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Saudi Food & Drug Authority

Guidelines for Production and Quality Control of Vaccines

Version 2.1

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Drug Sector
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Introduction

These guidelines give advice to manufacturers and regulatory authorities to ensure that new vaccines and vaccines that are new to a particular manufacturer meet the highest possible standards of the quality, safety and efficacy. These will enable the authorities to arrive at their decisions by reference to uniform criteria and will therefore avoid differences in evaluation.

Vaccines for human use are preparations that contain antigenic substances capable of inducing a specific and active immunity against the infecting agent or the toxin or the antigen produced by it.

Vaccines for human use may contain:

- organisms which have been inactivated by chemical or physical means and maintain adequate immunogenic properties
- living organisms that are naturally a virulent or that have been treated to attenuate their virulence whilst retaining adequate immunogenic properties
- antigens extracted from organisms, secreted by them, or produced by recombinant DNA (rDNA) technology.

Antigens may be used in their native state or may be detoxified by chemical or physical means and may be aggregated, polymerized or conjugated to a carrier to increase their immunogenicity.

Part 1 - Manufacturing and Control Requirements of Vaccines

I- Active Substance

Production of active substance, whether by fermentation, cultivation, isolation, or synthesis, usually starts with raw materials. Subsequent steps of the procedure involve preparation, characterization and purification of intermediates eventually resulting in the active substance. The quality and purity of the active substance cannot be assured solely by downstream testing, but depends on proper control of the manufacturing and synthetic process as well.

Proper control and attainment of minimal levels of impurities depends on:

- appropriate quality and purity of the starting materials, including the seed organisms, and reagents;
- establishment and use of in-process controls for intermediates;
- consistent adherence to validated process procedures; and
- adequacy of the final (release) control testing of the active substance.

A. Description and Characterization

For single or combination vaccine, this section should be completed for each active substance identified as being present in the final drug product.

A.1 Description

This section should contain a clear description of the active substance. The biological name (including strain and/or clone designation) or chemical name, including any established name, should be provided. The description should also include the source of the cells, including microbes, from which the active substances were derived, the active components of the cell fractions or purified antigens, and the physical and chemical properties of the synthetic active substance. Any chemical modification or conjugation of the active substance should be described in detail. Also, a list of any inactive substances, which may be present in the active substance, should be provided.

A.2 Characterization

This section should contain a description of all analytical testing performed to characterize the active substance with respect to identity, purity, potency, and stability. Test results should include actual data such as tabular data, legible copies of chromatograms or spectra, photographs of gels or immunoblots, actual histograms of cytometric analysis, or other appropriate formats. Data should be well organized and fully indexed to enable easy access. Results for quantitative assays should be presented as actual data, not generally as "Pass" or "Fail." Some tests listed below may not be necessary or applicable for all substances.

A.2.1 Physicochemical Characterization

In general, characterization may include, but is not limited to the following:

- UV/visible or mass spectrometry;
- amino acid analysis;
- amino acid or nucleic acid sequencing;
- carbohydrate analysis and, if appropriate, sequencing;
- peptide mapping;
- determination of disulfide linkage;
- Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (reduced and non-reduced);
- isoelectric focusing (1D or 2D);
- various chromatographic methods such as HPLC, GC, LC, or thin layer chromatography;
- nuclear magnetic resonance spectroscopy; and/or
- assays to detect related proteins including deamidated, oxidized, processed, and aggregated forms and other variants, such as amino acid substitutions and adducts/derivatives, and other process contaminants such as sulfhydryl reagents, urea, residual host proteins, residual DNA, and endotoxin.

Additional physicochemical characterization may be required for modified active substance such as conjugates, multiple antigen peptides (MAP), or those undergoing further chemical or enzymatic modifications. The information provided should include the degree of derivatization or conjugation, the amount of unmodified substance, removal of free materials (e.g., toxins, linkers, etc.), and the stability of the modified substance.

A.2.2 Biological Activity

Further characterization of vaccines may include, but is not limited to the following:

- specific identity testing such as Western blot analysis or ELISA;
- cytometric analysis;
- neurovirulence testing, if appropriate;
- serotyping;
- electrophoretic typing;
- inactivation studies;
- neutralization assays; and
- titrations.

A description and results of all relevant in vivo and in vitro biological testing (bioassays) performed on the manufacturer's reference standard lot or other relevant lots to demonstrate the potency and activity(ies) of the active substance should be provided. This section should include a complete description of the protocol used for each bioassay, the control standards used, the validation of the inherent variability of the test, and the established acceptance limits for each assay. The characteristics of specific antibodies used in the immunochemical or serological assays should also be included.

B. Manufacturer

B.1 Identification

The application should include the name(s), address (es), SFDA registration number, and other pertinent organizational information for each manufacturer responsible for any portion of the manufacture or testing operations for the active substance. This may include independent contractors or other company subsidiaries serving as contractors, or other locations/sites owned and operated by the applicant. Also included in this section should be a discussion of the operations performed by each party and the responsibilities delegated to each party by the applicant.

B.2 Floor Diagram(s)

For each manufacturing location, a simple floor diagram of the general layout of the facilities, which traces the active substance through the manufacturing process, should be included. This diagram need to be a detailed engineering schematic or blueprints, but rather a simple drawing that clearly depicts the relationship of each manufacturing

area, suite, or room to the others. The uses made of adjacent areas that are not the subject of the application should also be included. The diagrams should be sufficiently clear to enable visualization of the production flow and to identify adjacent operations that may create particular concerns, e.g., the proximity of live viral cultures to inactivated intermediates or final products, segregation of animal facilities, etc. Room numbers or other unique identifiers should be clearly indicated.

B.3 Manufacture of Other Products

A comprehensive list of all additional products that are manufactured or manipulated in the same areas used to produce the active substance that is the subject of this application should be provided. This section should include a brief description of the type and developmental status of the additional active substances/products and indicate the areas into which these other products will be introduced, whether on an ongoing or campaign basis, and what manufacturing steps will be performed in the multiple-use area(s). Also, the applicant should indicate whether the production of other products will utilize the same product contact equipment and, if so, how that equipment will be cleaned and validated between operations for the manufacturing of different products. Data should be provided for the validation and cleaning in the appropriate section.

C. Method of Manufacture

This section should be completed for each active substance described in section I- A. A detailed description of the manufacturing and controls should be provided to demonstrate proper quality control and prevention of possible contamination with adventitious agents. The inclusion of a list of all relevant Standard Operation Procedures (SOPs) is recommended; however, actual copies of the SOPs are not required.

C.1 Biological Raw Materials

A list of all materials (culture media, buffers, resins for peptide synthesis, chemicals, columns, etc.) used in the manufacture of the active substance, and their tests and specifications, or reference to official compendia, should be provided. For purchased materials, representative certificates of analysis from the supplier(s) and/or manufacturer's acceptance criteria should be provided. Redundant testing at the

purchasing manufacturer may not be necessary if the testing methods at the vendor are approved by the purchasing manufacturer. Custom reagents, such as monoclonal antibodies, enzymes, other proteins, uncommon amino acids and derivatives, or glycolipids, used in purification or production of the active substance, should be described in detail, including identification of the vendor/supplier, specificity, and origin, including the manufacturing scheme, if applicable. Results of adventitious agent testing of raw materials used in propagation, e.g., serum, trypsin, amino acids and other biological reagents should be provided including bacterial and fungal agents, cultivatable and non-cultivatable mycoplasmas, mycobacteria, and viruses. 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 are considered raw materials. This list should be referenced in parts of the application which provide detailed descriptions of the use of each component.

For human-derived raw materials and reagents, your 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-derived and porcine-derived materials and reagents should be done in a manner that minimize the risk of contamination from bovine spongiform encephalopathy (BSE) and porcine adventitious viruses respectively.

C.2 Flow Charts

In this section, a complete visual representation of the manufacturing process flow should be provided for each active substance. For multiple active substances 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 the steps in production, equipment and materials used, room or area where the operation is performed (may reference diagrams in other sections of the application), and a complete list of the in-process controls and tests performed on the product at each step. In-process holding steps should be included, with time and temperature limits indicated. For chemical synthesis, a flow chart should include all the steps in a general synthesis cycle with other specific steps, such as fragment condensation or peptide cleavage, indicated. 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 units). Such transfers should be described for movement of product between equipment, areas, rooms, buildings and sites. Manufacturing steps which are computer controlled should be identified. Reference may be made to other sections of the application for more detailed process information. If equipment is dedicated to specific areas or products, it should be identified.

C.3 Origin and Source of Cells

The source of cells (laboratory or culture collection) from which the cell substrate was derived should be stated, and relevant references from the scientific literature should be cited. Information obtained directly from the source laboratory is preferred. When this is not available, literature references may be utilized.

