitious agents. It is recommended to include a list of all relevant standard operating procedures (SOPs), but inclusion of a copy of these SOPs is not necessary.

C.1 Biological Raw Materials[2]

Provide a list of all materials (culture media, buffers, resins for peptide synthesis, chemicals, columns, etc.) required in manufacturing the active pharmaceutical ingredient (API), and their tests specifications and test method. Reference either the official compendia (BP/USP/EP) or in-house specification and method. For purchased materials, provide representative certificates of analysis (CoAs) from the supplier(s) and/or manufacturer's acceptance criteria. Redundant testing at the purchasing manufacturer of vaccine may not be necessary if the purchasing manufacturer has approved the specification and testing methods at the vendor.

Describe in detail the custom reagents, such as monoclonal antibodies, enzymes, other proteins, uncommon amino acids and derivatives, or glycolipids, used in purification or production of the API. This should include identification of the vendor/supplier, specificity, and origin, including the manufacturing scheme if applicable. Provide the results of adventitious agent testing of raw materials used in propagation (e.g., serum, trypsin, amino acids, and other biological reagents), including bacterial and fungal agents, cultivatable and/or non-cultivatable mycoplasmas, mycobacteria, and viruses (if applicable). If your process includes removal or inactivation of potential infectious contaminants from biological raw materials, you should validate this process.

Process gases (compressed air, carbon dioxide, nitrogen) and water (purified and water for injection) are considered raw materials. Reference this list in parts of the application that provide detailed descriptions of the use of each component. For human-derived raw materials and reagents, documentation should include sourcing from appropriately screened and tested donors or use of products that are already licensed for human use. Testing and sourcing of bovine- and porcine-derived materials and reagents should occur in a manner that minimizes the risk of contamination from bovine spongiform encephalopathy (BSE) and porcine adventitious viruses.

C.2 Flow Charts[2]

For each active substance, provide the manufacturing process flow with a complete visual representation.

If multiple active substances are prepared from a single strain, a common flow chart is acceptable through the propagation and harvest cycle, with indications of where the processing diverges. This flow chart should show every step-in production, equipment, and materials used in the production process, the room or area where the operation is performed (provide reference diagrams), and the complete list of the in-process controls and tests performed on the product at each step. Include in-process holding steps with time and temperature limits indicated where applicable. For chemical synthesis, the flow chart should include all the steps in a general synthesis cycle with other specific

steps indicated, such as fragment condensation or peptide cleavage. This diagram should also include information (or be accompanied by a descriptive narrative) on the methods used to transfer the product between steps (e.g., open transfers under laminar flow/mobile laminar airflow units). Document such transfers for product movement between equipment, areas, rooms, buildings, and sites. Identify computer-controlled manufacturing steps and any equipment that is dedicated to specific areas or products.

C.3 Origin and Source of Cells[2]

Indicate the source of the cells (laboratory or culture collection) from which the cell substrate was obtained and cite relevant references from the scientific literature. Information obtained directly from the source laboratory is recommended. If these are not available, use literature references.

C.3.1 Human Cells[2]

Describe the source of cells for human cell substrates, as apposite for the given cells, including:

- The materials and methods used.
- The tissue or organ of origin.
- The donor's age, gender, and ethnic and geographical origin.
- General physiological condition.
- Health or medical history, as well as the results of any pathogenic agent tests.

The donor's age may influence the in vitro lifespan of the cell line; it focuses on human diploid fibroblasts, so provide this information if available.

C.3.2 Animal Cells[2]

Provide descriptions of the animal source (including fertilized avian eggs) used for the propagation of microorganisms, or production of recombinant proteins used for vaccine manufacturing. Include information about species, strains, age, sex, geographical location, health status (specific pathogen-free), the result of adventitious agent screening, husbandry practices (quarantine procedure), inoculation description, description of the tissues harvested and the method of the harvest of the animals. Animal cells may be adventitious agents, posing a potentially greater risk to humans if not properly controlled. Describe the steps taken to remove, inactivate, or prevent contamination of the product by any adventitious agent present in the cell substrate.

C.3.3 Virus/Bacteria/Other Sources[2]

Provide a detailed description of the virus/bacteria/other seeds used for vaccine manufacturing. The information submitted should include, at minimum: the original source, passage history, seed lot system, culture technique, genotype, and strain/variant of virus/bacteria/other.

C.3.4 Microbial Cells[2]

Provide a description of the microorganism(s) from which the cell substrate is derived, including the species, strain, and known genotypic and phenotypic characteristics. Microbial cells and their derivatives used as the vaccine active substance include whole-cell vaccines (live or killed), crude lysate or purified immunogens, rDNA products, conjugates, and plasmid DNA vaccines.

Include the history and characteristics of each strain used to produce the vaccine and a complete strain description. This should include the origin of the isolate, species, biochemistry (fermentation profile, etc.), strain identifier, specific identifying characteristics (serotype, etc.), virulence (attenuation method, if applicable), genetic characterization if known (markers, inserts, deletions, etc.), plasmids. genetic stability, pathogenicity, toxin production, and any other biological hazard.

C.4 History of Cells[2]

Provide a description of the cultivation of the cells. Detail the technique/method used for the isolation of the cells, procedures employed in the culturing of the cells in vitro, procedures used to establish cell lines (for example, the use of any physical, chemical, or biological procedure, or added nucleotide sequences). Describe any genetic manipulation or selection and provide all information on the identification, characteristics, and results of testing of these cells for endogenous and adventitious agents.

Continuous cell lines usually include quantitating culture duration by estimation of either a number of population doublings or a number of sub cultivations at a defined dilution ratio, or time in days. The accurate number of population doublings based on estimation during all stages of research, development, and manufacturing is an important point of consideration for diploid cell lines possessing a finite in vitro lifespan. For microbial cells, document sub cultivation frequency after adequately accounting for cell substrate generation.

Applicants should provide a rigorous discussion of procedures that might create exposure to infectious agents regarding the generation of cell substrates. Describe constituents of the culture medium, particularly information regarding exposure of the cells to materials of human or animal origin like sera, enzymes, hydrolysates, or other living cells. The outline should include the source, method of preparation and control, test results, and quality assurance. Reference relevant literature on these points when available. This information will allow an in-depth analysis of potential entry routes for adventitious agents from these sources and can be part of the risk-benefit analysis of the product.

C.5 Generation of the Cell Substrate[2]

The selection of an acceptable parental cell line is a critical step. A parental cell line is often the unfransfected recipient cell line for recombinant products. The use of characterized parental cell banks is recommended but not essential. A characterized parental cell bank could also be useful, particularly when multiple cell substrates are generated from the identical parental cell type, by providing a group of data on which the master cell bank (MCB) quality assessments are often based. The myeloma cell line, for example, might be saved as a parental cell line for hybridomas.

Thoroughly document all specific procedures deployed to generate the precise cell substrate, for example: cell fusion, selection, transfection, colony isolation, cloning, gene amplification, and adaptation to specific culture conditions or mediums. Submit the expansion pattern and morphological appearance and characteristics of the cell lines, from the MCB to the end-of-production cells. Provide thorough discussion of any cell substrate's adventitious agent profile. Information on the methodologies employed in the event of the cell substrate can aid in providing a transparent understanding of the cell substrate's history. Before cell banking, some cell substrates like human diploid fibroblasts might not require extensive manipulation or cloning.

The cell substrate for recombinant products is the transfected cell containing the required sequences that have been cloned from one cell progenitor. Consult other relevant international guidelines for more information on the generation of rDNA modified cell substrates. For nonrecombinant products or nonrecombinant vaccines, the cell substrate is the cell from the parental line chosen for preparation of the MCB without further modification. For products derived from hybridomas, the cell substrate is the hybridoma cell line derived by the fusion of the parental myeloma cell line with other parental cells, e.g., immune spleen cells.

C.6 Cell Banking [2]

Cell banking ensures that an adequate supply of equivalent, well-characterized cells are available for production over the expected lifetime of the product. Additionally, to provide a continuous supply of biological starting material, cell banking provides a comprehensive characterization of the cell substrate and reduces the possibility of adventitious agent contamination, and/or maximizes the possibility of detection of contamination. Manufacturers are accountable for ensuring the quality of every cell bank and of the testing performed on each bank.

Generally, the cell bank system consists is two-pronged: the MCB and a working cell bank (WCB), often called a manufacturer's working cell bank (MWCB). The MCB represents a group of cells of uniform composition derived from one source prepared under defined culture conditions. The WCB derives from one or more vials of cells from the MCB that are expanded by serial subculture. The pooled cells are dispensed into individual vials and cryopreserved to create the WCB for vaccine manufacturing purposes.

The MCB and WCB may differ in certain respects, e.g., culture components and conditions. Similarly, the culture conditions accustomed to preparing the MCB and WCB may vary from those used for the production process. If changes in the cell culture process don't affect product quality, do not reclone the cells or rebank the MCB or WCB. It is essential that a characterized bank provides a homogenous product.

A single-tiered banking system comprises only the MCB, with no WCBs (for example, if relatively few containers were needed every year to supply the required product). In some microbial expression systems, a brand-new transformation is performed for every new cell substrate container lot, based on using aliquots of thoroughly tested host cell and plasmid banks for every new transformation and on testing of every transformed cell substrate bank. The MCB takes into account this transformed cell substrate bank, used because of the source of cell substrate for production. Host cell banks, plasmid banks, and MCBs maintenance involves appropriate preservation methods. This alternative system is adequate because the transformation of bacteria and yeast is mostly a reproducible and simple process, unlike the events needed for the transfection of metazoan cells. Manufacturers should provide information on the host cells, rDNA molecules (such as plasmids), method of transformation, and cell banking, and therefore the results of characterization studies.

However, the manufacturer meeting the product-specific requirements mentioned in WHO TRS or compendia (BP/USP/EP) will be considered adequate for cell banking.

C.6.1 Master Cell Bank [2]

Identify the cells that constitute the MCB and provide a complete history and characterization of the MCB for market authorization (including, as appropriate, for the given cells):

The biological or chemical method used to derive the cell bank.

Identity by any suitable validated method.

Specific identifying characteristics (morphology, serotype, etc.).

Karyology and/or tumorigenicity if applicable.

Virulence markers if applicable.

Genetic markers if applicable.

Purity of culture.

Media and components (e.g., serum).

C.6.2 Working Cell Bank [2]

Provide a detailed description of the procedures used to derive a WCB from the MCB. This should include the identification system used for the WCB as well as its procedures for storage and cataloging. The assays used for qualification and characterization of each new WCB should be included with the results of those assays for the working cell bank currently in use. A description of animal passage, if applicable, of the WCB performed to assure the presence of virulence factors which are protective antigens should be supplied. This section should also contain a description of the methods and procedures used to assure culture purity and identity.

C.6.3 Primary Cell Bank[2]

In some cases, another tier of the cell bank system, a primary cell bank, is also established, allowing manufacturers to perform extensive testing on a pool of cryopreserved primary cells before their use in vaccine production.

Because primary cell cultures are used within the first passage after establishment from the tissue of origin, extensive characterization of the cells before use isn't possible, as it is with banked cell substrates. Furthermore, biological products derived from cell substrates are frequently not subjected to extensive processing (e.g., purification).

Discuss the rationale for the employment of primary cells. The information submitted for every primary cell line used should include, at a minimum, the following:

- Species and ages of the animals, in addition to the source of cell derivation.
- Health status of the animals from which the cells are derived, e.g., specific pathogen-free.
- Husbandry practices (quarantine, etc.) used to ensure the suitability of the animals.
- Veterinary and laboratory monitoring used to ensure the suitability of the animals.
- A description of the preparation of primary cell substrates including materials, components used, the identity and source of all reagents of human or animal origin.
- An explanation of concurrent testing, and the results, demonstrating the absence of detectable contaminants and adventitious agents in these substrates, which can include:

- Observation of production or uninfected control cultures before, during, and beyond the amount of production,
- Inoculation of culture fluids from production and uninfected control cultures into various susceptible indicator cell cultures capable of detecting a wide range of relevant viruses, followed by examination for cytopathic changes and testing for the presence of hem-adsorbing viruses; and
- Other tests for specific agents (such as relevant retroviruses) as necessary.

- Methods used for isolation of cells from tissue, the establishment of primary cell cultures, and maintenance of cultures.
- Tests performed on primary cell substrates to qualify them to be used in production.

C.6.4 Diploid Cell Strains[2]

Expansion and cell banking of primary cell culture resulting the established of diploid cell strains. These cells have a limited lifespan and don't seem to be immortal like primary cell lines. The diploid cells normally retain a diploid or near diploid karyotype, a characteristic that also differs from cell lines, which are generally aneuploid or non-diploid. The karyotype of the cell source should be determined because it could be helpful to ascertain the identity and characterize a cell strain. Such analyses will establish the diploid character of the cells and determine their freedom from contamination with other cell lines. It'd even be useful to observe the genetic stability of the diploid cell strain throughout the production.