C.3.1 Human Cells

For human cell substrates, the source of cells should be clearly described, including:

- the materials and methods used;
- the tissue or organ of origin;
- age, gender, ethnic and geographical origin;
- general physiological condition; and
- health or medical history of the donor must be provided along with the results of any tests for pathogenic agents.

Specifically for human diploid fibroblasts, the age of the donor may influence the *in vitro* lifespan of the cell line and this information should be provided if available.

C.3.2 Animal Cells

Detailed descriptions of the animal source (including fertilized avian eggs) used for the propagation of microorganisms, or production of recombinant proteins for use as vaccines should include, but is not limited to:

- the species, strains, age, sex and geographical origin;
- the health status of the animals, e.g., specific pathogen free;
- the results of adventitious agent screening;
- the animal husbandry practices, e.g., quarantine procedures, used to ensure the suitability of the animals;
- the veterinary and laboratory monitoring used to ensure the suitability of the animals;

- a description of the inoculation of the animals; and
- a description of the tissues harvested and the method of harvest.

Cells of animal origin may harbor adventitious agents and consequently pose a potentially greater risk to humans if not properly controlled. The measures taken to remove, inactivate, or prevent contamination of the product from any adventitious agent present in the cell substrate should be described.

C.3.3 Virus Sources

This section should include a detailed description of the virus seed used for vaccine production. The information submitted should include, but is not limited to:

- the original source of the virus;
- the passage history of the virus strains;
- details of the seed lot system; and
- the culture techniques for virus seed maintenance.

C.3.4 Microbial Cells

This section should contain a description of the species, strain and known genotypic and phenotypic characteristics of the microorganism from which the cell substrate is derived. 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.

The history and characteristics of each strain used to produce the product and a complete strain description should be provided, including:

- origin of isolate;
- species;
- biochemistry (fermentation profile, etc.);
- strain identifier and specific identifying characteristics (serotype, etc.);
- virulence (attenuation method, if performed);
- genetic characterization, if known (markers, inserts, deletions, etc.);
- plasmids;
- genetic stability; and
- pathogenicity, toxin production and other biohazard information, if any.

C.4 History of Cells

The cultivation history of the cells should be documented. The method originally used for the isolation of the cells should be described as well as the procedures used in the culturing of the cells in vitro and any procedures used to establish cell lines (for example, use of any physical, chemical, or biological procedure, or added nucleotide sequences). A description of any genetic manipulation or selection should be provided. All available information regarding the identification, characteristics, and results of testing of these cells for endogenous and adventitious agents should be provided.

For continuous cell lines of metazoan origin, it is usually adequate to quantitate culture duration by estimation of either number of population doublings, or number of subcultivations at defined dilution ratio, or time in days. For diploid cell lines possessing finite in vitro lifespan, accurate estimation of the number of population doublings during all stages of research, development, and manufacturing is important. For microbial cells, documentation of subcultivation frequency after cell substrate generation is considered adequate.

Regarding the generation of cell substrates, applicants should provide a thorough discussion of procedures which would provide exposure to infectious agents. Constituents of the culture medium should be described, in particular, information regarding exposure of the cells to materials of human or animal origin such as serum, enzymes, hydrolysates, or other living cells. The description should include the source, method of preparation and control, test results, and quality assurance. Relevant literature on these points may be referenced when available. This information will allow a detailed analysis of potential entry routes for adventitious agents from these sources, and will be part of the risk-benefit analysis of the product.

C.5 Generation of the Cell Substrate

A crucial step is the choice of a suitable parental cell line. For recombinant products, a parental cell line is typically the untransfected recipient cell line. The use of characterized parental cell banks is suggested, but is not considered essential. A characterized parental cell bank may be of benefit, especially when multiple cell substrates are generated from the same parental cell type, by providing a set of

information on which the quality assessment of the Master Cell Bank (MCB) can be based. For example, the myeloma cell line may be banked as a parental cell line for hybridomas.

All specific procedures used to generate the cell substrate should be well documented. These may include, for example, cell fusion, selection, transfection, colony isolation, cloning, gene amplification, and adaptation to specific culture conditions or media. The growth pattern and morphological appearance of the cell lines, from the master cell bank to the end-of production cells, should be submitted. A thorough discussion of the adventitious agent profile of any cell substrate should be provided. Information regarding the methodologies utilized in developing the cell substrate can help to provide a clear understanding of the history of the cell substrate. Some cell substrates such as human diploid fibroblasts may not need extensive manipulation or cloning prior to cell banking.

For recombinant products, the cell substrate is the transfected cell containing the desired sequences, which has been cloned from a single cell progenitor. For further information on generation of rDNA modified cell substrates, consult other relevant international guidelines. For non-recombinant products or non-recombinant vaccines, the cell substrate is the cell from the parental cell 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 fusion of the parental myeloma cell line with other parental cells, e.g., immune spleen cells.

C.6 Cell Banking
Cell banking assures that an adequate supply of equivalent; well-characterized cells exist for production over the expected lifetime of the product. In addition to providing a constant supply of biological starting material, cell banking provides you with the opportunity to undertake a comprehensive characterization of the cell substrate and to minimize the chance of adventitious agent contamination and/or to maximize the chance of detection of a contamination. Manufacturers are responsible for ensuring the quality of each cell bank and of the testing performed on each bank. Manufacturers should describe their strategy for providing a continued supply of cells from their cell bank(s), including the anticipated utilization rate of the cell bank(s) for

production, the expected intervals between generation of new cell bank(s), and the criteria for qualification of cell bank(s).

Ordinarily, the cell bank system consists of two tiers: a master cell bank (MCB) and a working cell bank (WCB), often called a manufacturer's working cell bank (MWCB). The MCB represents a collection of cells of uniform composition derived from a single source prepared under defined culture conditions. The WCB is derived from one or more vials of cells from the MCB which are expanded by serial subculture. The pooled cells are dispensed into individual vials and cryopreserved to form the WCB for vaccine manufacturing.

It should be noted that the MCB and WCB may differ from each other in certain respects, e.g., culture components and culture conditions. Similarly, the culture conditions used to prepare the MCB and WCB may differ from those used for the production process. If changes in cell culture process do not affect product quality, it is not considered necessary to reclone the cells or to rebank the MCB or WCB. It is important that a characterized bank provides a consistent product.

A single-tiered banking system consisting only of the MCB but no WCBs could be used in principle, for example, if relatively few containers were needed each year to produce the desired product. In some microbial expression systems, a new transformation is performed for each new cell substrate container lot, based upon using aliquots of thoroughly tested host cell banks and plasmid banks for each new transformation and on testing of each transformed cell substrate bank. This transformed cell substrate bank is considered the MCB, and it is used as the source of cell substrate for production. Host cell banks, plasmid banks, and MCBs are maintained by appropriate preservation methods. This alternative system is considered adequate because the transformation of bacteria and yeast is generally a very reproducible and easily performed process, unlike the events needed for transfection of metazoan cells. Manufacturers should provide information on the host cells, r-DNA molecules (such as plasmids), method of transformation and of cell banking, and the results of characterization studies.

C.6.1 Master Cell Bank

The cells comprising the MCB should be identified and a complete history and characterization of the MCB should be provided, including, as appropriate for the given cells:

- the biological or chemical method used to derive the cell bank;
- biochemistry (cell surface markers, isoenzyme analysis, specific protein or mRNA, etc.);
- specific identifying characteristics (morphology, serotype, etc.);
- karyology and tumorigenicity;
- virulence markers;
- genetic markers;
- purity of culture; and
- media and components (e.g., serum).

C.6.2 Working Cell Bank

This section should contain a description of the procedures used to derive a WCB from the MCB. The description should include the identification system used for the WCB as well as the procedures for storage and cataloging of the WCB. The assays used for qualification and characterization of each new WCB should be included with the results of those assays for the WCB currently in use. If applicable, a description of animal passage 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

In some instances, another tier of cell bank system 'Primary Cell Bank' may be established which allows the manufacturers to perform extensive testing on a pool of cryopreserved primary cells prior to their usage in vaccine production.

Because primary cell cultures are used within the first passage after establishment from the tissue of origin, it is not possible to carry out extensive characterization of the cells prior to their use as is done for banked cell substrates. In addition, biological products produced using primary cell substrates often do not undergo extensive processing (e.g., purification). A discussion of the rationale for the use of primary cells should be provided.

The information submitted for each primary cell line used should include, but is not limited to:

- the species and age of the animals and the source tissue from which the cells are derived;
- the health status of the animals from which the cells are derived, e.g., specific pathogen free;
- the animal husbandry practices used to ensure the suitability of the animals (animals should be adequately quarantined for an appropriate period of time prior to use for the preparation of cells);
- the 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 the source of all reagents of human or animal origin;
- an explanation of the concurrent testing done to demonstrate the absence of detectable contaminants and adventitious agents in these substrates which may include: observation of production or uninfected control cultures before, during, and beyond the period 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 hemadsorbing viruses; and other tests for specific agents (such as relevant retroviruses) as necessary. The results of those tests should be included;
- Methods used for isolation of cells from tissue, establishment of primary cell cultures and maintenance of cultures should be described; and
- Tests performed on primary cell substrates to qualify them for use in production should be described.

C.6.4 Diploid Cell Strains

Diploid cell strains are established from primary cell cultures by expansion and cell banking. These types of cells have a finite life span and are not immortal like cell lines. Diploid cells usually 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, as it might be helpful to establish identity and to characterize a cell strain. Such analyses will establish the diploid character of the cells and determine its freedom from contamination with other cell lines. It might also be useful to monitor the genetic stability of the diploid cell strain throughout production.

C.6.5 Tumorigenic Cell Lines

Tumorigenic cell lines are cells that are derived from tumor. Additional testing should be performed if the cell lines are tumorigenic. Tumorigenic cell lines should be assessed for potential oncogenic viruses and oncogenic substances (including nucleic acids), which could be associated with induction of a neoplastic process in a vaccine recipient. Test strategies for potential oncogenic viruses or oncogenic substances may be determined case-by-case, depending on the tissue type, source species, passage history, and extent of knowledge about the transforming event(s). (See section I-C.8.3 for a discussion of tumorigenicty testing and section I- C.8.4 for a discussion of oncogenecity testing.)

In cases where the transforming event is known (for example, if the cells were transformed by a known oncogene), testing should demonstrate that the final product is free of the transforming agent. For example, if adenovirus sequences are used to transform a primary human cell to produce a cell line (e.g., 293 cells), then testing should demonstrate that the final product is free of the introduced viral sequences. 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 provide assurance of safety. Tumorigenic or tumor-derived cell lines for which the mechanism of transformation is unknown will require additional testing to ensure the absence of potential transforming and oncogenic agents.

The testing recommended for qualification of cell substrates and cell banks should also be applied to rodent cell lines. However, because most rodent cell lines used for the production of biologicals are known to be tumorigenic, it is considered unnecessary to test rodent cell lines for tumorigenicity. A presumption is made that they will be tumorigenic, and the considerations described above apply. In addition, rodent cell lines are presumed to be capable of producing endogenous retroviruses. Assessment of the quantity and type of retroviruses produced should be performed. Infectivity assays for retroviruses are also recommended. Rodent cell lines should only be used if the product can be sufficiently purified to demonstrate levels of viral clearance that assure the final product is not contaminated with retroviral particles.

C.6.6 End of Production Cells (EPC)

Cells should be characterized from the actual end-of-production of one or more lots or cells that are expanded to or beyond the end of production passage level. Such cells are referred to as End-of-production cells (EPC). The stability of the cell substrate characteristics should be demonstrated using the EPC. The characterization should include growth characteristics, tumorigenic phenotype, expression of endogenous viruses, stability of expression of the inserted or engineered genes, and genetic stability.

For rDNA derived active substances, a detailed description of the characterization of the EPC that demonstrates that the biological production system is consistent during growth should be provided. The results of the analysis of the EPC for phenotypic or genotypic markers to confirm identity and purity should be included. This section should also contain the results of testing supporting the freedom of the EPC from contamination by adventitious agents. The results of restriction enzyme analysis of the gene constructs in the EPC should be submitted. Further guidance can be obtained from ICH document on "Analysis of Expression Construct in Cell Used for Production of rDNA Derived Protein Products".

C.7 Cell Banking Procedures

It is important to prevent a contaminated cell substrate (or bank) from being used in production and to avoid a loss of product availability or development time resulting from the need to recreate a cell bank found to be unusable due to contamination. It is recognized that no cell bank testing regimen is able to detect all potential contaminants; therefore, use of these preventive principles during cell banking is important to provide reasonable assurance of the absence of contamination and to provide a reliable source of the cell substrate.

A description of the cell banking procedures used should be provided, including:
- the banking system used;
- the size of the cell banks;
- the container and closure system used;
- a detailed description of the methods, cryoprotectants, reagents and media used for preparation of the cell banks;
- the conditions employed for cryopreservation and storage;
- in-process controls; and

- storage conditions.

A description should be provided of the procedures used to avoid microbial contamination and cross-contamination by other cell types present in the facility, and the procedures that allow the banked cells to be traced. This should include a description of the documentation system as well as that of a labeling system which 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 vessel until a pool of cells can be obtained which is sufficient to generate enough containers for the bank. To ensure the uniform composition of the contents of each container, a single pool of cells for banking should be prepared by combining the cells from all of the culture vessels, if more than one vessel is used.

Cells suspended in preservation medium are aliquoted from the single pool into sterilized containers which are then sealed and stored under appropriate conditions. For example, animal cells in media containing a cryoprotectant are frozen in the sealed containers under defined and controlled conditions, and then transferred to storage in the vapor or liquid phase of liquid nitrogen or at equivalent ultra low temperatures. Other methods of preservation and storage may be adequate depending on the organism used, but they should be capable of maintaining a level of cell viability upon reconstitution which is both consistent and adequate for production use.

To ensure continuous, uninterrupted production of pharmaceuticals, manufacturers should carefully consider the steps that can be taken to provide for protection from catastrophic events that could render the cell bank unusable. Examples of these events include fires, power outages and human error. Manufacturers should describe their plans for such precautions; for example, these may include redundancy in the storage of bank containers in multiple freezers, use of back-up power, use of automatic liquid nitrogen fill systems for storage units, storage of a portion of the MCB and WCB at remote sites, or regeneration of the MCB.

The starting point of reference for estimates of in vitro cell age during manufacturing should be the thawing of one or more containers of the MCB. For diploid cell lines, in vitro lifespan should be estimated in terms of population doubling levels. The

population doubling level at which senescence occurs should be determined for diploid cells.

C.8 Characterization and Testing of Cell Banks

The characterization and testing of banked cell substrates is a critical component of the control of vaccines. Characterization of the MCB allows the manufacturer to assess this source with regard to presence of cells from other lines, adventitious agents, endogenous agents and molecular contaminants (e.g., toxins or antibiotics from the host organism). The objective of this testing is to confirm the identity, purity, and suitability of the cell substrate for manufacturing use.

Detailed information on the characterization and testing of banked cell substrates should be submitted. In some cases, additional testing such as tumorigenicity or karyology may be useful. The testing program chosen for a given cell substrate will vary according to the biological properties of the cells (for example, growth requirements), its cultivation history (including use of human-derived and animal-derived biological reagents) and available testing procedures. The extent of characterization of a cell substrate may influence the type or level of routine testing needed at later stages of manufacturing. Manufacturers should perform tests for identity and purity once for each MCB, and tests of stability during cell cultivation once for each product to be registered. In addition, tests of purity and limited tests of identity should be performed once on each WCB. Also applicants should consult the ICH guideline on viral safety. Relevant tests among those described below should be performed and described in the market application, along with the results of the testing.

For cell lines containing exogenously assembled expression constructs, characterization of nucleotide and amino acid sequences are required. It may also be useful to examine the coding sequences in some non-rDNA-derived cell lines where the gene sequences have been characterized and are well understood. However, it is not considered necessary to carry out investigations of the sequences encoding complex natural products, for example, families of related gene products, microbial vaccine antigens, or monoclonal antibodies from hybridomas.

C.8.1 Testing for Identity and Purity

Appropriate tests should be performed to determine that the banked cell is what it is represented to be. Either phenotypic or genotypic characteristics may be used in identity testing. It is not considered necessary to do all the possible tests. Tests of identity are generally performed on the MCB. In addition, limited identity testing is generally performed on each WCB. Relevant tests should be described in the application, along with the results of the testing. In general, the methods described in section I- A.2.1 are considered adequate tests to confirm the identity and purity.

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

For most microbial cells, analysis of growth on selective media is usually adequate 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 may be used, biological characterization methods such as phage typing should be considered as supplementary tests of identity. For plasmid banks, identity assessment can be accomplished as described by the ICH document on analysis of the expression construct. Expression of the desired product is also considered adequate to confirm the identity of the microbial expression system.

A critical aspect of cell development and banking is the assessment that the MCB and WCB are biologically pure, i.e., are free from adventitious microbial agents and adventitious cellular contaminants. The impact of selective agents and antibiotics on

the detection of adventious microbial contaminants should be considered when planning and performing these tests.

Results of tests for the presence of bioburden (bacteria and fungi) and mycoplasma in metazoan cells should be submitted for the MCB and WCB. The results of virus testing of metazoan cell substrates to detect possible contaminating viruses, using appropriate screening tests designed to detect a wide spectrum of viruses and relevant specific tests based on the cultivation history of the cell line, should be submitted.