C.6.5 Tumorigenic Cell Lines[2]

Tumorigenic cell lines are the cells derived from tumors. If the cell lines are tumorigenic, additional testing should be performed. Tumorigenic cell lines should be assessed for potential oncogenic viruses and oncogenic substances (including nucleic acids) that might cause the induction of a neoplastic process in a vaccine recipient. Depending on the tissue type, source species, passage history, and knowledge of transforming event(s), test strategies for potential oncogenic viruses or substances are often determined on case-by-case bases.

Testing should demonstrate that the finished product is free from the transforming agent in cases where the transforming event is known (e.g., if the cells were transformed by a known oncogene). For instance, if adenovirus sequences are wont to transform a primary human cell to produce a cell line (e.g., 293 cells), testing should confirm that the final product is free from the introduced viral sequence. Similarly, if a virus is used to transform cells, that virus and its genetic material should not be detectable in the final product using an assay with sensitivity sufficient to produce assurance of safety. In tumorigenic or tumor-derived cell lines where the mechanism of transformation is unknown, additional testing is required to confirm the absence of potential transforming and oncogenic agents.

The testing recommended for the qualification of cell substrates and cell banks should be applied even to rodent cell lines. However, because most rodent cell lines used in the production of biologicals are known to be tumorigenic, it is considered unnecessary to check rodent cell lines for tumorigenicity. Assuming they will be tumorigenic, the considerations described above apply. Additionally, rodent cell lines are presumed to be capable of manufacturing endogenous retroviruses. Perform an assessment of the number and type of retroviruses produced. Infectivity assays for retroviruses are recommended. Use rodent cell lines only if the product will be sufficiently purified

to demonstrate levels of viral clearance, assuring the ultimate product is not contaminated with retroviral particles.

C.7 Cell Banking Procedures[2]

Avoid using a contaminated cell substrate (or bank) in production and avoid a loss of product availability or development time resulting from the need to re-establish a cell bank due to contamination. No cell bank testing regimen can detect all potential contaminants; thus, use these preventive principles during cell banking to reasonably assure the absence of contamination and a reliable source of the cell substrate.

Provide detailed information on the cell banking procedures used. This should include: the system; size; container and closure system; methods, cryoprotectants, reagents, and media for preparation; cryopreservation and storage conditions; in-process controls; and storage.

Explain the procedures to avoid microbial contamination and cross-contamination by other cell types present in the facility, as well as steps that allow the banked cells to be traced.

Ensure that the documentation system and labeling system can withstand the process of preservation, storage, and recovery from storage without loss of labeling information on the container.

Cells are generally prepared for banking by expanding cultures in a progressively greater number or larger size of the vessel until a pool of cells large enough to generate enough containers for the bank can be obtained. To ensure that the contents of each container are uniformly composed, prepare a single pool of cells for banking by combining the cells from all of the culture vessels, if using more than one vessel.

Cells suspended in the preservation medium are kept into sterilized containers, which are then sealed and stored under appropriate conditions. For instance, animal cells in media containing a cryoprotectant are frozen within the sealed containers under defined and controlled conditions, then transferred to storage within the vapor or liquid phase of cryogen or equivalent ultra-low temperatures. Other methods of preservation and storage could also be adequate, depending on the organism used, but they must be capable of maintaining a level of cell viability upon reconstitution that is both consistent and enough for production use.

To ensure continuous, uninterrupted production of pharmaceuticals, manufacturers should carefully consider the steps they will take to prepare for catastrophic events that would render the cell bank unusable. These events could include fires, power outages, and human error. Manufacturers should describe their plans for such precautions; for instance, these may include redundancy within the storage of bank containers in multiple freezers, use of back-up power, use of automatic cryogen fill systems for storage units, storage of some of the MCB and WCB at remote sites, or regeneration of the MCB.

Use the thawing of one or more MCB containers as the starting point for estimates of in vitro cell age during production. Calculate in vitro lifespan for diploid cell lines in terms of population-doubling levels.

C.8 Characterization and Testing of Cell Banks[2]

Characterization and testing for banked cell substrates is a crucial part of vaccine control. Characterization of the MCB allows the manufacturer to assess this source concerning the presence of cells from other lines, adventitious agents, endogenous agents, and molecular contaminants (e.g.,

toxins or antibiotics from the host organism). This testing aims to verify the identity, purity, and suitability of the cell substrate for manufacturing use.

Submit detailed information on banked cell substrate characterization and testing including, in some cases, additional tests like tumorigenicity or karyology studies. The test method chosen for a specific cell substrate depends on the biological characteristics of the cell (such as growth requirements), the cultivation history of the cell (including the employment of human and animal derived biological reagents), and therefore the available the test method. The extent of cell substrate characterization can influence the kind or level of routine testing required at later stages of production. Manufacturers are required to perform identity and purity testing once for every MCB and a test of stability during cell cultivation once for every product to be registered. Applicants also consult the International Council on Harmonization (ICH) guideline on viral safety. Perform the relevant tests among those described below and describe them within the market application, along with the results of the testing.

Cell lines containing exogenously assembled expression constructs require nucleotide and/or organic compound sequence characterization or other phenotypic analyses. It should even be useful to look at the coding sequences of several non-rDNA-derived cell lines whose gene sequences are characterized and well understood. However, it is not needed to review sequences encoding complex natural products like families of related gene products, microbial vaccine antigens, or monoclonal antibodies from hybridomas.

However, if manufacturers can meet product specific requirements mentioned in WHO TRS or compendia, it will be considered adequate for cell banking.

C.8.1 Testing for Identity and Purity[2]

Perform appropriate tests to verify that the banked cell is accurately represented. Use of either phenotypic or genotypic characteristics is acceptable in identity testing; it is not necessary to conduct all possible tests. Perform identity tests on the MCB, with limited identity testing on each WCB. Describe the relevant tests in the application along, with the testing results. In general, the methods described in section I- A.2.1 are adequate to substantiate identity and purity.

For metazoan cells of either human or animal origin that grow attached to the substratum, the morphological analysis could be a useful tool in conjunction with other tests. In most cases, isoenzyme analysis is sufficient to verify the species of origin for cell lines derived from human or animal sources; other tests could also be appropriate depending on the history of the cell line. Other technologies are also substituted to verify species of origin; these include, for example, banding cytogenetics or the use of species-specific antisera. An alternative strategy would be to demonstrate the presence of unique markers, for instance, by using banding cytogenetics to detect a unique marker chromosome, or DNA analysis to detect a genomic polymorphism pattern (for example, fragment length polymorphism, variable number of tandem repeats, or genomic dinucleotide repeats). Either confirmation of species of the origin or the presence of known unique cell line markers is considered an adequate test of identity. Expression of the specified product may represent a complementary approach to confirmation of identity.

Most microbial cells typically require analysis of growth on selective media to confirm host cell identity at the species level for the host cell bank and the transformed cell bank. For E. coli, where a variety of strains could also be used, biological characterization methods like phage typing should be considered as supplementary tests of identity. For plasmid banks, identity assessment may be accomplished as described by the ICH document on analysis of the expression construct.

Expression of the required product is additionally considered equal to confirm the identity of the microbial expression system.

The assessments of MCB and WCB are biologically pure (i.e., free from adventitious microbial agents and adventitious cellular contaminants, where applicable). This assessment is a critical aspect of cell development and banking. Consider the effect of antibiotics and selective agents for the detection of adventitious microbial contaminants when planning and performing these tests. Submit the results of tests for the presence of bioburden (bacteria and fungi) and mycoplasma in metazoan cells for the MCB and WCB. Also provide the results of virus testing of metazoan cell substrates to detect possible contaminating viruses, using appropriate screening tests designed to detect a good spectrum of viruses and relevant specific tests that supported the cultivation history of the cell line.

The purity of cell substrates is often compromised through contamination by cell lines of the identical or different species of origin. The selection of tests to be performed depends on whether opportunities have existed for cross-contamination by other cell lines. In some cases, it will be necessary to take care of growing cultures of various cell lines within the same laboratory.

Open manipulations are performed during procedures in cell banking; take care to avoid simultaneous open manipulations of other cell lines to stop cross-contamination.

Whenever another cell line was present within the cell banking room at the identical time that open cell banking procedures were performed (such as cell expansion, pooling, or aliquoting of the chosen cell lines), test the cell banks for the presence of cells from (or products derived from) the second cell line. In general, the methods described above to assess cell identity are considered adequate tests to detect cross-contamination by other cell lines. Additional assurance of lack of cross-contamination may be provided by successful preparation of the intended product from the cell substrate.

The appearance and performance of specific tests for adventitious microbial agents in cell banks should consider the properties of the banked cell for microbial cells. The likely contaminants are based on scientific literature, source, methods, materials used for cultivation, and other organisms present within the banking laboratory. For example, perform a visual examination of the characteristics of well-isolated colonies using several microbiological media, some of which do (while a few do not) support the growth of the cell substrate. However, manufacturers do not necessarily need to characterize resistant mutants of the cell substrate arising from such studies, or other artifacts of such assays. The aim of these assays is to detect existing contaminants.

C.8.2 Testing for Cell Substrate Stability[2]

Another aspect of cell characterization is suitability for intended production use. There are two concerns for cell substrate stability consistent production of the intended product and retention of production capacity during storage under specified conditions.

Examine at least two time points during cultivation for production for the evaluation of stability. The one-time point using cells has received a minimal number of cell sub-cultivations. Other time point uses cells at or beyond the limit considering in vitro cell age for production use described within the marketing application. Data derived from production cells expanded under pilot plant scale or commercial scale conditions to the proposed limit in vitro cell age for production use or beyond the limit should be supported by the limit of in vitro cell age for production use.

The primary focus should be on evaluating the cell substrate according to the consistent production of the required product of interest. The character of the cell substrate, cultivation methods, and products play a significant role in testing and test article(s) used for such assessments. The consistency of the coding sequence of the expression construct should be verified in several ways, including the limit of in vitro cell age derived from cells cultivation for production use; product evaluation in cell lines (rDNA expression constructs); and macromolecule testing.

For non-recombinant cell lines during which the coding sequence for the required product has already been analyzed at the MCB or WCB level, use supermolecule testing or analysis of the purified protein product to confirm the invariability of the protein coding sequence during production. This is applicable to production cells cultivated to the proposed limit of in vitro cell age for production use or beyond.

Where the products cannot be analyzed as described above, other specific traits like morphological characteristics, growth characteristics, biochemical markers, immunological markers, desired product productivity, or other relevant genotypic or phenotypic markers are also useful for assessing cell substrate stability. In some cases, direct comparison of MCB characteristics with the limit of in vitro cell age from production cells within or beyond is difficult to assess cell stability during production. Oxygen or glucose consumption rates and ammonia or lactate production rates are important to know for testing. Maintain data from cells that expand to the proposed increased limit of in vitro cell age. This maintenance occurs by increasing within the specified limit of in vitro cell age for production use.

For diploid cell lines, present data establishing the finite in vitro lifespan of the cells from the WCB under conditions representative of these used for manufacturing.

Typically, evidence for banked cell stability under defined storage conditions is generated during the production of trial material from the banked cells. Data from cell viability determination when preserved cells are reconstituted for production of trial supplies will confirm that the revived cells survived the preservation process. Data from clinical materials preparation will show that the revived cells are can be used to make the required product. In the application dossiers, clearly document available data and provide a proposal for monitoring banked cell stability.

The proposed monitoring will be distributed when one or more cryopreserved bank containers are thawed for production use, when the product or production consistency is monitored in a relevant way, or when one or more cryopreserved MCB containers are thawed for preparation of a new WCB (and the new WCB is correctly qualified), as appropriate. When production is halted for an extended period, perform viability testing on the cell bank used as a source of the assembly substrate on a daily basis. No additional testing of the MCB or WCB is usually sought if the viability of the cell substrate is not significantly reduced.

C.8.3 Testing for Karyology and Tumorigenicity[2]

Tumorigenicity is a process by which cells form tumors when inoculated into an animal (generally in syngeneic, immunocompromised allogeneic, or immunosuppressed xenogeneic hosts). Tumorigenicity is a characteristic of the immortalized cells themselves, instead of the agents or components present in them.

Tumorigenic cells haven't been employed in the production of prophylactic viral vaccines, mainly theoretical concerns that components within tumorigenic cells may induce tumors in vaccine

recipients. These concerns include the potential presence of exogenous agents like oncogenic viruses and potential risks from such endogenous agents. like Endogenous viruses or oncogenic nucleic acids. Furthermore, intact human cells derived from human tumors are shown to make tumors in allogeneic humans.

The purpose of tumorigenicity studies is to work out whether the cell matrix can form tumors after the inoculation of animals. The TPD 50 (tumor-producing dose in 50% of animals) and capacity to form metastases are characteristic properties of a cell line, and these characteristics might be used to further define the tumorigenic phenotype of a cell line. Considerations associated with tumorigenicity testing include:

:

- Selection of appropriate animal models.
- Definition of a positive result.
- Determination of appropriate test duration.
- Determination of the suitable number of cells to be tested.
- Selection of appropriate controls.

Use an animal model known to be susceptible to tumor formation by tumorigenic cells. Because immunocompromised adult and newborn rodents are relatively sensitive to revealing a tumorigenic phenotype, consider these animal models.