The purity of cell substrates can be compromised through contamination by cell lines of the same or different species of origin. The choice of tests to be performed depends upon whether opportunities have existed for cross-contamination by other cell lines. In some cases, it may be necessary to maintain growing cultures of different cell lines in the same laboratory. During procedures in cell banking where open manipulations are performed, care should be taken to ensure that simultaneous open manipulations of other cell lines are avoided to prevent cross-contamination. Whenever another cell line was present in the cell banking room at the same time that open cell banking procedures were being performed (such as cell expansion, pooling, or aliquoting of the chosen cell line), the cell banks should be tested 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 also considered adequate tests to detect cross-contamination by other cell lines. Additional assurance of lack of cross-contamination can be provided by successful preparation of the intended product from the cell substrate.

For microbial cells, the design and performance of specific tests for adventitious microbial agents and adventitious cellular contaminants in cell banks should take into account the properties of the banked cell, the likely contaminants based upon scientific literature, source, methods and materials used for cultivation, and other organisms present in the banking laboratory. For example, visual examination of the characteristics of well-isolated colonies is suggested, using several microbiological media, of which some do and some do not support growth of the cell substrate. However, it is not intended that manufacturers necessarily characterize resistant mutants of the cell substrate arising from such studies, or other artifacts of such assays. Rather, the purpose of such assays is to detect existing contaminants.

C.8.2 Testing for Cell Substrate Stability

Another dimension to cell characterization is appropriateness for intended use in production. There are two concerns for cell substrate stability: Consistent production of the intended product and retention of production capacity during storage under defined conditions.

For the evaluation of stability during cultivation for production, at least two time points should be examined, one using cells which have received a minimal number of subcultivations, and another using cells at or beyond the limit of in vitro cell age for production use described in the marketing application. The limit of in vitro cell age for production use should be based on data derived from production cells expanded under pilot plant scale or commercial scale conditions to the proposed limit of in vitro cell age for production use or beyond. Generally, the production cells are obtained by expansion of cells from the WCB; cells from the MCB could be used with appropriate justification. This demonstration of cell substrate stability is commonly performed once for each product marketing application.

Evaluation of the cell substrate with respect to the consistent production of the intended product of interest should be the primary subject of concern. The type of testing and test article(s) used for such assessments will depend on the nature of the cell substrate, the cultivation methods, and the product. For cell lines containing rDNA expression constructs, consistency of the coding sequence of the expression construct should be verified in cells cultivated to the limit of in vitro cell age for production use or beyond by either nucleic acid testing or product analysis. For nonrecombinant cell lines in which the coding sequence for the desired product has already been analyzed at the MCB or WCB level, invariability of the protein coding sequence during production should be verified in the production cells cultivated to the proposed limit of in vitro cell age for production use or beyond by either nucleic acid testing or analysis of the purified protein product.

Where the product cannot be analyzed as described above, other specific traits which may include, for example, morphological characteristics, growth characteristics, biochemical markers, immunological markers, productivity of the desired product, or other relevant genotypic or phenotypic markers may be useful for the assessment of cell substrate stability. In some cases, where direct comparison of the characteristics of the MCB with those of the production cells at or beyond the limit of in vitro cell age is difficult or impossible, one may compare the characteristics of cells at the

initial stages of cultivation or production to those of cells at or beyond the limit of in vitro cell age for production use in order to assess cell stability during production. Indices such as, for example, oxygen or glucose consumption rates, ammonia or lactate production rates may be useful for such testing. Increases in the defined limit of in vitro cell age for production use should be supported by data from cells which have been expanded to the proposed new limit of in vitro cell age. For diploid cell lines, data should be presented that establish the finite in vitro lifespan of the cells from the WCB under conditions representative of those employed for manufacturing use.

Evidence for banked cell stability under defined storage conditions will usually be generated during production of clinical trial material from the banked cells. Data from the determination of cell viability when the preserved cells are reconstituted for production of clinical trial supplies will verify that the revived cells have survived the preservation process. Data from the preparation of clinical materials will demonstrate that the revived cells can be used to prepare the desired product. Available data should be clearly documented in the application dossiers, plus a proposal for monitoring of banked cell stability should be provided. The proposed monitoring can be performed at the time that one or more containers of the cryopreserved bank is thawed for production use, when the product or production consistency is monitored in a relevant way, or when one or more containers of the cryopreserved MCB is thawed for preparation of a new WCB (and the new WCB is properly qualified), as appropriate. In the case when production does not take place for a long period of time, viability testing on the cell bank used as a source of the production substrate should be performed at an interval described in the marketing application. If the viability of the cell substrate is not significantly decreased, generally no further testing of the MCB or WCB is considered necessary.

C.8.3 Testing for Karyology and Tumorigenicity

Tumorigenicity is defined as the process by which cells form tumors when inoculated into animals (generally a syngeneic, an immunosuppressed allogeneic or an immunosuppressed xenogeneic host) (see Glossary). Tumorigenicity is a characteristic of the immortalized cells themselves, rather than of agents or components present in them.

Tumorigenic cells have not traditionally been used for the production of prophylactic viral vaccines, primarily because of theoretical concerns that components within tumorigenic cells could induce tumors in vaccine recipients. These concerns include the potential presence of exogenous agents, such as oncogenic viruses, and the potential risk from endogenous materials, such as endogenous viruses or oncogenic nucleic acids. In addition, intact human cells derived from human tumors have been shown to form tumors in allogeneic humans.

The goal in tumorigenicity testing is to determine whether the cell substrate is capable of forming tumors after inoculation into 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:

- choice of appropriate animal models;
- definition of a positive result;
- determination of the appropriate duration of testing;
- determination of appropriate numbers of cells to be tested; and
- selection of appropriate controls.

One should use an animal model that is known to be susceptible to tumor formation by tumorigenic cells. Because immunocompromised adult and newborn rodents are relatively sensitive for revealing a tumorigenic phenotype, these animal models should be considered. Thus, the most commonly used animals for tumorigenicity testing are nude (nu/nu) mice because they are T-cell deficient. Newborn nude mice appear to be more susceptible to tumor formation than adult nude mice, suggesting that newborn nude mice might be the best choice to use when identification of a weakly tumorigenic phenotype is important. One might choose to use another animal model if it has been shown to have comparable sensitivity to the nude mouse model.

Selection of the appropriate duration of testing requires balancing the increased sensitivity that might be obtained using a longer test, against the likelihood of false-positive results due to spontaneous tumor formation. Weakly tumorigenic cells might require between 4 and 7 months to form tumors in nude mice. Thus, extended observation periods might be necessary in some cases.

Utilization of karyology and tumorigenicity testing for evaluating the safety of a diploid cell line or characterizing a new cell line may be useful depending on the cells, the nature of the product and the manufacturing process. Extensive analysis to determine the relative abundance of aneuploid cells has not been found to be useful. Karyology need not be determined for rodent cell lines or new cell lines known to be non-diploid. However, cytogenetic analysis may be an adequate method to assess cell substrate identity or purity as described in sections I- C.8.1. Repetition of tumorigenicity testing for cells with already documented evidence of tumorigenicity is not considered necessary.

For products that are highly purified and that contain no cells, karyology and tumorigenicity testing are generally not considered necessary, provided that appropriate limits for residual host cell DNA are shown to be consistently met by either process validation studies or by lot release testing.

In general, products for which the presence of live cells cannot be excluded or which have little downstream purification (for example, some conventional live virus vaccines) will need such characterisation of the cell substrate. The utility of tumorigenicity testing and chromosomal analysis for new cell substrates for unpurified products should be evaluated on a case-by-case basis. Use of cell lines known to be tumorigenic or to possess abnormal karyology should be evaluated in terms of risk-benefit for each product application when the product contains cells or when not highly purified.

Products that are manufactured in genetically unmodified MRC-5 or WI-38 cells do not need characterisation of these cell substrates by karyology or tumorigenicity since extensive characterisation has already been performed and published for these cell lines. However, for each MRC-5 and WI-38 WCB generated, manufacturers should confirm, once, that the cells grown in the manner to be used in production are diploid and have the expected lifespan. For new or previously uncharacterised diploid cell

substrates, confirmation of diploid karyology should be presented and tumorigenic potential should be established, using cells from the MCB.

C.8.4 Testing for Oncogenicity

Oncogenicity is defined as the process by which agents immortalize cells and endow them with the capacity to form tumors (see Glossary). It is important to ensure that a cell substrate does not contain potentially oncogenic components that could contaminate the product. If the vaccine is manufactured in a cell substrate that was derived from a tumor, or that has developed a tumorigenic phenotype through an unknown mechanism, it might carry a higher theoretical risk of containing oncogenic substances.

If the presence of an oncogenic virus is suspected because of the cell phenotype or the origin of the cell substrate, it might 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, in order 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 Viral Seeds

The methods used to bank stocks of vaccine viral seeds are similar to those used in cell banking. As with cell banks, passage history and derivation history of viral seeds should be documented. The description should include donor screening, testing and donor medical history. Any manipulation of the viral phenotype, such as cold-adaptation, development of temperature-sensitivity, or attenuation of virulence, should be well documented and described. Any genetic manipulations, such as reassortment or recombination, should also be well documented and described, including determining the nucleic acid sequences and sourcing of each biological starting material (e.g., plasmids, parental viruses).

These vaccine virus banks are commonly referred to as the master viral seed (MVS) and working viral seed (WVS). Viral seeds should be stored in liquid nitrogen and in

more than one location within a manufacturing facility or at a distant site for security reasons. The viral seed should be assessed for its growth characteristics on the production cell substrate, tissue tropism, genetic markers, viability during storage, genetic stability through production, attenuation (if applicable), and its absence of adventitious agents. If attenuation or derivation is achieved by passage through different cell types from different species your viral seed should be assessed for absence of adventitious agents from all species that they might have been exposed to from isolation, through passage, and during production, including those that might be present in the raw materials used at each of these stages.

C.9.1 Master Viral Seed

The Master Viral Seed (MVSs) should be extensively characterized. In addition, the stability of genotype and phenotype should be demonstrated for a number of passages beyond the level used in the production. For example, if the MVS is considered to be at passage 47, the WVS at passage 48, the production cultures inoculated with WVS at passage 49, and the production process allows for only a single round of viral replication, then it would be appropriate to assess stability of the vaccine virus at passages 47 through 51 (5 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.

In some cases, the virulence and the broad host range of the vaccine virus might complicate in vitro and in vivo adventitious agent testing. Testing for adventitious agents might then require neutralization of viral seed. Preferably, neutralizing antibodies should be monoclonal and prepared in a species other than the cells in which the MVS 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. It is important to demonstrate that the neutralization procedure does not interfere with detection of adventitious viruses. Sometimes, it is not possible to efficiently neutralize a viral seed. In such cases, one

may choose alternative strategies, including testing smaller quantities of seed, subculture onto fresh target cells in the in vitro adventitious agent test, or introduction of additional tests (e.g., PCR, Antibody Production assays).

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

C.9.2 Working Viral Seed

Working virus seeds (WVSs) may be subjected to less rigorous characterization than the MVSs from which they were derived. Once it is demonstrated that MVS is free of adventitious agents from the species to which the vaccine virus had been exposed during its isolation and passage history, then it is only needed to show that the WVS is free of adventitious agents from the species used to generate the WVS (e.g., production cells and raw materials used in propagation and processing).

C.10 Genetic Constructs and Recombinant Cell Lines

For rDNA derived products and rDNA-modified cell substrates, detailed information should be provided regarding the host cells, and the source and function of the component parts of the recombinant gene construct including:

C.10.1 Host Cells

A description of the source, relevant phenotype, and genotype should be provided for the host cell used to construct the biological production system. The results of the characterization of the host cell for phenotypic and genotypic markers, including those that will be monitored for cell stability, purity, and selection should be included.

C.10.2 Gene Construct

A detailed description of the gene which was introduced into the host cells, including both the cell type and origin of the source material, should be provided. A description of the method(s) used to prepare the gene construct and a restriction enzyme digestion map of the construct should be included. The complete nucleotide sequence of the

coding region and regulatory elements of the expression construct, with translated amino acid sequence, should be provided, including annotation designating all important sequence features.

C.10.3 Vector

Detailed information regarding the vector and genetic elements should be provided, including a description of the source and function of the component parts of the vector, e.g. origins of replication, antibiotic resistance genes, promoters, enhancers. A restriction enzyme digestion map indicating at least those sites used in construction of the vector should be provided. The genetic markers critical for the characterization of the production cells should be indicated.

C.10.4 Final Gene Construct

A detailed description should be provided of the cloning process which resulted in the final recombinant gene construct. The information should include a step-by-step description of the assembly of the gene fragments and vector or other genetic elements to form the final gene construct. A restriction enzyme digestion map indicating at least those sites used in construction of the final product construct should be provided.

C.10.5 Cloning and Establishment of the Recombinant Cell Lines

Depending on the methods to be utilized to transfer a final gene construct or isolated gene fragments into its host, the mechanism of transfer, copy number, and the physical state of the final construct inside the host cell (i.e. integrated or extrachromosomal), should be provided. In addition, the amplification of the gene construct, if applicable, selection of the recombinant cell clone, and establishment of the seed should be completely described.

C.11 Cell Growth and Harvesting

This section should contain a description of each of the following manufacturing processes, as appropriate. The description should contain sufficient detail to support the consistency of manufacture of the active substance. It is understood that all of the processes listed below may not be performed on every active substance, or be

performed in the order given. A description of the assignment of batch numbers and how each batch of a stabilized intermediate containing multiple active substances can be related to its component harvests and batches of individual active substances should be included.

C.11.1 Propagation

This section should contain descriptions of:

- each step in propagation from retrieval of the WCB to culture harvest (stages of growth);
- the media used at each step (including water quality), with details of their preparation and sterilization;
- the inoculation and growth of initial and sub-cultures, including volumes, time and temperature of incubation(s);
- how transfers are performed;
- precautions taken to control contamination;
- in-process testing which determines inoculation of the main culture system;
- in-process testing to ensure freedom from adventitious agents, including tests on culture cells, if applicable; the nature of the main culture system including operating conditions and control parameters (e.g., temperature of incubation, static vs. agitated, aerobic vs. anaerobic, culture vessels 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; and
- the use of antibiotics in the medium and rationale, if applicable.

A brief description of all process parameters which are monitored and a typical growth curve or growth description (see section I- D.2, Process validation) should be provided. A list of in- process controls and testing for purity, viability, antigen yields, and phenotypic identity; as well as the time points at which testing is performed should be included in both the Flow Chart (Section I- C.2) and the Batch Records (Section I- C.14). A description should be provided of the precautions taken to control contamination, e.g., during sample removal and transfers, and whether these are "closed" or "open" procedures.

C.11.2 Harvest

A description of the method(s) used for separation of crude active substance from the propagation system (precipitation, centrifugation, filtration, etc.) should be provided. Brief descriptions should be given for the following:

- the process parameters monitored;
- the criteria for harvesting;
- the determination of yields; and
- the criteria for pooling more than one harvest, if applicable.

This section should include a working definition of a harvest "batch." A description should be provided of the precautions taken to maintain aseptic conditions and prevent contamination during harvesting. A description of the procedures used to monitor bioburden (including acceptance limits) or sterility should be included. If the harvested crude active substance is held prior to further processing, a description of storage conditions and time limits should be provided.

C.12 Inactivation, Purification and Downstream Processing

This section should contain a description of the methods and materials by which intermediate forms and the final bulk of the active substance are separated and concentrated from the cells, media, solvents or solutions used in the production process. The description of each step of the purification process should also include the accompanying analytical tests developed or adopted by the manufacturer to show identity, purity, and concentration, and the levels of product related and non-product related impurities. This is particularly important if the latter materials are determined to be toxins, carcinogens, teratogens, or allergens. Antibiotics and other components (e.g., growth factors, antibodies) used in the culture but neither required nor specifically intended to be in the final vaccine product should be removed before use. Procedures to assure containment and prevention of contamination or cross-contamination should be provided.

Because live attenuated viruses, whole inactivated virions, or virus-like particles often cannot be purified as rigorously as viral subunit vaccines, their potential for contamination might be greater than that of subunit vaccines. The generation of live viral vaccines often involves cell disruption. In addition, such vaccines often are minimally purified and are not subjected to any inactivation steps. Comprehensive testing and qualification of the biological starting materials and raw materials should

be performed, and lot-by-lot testing for adventitious agents might be necessary, because it might not be possible for a manufacturer of live viral vaccines to validate clearance of any adventitious agents.

For inactivated vaccines, the process used to inactivate the vaccine virus might not inactivate all adventitious agents that might be present (as occurred with early inactivated poliovirus vaccines). Therefore, documentation of the validation should be provided for inactivation of adventitious agents. The choice of tests and the stages at which the tests are applied will depend on the inactivation process. The degree of viral clearance that is feasible might influence the sensitivity of the testing that should be performed to demonstrate the absence of contaminants in the product.

One should validate any methods used to inactivate or clear potential viral contaminants during production of the vaccine including the starting materials used to produce it, as the purity of the product could be affected by the purity of reagents and biological raw materials used to produce the vaccine. For example, inactivation of viruses by irradiation of serum could provide additional assurance regarding the purity of the final product. Certificates of Analysis (COA) for all reagents and biological raw materials used for vaccine production should be included in the submission.

If a seed was exposed to a known adventitious agent or if the passage history of a virus seed is unclear, viral seeds should be purified (e.g., by molecular cloning, serial passage in 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 a viral seed to within an acceptable safety margin, this approach could be used to qualify a seed.