Thus, the foremost commonly used animals for tumorigenicity testing are nude (nu/nu) mice because they are T-cell deficient. Newborn nude mice appear to be more vulnerable to tumor formation than adult nude mice, suggesting that neonatal nude mice are the most suitable option to use when the identification of a weakly tumorigenic phenotype is most important. Another animal model might be preferable if shown to own comparable sensitivity to the mouse model.

Selection of the acceptable study duration of testing requires balancing the increased sensitivity, which may be obtained by employing a longer test, against the likelihood of false-positive results because of spontaneous tumor formation. Weakly tumorigenic cells might require four to seven months to make tumors in nude mice.

Therefore, in some cases, longer observation periods are also required using karyology and tumorigenicity testing to assess the safety of a diploid cell line or characterizing a brand new cell line, depending on cells, product type, and manufacturing process.

Extensive analysis to see the relative abundance of aneuploid cells has not proven useful. Karyology need not be determined for rodent cell lines or new cell lines known to be non-diploid. However, the cytogenetic analysis could be an adequate method to assess cell substrate identity or purity as described in section I - C.8.1. Repetition of tumorigenicity testing for cells with documented evidence of tumorigenicity is not desirable. For highly purified, cell-free products, karyology and tumorigenicity testing are generally not considered necessary, provided that appropriate limits for residual host cell DNA are shown to be consistently met. This needs to be proved either through process validation studies or by lot release testing.

In general, products where the presence of live cells cannot be ruled out or where downstream purification is minimal (for example, some conventional live virus vaccines) will necessitate such characterization of the cell substrate. Tumorigenicity testing and chromosomal analysis for new cell substrates for unpurified products should be evaluated on a case-by-case basis. When a product contains cells or is not highly purified, the employment of cell lines known to be tumorigenic or to possess process abnormal karyology should be evaluated in terms of risk-benefit for every product application.

Products manufactured in genetically unmodified MRC-5 or WI-38 cells do not require karyology or tumorigenicity characterization because extensive characterization of those cell lines has already been performed and published. Manufacturers should confirm once that the cells grown within the manner to be used in production are diploid and have the expected lifespan for every MRC-5 and WI-38 WCB generated. For new or previously uncharacterized diploid cell substrates, present confirmation of diploid karyology and establish tumorigenic potential using cells from the MCB.

C.8.4 Testing for Oncogenicity[2]

Oncogenicity is the process by which agents immortalize cells and enable them to form tumors. It is important to ensure that the cell substrate is free of potentially oncogenic components that could contaminate the product. The theoretical risk of containing oncogenic components may be higher if vaccines are produced in tumor-derived cell substrates or cell substrates that have developed a tumorigenic phenotype by unknown mechanisms.

If the presence of an oncogenic virus is suspected because of the cell phenotype or the origin of the cell substrate, it may be appropriate to perform oncogenicity testing in animals using lysates of the cell substrate. For cell substrates with a tumorigenic phenotype, it might be appropriate to perform oncogenicity testing in animals using DNA from the cell substrate to provide assurance that residual DNA is non-oncogenic. Oncogenicity testing might also be appropriate for products with high quantities of residual cellular DNA. Existing assays for the presence of oncogenic agents in certain cell substrates might not be adequate to provide sufficient assurance of the safety of the vaccine for clinical use.

C.9 Seeds[2]

The methods used to store viral/bacterial/recombinant vaccine seed stocks are like those employed in cell banking. Like for cell banks, document passage history (viral) and seed origin history. The outline should include donor screening, testing, and case history where available/required. If applicable, document and explain phenotypic manipulations like cold adaptation, development of temperature sensitivity, or attenuation of virulence/pathogenicity. Describe and document genetic manipulations like reassortment and recombination, including macromolecule sequencing and sourcing (if applicable) of starting biological material (plasmids, parental viruses, etc.).

These vaccine virus/bacteria/recombinant organism banks are commonly cited because the master seed (MS). Seeds should be stored at -70ºC or lower (per requirements) and stored in multiple locations within a production facility or at an overseas site for security reasons. Test the seed for its growth characteristics on the assembly cell substrate, tissue tropism, genetic markers, viability during storage, genetic stability through production (if required), attenuation (if applicable), and its absence of adventitious agents. If attenuation or derivation is achieved by passage through different cell types from different species, test the seed for the absence of adventitious agents from all species

that they could be exposed to from isolation, through passage, and through production, including those that may be present within the raw materials used at each stage.

However, if manufacturers can meet product specific requirements mentioned in the WHO TRS or compendia, it will be considered adequate for seed banking.

C.9.1 Master Seed[2]

The master seed (MS) should be fully characterized. Additionally, stability genotype and phenotype should be demonstrated for passages beyond the level used in the production. For example, if the MS (virus) is considered to be at passage 47, the WS (virus) at passage 48, the production cultures inoculated with WS at passage 49, and the production process allows for only a single round of replication, then it would be appropriate to assess the stability of the vaccine virus at passages 47 through 51 (five passages).

Tests should be performed for identity (which could necessitate sequencing the entire vaccine virus), bacterial and fungal sterility, the presence of mycoplasmas, Mycobacterium tuberculosis (if appropriate), adventitious viruses (in vitro and in vivo tests), the viral phenotype (e.g., tissue tropism, attenuation properties, temperature sensitivity), and genetic stability. Specific tests should also be considered for agents that might be present in the seed due to its passage history.

The virulence/pathogenicity and the broad host range of the vaccine virus/bacteria/ recombinant organism might complicate in vitro and in vivo adventitious agent testing in some cases. Testing for adventitious agents might then require the neutralization of the seed. Preferably, neutralizing antibodies should be monoclonal and prepared in a species other than the cells in which the MS was prepared. In addition, due to the potential for cross-neutralization of adventitious human viruses, neutralizing antibodies should generally not be prepared from human or primate serum.

Sometimes, it is not possible to efficiently neutralize a seed. In such cases, alternative strategies, including testing smaller quantities of seed or introducing additional tests (e.g., PCR, antibody production assays), may be preferable.

Assessment of neurovirulence is often appropriate, and it is recommended that one uses an appropriate animal model, methods, and scoring systems for this assessment before initiating such studies. For viruses that are neurovirulent or might revert to neurovirulence (e.g., polio viruses), it might be necessary to assess neurovirulence not only on the MS or an end-of-production passage level virus stock, but also on the product lot-by-lot (if applicable).

C.9.2 Working Seed[2]

A working seed (WS) can undergo less rigorous characterization than the original MS. Once it is demonstrated that the MS is free of adventitious agents from the species to which the vaccine virus/bacteria/recombinant organism had been exposed during its isolation and passage history, it is necessary only to show that the WS is free of adventitious agents from the species used to generate the WS (e.g., production cells and raw materials used in propagation and processing).

C.10 Genetic Constructs and Recombinant Cell Lines[2]

For rDNA-derived products and rDNA-modified cell substrates, give detailed information about the host cells and the source and function of the component parts of the recombinant gene construct.

C.10.1 Host Cells[2]

Provide the sources, relevant phenotype, and/or genotype of the host cell used to build the biological production process. Include the results of the phenotypic and/or genotypic characterization markers of the host cell, as well as the monitoring procedure for cell stability, purity, and selection, should be included (if applicable).

C.10.2 Gene Construct[2]

Provide information on gene sequences that were introduced into the host cells and the method(s) adapted to prepare the gene construct and a restriction enzyme digestion map of the construct. Include the entire nucleotide sequence of the coding region and regulatory elements of the expression construct, with translated amino acid sequence, with annotation designating all important sequence features.

C.10.3 Vector[2]

Give detailed information regarding the vector and genetic elements and description of the source and role of the component parts of the vector (e.g., origins of replication, antibiotic resistance genes, promoters, and enhancers). Include a restriction enzymes digestion map indicating a minimum of those sites employed in the construction of the vector and the genetic markers critical for the characterization of the production of cells.

C.10.4 Final Gene Construct[2]

Provide a detailed description of the cloning process that resulted in the final recombinant gene construct and a step-by-step description of the assembly of the gene fragments and vector or other genetic elements to make the ultimate gene construct. Also provide an endonuclease digestion map indicating a minimum of the sites employed in the ultimate product construct.

C.10.5 Cloning and Establishment of the Recombinant Cell Lines[2]

Depending on the methods to be used to transfer a final gene construct or isolated gene fragments into its host, provide the mechanism of transfer, copy number, and the physical state of the closing construct inside the host cell (i.e., integrated or extrachromosomal). Completely describe the amplification of the gene construct, if applicable, selection of the recombinant cell clone, and establishment of the seed.

C.11 Cell Growth and Harvesting[2]

C.11.1 Propagation[2]

In the propagation section, provide the following descriptions:

- Every step-in propagation from retrieval of the WCB to culture harvest (stages of growth).
- The medium used at each step (including water quality with specification) and details of preparation and sterilization (time, temperature, and pressure).
- The inoculation and growth of initial and subcultures (passages number), including volumes, time, and temperature of incubation(s).
- The procedure of transfer precautions to manage contamination and cross contamination.

- In-process testing parameters, which determine inoculation of most culture systems.
- In-process testing to ensure freedom from adventitious agents, including tests on culture cells, if applicable; the character of the culture system, including the operating system and control parameters (e.g., incubation temperature, static vs. agitated, aerobic vs. anaerobic, culture vessel vs. fermenter, volume of fermenter, or number and volume of culture vessels).
- The parallel control cell cultures, if applicable, including number and volume of culture vessels.
- Induction of antigen, if applicable.
- The use of antibiotics within the medium and rationale, if applicable.

Provide a brief description of all process parameters that are monitored and a typical growth curve or growth description; describe the in-process controls and testing for purity, viability, and phenotypic identity; and, because the times of testing should be included in both the Flow Chart and the Batch Records, include an outline of the precautions taken to regulate contamination (e.g., during sample removal and transfers) and whether they are “closed” or “open” procedures.

C.11.2 Harvest[2]

Provide a detailed description of the methods (precipitation, centrifugation, filtration, etc.) used to separate the crude active substance from the propagation system, including:

- The process monitored parameters.
- Harvesting criteria.
- If more than one harvest, the criteria for pooling

Include a working definition of a “harvest batch” and a description of precautions taken to maintain aseptic conditions (avoiding contamination and cross-contamination) during harvesting. Provide a description of the procedures used to monitor bioburden (including acceptance limits), sterility, and endotoxin (as appropriate). If the harvested crude active substance is held prior to further processing, describe storage conditions and time limits.

C.12 Inactivation, Purification and Downstream Processing[2]

Describe the methods and materials used to separate and concentrate intermediate forms and therefore the final bulk of the active substance from cells, media, solvents, or solutions employed in the manufacturing process.

Before use, remove antibiotics and other components (growth factors, antibodies, etc.) utilized in the culture but neither required nor specifically intended to be in the final vaccine product.

Provide a system to assure containment and prevention of contamination or cross-contamination. This is important because live attenuated viruses, whole inactivated virions, or virus-like particles often cannot be purified as rigorously as viral subunit vaccines, and their potential for contamination may be greater than that of subunit vaccines. The generation of live viral vaccines often involves cell disruption.

Within the submission, include CoAs for all reagents and biological raw materials used for vaccine production, as well as the methods of research and validation or verification.

Purify any seeds that were exposed to a known adventitious agent or that have an unclear passage history (e.g., by molecular cloning, the serial passage in a medium containing neutralizing antibody directed against the adventitious agent, or plaque purification). If the purification method is demonstrated to be capable of removing all adventitious agents from the seed to within an appropriate margin of safety, this approach may be used to qualify a seed.

However, the manufacturer meeting product-specific requirements mentioned in the WHO TRS or compendia will be considered adequate for inactivation and purification.

C.12.1 Inactivation[2]

If applicable, provide inactivation descriptions for:

- The method(s) to verify culture purity before inactivation;
- The method(s) and agent(s) used for inactivation;
- The method(s) undertaken to control aggregation and assure homogeneous access of inactivating agent(s);
- The stage in production where inactivation or killing is performed; and
- The parameters that are monitored.

Provide verification of the adequacy of and margin of safety achieved by the procedure of inactivation or killing. In the case of inactivating agents used in the manufacturing of bacterial vaccines, minimize and control traces appearing in the finished product.

C.12.2 Purification[2]

Explain the objectives and rationale for purification of component antigens from crude harvest, with descriptions, as appropriate, provided for:

- Methods used, including specialized equipment like columns; ultracentrifugation, ultrafiltration, and custom reagents like monoclonal antibodies;
- The process parameters monitored;
- The determination of yields;
- In-process testing (e.g., sensitivity and specificity of ELISA)
- The criteria for pooling over one batch;
- Sterility or bioburden monitoring and the precautions taken to prevent contamination and cross-contamination during purification;
- The reuse and/or regeneration of columns and adsorbents; and
- Monitoring for residual impurities and leachable reagents.

List the in-process controls and tests for purity, identity, and biological activity, and include the time points at which testing is performed in both the flow chart and batch records. List the ultimate acceptance criteria for the purified active substance; if the purified active substance is held before further processing, outline of the storage conditions and deadlines. Include verification of the soundness of the purified substance under the conditions described.