C.12.1 Inactivation

Descriptions should be provided for:

- how culture purity is verified before inactivation;
- the method(s) and agent(s) used for inactivation;
- the method(s) undertaken to prevent aggregation and assure homogeneous access of inactivating agent(s);
- the stage in production where inactivation or killing is performed; and
- the parameters which are monitored.

Verification of the adequacy and margin of safety achieved by the method of inactivation or killing should be provided (see section I- D.2., Process Validation).

In the case of inactivating agents used in the manufacturing of bacterial vaccines, 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.

C.12.2 Purification

This section should contain an explanation of the objectives and rationale for purification of component antigens from crude harvest. Descriptions should be provided for:

- the methods used, including specialized equipment such as columns; ultracentrifugation, ultrafiltration, and custom reagents such as monoclonal antibodies;
- the process parameters monitored;
- the determination of yields;
- in-process testing (e.g., sensitivity and specificity of ELISA);
- the criteria for pooling more than one batch, if applicable;
- sterility or bioburden monitoring and the precautions taken to prevent contamination during purification;
- the reuse and/or regeneration of columns and adsorbents; and
- monitoring for residual impurities and leachable reagents.

A list of in-process controls and tests for purity, identity, and biological activity should be provided. The time points at which testing is performed should be included in both the Flow Chart (Section I- C.2) and the Batch Records (Section I- C.14.). A list of the final acceptance criteria for the purified active substance should be provided. If the purified active substance is held prior to further processing, a description of the storage conditions and time limits should be included. Verification of the stability of the purified substance under the conditions described should be included (see section I- D.2, Process Validation).

C.12.3 Stability Processing

A description should be provided for 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. A description of precautions taken to monitor bioburden and prevent contamination during these processes should also be given. If the stabilized intermediate is held prior to further processing, a description of storage conditions and time limits should be included. Verification of the stability of the active substance under the conditions described should be provided (see section I-D.2, Process Validation).

C.12.4 Detoxification

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

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

Verification of the adequacy of the method for detoxification should be provided (see section I- D.2, Process Validation).

C.13 Synthetic active Substance

For the purposes of this guidance, synthetic active substance includes: 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.

C.13.1 Synthetic Peptides

The detail of the peptide synthesis including purification procedures should be provided as outlined in the "Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances".

C.13.2 Conjugates and Modified Active Substance

This section of the guidance refers to active substances derived from another active substance or intermediate through chemical or enzymatic modification, e.g., conjugation of an immunogenic to a carrier molecule, enzymatic or chemical cleavage

and purification of the non-toxic subunit of a toxin, or 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

This section should provide a detailed description of:

* the specifications and acceptance criteria, for the native active substance starting materials, which assure suitability for conjugation or modification;
* the conditions of all reactions and/or syntheses used to produce a semi-synthetic conjugated molecule, derivatized molecule, or subunit, including intermediate forms of the reactants and active substance; also include the process parameters which are monitored, in-process controls, testing for identity and biologic activity, and any post-purification steps performed to produce a stabilized derived active substance.

The application should include a description of the methods and equipment used for separation of unreacted materials and reagents from the conjugate, derivative, or subunit, and a rationale for the choice of methods.

C.13.2.2 Specifications

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

C.14 Batch Records

A completed (executed) representative batch record of the process of production of the active substance should be provided.

D. Process Controls

D.1 In-process Controls

For all in-process testing indicated in the Flow Charts, a brief description of the sampling procedures and the test methods used should be provided. For testing performed at significant phases of production, the criteria for accepting or rejecting an in-process batch should be specified.

If primary cell cultures are used to propagate the vaccine virus, complete testing of the primary culture might not be feasible prior to inoculation of virus. In this situation, one should produce and test uninfected control-cell cultures that are derived in parallel with and handled in the same manner as the production culture. Control-cell cultures should be processed simultaneously with the production culture, but left uninfected and tested for the presence of adventitious agents by direct observation and testing of the cell sheet and by testing the culture fluid using appropriate tests. Tests for adventitious agents should be performed at times at which one would perform similar tests on the manufactured product if it were possible to do so.

Use of control-cell cultures is important when the vaccine virus might interfere with the results of in-process testing of the product; for example, when the virus cannot easily be neutralized to permit testing for adventitious agents. One should propagate control-cell cultures under conditions similar to production for a suitable period to allow for possible reactivation and detection of latent or endogenous adventitious agents and poorly replicating adventitious agents. One should use a culture period of at least 14 days beyond the time of inoculation of the production vessels prior to testing. A longer period might be needed to detect some agents. Testing of control cells does not always eliminate the need for testing end-of-production cells, which might be required to demonstrate the absence of agents induced during vaccine manufacture.

D.2 Process Validation

A summary report, including protocols and results, should be provided for the validation studies of each critical process or factor that affects active substance specifications, i.e., 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

The reliability of assays or tests used to evaluate the cell substrate in the context of intended use should be demonstrated. Test methods should be validated according to the principles defined in ICH Q2A and Q2B documents and U.S. pharmacopoeia.

Certain "compendial" methods might not require full validation, as long as their suitability under the actual conditions of use is verified. Verification of suitability for the use may include a demonstration of comparability between one's limits of detection and those for methods described in the published literature.

Appropriate statistical analysis in the assay validation should be included. This may include assessment of assay accuracy, precision, limits of detection, limits of quantification, specificity, linearity and range, ruggedness and robustness, and system suitability.

D.2.2 Propagation

A growth curve or tabular representation of growth characteristics for each propagation step, based on historical performance under specified conditions, should be provided. Data should be included which demonstrate the efficiency of induction of antigen production, if applicable. Data should also be provided showing the stability of genetic markers under the conditions of propagation, if applicable.

D.2.3 Harvest

For each method or combination of methods, a tabulation should be provided of yields, purity, and viability (if applicable) of the crude harvest, based on historical performance.

D.2.4 Inactivation

Inactivation or killing curves, or a tabular representation, based on historical performance should be provided. Validation of the titration method to measure

residual live agents, including sensitivity in a background of inactivated agents, should be provided.

D.2.5 Purification

For each method or combination of methods used, a tabulation of yields, purity, and biological activity should be provided. Verification 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 should be included. A standard denominator (e.g., international units) should be used to facilitate comparison through processing, concentration, or dilution.

D.2.6 Sterility

A description and documentation of the validation studies for any processes used for media sterilization, effectiveness of preservatives, decontamination, inactivating cells prior to their release to the environment, if such inactivation is required, etc., should be provided. If the active substance is intended to be sterile, information of the sterilization process should be submitted.

D.3 Control of Bioburden

For each process which is not intended to be sterile, documentation of the control of extraneous bioburden by a tabulation of in-process testing for bioburden should be provided.

E. Manufacturing Consistency Testing

Problems related to antigen-antigen compatibility, formulation with the proper adjuvants and preservatives and the optimizing conditions that would maintain and ensure safety, purity and efficacy lead to the requirement of a proper demonstration and definition of consistency testing. After the final manufacturing procedures are established, consistency of manufacture should be demonstrated. This may be done by producing at least three, preferably consecutive, final bulk batches from which final containers are filled. It is recommended that consistency batches be produced in the manufacturing facility for which the corresponding Establishment License

Application will be submitted. The establishment and use of reference standards in assuring consistency in product characteristics should be described.

The consistency of combined vaccines should be evaluated by first of all demonstrating the consistency of their single individual valency. At least three consecutive production batches (bulk antigens) would be required for each valency of the combined vaccine to show consistency of valency production. For a pentavalent vaccine this amounts to: A1, A2, A3, B1, B2, B3, C1, C2, C3, Y1, Y2, Y3, Z1, Z2, Z3.

If consecutive lots are not used, a scientific explanation should be provided. At least three lots of combined vaccines should then be produced to demonstrate consistency of the combination. This amounts to:

A1………….Z1
A2………….Z2
A3………….Z3

The addition of one new valency (Z) to existing batches of a well established combination represents a common situation. For example, existing batches of diphtheria and tetanus will be combined with new batches of a cellular pertussis (aP). For this situation D1T1aP1, D1T1aP2, D1T1aP3, should generally adequately show consistency on the level of final bulk. In general, for the addition of a new valency (Z) to any number of existing and well established valencies (A1, B1, C1,….) consistency is to be shown according to:

A1 B1 C1...Z1
A1 B1 C1...Z2
A1 B1 C1...Z3

Where it is considered that the consistency of the well established combined vaccine has been already proven by numerous production batches that have been used in man.