C.12.3 Stability Processing[2]

Describe any post-purification steps performed to produce a stabilized intermediate (e.g., adsorption, addition of stabilizers, addition of preservatives, lyophilization (in bulk), desiccation) and the objectives and rationale for performing each process. Detail the precautions taken to monitor bioburden and prevent contamination during these processes. If the stabilized intermediate is held prior to further processing, describe storage conditions and time limits. Provide verification of the stability of the active substance under the conditions described.

C.12.4 Detoxification[2]

For toxoid or toxoid-containing vaccines, detail the detoxification procedures for the toxin component(s):

- The method(s) and agent(s) used for detoxification;
- The detailed stage in production in where detoxification is performed; and
- The parameters that are monitored.

Provide verification of the suitability of the strategy for detoxification.

C.13 Synthetic active Substance[2]

Linear or complex synthetic peptides, or modified synthetic or semi-synthetic immunogens such as lipopeptides, peptide to carrier protein, or polysaccharide to carrier protein conjugates, are the purpose of the guidelines. The synthetic active substance includes include the above said requirements, If the manufacturer can meet the product-specific requirements outlined in WHO TRS or compendia, the synthetic active substance will be considered adequate.

C.13.1 Synthetic Peptides[2]

Follow the “Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances” in terms of peptide synthesis details, including purification procedures.

C.13.2 Conjugates and Modified Active Substance[2]

This section of the guidance refers to active substances derived from another active substance or intermediate via chemical or enzymatic modification, such as immunogenic conjugation to a carrier molecule, enzymatic or chemical cleavage, and derivatization. The modification may change the fundamental immunogenicity, toxicity, stability, or pharmacokinetics of the source active substance. The derived active substance may include linking moieties and new antigenic epitopes.

C.13.2.1 Manufacturing Methods[2]

The manufacturing methods should provide a detailed description of:

- The specifications and acceptance criteria for the native active substance starting materials that ensure suitability for conjugation or modification.
- The conditions of all reactions and/or syntheses that produce a semi-synthetic conjugated molecule, derivatized molecule, or subunit, including intermediate styles of the reactants and active substance.

- The method parameters that are monitored, in-process controls, testing for identity and biologic activity, and any post-purification steps performed to provide a stabilized derived active substance.

Describe the methods and equipment used for separation of unreacted materials and reagents from the conjugate, derivative, or subunit, and a rationale for the selection of methods.

C.13.2.2 Specifications[2]

Provide specifications for each modified active substance, including identity, purity, potency, physical-chemical measurements, and stability measures. If test results for the derived substance will be reported for the final release of the drug product, provide a validation report for each specification, including estimates of variability and upper and lower limits. Unless their removal has been validated, specifications should include the amount of unreacted starting materials and process reagents.

C.13.3 mRNA Vaccine [13]

mRNA vaccines are classified as biologics. As a result, adequate control of the starting and raw materials, excipients, and manufacturing processes is required. Describe the intended clinical use of the mRNA vaccine, as with all vaccines, including the pathogen targeted, antigen(s) chosen, and disease to be prevented etc.

Describe relevant biological characteristics of the specific mRNA technology used, such as the ability of the given mRNA to trigger innate immune responses as well as target antigen-specific responses, and biostability.

To justify the vaccine design, describe all known or available information about the type of immunity considered relevant to the specific pathogen and disease. Include the rationale for selecting the target antigen sequence and any coding sequences added to or modified by the target antigen, such as those to ensure the target antigen folds into a specific conformation,

Provide the entire annotated sequence, identifying all ORFs and other sequence elements and justifying their use. Include justifications for the use of specific noncoding sequences (e.g., 5' UTR, 3' UTR, and poly A signal) and structural elements, such as the chosen 5' cap structure. Detail the complete annotated sequence identifying all ORFs and all other sequence elements, including their justification for use. Note justifications for use of specific noncoding sequence (e.g., 5` UTR, 3` UTR & poly A signal) and structural elements such as the chosen 5` cap structure.

For the mRNA vaccine, describe any viral replicase gene(s) encoded in the vaccine construct to allow amplification of the mRNA in human cells after delivery. Detail the formulation of the drug product and all excipients, including all components used to generate LNPs. Include the method of manufacture of LNPs and drug products, with information on critical quality attributes of intermediates and final product, as well as its in-process controls and any sterilization procedure.

C.14 Batch Records[2]

Document the active substance production process in a representative batch record. A summary report of a representative batch record is required.

D. Process Controls[2]

D.1 In process Controls[2]

Provide a detailed description of the in-process testing (flow charts), the sampling point during manufacturing, and the test parameters.

If primary cell cultures are used to propagate the vaccine virus, complete testing of the primary culture prior to virus inoculation may be impossible. In this case, generate uninfected control-cell cultures and test them in parallel with the production culture, handling them in the same way. Process control-cell cultures concurrently with production cultures, but leave them uninfected and test them for the presence of adventitious agents via direct observation and testing of the cell sheet, as well as examining the culture fluid using appropriate tests.

The use of control-cell cultures is critical when the vaccine virus may interfere with the results of in-process product testing, such as when the virus cannot be easily neutralized to allow testing for adventitious agents. Propagate control-cell cultures under production-like conditions for an appropriate period to allow for the possible reactivation and detection of latent or endogenous adventitious agents and poorly replicating adventitious agents. Prior to testing, the culture period after inoculating the production vessels should be at least 14 days.

Some agents may require a longer detection period. Control cell testing does not always eliminate the need for end-of-production cell testing, which may be required to demonstrate the absence of agents induced during vaccine manufacturing.

The manufacturer meeting the product-specific requirements outlined in WHO TRS BP/USP/EP will be deemed adequate for process control.

D.2 Process Validation[2]

Provide a summary report for the validation studies of each critical process or factor that affects active substance specifications, e.g., a decision to accept or reject a batch. The validation study reports with statistical rigor should document the variability in each process as it relates to final specifications and quality.

D.2.1 Assay Validation[2]

Validate the assays or tests used to evaluate the cell substrate in the context of intended use according to the ICH Q2A and Q2B documents, WHO TRS, U.S. Pharmacopeia, British Pharmacopoeia, or European Pharmacopoeia. Where applicable, perform the listed test to determine Accuracy, Precision (Repeatability, Interim Precision), Specificity, Detection Limit, Quantitation Limit, Linearity, and Range.

Certain “compendial” methods may not require full validation and need to perform verification.

If applicable, some microbiological tests are necessary to determine method suitability, such as a bacterial endotoxin test, sterility test, microbial limit test, etc., per pharmacopeial guidance (BP/USP/EP).

D.2.2 Propagation[2]

Based on historical performance under specified conditions, provide a growth curve or tabular representation of growth characteristics of each propagation steps (if appropriate). Include data demonstrating the efficiency of induction of antigen production, if applicable. Also note data demonstrating the stability of genetic markers under propagation conditions, if applicable.

D.2.3 Harvest[2]

Based on historical performance, provide a tabulation of crude harvest yields, purity, and viability (if applicable) for each method or combination of methods.

D.2.4 Inactivation[2]

Provide inactivation or killing curves, or a tabular representation, based on historical performance. Include validation of the method to measure residual live agents, including sensitivity in a background of inactivated agents.

D.2.5 Purification[2]

Provide the method or combination of methods used, as well as a tabulation of yields, purity, and biological activity. Include proof of the removal or dilution of product related and non-product-related impurities, e.g., processing reagents, endotoxin, contaminating cell proteins or nucleic acids, and other residual contaminants for the market authorization documents.

D.2.6 Sterility[2]

Describe and document the validation studies for any processes used for media sterilization, the effectiveness of preservatives, decontamination, inactivating cells before their release to the environment, if such inactivation is required, etc. If the active substance is intended to be sterile, submit information on the sterilization process.

D.3 Control of Bioburden[2]

The process which is not intended to be sterile, where applicable, document each step of the control of extraneous bioburden by a tabulation of in-process testing for bioburden should be provided.

E. Manufacturing Consistency Testing [2]

After successfully manufacturing at least three consecutive batches of drug substance, demonstrate the consistency of the manufacturing process for each vaccine component.

Describe the establishment and use of reference standards in assuring consistency in product characteristics.

E.1 Reference Standards[2]

Provide a certificate of analysis of primary reference standards and working standard standards, along with the detailed procedure for preparing the working standards.

E.2 Release Testing[2]

Submit the release specification and testing method with validation/verification (if compendial method) and the result and other (for information only) characterization data (e.g., certificates of analysis) for each batch.

E.3 General policy of DGDA for independent lot release

The processes of protocol review and independent lab testing for lot release of vaccines are carried out according to procedures mentioned in the vaccines lot release guideline in Bangladesh, as well as SOPs established in the National Control Laboratory of DGDA.

Emergency fast-track lot release could be acceptable in the event of a public health emergency, provided that legal procedures and a clearly defined purpose are in place. Exemption from lot release shall be documented for special situations such as UN-provided vaccines and in emergency situations such as an outbreak.

F. Active Substance Specifications and Impurities Profile[2]

F.1 Specifications [2]

Provide each active substance's specifications, methods, and tests result. Include assays for identity, purity, potency (biologic effect), physicochemical measurements that predict potency, and (where applicable) measures of stability. Present purity in relation to the theoretical composition for highly purified substances. Include test results for stabilized intermediates of component antigens, as appropriate, in the drug product's final release. Provide results of the validation studies for each of these specifications, including estimates of variability and upper and lower limits.

F.2 Impurities Profile[2]

Provide detailed impurities data of the active substance, including the identity and quantity of impurities (where applicable), along with the analytical data that supports the impurities profile (gels, elution profiles, Western blots, etc.).

The manufacturer meeting the product-specific requirements outlined in WHO TRS or compendia (BP/USP/EP) will be deemed adequate for supporting the impurities profile.

F.3 Active Substance Stability[2]

Provide detailed information on the stability of the active substance and any in-process material at each holding step (according Guidelines on Stability Evaluation of Vaccines, WHO).

G. Container Closure System[2]

Submit detailed information of the container and closure system, and its compatibility with the drug substance. The description should include details about the supplier/manufacturer, the address of the manufacturing site (if more than one factory), and the results of compatibility, toxicity, and biological tests. A drug master file (DMF) of the container and closure system may be referenced for this information (alternatively). If the drug substance is intended to be sterile, provide evidence (sterility test) of container and closure integrity for the duration of the proposed expiry period.

The manufacturer meeting product-specific requirements mentioned in compendia will be considered adequate for supporting the integrity of the container closure system.

II. Drug Product[2]

Provide information on the final drug product, including all active ingredients and excipients. If using proprietary preparations or mixtures, the information provided should include a complete composition statement as well as other information that will properly describe and identify these materials. Supply testing results or certificates of analysis demonstrating the absence of adventitious agents for all ingredients of human or animal origin. Cross-reference appropriate information to the information under I. Active Substance.

A. Composition and Characterization[2]

A.1 Composition[2]

The submission should provide all components in the drug product (finished product), including drug substance(s) and other ingredients, with their unit doses and batch quantities specified. For inactive ingredients (if applicable), the quantity may be expressed as percent or molarity.

The manufacturer meeting product-specific requirements mentioned in compendia (PB/USP/EP) will be considered as adequate for the composition of the drug product.

A.1.1 Active Substance(s) [2]

Provide a list of each active substance for market authorization.

A.1.2 Excipient [2]

The excipient section should contain a list of all inactive components with the rationale for the inclusion of each within the ultimate product. The information provided should include CoAs, results of analytical testing, or other information that can describe or identify each excipient. If compendial excipients are used, consider including citations in lieu of analytical testing. Excipients may include, but are not limited to:

- Diluents (include molarity and pH for these);
- Bulking agents;
- Adsorbents (other than adjuvants); and
- Stabilizers (e.g., sugars, wetting agents).

During formulation development, a manufacturer should determine the effect of using different buffers, salts, and other chemical factors on the safety, purity, and potency of the final monovalent or combined vaccine. Similarly, ascertain if the stabilizers will interact to the detriment of the safety, purity, or potency of the vaccine.

A.1.3 Adjuvant[2]

The adjuvant section should contain a listing of the formula and precise quantity of every adjuvant per unit dose. Indicate the number of adjuvants decided by assay or by calculation and describe the method used. Including satisfactory evidence that the adjuvant does not adversely affect the safety

or potency of the product will permit the use of adjuvants. The adjuvant used should be compatible with other ingredients in the final formulation. The drug application should describe:

- Changes in manufacture concerning adsorption, like the stage at which the adsorption takes place for a previously licensed component;
- The efficiency of simultaneous adsorption (if applicable); and
- The efficiency of adsorption of components associated with changes within the adjuvant, or relative concentrations.

A.1.4 Preservative[2]

Identify each preservative should be identified by chemical similarly to any brand name, or reference to compendial sources (BP/USP/EP).

Provide a rationale for the inclusion of a preservative in drug products. Include the results of the preservative effectiveness studies in market authorization documents.

All useful antimicrobial preservative generally are toxic substances. For optimum protection of patients, the effective concentration of the preservative within the final product should be below the level of toxicity. Do not include antimicrobial preservatives in the finished product unless their use is justified by quality and/or safety considerations. Preservatives should not use live vaccines, but it is justified to use of inactivated vaccines that:

- Are present in multi-dose containers; and/or
- Are presented as suspensions to render sterilization/filtration impossible.