The addition of more than one new valency (Y, Z) to a well established combination may arise, for example, when Hepatitis A and Hepatitis B are combined with diphtheria, tetanus and pertussis to form a pentavalent vaccine. In this situation the possible combination of batches may easily become very large (different production scales, expiry dates, etc.). In this case consistency should be shown by three consecutive final bulks as follows:

A1 B1 C1 ...Y1 Z1…
A1 B1 C1…Y2 Z2…
A1 B1 C1 ...Y3 Z3…

This procedure may be extended to any combination of existing and new valencies and does not depend on the presence of any existing and well established valency in the combined vaccine (provided that the consistency of each valency has been shown separately).

For certain products, such as multivalent polysaccharide vaccines, the testing of combinations using fewer than three monovalent lots of each type may be adequate in some cases, since the testing of all possible combinations for separate lots would be prohibitive and also would not contribute significantly to the evaluation of the product.

E.1 Reference Standards
A description of the preparation, characterization, and stability of primary and working reference standards should be provided. A detailed description of the procedures to qualify new lots of reference standards and acceptance criteria for a new reference standard should be included.

E.2 Release Testing
The aim of the release testing of a given vaccine batch is to show that this batch is consistent with and equivalent to the successive batches produced by a given manufacturer and to the batches that have been shown safe and efficacious in clinical trials in man. Among all tests included in the specifications for batch release most have to be carried out on the product in its final container i.e. volume, pH, sterility, endotoxins, preservatives / adjuvants / excipient contents, qualitative and quantitative

identification of the adsorbed antigens, freedom from abnormal/specific toxicity, toxicity reversal and potency or antigen content.

However, on a case by case basis and provided that adequate data have been presented some of the tests may be performed on the final bulk product, e.g.

- the quantification of preservatives and excipients;
- the abnormal toxicity test;
- the in vivo potency testing;
- the pyrogenicity test for vaccine components where it is required;
- toxicity reversal testing; and
- physico-chemical analysis for purity and integrity in cases where it is not required for the final product but for the bulk component. In such cases the additional manufacturing process should be demonstrated to have no effect on the release specifications of the finished product.

The tests for sterility, freedom from abnormal/specific toxicity and identity should be performed according to the European pharmacopoeial monographs or WHO requirements on both the products single components or on the combined product depending on the presentation. Similarly, each vaccine component should meet the purity characteristics according to the European pharmacopoeial monographs or WHO requirements at the appropriate stages of manufacture.

When a combined vaccine is composed of two separate preparations which shall be reconstituted with each other at the time of administration, full approved release testing operations carried out on each of the two preparations separately shall be deemed as sufficient for routine release testing purposes.

Repeating the tests on the reconstituted product, particularly those involving animals such as potency testing, is not required, provided that during development, duly validated studies demonstrating compatibility of the two components following reconstitution have been shown satisfactory by the manufacturer with due consideration of batch consistency, batch size and frequency of production.

These studies will show that components and the final reconstituted vaccine have sufficient comparability of purity and immunogenicity to meet the release specifications.

Any further requirements should be analysed on a case by case basis.

Release (acceptance criteria) testing results and other (for information only) characterization data (e.g., certificates of analysis) for each batch should be submitted.

E.3 General policy of SFDA for independent lot release

The process of protocol review and independent lab testing for lot release of vaccines are carried out according to procedures mentioned in WHO Guidelines titled by "Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities" as well as standard operating procedures established in our national control laboratory.

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

F. Active Substance Specifications and Impurities Profile

F.1 Specifications

This section should contain the specifications and tests for each active substance. These should include assays for identity, purity, potency (biologic effect), physicochemical measurements which predict potency, and where applicable, measures of stability. For highly purified substances, purity in reference to the theoretical composition should be presented. In some cases test results for the stabilized intermediates of component antigens should be included in the final release of the drug product. The results of the validation studies for each of these specifications, including estimates of variability and upper and lower limits, should be provided.

F.2 Impurities Profile

This section should include a discussion of the impurities in the active substance. The identity and quantity of impurities should be provided along with the analytical data (gels, elution profiles, Western blots, etc.) which support the impurities profile. Impurities that should be characterized and quantitated include:

- product related impurities (variants or alterations of antigen occurring during processing or storage)
- Process related impurities:
  - media components;
  - cell substrate proteins or nucleic acids; or
  - process reagents which have not been removed by the purification process (see D., Process Controls).

F.3 Active Substance Stability

This section should contain 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. Reprocessing

This section should include detailed information on any reprocessing that may be done on each active substance. The information provided for each reprocessing procedure should include:

- a description of the conditions or criteria, determined from process controls or specifications, which indicate the need for re-processing;
- a description of the reprocessing step;
- the Standard Operating Procedure for the step;
- a description of any additional or modified in-process controls or specifications which are included to monitor re- processing steps;
- a description of the modifications in batch numbers and documentation of re-processing in the Batch Production Record (BPR); and
- the evidence derived from validation studies which assures that product identity, purity, potency, and stability is preserved for re-processed batches.

H. Container Closure System

A description of the container and closure system, and its compatibility with the active substance should be submitted. The submission should include detailed information concerning the supplier, address, and the results of compatibility, toxicity and biological tests. Alternatively, a Drug Master File (DMF) may be referenced for

this information. If the active substance is intended to be sterile, evidence of container and closure integrity for the duration of the proposed expiry period should be provided.

II. Drug Product

This section should contain information on the final drug product including all active substances and excipients in the final product. If any proprietary preparations or mixtures are used as components, the information provided should include a complete statement of composition and other information that will properly describe and identify these materials. For all ingredients of human or animal origin, testing results or certificates of analysis demonstrating their freedom from adventitious agents should be provided. Appropriate information may be cross-referenced to those under Active Substance.

A. Composition and Characterization

A.1 Composition

A list should be provided of all components in the drug product, including active substance(s) and other ingredients, with their unit doses and batch quantities specified. For some inactive ingredients, the quantity may be expressed as percent or molarity.

A.1.1 Active Substance(s)

A list of each active substance should be provided.

A.1.2 Excipient

This section should contain a list of all inactive components with the rationale for the inclusion of each in the final product. The information provided should include certificates of analysis, results of analytical testing, or other information that will describe or identify each excipient. If compendial excipients are used, citations may be included in lieu of analytical testing. Excipients may include, but not be limited to:

- diluents (molarity and pH should be included);
- bulking agents;
- adsorbents (other than adjuvants); and
- stabilizers (e.g., sugars, wetting agents, etc).

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, the manufacturer should ascertain if the stabilizers will interact to the detriment of the safety, purity or potency of the vaccine.

A.1.3 Adjuvant

Adjuvants are agents that augment specific immune responses to antigens. An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product. As with other ingredients in the final formulation, the adjuvant should be shown to be compatible with all components in the formulation. If appropriate, the manufacturer should demonstrate how much of each component is being adsorbed to the adjuvant. This section should also contain the chemical formula and precise quantity of each adjuvant per unit dose. Whether the quantity of adjuvant is determined by assay or by calculation should be indicated and the method used should be described.

The drug application should describe:

- changes in manufacture concerning adsorption, such as the stage at which the adsorption takes place for a previously licensed component;
- the efficiency and kinetics of simultaneous adsorption (if applicable);
- the efficiency and kinetics of adsorption of components related to changes in the adjuvant, or relative concentrations;
- the assessment of post-formulation adsorption of components not previously present as adsorbed; and
- the effect of the adjuvant on the ability to assay components that were not previously adsorbed; immunologic identity tests or pyrogenicity tests should also be addressed.

Manufacturers should consider whether the following have an impact on the safety, purity and potency of the new combination in comparison to that of the monovalents:

- whether a non-adsorbed component becomes adsorbed;
- whether de-adsorption of the adsorbed component occurs;
- for a previously licensed vaccine, changing the stage of manufacture at which adsorption occurs;
- chemical forms (e.g., aluminum hydroxide and aluminum phosphate) of the adjuvant and buffers which are different from prior manufacturing;
- the effects of mixing different adjuvants; and

- how time affects the adsorption of antigen(s) to the adjuvant. (Demonstration that no significant desorption takes place in the course of the shelf-life of the product, where relevant; and demonstration that the adjuvant is non-toxic).

A.1.4 Preservative

Each preservative should be identified by chemical as well as any trade name or reference European and U.S. pharmacopoeias.

Antimicrobial preservatives should not be included in the finished product unless their use is justified by quality and/or safety considerations. Their use is never acceptable in live vaccines, but may be justified in the case of inactivated vaccines which:

- are present in multi-dose containers; and/or
- are presented as suspensions so that sterilization/filtration is impossible.

In all cases where products are proposed to contain antimicrobial preservatives:

- a benefit risk analysis should be presented in the application dossier; any potential toxicity and/or potential allergenicity should be addressed in this presentation;
- the concentration of the antimicrobial preservatives should be controlled in the bulk and in the finished product specifications in accordance with the European and U.S. pharmacopoeial limits;
- the efficacy of preservation should be tested according to the European and U.S. pharmacopoeial requirements for human vaccines;
- the maintenance of preservative concentration or efficacy throughout the period of validity should be demonstrated; and
- the name and concentration of the antimicrobial preservatives should be stated on the labeling.