If proposing that products contain antimicrobial preservatives, follow the below:

- Present a benefit-risk analysis within the application dossier; address any potential toxicity and/or potential allergenicity during this presentation.
- Control the concentration of the antimicrobial preservatives within the bulk and within the finished product specifications in accordance with the EU, British, and U.S. pharmacopeial limits.
- Test the efficacy of preservation in step with the EU, British, and U.S. pharmacopeias' requirements for human vaccines.
- Demonstrate the maintenance of preservative concentration or efficacy throughout the period of validity.
- State the name and concentration of the antimicrobial preservatives on the labeling.

When selecting a preservative, the applicant should consider:

- The effectiveness against potential microbial contaminants;
- Possible interaction with the formulation or container (for example, thiomersal could also be ineffective within the presence of sera, and might bind to -SH groups and polymeric material; for toxoid vaccines, phenol might impair the antigenicity); and
- Possible effects on testing in biological systems.

If replacement of preservatives is needed based on effects or for other reasons, include a risk/benefit evaluation, taking into consideration that such a change implies a replacement formulation with, on

a case-by-case basis, the necessity for extra studies for sterility, potency, stability, and their clinical implications.

A.2 Compatibility of Components[2]

Combining monovalent vaccines has been shown to produce a new combination that is less safe or effective than desired. Inactivated vaccine components may sometimes interfere with one or more active components. For example, when whole cell pertussis vaccine and inactivated poliovirus vaccine (IPV) were combined, the result was a vaccine with lower pertussis potency.

When live vaccines are combined, immunological interference between vaccine viruses or virus subtypes has been observed. As a result, the combined components stimulated weaker immune responses than viruses given separately. Component cross-reactivity may also occur in a combination of live vaccines, where recombinational events may allow attenuated organisms to be reconstituted into virulent forms.

As a result, it is necessary to validate the compatibility of the combined components before starting clinical trials. It is recommended that the product be characterized and the components' integrity be assessed using a battery of physicochemical, biochemical, and biological assays.

To further demonstrate the components' compatibility, preclinical studies in an appropriate animal model are recommended to determine the effects of combinations on potency and immunogenicity. The manufacturer should consider that the product's components may revert to toxicity or virulence and quantify any such tendency with both monovalent and combined vaccines. Similarly, evaluate the physical characteristics of the combination product, including resuspension, as well as the suitability of the container and closure.

The manufacturer may investigate reducing the dose of some or all components if the combination of component vaccines results in a volume that is too large to be safely administered. For example, the manufacturer may use concentrated intermediate bulks to achieve final concentrations equal to the monovalent component vaccines to restore an optimum final volume. Perform preclinical testing to assess the effects of such formulation changes.

A manufacturer meeting product-specific requirements specified in compendia (British/U.S./European pharmacopoeia) will be considered adequate for component compatibility.

A.3 Specifications and Analytical Methods for Drug Product Ingredients[2]

Provide a detailed description of tests, specifications, and methods for all ingredients, if not specified in the active substance.

The manufacturer meeting product-specific requirements as compendia (BP, USP, or EP) will be considered as adequate for specifications and analytical methods for drug product ingredients.

A.3.1 Description[2]

Provide a qualitative statement describing the physical state (lyophilized solid, powder, liquid), color, and clarity of the drug product and other ingredients.

A.3.2 Identity[2]

On labeled final vaccine containers, perform an identity test. Unless demonstrating that this is not necessary, perform an identity test for every monovalent, in addition to each active component present in the combination. The test's purpose is to identify product as specified on the product labeling and to differentiate it from the other product being processed within the same laboratory. To ensure that mislabeling has not occurred, identity tests required to differences that exist between different final product container.

Each assay's description should include an assessment of its specificity and sensitivity.

A.3.3 Purity and Impurities[2]

Provide information on the purity of the ultimate product, including identification and quantitation of impurities, such as degradation products, inherent in the final dosage form. Determine the appropriate limits of those impurities and include them within the specifications if impurities form during the manufacturing of the drug product or are known to be introduced.

A.3.4 Potency[2]

Potency is the capacity or specific ability of the product to determine by laboratory test results as per predefined specifications or by adequately controlled clinical data obtained through the administration of the intended vaccine, to effect a given result.

A description should be provided of the potency for the drug product. Information on the assay's sensitivity, specificity, and variability should be submitted, as well as data from the material used to prepare clinical/preclinical lots that were used to set the acceptance limits for the assay.

The potency of each component for which an efficacy claim is made should be determined for a combination product. The potency of each component should comply with the potency requirement for the monovalent product unless it can be determined that any reduction in potency due to interaction with other components of the combination product does not result in a lowering of the efficacy in humans.

When additional processing has been shown, there is no effect on the potency of the final product. In this case, testing in the final container may be done instead of testing the final formulated bulk vaccine. However, in some cases, such as with lyophilized products, a demonstration of the product's potency in the final container is required. Describe the drug product's potency assay. Submit information on the assay's sensitivity, specificity, and variability, as well as data from the material for preparing clinical/preclinical lots that set the acceptance limits for the assay.

A.3.5 Other tests[2]

A.3.5.1 Testing for the Presence of Residual Cells[2]

Test the presence of residual cells in the final vaccine product (if appropriate). For example, implement and validate filtration to ensure that no intact cells remain in the final product. Residual cell removal processes must be robust, which is especially important for immortalized cells. The extent to which these processes clear intact cells is an important part of this validation.

A.3.5.2 Testing for Residual Cellular DNA [2]

Residual DNA may be a risk to the ultimate product thanks to oncogenic and/or infectivity potential. It may be oncogenic because of several potential mechanisms, including the mixing and expression of encoded oncogenes or insertional mutagenesis following DNA integration. Residual DNA can also be capable of transmitting viral infections if retroviral proviruses, integrated copies of DNA viruses, or extrachromosomal genomes are present.

The risk of oncogenicity and infectivity of the cell-substrate DNA is diminished due to decreasing its biological activity. This will be accomplished because of decreasing the number of residual DNA and reducing the scale of the DNA (e.g., by deoxyribonuclease, or DNase, treatment or other methods) to below the dimensions of a functional gene.

Chemical inactivation can decrease both the scale and biological activity of DNA. If undertaking DNA removal, digestion, or inactivation, validate the methods used.

Measure the amount of residual DNA within the final product. For widely used human diploid cell strains, like MRC-5 and WI-38 cells, measurement of residual DNA may be unnecessary because it is not considered residual DNA from these human diploid cells to be a security issue.

Limiting the number of residual DNA may be required, considering the potential risks related to that DNA, for human diploid or primary cell types for which there is less study. Residual DNA should be limited for continuous non-tumorigenic cells, like low-passage Vero cells, to just 10 ng/dose for parenteral inoculation, as recommended by WHO. When using cells with tumorigenic phenotypes or other characteristics that create special concerns, more stringent limitations of residual DNA quantities could be needed to assure product safety.

The manufacturer meeting product-specific requirements mentioned in compendia will be considered adequate for residual cellular DNA.

B. Manufacturer and Facilities[2]

Submit the name(s) and address(es) of all manufacturers involved in the manufacture and testing of the drug product, including contractors, and a description of the responsibility (ies). A list of all other products (research & development, clinical or approved) made in the same rooms should be provided.

C. Manufacturing Methods [2]

Provide a detailed description of the manufacturing process flow of the formulated bulk (API) and finished drug product (vaccines), including sterilization operations (e.g., filtration), aseptic processing procedures and process simulation, lyophilization (time and temperature), and packaging materials details.

Accompanying this narrative, include a flow chart of the production step, the equipment and materials used in the manufacturing process of bulk and finished products, the manufacturing area, and in-process controls and tests performed on the product at each step. Create a master production record (MPR) for the drug product, including complete manufacturing instructions for adsorption (if applicable), formulation (if applicable), filtration, filling, labeling, and packaging. References may be made to other sections for more detailed information. Provide the results of studies validating the

compatibility of the components, including adjuvants and/or preservatives and demonstrate the drug product's consistency from lot to lot.

D. Drug Product Specifications[13]

D.1 Sampling Procedures[2]

Include the sampling procedures, detailing the number of samples for monitoring a batch of the finished drug product.

D.2 Specifications and Methods[2]

Submit approved and validated test methods to assure the identity, purity, strength and/or potency, as well as the lot-to-lot consistency of the finished product (vaccine) and the specifications used for the vaccine. Provide certificates of analysis and analytical results for at least three consecutive batches for market authorization.

D.3 Validation Results[2]

Provide the results of studies validating the accuracy, precision (repeatability, interim precision), specificity, detection limit, quantitation limit, linearity, and range of each method used for release testing (if applicable). Where applicable, this should include descriptions of reference standards/working standard and their validation. For analytical methods available in the BP/USP/EP sources, provide the appropriate citations.

E. Container Closure System[2]

Submit a detailed description of the container (e.g., vial, pre-filled syringe) and closure system, as well as its compatibility with the drug product. Provide detailed information about the supplier(s)/manufacturers, including address(es), and the results of compatibility. Alternatively, reference a drug master file (DMF) for this information. For sterile products, both container and closure should be sterile. Submit the limit of endotoxin and provide evidence of container and closure integrity for the duration of the proposed expiry period.

F. Sterility[2]

The final product must meet the sterility requirements per EP, BP, and USP test methods.
Describe the sterility test methods and document any processes used for sterilization, and provide the effectiveness of preservatives in the validation studies.

Antibiotic generally not using during the production of vaccine for the confirm of sterility or reduce or inhibit the bioburden. In the case of any vaccine in which antibiotic is proposed to include in production:

- traces appearing in the finished product should be minimized and controlled; and
- the labeling and package leaflet texts should state the presence of any traces present.

G. Bacterial Endotoxins test[2]

Describe the bacterial endotoxins test (BET) method used for the product. The outline should include qualification of the laboratory, inhibition and enhancement testing and results, determination of non-inhibitory concentration, and maximum valid dilution.

The BET may detect or quantify endotoxins from Gram-negative bacteria using amoebocyte lysate from the Xiphosurus polyphemus (Limulus polyphemus or Tachypleus tridentate). There are three techniques for this test:

1) The gel-clot technique, which relies on gel formation.
2) The turbidimetric technique, supported in the event of turbidity after cleavage of an endogenous substrate.
3) The chromogenic technique, supported in the event of color after cleavage of an artificial peptide-chromogen complex.

H. Lyophilization[2]

Validate the lyophilization process used for manufacturing vaccines, if appropriate

A validation summary for lyophilization of the drug product should be given which includes:

• A narrative description of the validation (or protocol);

• Certification that installation qualification (IQ) and operational qualification (OQ) have been completed; • A validation data summary

• Explanation of all excursions or failures; and

• Deviation reports and results of investigations of all excursions or failures

I. Drug Product Stability [2]

I.1 Stability Protocol[2]

Depending on the nature of the antigen and other components as well as on the manufacturing process, include stability-indicating parameters and frequency of testing in the protocol. Stability-indicating parameters should be selected on a case-by-case basis.

Provide a stability study protocol that includes, as applicable:

- Identification
- Potency
- Physicochemical measurements (which are potency-indicating)
- Moisture, if lyophilized
- pH, if appropriate
- Sterility and control of bioburden
- Viability of cells, if frozen and thawed

- Pyrogenicity and Bacterial endotoxin
- General safety
- Appearance
- Specific toxicity
- Antimicrobial agent content
- Completeness of adsorption
- Adjuvant (adsorbent) content
- Changes in physicochemical properties

I.2 Stability Data[2]

Provide the summary results that support the proposed recommended expiry date. Separately conduct stability tests of each dosage form of vaccines and submit a summary report for market authorization. For lyophilized products, including the data supporting the shelf-life of the product following reconstitution (in use of stability data). If the drug product is frozen, provide data supporting the stability of the product through a stated number of freeze-thaw cycles.

I.3 Stability Program[13]

Provide a plan for an ongoing stability program. This could include the protocol to be used, the number of ultimate lots to be entered into the soundness protocol annually, and the method for selecting the lots.

The current approach for testing frequency (at Initial, 3, 6, 9, 12, and 18 months, and every six months afterward) described for pharmaceuticals product are not applied to all vaccines. Therefore, determine appropriate intervals for testing with consideration for the characteristics of the vaccine in question, the rate of change of the parameter measured, the aim of testing, study design, and subsequent data analysis. Generally, the soundness testing of vaccines should comply with all requirements mentioned in WHO's guideline titled "Draft Guidelines on Stability Evaluation of Vaccines."

J. Labeling Requirements

The label may state:

- The name of the preparation.
- A reference identifying the ultimate lot/batch number.
- The prescribed human dose and route of administration.
- The storage conditions.
- The expiry dates.
- The name and the amount of antimicrobial preservative (if any).
- The name of any antibiotic, adjuvant, flavor, or stabilizer in the vaccine for freeze-dried vaccines:

- The name or composition, and thus the volume, of the reconstituting liquid to be added.

In addition, specific labeling requirements for every vaccine mentioned under an individual monograph should include the following:

- Marketing authorization (MA) number;
- Generic and commercial names; and
- Adherence to DGDA labeling and packaging guidelines.

Part 2 – Preclinical Testing of Vaccines [11]

The main objective of the pre-licensure clinical development program is to accumulate adequate data to support licensure. The most recommended elements of the program are to:

- Describe the interaction between the vaccine and the host immunologic response;
- Identify safe and effective dose regimens and schedules;
- Estimate vaccine efficacy by directly measuring efficacy and/or to supply evidence of vaccine efficacy supported immune responses;
- Describe the security profile; and
- Assess co-administration with other vaccines, as is often relevant.

Consideration of the content of pre-licensure clinical development program is undertaken on a product-specific basis. Requirements may differ depending on the kind of vaccine, its manufacturing process, its mechanism of action, the disease to be prevented, and the target population.

Preclinical trial should be conducted per WHO TRS 927 Annex-1 and/or WHO TRS 987, Annex-2, and/or ICH specific safety guidelines and evidence to be provided.

Part 3 - Clinical Evaluation of Vaccines [11]

Clinical trials should be conducted per Guidelines for Clinical Practice (GCP) for trials on pharmaceutical products in Bangladesh; ICH E6 guidelines and WHO TRS 924 Annex-1; WHO TRS 1004, Annex-9; and/or any vaccine-specific WHO TRS and evidence to be provided.

The main objectives of the clinical program before receiving market authorization are to:

- Accumulate required data to support market authorization.
- Describe the interaction between the vaccine and the host immune reaction.
- Identify safe and effective dose regimens and schedules.
- Estimate vaccine efficacy by directly measuring efficacy and/or to supply evidence of vaccine efficacy supported immune responses to describe the security profile to assess co-administration with other vaccines, as is often relevant.

Consider the content of the pre-licensure clinical development program on a product-specific basis. Requirements may differ depending on the type of vaccine, its manufacturing process, its mechanism of action, the disease to be prevented, and the target population.

Phase I trials are generally conducted in healthy adults. In phase I clinical trials, check the safety of various amounts of the antigen(s) in each dose of candidate vaccine formulations, with or without an adjuvant, and explore immune responses to the antigenic components. Safety and immunogenicity trials that are conducted as part of the clinical test trial.

Phase II trials cover a broad population age range and it will not be necessary in all instances to use an age de-escalation approach (for example, to maneuver from adults to adolescents, then to children aged 6–12 years, followed by younger children, toddlers, and infants) to sequential trials or to groups within trials. For example, if a vaccine has a negligible potential benefit for older children, it will be acceptable in some cases to proceed directly from trials in adults to trials in younger children, including infants and toddlers. These trials are usually designed to produce sufficient safety and immunogenicity data to support the choice of one or more candidate formulations for evaluation in pivotal trials (that is, to pick the number of antigenic components and, where applicable, adjuvant in each dose). Pivotal trials are intended to produce robust clinical evidence in support of licensure. They are commonly called Phase III trials.

In exceptional cases, licensure relies on a clinical test trial designed to produce robust statistical conclusions. Usually, the investigational formulations in pivotal trials use validated processes and undergo lot release in the same way as intended for the commercial product. Pivotal trials are also designed to produce an estimate of vaccine efficacy or to produce a sign of the power of the vaccine to stop clinical disease on the idea of immunogenicity data. Infrequently, an assessment of a selected safety aspect could also be the first (or a co-primary) objective during a pivotal trial.

Post-licensure clinical evaluations are important to observe vaccine safety in routine use. Studies designed to handle specific questions of safety that were identified as potential concerns from pre-licensure trials might have to be conducted. It will be appropriate to conduct studies specifically intended to estimate the vaccine effectiveness. Some jurisdictions may require conducting one or more trials after licensure to deal with specific issues.

A. Special Consideration for Vaccine Development [13]

A.1 Vaccines that contain more than one antigen [13]

A.1.1 Immune interference [13]

Each antigen during a vaccine has the potential to interfere with immune responses to one or more other antigens in the same product. Immune interference may be caused by chemical and/or immunological interactions, and it may result within the enhancement or depression of responses to one or more antigens, changing the character of the immunologic response.

Responses to antigens that are conjugated to protein carrier molecules will be unpredictable when multiple antigens are included within the same vaccine. Inclusion of a conjugated antigen during a vaccine may affect responses to other antigens that are identical (e.g., tetanus toxoid) or similar (e.g., diphtheria toxoid and CRM197) to the carrier protein. If significant enhancement or interference is detected, other formulation changes may be needed and/or a change in dosing regimen may need to be explored. These phenomena could affect the local and systemic tolerability of vaccination.

It is usually necessary to conduct adequate investigation of the results, if any, of mixing the antigens in one vaccine on the immune responses to every component is required. However, in some circumstances it is unnecessary to administer antigens during a novel combination separately and together if the final product is shown to be satisfactorily immunogenic, safe, and efficacious. Therefore, consider the requirement for and extent of immune interference studies on a case-by-case basis.

In most cases, the assessment of immune interference will use serological data. When no immunological correlates of protection exist for a few or all the antigens of interest, assessing immune interference becomes particularly difficult and can be done only through simple comparisons. It is recommended that, whenever possible, to focus on parameters that are possible to reflect clinical protection, like functional antibody levels.

The nature of the antigens to be combined will influence the planning of studies to evaluate interference. If two antigens haven't been combined before for formulation, the reaction to every antigen when given alone should be compared to administration during a combined product. However, comparing the separate and combined administration of every antigen in a product might not be necessary or feasible if several of those have already been formulated together in licensed products or if there are many antigens involved. In such cases, the consequences of adding one or more antigens to a well-established combination product will be assessed by comparing responses to the novel combination with separate administrations of the extra antigens plus the licensed combination. All such studies should include a radical comparison of safety data.

A.1.2 Cross-reacting immune responses [13]

Cross-reacting immune responses may occur when a vaccine contains one or more antigens that produce immune responses that cross-react with other antigens.

When an antibody to an antigen from a specific microorganism (species or type within a species) has a high affinity for antigens from one or more other species or types within a species, a beneficial cross-reaction may occur. In some cases, studies of protective efficacy and/or studies of functional

immune responses may provide enough evidence to support a claim of protection against species or subtypes not included in the vaccine.

In contrast, antibodies elicited by a vaccine with cross-reactivity to human antigens may be harmful. It may be difficult to investigate the possibility of this occurring prior to initial authorization. If there are grounds to anticipate such issues, post-marketing safety studies require special consideration.

A.2 Concomitant administration of vaccines[13]

When administering two or more vaccines concurrently but separately (via any route), the risk of immune interference and the effects on overall safety are also important considerations. In the absence of specific data, general principles can be applied; however, several examples of unexpected immune interference have emerged in recent years.

These include the effects of acellular pertussis vaccines on responses to conjugated saccharides, as well as the variable enhancement or depression of immune responses to conjugated saccharides when the carrier proteins are the same or different.

It is critical to justify the criteria used to determine whether concomitant administration has potentially clinically significant effects on immune responses to individual antigens when assessing the potential for immune interference. If any studies reveal significant immune interference or an unacceptable increase in unwanted effects, applicants should investigate the shortest possible interval between administrations to avoid these issues.

It would be preferable to have safety and immunogenicity data on concomitant administration with at least one type of licensed vaccine that would almost certainly be given at the time of a novel vaccine's first approval. In many cases, satisfactory results would suffice to make a broad statement about co-administration with specific types of antigens without mentioning brand names. However, sometimes product-specific issues are anticipated or discovered that necessitate distinguishing between brands in the prescribing information.

Protocols must allow for the usual recommended antigens to be given on time, clinical trials for some vaccines, such as those intended for the primary series in infants, will inevitably involve co-administration with certain products at one or more schedules. As a result, information on the safety of co-administration, as well as some data on immune responses to all antigens before and after the primary series, are likely to be available. If it can be established that the antigens adequately prime infants and elicit acceptable antibody responses for at least short-term protection, a formal assessment of immune interference may not be required. However, studies involving the omission of the new vaccine from one group may need to compare concomitant administration with staggered administrations (e.g., together at 2, 4, and 6 months, compared to the usual antigens at this schedule and the new vaccine at 3, 5, and 7 months).

Immune interference data from one schedule cannot always be extrapolated to other schedules. Potentially clinically significant interference, for example, may be detected on an accelerated schedule but not on a less concentrated schedule. As a result, if only the latter is studied, any immune interference that may occur may go undetected.

Studies that evaluate immune interference for routine vaccinations administered later in life or for travel purposes should usually compare concomitant with separate administrations of products.

In the case of primary series studies, it may be acceptable if the data is derived from co-administration with only one brand of a specific type of vaccine that is likely to be co-administered.

A.3 Interchange of vaccines within a schedule[13]

Most inactivated vaccines require more than one dose of an antigen to achieve adequate priming and maintain protection against infection. As a result, the question for primary series and booster doses is whether the first and all subsequent doses administered with the same product or whether other products containing similar antigens can be used interchangeably.

If required, use appropriate data to support active endorsements in prescribing information for switching. Tailor the design of studies intended to support interchangeability claims to the exact claim required and include data on safety and immunogenicity. The final wording of the prescribing information will have to be considered in the light of the potential for extrapolating data on interchangeability obtained with one brand to other similar vaccines.

A.4 Vaccine lots and lot-to-lot consistency studies[13]

During the pre-licensure clinical development program, the national regulatory authority may recommend lot-to-lot consistency trials for all new candidate vaccines. Where these trials are not routinely recommended, they may be considered for certain types of vaccines with inherent variability in product manufacture. If requested, the rationale for conducting the trial and the objectives should be clear.

Furthermore, manufacturers should establish that the clinical trial lots, particularly those in the later stages of development, are adequately representative of the formulation intended for marketing throughout its shelf life.

The need for a formal lot-to-lot consistency study should be evaluated on an individual basis. Such a study may be necessary when there is inherent and unavoidable variability in the vaccine's final formulation in one or more respects. However, for vaccines with a highly reproducible manufacturing method, such studies may not provide additional information to that generated during the rest of the clinical program.

Besides determining the number of lots to be compared, one consideration is whether the lots tested should be produced consecutively or at random.

The pre-defined criteria for determining lot comparability will typically be based on one or more immunological parameters, and a comparison of safety data is also necessary in these cases. Careful consideration is required to determine which immunological parameters are the most valid and clinically relevant, and the gap between lots may be potentially clinically significant.

A.5 Bridging studies[13]

Clinical bridging studies generate immunogenicity data to support the extrapolation of data on safety and protective efficacy obtained under specific conditions of use to other situations (e.g., different formulations, additional schedules, populations). It is important to consider the important immunological parameters for determining immune response comparability when designing such studies.

When there is an established immunological correlate for protection, the proportions reaching this level should be not only comparable across treatment groups, but also acceptable in light of all previous experience with responses to the antigen. When the correlate is unknown or uncertain (for example, when predicting long-term efficacy), comparing proportions reaching a pre-defined cut-off for functional antibody may be more relevant than comparing geometric mean concentrations (GMCs).

On occasion, the term may be applied more loosely to simple comparisons of immunogenicity data sets. For example, compare data from premature infants to full-term infants, immunocompromised individuals to healthy individuals, or different formulations of the same vaccine. The assessment of the findings is subject to the same considerations as described above. Comparisons between studies, rather than within a single study, may necessitate extra caution.

A.6 Circumstances in which approval might be based on very limited data[2]

Special consideration is required for the clinical development of vaccines when protective efficacy studies are not reasonable and when there is no established immunological correlate of protection.

For example, vaccines are intended to prevent rare infections that carry considerable morbidity and mortality, including pathogens. In case of an epidemic or deliberate release, these pathogens cause widespread disruption to humanity.

In principle, there are several perspectives to this scenario. Relevant data on protective efficacy may be found from challenge studies in animal models. There may be immunological correlates of protection established for similar but not identical antigens. It can be used as a guide to likely efficacy for the time being. Immunological research should, whenever possible, focus on measuring functional immune responses.

Taking the results of these and any other relevant investigations into account, it is possible to construct a reasonable case for likely efficacy. A presumptive risk-benefit relationship that could support authorization could be derived. The prescribing information, on the other hand, should explain the basis for the opinion.

If authorization had to be based on such limited data, estimating vaccine effectiveness in the post-authorization period may be impossible unless a significant natural epidemic or deliberate release occurs. In any case, it is likely that only national surveillance programs run by public health authorities will provide reliable data. As a result, applicants should collaborate with public health authorities to develop plans to collect data on safety and efficacy if the opportunity (e.g., a significant outbreak or major epidemic) arises.

B. Consideration for the Summary of Product Characteristics (SPC)[2]

B.1 Therapeutic indications

Indications should include the following, at a minimum:

- Disease(s) to be prevented (including specific species, if suitable for vaccine content).
- Minimum age for use (e.g., infants from 2 months).
- Appropriate age categories (newborn, infant, child, adolescent, adult, etc.).

- The maximum age of use where such restrictions are appropriate, based on factors such as the epidemiology of the disease and the antigenic content of the vaccine.

It may also be necessary to mention:

- Particular populations for which vaccine is appropriate (e.g., naive, primed, at-risk).
- Populations for which vaccine is not suitable should be addressed.

B.2 Posology and method of administration [13]

B.2.1 Posology [13]

For each individual vaccines, clearly describe the doses and schedule for primary and booster doses. In general, recommendations reflect minimum age initially dose, minimum dosing interval, and minimum interval between the last dose of the first series and the first (and perhaps sequential) booster dose(s). All dosing and intervals should be supported by clinical study.

For most vaccines intended for use in infancy, and for several intended to boost up antigens routinely delivered in infancy, it will be necessary to incorporate a general statement regarding the necessity to follow official guidance on the precise timing of those doses.

Advice on dose and schedule might have to be separately produced for various age groups or other defined populations (e.g., the immunosuppressed). It may be appropriate to state whether exchangeability of vaccines within a schedule are often recommended.

B.2.2 Method of administration [13]

Indicate the injection route, preferably with the site of initial choice (e.g., deltoid muscle). The key statements are:

- Do not inject intravascularly.
- Exceptional subcutaneous administration is necessary in patients with thrombocytopenia or bleeding disorders.

B.3 Contraindications [13]

Contraindications should usually be restricted to absolutes applied at the time of administration.

The following should usually appear, at minimum:

- TRADENAME should not be administered to subjects with known hypersensitivity to any component of the vaccine.
- As with other vaccines, TRADENAME should be postponed in subjects suffering from acute severe febrile illness.

B.4 Special warnings and precautions for use [13]

Appropriate common statements might include:

- Appropriate medical treatment and monitoring must be immediately available in the event of adverse events following immunization (AEFI) following all injectable vaccine administrations.
- (TRADENAME) should never be administered intravenously.

- Thiomersal was used in the manufacturing process of this vaccine, and residue of it is present in the final product, Therefore, sensitization reactions may occur.
- As with any vaccine, a protective reaction may not occur.

This section may additionally describe:

- Lack of protection or the bounds of any cross-protection against strains or serotypes not included within the vaccine.
- Situations (e.g., administration to those who are already within the incubation stage) or populations (e.g., the elderly) during which the vaccine's efficacy not investigated or couldn't be anticipated.
- Factors that may be related to an impaired response.
- For live attenuated vaccines, the potential for vaccine strain transmission and the chance of reversion to virulence or reassortment, or of re-assortment with wild-type strains, should be described.

B.5 Interaction with other medicinal products and other forms of interaction[13]

The section should clearly differentiate endorsements for concomitant administration that are based on clinical data as opposed to statements based on general principles. In general, satisfactory data obtained on concomitant administration with a representative vaccine of a certain type (e.g., giving a combination vaccine against diphtheria, tetanus, pertussis and other antigens vaccine with one of the MMRs on the market) should serve to support a general statement for co-administration. Immune interference that's clinically significant or potentially clinically important immune interference should be mentioned. If there's no data on co-administration with a kind of vaccine that is likely to require co-administration, this could be stated.

Appropriate common statements may include:

- It could also be expected that in patients receiving immunosuppressive treatment or patients with immunodeficiency, an adequate immune response may not be elicited;
- Immunoglobulin is not to be used with TRADENAME; and
- If it is necessary to supply immediate protection, TRADENAME could also be given at the same time as (normal/x-specific) immunoglobulin. Injections of TRADENAME and immunoglobulin must be made into separate limbs.

B.6 Pregnancy and lactation[13]

For vaccines that will be administered only within the pre-pubertal years. It is sufficient to state:

- TRADENAME is not intended for use in adults. Data on use in pregnancy or lactation and animal reproduction studies is not available for humans.
- For vaccines to be used in individuals of childbearing age, the section should include preclinical and clinical experience.
- For live attenuated vaccines, contraindication during pregnancy is common. However, for a well-known vaccine with reported experience, it should be sufficient to discourage vaccination during pregnancy unless clearly necessary.

- Regarding lactation, absent sufficient data, it is common to state with an inactivated vaccine: “If administering TRADENAME to breastfeeding mothers, note that the effect on their infants has not been studied.”
- Recommendations for live attenuated vaccines should be considered on a case-by-case basis.

B.7 Effects on ability to drive and use machines[13]

For vaccines that will be administered only in the pre-pubertal years, it may be sufficient to state:

- TRADENAME is not intended for use in adults.

When making statements when the vaccine is intended for adults, the usual considerations apply.

B.8 Undesirable effects[13]

Vaccine-specific considerations may include:

- Details of local and systemic reactions.
- Special notes on certain adverse drug reactions (ADRs like fevers and febrile convulsions.
- ADRs and ADR rates separated by age bracket, number of doses, previous vaccination history, occurring in studies or reported from post-marketing surveillance.
- Special notes should address any increased rate of ADR(s) observed on concomitant administration with other vaccines.

B.9 Overdose[13]

Any overdose experience should be mentioned. It should be appropriate to say that overdose is unlikely because of the mode of presentation (e.g., single dose pre-filled syringe).

B.10 Pharmacodynamic properties [13]

This section should briefly summarize (tabulation is also appropriate) the foremost pertinent immunological data (using the foremost relevant parameters) and any estimates of efficacy or effectiveness considered to be valid (with caveats regarding the population in which these were measured). As necessary, the information should be attenuated by primary series and boosting, by cohort or by other factors, like immunosuppression.

The section also includes details of the established or reputed immunological correlate of protection. The foremost recent WHO guidelines for particular vaccines must be followed.

C. Role and Responsibilities of Directorate General of Drug Administration[13]

One of the responsibilities of the DGDA is to set up appropriate regulatory oversight for the marketing authorization and post-marketing surveillance of vaccine products that are developed and/or authorized for use in its area of jurisdiction. The experience and expertise of the DGDA in evaluating vaccine products is a key prerequisite for appropriate regulatory oversight of these products. The DGDA is responsible for determining a suitable regulatory framework for the marketing authorization of vaccine products. It may choose to use or amend existing pathways or

develop a new pathway for this purpose. The annexes detail marketing authorization pathways for vaccine products.

Annexures

Annexure-1: Registration flowchart for vaccine product manufactured by imported bulk antigen

Steps for registration:

*If the vaccine is unintroduced or new for the country then DCC approval will be needed.

Step-1: Source Validation for Vaccines

a) Principal company profile (Attested by Bangladesh Embassy/Chamber of commerce)

b) A valid copy of principal company manufacturing license (Attested by Bangladesh Embassy/ Chamber of commerce)

c) GMP certificate issued by the licensing authority of the country concerned (Attested by Bangladesh Embassy/ chamber of commerce)

d) List of countries where companies export their produced raw materials which will be imported in Bangladesh (Attested by Bangladesh Embassy/ chamber of commerce)

e) Certificate of analysis mentioning specification of each active substances (Attested by Bangladesh embassy/ chamber of commerce)

f) Form -9 (Signed by the manufacturer mentioning the product name)

g) Authenticated pre-clinical, clinical completed study report and copy of clinical trial protocol approval by Drug regulatory authority (in case of biological/ / vaccine)

h) Manufacturing companies have to submit certifications registered from their local drug regulatory authorities in terms of their product/products.

i) As biological / vaccine bulk product/products are sensitive, in that case proof are to be submitted regarding required facilities for transportation, storage and distribution of the mentioned products.

Step-2: Application for Registration / Formulation Approval and Marketing Authorization (MA)

a) Dossier in CTD format with 5 modules

b) Sample and lab document submitted to NCL

Step-3: Price certificate Issuance

After approval of formulation / annexure, subsequently Price certificate is issued.

Step-4: MA Certificate Issuance

After issuance of Price certificate, MA certificate is issued.

Step-5: Post marketing documents:

a) Real time stability data up to shelf-life

b) Post marketing observational study report on reasonable number of subjects within 06 months to 01 year.

c) Any change should be submitted as per ICH Q5E guideline.

Annexure-2: Registration flowchart for vaccine product manufactured by indigenous or locally developed bulk

*If the vaccine is unintroduced or new for the country then DCC approval will be needed.

Step-1: Application for NOC to import or permission to develop Host cell/Cell line/Master cell

a) Host cell / cell line / master cell identification and characteristics document.
b) Certificate of analysis

Step-2: Application to DGDA for the permission to start Preclinical study

For Pre-clinical study to be conducted inside Bangladesh

2.1 Information about product development

a) Information about cell bank
b) Procedure to prepare working cell bank
c) Data generated from development of R&D batch, Manufacturing flowchart, Cell bank history, Preliminary characterization and manufacturing process in brief.
d) Analytical specifications

2.2 Information about pre-clinical study

a) Protocol for preclinical study for local study

For Pre-clinical study to be conducted outside Bangladesh

2.1 NOC application to send the sample for overseas CRO

a) Description of the test product
b) Overseas CRO information
c) Non-commercial Invoice

Step-3: Application to DGDA for research / manufacturing license to prepare Clinical trial batches

3.1 Information about production of pre-clinical trial batch

a) Preclinical batch summary report
b) Certificate of Analysis (COA)
c) Stability study of at least 3 months of development batch

Step-4: Application to DGDA for the permission to start Clinical trials

4.1 If clinical trials be conducted in overseas, application to DGDA for NOC to send sample.
4.2 If clinical trials to be conducted in Bangladesh, the trial permission and conduction of trial would be according to guidelines for Good Clinical practice (GCP) for trial on pharmaceutical products in Bangladesh.

Step-5: Application for Registration / Formulation Approval and Marketing Authorization (MA)

a) Dossier in CTD format with 5 modules
b) Sample and lab document submitted to NCL

Step-6: Price certificate Issuance

After approval of annexure, subsequently price certificate is issued.

Step-7: MA Certificate Issuance

After issuance of Price certificate, MA certificate is issued.

Step-8: Post marketing documents:

a) Real time stability data up to shelf-life
b) Post marketing observational study report on reasonable number of subjects within 6 months to 1 year.
c) Any change should be submitted as per ICH Q5E guideline.

Annexure-3: Registration flowchart for Introduced vaccine product imported as Finished product

Steps for registration:

*If the vaccine is unintroduced or new for the country then DCC approval will be needed.

Step-1: Application for registration

a) COPP from one of the 07 developed countries like USA, France, Germany, UK, Switzerland, Japan, Australia, or EMA certificate or WHO prequalified vaccine.

b) Principal company profile, Manufacturing license, List of countries where companies' product export,

c) Certificate of analysis mentioning specification of each lot/Batch of finished products

Step-2

a) Dossier in CTD format with 5 modules

b) Local agent must submit proof of availability of cold chain storage and supply facilities.

c) Sample and lab document submitted to NCL

Step-3: Price certificate Issuance

- After approval of the products, subsequently Price certificate is issued.

Step-4: MA Certificate Issuance

- After issuance of Price certificate, MA certificate is issued.

Annexure-4: General equipment list for Vaccine manufacturing.

a) General list of equipment for vaccine API manufacturing:

1. Balance
2. pH meter
3. Viscometer
4. Osmometer
5. Bioreactor
6. Fermenter
7. Centrifugation system
8. AKTA purification system
9. Filtration system
10. Different type of Chromatography system
11. Incubator
12. Shaker
13. Microscope
14. Spectrophotometer
15. Biosafety cabinet
16. Storage system, cell bank (Freezer, Refrigerator, Liquid Nitrogen tank)
17. Autoclave
18. Water system (Purified and WFI)
19. ETP.
20. Incinerator.
21. Sampling Booth
22. Dispensing Both
23. HVAC
24. Garments Storage Cabinet

b) General list of equipment for vaccine fill finishing manufacturing

1. Blending vessels.
2. Sterile filtration system.
3. Filling machine (Vial, Ampoule, Pre-filled syringe).
4. Lyophilizer.
5. Sealing machine.
6. Labelling machine.

7. Printing machine.
8. Water system (Purified & WFI).
9. Autoclave.
10. Dry heat sterilizer.
11. Balance.
12. Storage system (Cold room, Freezer, Refrigerator). etc.,
13. HVAC

Annexure-5: General equipment list for Quality Control.

Major Equipment list for Quality control:

1. Spectrophotometer
2. Image analyzer
3. Biosafety cabinet
4. Storage system, cell bank (Freezer, Refrigerator, Liquid Nitrogen tank)
5. Gel electrophoresis system (IEF, SDS PAGE)
6. Western/Southern/Northern blot
7. PCR/RT-PCR
8. pH meter
9. Viscometer
10. Osmometer
11. TOC analyzer
12. Centrifugation system
13. HPLC/ uHPLC
14. Shaker
15. Incubator
16. Autoclave
17. Stability chamber
18. Moisture analyzer
19. Conductivity meter
20. Balance
21. ELISA
22. UV
23. Size-exclusion chromatography
24. CO_2 Incubator
25. Freezer
26. Refrigerator
27. Inverted Microscope
28. Hot Air Oven

Annexure-6: Human resources area of expertise.

1. Pharmacy
2. Biotechnology
3. Genetic Engineering
4. Biochemistry
5. Microbiology
6. Bioinformatics
7. Chemistry
8. Applied Chemistry
9. Molecular Biology

Annexure-7: Form-9

Form 9 [Sec rule 241]

Form of undertaking to accompany an application for an import license

Whereas…..of……. intends to apply for a license under the Drugs Rules. 1945 for the import into Bangladesh of the substances specified below manufactured by us. We ……. of……. hereby give this undertaking that for the duration of the said license -

(I) The said applicant shall be our agent for the import of the substances into Bangladesh;

(2) We shall comply with the conditions imposed on a licensee by clauses (a) to (e) of Rule 78 of Drugs Rules,1945;

(3) We declare that we are carrying on the manufacture of the substances mentioned in this undertaking at the premises specified below and we shall from time to time report any change of premises on which the manufacture will be carried on and in cases where manufacture is carried on in more than one factory any change in the distribution of functions between the factories;

(4) We shall comply with the provisions of Part IX of the Drugs Rules, 1945:

(5) Every substance manufactured by us for import under license into Bangladesh shall as regards strength. quality and purity conform with the provisions of Chapter III of the Drugs Act, 1940 and of the Drugs Rules, 1945;

(6) We shall comply with such further requirements, if any, as may be specified by rules made by the central government under the Act and of which the licensing authority has given to the licensee not less than four months' notice.

List of Substances

Particulars of premises where manufacture is carried on.

Date: ……. Signed by or on behalf of the manufacturer…….

Amended *vide* Ministry of Health and Works (Health Division) Notification No F. *27·34/49·M.* S. & G. dated the 21st September. 1950.

Glossary

Adventitious Agent: A microorganism (including bacteria, fungi, mycoplasma/spiroplasma, mycobacteria, rickettsia, viruses, protozoa, parasites, TSE agent) that is inadvertently introduced into the production of a biological product.

Adjuvants: Adjuvants are substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine.

mRNA Vaccines: A type of vaccine that uses a copy of a molecule called messenger RNA (mRNA) to produce an immune response. The vaccine delivers molecules of antigen-encoding mRNA into immune cells, which use the designed mRNA as a blueprint to build foreign protein that would normally be produced by a pathogen (such as a virus).

DNA Vaccine: A type of vaccine that transfects a specific antigen-coding DNA sequence into the cells of an organism as a mechanism to induce an immune response. DNA vaccines work by injecting genetically engineered plasmid containing the DNA sequence encoding the antigen(s) against which an immune response is sought, so the cells directly produce the antigen, thus causing a protective immunological response.

Protein Subunit Vaccine: Subunit vaccines, like inactivated whole-cell vaccines, do not contain live components of the pathogen. They differ from inactivated whole-cell vaccines, by containing only the antigenic parts of the pathogen. These parts are necessary to elicit a protective immune response. This precision comes at a cost, as antigenic properties of the various potential subunits of a pathogen must be examined in detail to determine which particular combinations will produce an effective immune response within the correct pathway. Often a response can be elicited, but there is no guarantee that immunological memory will be formed in the correct manner.

Ancillary Product: Products that are used in the manufacture or production of a biological product that may or may not end up as part of the final product. Examples include: insulin, transferrin, growth factors, interferon, interleukins, other proteins, drugs or chemicals like dimethyl sulfoxide.

Aneuploid: Having an atypical number of chromosomes which is not an integer multiple of the haploid number.

Booster Vaccination: Vaccination given at a certain time interval after primary vaccination in order to enhance immune responses and induce long term protection.

Cell Bank: Vials of cells of uniform composition (although not necessarily clonal) derived from a single tissue or cell, aliquoted into appropriate storage containers, and stored under appropriate conditions, such as the vapor phase of liquid nitrogen.

Cell Line: Cells that have been propagated in culture since establishment of a primary culture and survival through crisis and senescence. Such surviving cells are immortal and will not senesce. Diploid cell strains have been established from primary cultures and expanded into cell banks but have not passed through crisis and are not immortal.

Combination Vaccine: For this document, a combination vaccine consists of two or more live organisms, inactivated organisms or purified antigens combined either by the manufacturer or mixed immediately before administration and intended to:

1) prevent multiple diseases, or
2) prevent one disease caused by different strains or serotypes of the same organism.
3) Vectored vaccines and conjugated vaccines are combination vaccines if the prevention of the disease caused by the vector organism or the carrier moiety is to be one of the combination's indications.

Control Cells: Cells that are split off from the production culture and maintained in parallel under the same conditions and using the same reagents (e.g., culture medium) in order to perform quality control tests on cells that have not been exposed to the vaccine virus (which may interfere with some tests).

Diploid: Having the expected number of chromosomes for a species, (i.e., two of each autosomal chromosome and two sex chromosomes).

Diploid Cell Line: A cell line having a finite in vitro lifespan in which the chromosomes are paired (euploid) and are structurally identical to those of the species from which they were derived.

Drug Product: It is the finished dosage form of the product. The drug product contains the active substance(s) formulated with other ingredients in the finished dosage form ready for marketing. Other ingredients, active or inactive, may include adjuvants, preservatives, stabilizers, and/or excipients. For vaccine formulation, the active substance(s) may be diluted, adsorbed, mixed with adjuvants or additives, and/or lyophilized to become the drug product.

Active Substance: It is the unformulated active (immunogenic) substance which may be subsequently formulated with excipients to produce the drug product. The active substance may be whole bacterial cells, viruses, or parasites (live or killed); crude or purified antigens isolated from killed or living cells; crude or purified antigens secreted from living cells; recombinant or synthetic carbohydrate, protein or peptide antigens; polynucleotides (as in plasmid DNA vaccines); or conjugates.

Endogenous Virus: A virus whose genome is present in an integrated form in a cell substrate by heredity. Endogenous viral sequences may or may not encode for an intact or infectious virus.

End-Of-Production Cells (EOPC): Cells harvested at the end of a production run or cells cultured from the Master Cell Bank (MCB) or Working Cell Bank (WCB) to a passage level or population doubling level comparable to or beyond the highest level reached in production.

End-Of-Production Passage Level: The maximal passage level achieved during manufacturing at final vaccine harvest. Cells may be evaluated at this level or beyond.

Final Bulk: The stage of vaccine production directly prior to filling of individual vials.

Harvest: At the end of vaccine virus propagation in cell culture, material is collected from which vaccine will be prepared. This material may be the culture supernatant, the cells themselves (often in disrupted form), or some combination thereof.

Host Cells: See Parental cells.

Genetically Modified Organism (GMO): an organism or a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/ or natural recombination. This definition covers micro-organisms including viruses, viroids, cell cultures including those from animals but does not cover naked rDNA and naked recombinant plasmids.

Good Clinical Practice (GCP): A standard for clinical studies which encompasses the design, conduct, monitoring, terminations, audit, analyses, reporting and documentation of the studies and which ensures that the studies are scientifically and ethically sound and that the clinical properties of the pharmaceutical product (diagnostic, therapeutic or prophylactic) under investigation are properly documented.

Good Laboratory Practice (GLP): A quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived, and reported. GLP principles may be considered as a set of criteria to be satisfied as a basis for ensuring the quality, reliability and integrity of studies, the reporting of verifiable conclusions and the traceability of data.

Good Manufacturing Practice (GMP): A part of the pharmaceutical quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and a required by the marketing authorization.

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

Immortalization: The process by which cells with finite lifespan (e.g., primary cells, diploid cell strains) are converted to those with infinite lifespan.

Latent: A virus that is present in a cell, without evidence of active replication, but with the potential to reactivate, is considered to be microbiologically latent.

Manufacturer's Working Cell Bank (MWCB) or Working Cell Bank (WCB): A cell bank derived by propagation of cells from MCB under defined conditions and used to initiate production cell cultures on a lot-by-lot basis.

Master Cell Bank (MCB): a quantity of well-characterized cells of animal or other origin, derived from a cell seed at a specific population doubling level (PDL) or passage level, dispensed into multiple containers and stored under defined conditions. The MCB is prepared from a single homogeneously mixed pool of cells. In some cases, such as genetically engineered cells, the MCB may be prepared from a selected cell clone established under defined conditions. However, the MCB may not be clonal. The MCB is used to derive a working cell bank (WCB).

Master Seed (MS): A seed of a selected vaccine virus/bacteria/other from which all future vaccine production will be derived, either directly, or via Working Seeds.

Metazoan: Organism of multicellular animal nature.

Oncogenicity: The property of certain biological agents (e.g., viruses) or materials (e.g., nucleic acids) that are capable of immortalizing cells and endowing them with the capacity to form tumors. Oncogenicity is distinct from tumorigenicity (See Tumorigenicity).

Parental Cells: Cell to be manipulated to give rise to a cell substrate or an intermediate cell line. For microbial expression systems, it is typical to also describe the parental cells as the host cell. For hybridomas, it is typical to also describe the parental cells as the cells to be fused.

Parental Virus: Virus that has been manipulated in some manner to generate a viral seed with characteristics needed for production.

Parent Cell Bank: A few vials consisting of cells from which the Master Cell Bank was derived. Parental Cells may be manipulated to derive a cell substrate with desired characteristics.

Passage Level: The number of times, since establishment from a primary cell culture, a culture has been split or re-seeded.

Plasmid: Double-stranded circular DNA molecules capable of replicating in bacterial cells.

Population Doubling Level: The number of times, since establishment from a primary cell culture, a culture has doubled in number of cells.

Potency: The measure of biological activity, using a suitably quantitative biological assay, based on the attribute of the product that is linked to the relevant biological properties.

Preclinical Evaluation of Vaccine: All *in vivo* and *in vitro* testing prior to first testing of vaccines in humans. This is prerequisite to the initiation of clinical trials and includes product characterization, proof of concept/ immunogenicity studies and animal safety testing conducted prior to introducing the product into the humans.

Preclinical Toxicity Study: A study designed with the primary purpose of demonstrating the safety and tolerability of a candidate vaccine product.

Primary Cells: Cells placed into culture immediately after an embryo, tissue, or organ is removed from an animal or human and homogenized, minced, or otherwise separated into a suspension of cells. Primary cells may be maintained in medium, but are not passaged (split).

Primary Vaccination: First vaccination or series of vaccinations given within a predefined period, with an interval of less than 6 months between doses, to induce clinical protection.

Product Characterization: Full battery of physical, chemical, and biological tests conducted for a particular product. These tests include but are not limited to in process control testing, testing for adventitious agents, testing process additives and process intermediates, and lot release.

Protocol or Study Plan: A document that states the background, rationale and objectives of the studies and describes its designs, methodology and organization, including statistical considerations, and the conditions under which it is to be performed and managed.

Purity: Relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product.

Qualification: Determination of the suitability of a cell substrate for manufacturing based on its characterization.

Relevant Animal Model: is an animal which develops an immune response similar to the expected human response after vaccination. It is acknowledged that species specific differences in immune responses will likely exist. Ideally, the animal species used should be sensitive to the pathogenic organism or toxin.

Route of Administration: The means by which the candidate vaccine product is introduced to the host. Routes of administration may include the intravenous, intramuscular, subcutaneous, transcutaneous, intradermal, transdermal, oral, intranasal, intranodal, intravaginal and intrarectal routes.

Seroconversion: Predefined increase in antibody concentration, considered to correlate with the transition from seronegative to seropositive, providing information on the immunogenicity of a vaccine. If there are pre-existing antibodies, seroconversion is defined by a transition from a predefined low level to a significantly higher defined level, such as fourfold increase in geometric mean antibody concentration.

Tumorigenic: A cell type is tumorigenic if it forms tumors when inoculated into animals (generally a syngeneic, an immunosuppressed allogeneic, or an immunosuppressed xenogeneic host). These tumors may be at the injection site or a different site and may also metastasize to other sites.

Non-Tumorigenic: A cell type is non-tumorigenic if it is shown not to form tumors in appropriate animal models.

TRADENAME: Brand or commercial name of the vaccine product.

Tumorigenicity: Tumorigenicity is the process by which immortalized cells form tumors when inoculated into animals (see Tumorigenic). Tumorigenicity is distinct from Oncogenicity (See Oncogenicity).

Tumorigenicity Testing: An assay/test that determines whether or not immortalized cells are tumorigenic when injected into animals.

Vaccine: A vaccine is an immunogenic, the administration of which is intended to stimulate the immune system to result in the prevention, amelioration or therapy of any disease or infection. A vaccine may be a live attenuated preparation of bacteria, viruses or parasites, inactivated (killed) whole organisms, living irradiated cells, crude fractions or purified immunogens, including those derived from rDNA in a host cell, conjugates formed by covalent linkage of components, synthetic antigens, polynucleotides (such as the plasmid

DNA vaccines), living vectored cells expressing specific heterologous immunogens, or cells pulsed with immunogenic. It may also be a combination of vaccines.

Validation: Validation defines the performance characteristics of an analytical procedure, based on the demonstration that the procedure is suitable for its intended purpose or use. Validation of a process is the determination of what characteristics the process is capable of performing and the demonstration that the process uniformly performs to defined characteristics.

Viral Clearance: The combination of the physical removal of viral particles and the reduction of viral infectivity through inactivation.

Seed: A live viral/bacterial/other preparation of uniform composition (although not necessarily clonal) derived from a single culture process, aliquoted into appropriate storage containers, and stored under appropriate conditions.

Working Cell Bank: See Manufacturer's Working Cell Bank (MWCB).

Working Seed (WS): A seed derived by propagation of microorganism from the MS under defined conditions and used to initiate production cell cultures lot-by-lot.

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