In selecting a preservative system the applicant should consider:

- the effectiveness against potential microbial contaminants;
- possible interaction with the formulation or container (for example, thiomersal may be ineffective in the presence of sera, and can 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 considered on the basis of side effects or for other reasons, a risk/benefit evaluation should be made, taking into consideration that such a change implies a new formulation with on a case by case basis the need for additional studies for sterility, potency, stability and their clinical implications.

A.2 Compatibility of Components

Experience has shown that combining monovalent vaccines may result in a new combination which is less safe or effective than desirable. Sometimes the components of inactivated vaccines may act adversely on one or more of the active components. One such instance occurred when the combining of whole cell pertussis vaccine and inactivated poliovirus vaccine (IPV) resulted in a vaccine with a decreased pertussis potency.

Additionally, immunological interference between vaccine viruses or virus subtypes has been observed when live vaccines are combined. Consequently, the combined components stimulated weaker immune responses than did viruses administered separately. Component crossreactivity could also occur with a combination of live vaccines where recombinational events may allow attenuated organisms to be reconstituted to virulent forms.

Therefore, it is of outmost importance to validate the compatibility of the combined components before any clinical trials begin. It is advised that the product be characterized and the integrity of the components be assessed by performing a battery of physicochemical, biochemical and biological assays.

To further demonstrate the compatibility of the components, it is recommended that preclinical studies, in an appropriate animal model, be conducted to determine the consequences of combinations on potency and immunogenicity. The manufacturer should consider that the components of the product may revert to toxicity or virulence and should quantify any such tendency both with the monovalent and the combined vaccines. Similarly, the physical characteristics, including resuspension and the suitability of the container and closure for the combination product should be assessed.

If the combination of component vaccines results in a volume too large to be safely administered, the manufacturer may investigate dose-reduction of some or all components. For instance, the manufacturer may restore an optimum final volume by utilizing concentrated intermediate bulks to achieve final concentrations equal to the monovalent component vaccines. The effects of such formulation changes should be evaluated preclinically.

A.3 Specifications and Analytical Methods for Drug Product Ingredients

This section should contain a description of tests and specifications for all ingredients, if not specified in the active substance section.

A.3.1 Description

A qualitative statement describing the physical state (lyophilized solid, powder, liquid) and color and clarity of the drug product and other ingredients should be provided.

A.3.2 Identity

An identity test shall be performed on labeled final containers of vaccines. An identity test should be performed for each monovalent as well as for active component present in the combination unless it can be shown that this is not necessary. The purpose of the test is to identify the product as the one designated in the product labeling and to distinguish it from any other product being processed in the same laboratory. Identity tests should focus on those differences that exist between different final container products to assure mislabeling has not occurred.

The assays used to establish the identity of the drug product should be described. The description of each assay should include an evaluation of its specificity and sensitivity.

A.3.3 Purity and Impurities

This section should include information on the purity of the final product including identification and quantitation of impurities, including degradation products, inherent in the final dosage form. If impurities are known to be introduced or formed during the production of the drug product, the acceptable limits of these impurities should be determined and included in the specifications.

A.3.4 Potency

Potency is the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through administration of the product in the manner intended, to affect a given result.

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

For a combination product, the potency of each component for which a claim of efficacy is made should be determined. 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. Testing of the final formulated bulk vaccine may be substituted for testing in the final container when additional processing has been shown to have no effect on the potency of the final product. However, in some cases such as a lyophilized product, demonstration of the potency of the product in the final container is necessary. Tests for potency should detect any component interactions that may have a potentiating or interfering effect on any other component.

A.3.5 Other tests

A.3.5.1 Testing for the Presence of Residual Cells

The final vaccine product should be tested for the presence of residual cells. Processes, such as filtration, should be implemented and validated to ensure that intact cells are not present in the final product. Validation that residual cell removal processes are robust is important for immortalized cells. Determining the extent to which intact cells are cleared by these processes is an important part of this validation.

A.3.5.2 Testing for Residual Cellular DNA

Residual DNA might be a risk to the final product because of oncogenic and/or infectivity potential. There are several potential mechanisms by which residual DNA could be oncogenic, including the integration and expression of encoded oncogenes or insertional mutagenesis following DNA integration. Residual DNA also might be capable of transmitting viral infections if retroviral proviruses, integrated copies of DNA viruses, or extrachromosomal genomes are present.

The risks of oncogenicity and infectivity of the cell-substrate DNA can be lessened by decreasing its biological activity. This can be accomplished by decreasing the amount of residual DNA and reducing the size of the DNA (e.g., by DNAse treatment or other methods) to below the size of a functional gene.

Chemical inactivation can decrease both the size and biological activity of DNA. If DNA removal, digestion, or inactivation is undertaken, these methods should be validated.

The amount and size distribution of residual DNA in the final product should be measured. For widely used human diploid cell strains, such as MRC-5 and WI-38 cells, measurement of residual DNA might be unnecessary because it is not considered residual DNA from these human diploid cells to be a safety issue. Limitation of the amount of residual DNA might be required, depending on the potential risks associated with that DNA, for human diploid or primary cell types for which there is less experience. Residual DNA should be limited for continuous non-tumorigenic cells, such as low-passage Vero cells, to less than 10 ng/dose for parenteral inoculation as recommended by WHO. When using cells with tumorigenic phenotypes or other characteristics that give rise to special concerns, more stringent limitation of residual DNA quantities might be needed to assure product safety.

B. Manufacturer and Facilities

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

C. Manufacturing Methods

This section should include a detailed description of the manufacturing process flow of the formulated bulk and finished drug product including the sterilization operations, aseptic processing procedures, lyophilization, and packaging. Accompanying this narrative, a flow chart should be provided that indicates the production step, the equipment and materials used, the room or area where the operation is performed (may reference the simple floor diagram) and a listing of the in- process controls and tests performed on the product at each step. A Master Production Record (MPR) for the drug product should be provided, including complete manufacturing instructions for adsorption (if applicable), formulation, filling, labeling, and packaging. References may be made to other sections for more detailed information. Results of studies validating the compatibility of the

components including the adjuvants and/or preservatives, if applicable, should be provided. Lot-to-lot consistency of the drug product should be demonstrated.

D. Drug Product Specifications

D.1 Sampling Procedures
The sampling procedures for monitoring a batch of finished drug product should be included.

D.2 Specifications and Methods
A description of all test methods selected to assure the identity, purity, strength and/or potency, as well as the lot-to- lot consistency of the finished product and the specifications used for the drug product should be submitted. Certificates of analysis and analytical results for at least three consecutive batches should be provided.

D.3 Validation Results
The results of studies validating the specificity, sensitivity, and variability of each method used for release testing should be provided. Where applicable this should include descriptions of reference standards and their validation. For analytical methods in the European and U.S. pharmacopoeias sources, the appropriate citations should be provided.

E. Container Closure System

A description of the container and closure system, and its compatibility with the drug product should be submitted. Detailed information concerning the supplier(s), address(es), and the results of compatibility, toxicity and biological tests should be included. Alternatively, a Drug Master File (DMF) can be referenced for this information. For sterile product, evidence of container and closure integrity should be provided for the duration of the proposed expiry period.

F. Sterility

The final product shall meet the sterility requirements outlined in European and U.S. pharmacopoeial test methods. Sterility test methods should be described. A description and documentation of the validation studies for any processes used for sterilization, effectiveness of preservatives should be provided.

Antibiotics should not normally be used during vaccine production to ensure bacterial sterility or to reduce bioburdens. In the case of any vaccine in which it is proposed to include antibiotics 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

The bacterial endotoxins test used for the product should be described. The description should include qualification of the laboratory, inhibition and enhancement testing and results, determination of non-inhibitory concentration and maximum valid dilution.

H. Lyophilization

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

This section should state the proposed expiration dating period for the drug product and the recommended storage conditions. The criteria for determining the date of manufacture, from which the expiration dating period begins, should be defined. For lyophilized products, a shelf-life after reconstitution should be proposed.

Stability testing requires that primary data to support a requested storage period for either active substance (bulk material) or drug product (final container product) should be based on long-term, real-time, real-condition stability studies. Accelerated stability testing data may be used as supporting data but cannot be used to assign the expiry date. In the case of combined vaccines there are additional points to be considered: stability data are requested for each individual component before

combination, after combination into the bulk product, and for the combined vaccine as finished product; stability data on at least three batches of each of these three manufacturing steps of the combined vaccine should be provided for the requested dating period. The period of validity for a given batch begins on the day on which the assay is started. Where there is no assay for a given vaccine, some other date should be specified in the marketing application, for example, the date of an approved stability-indicating test, the date of freeze-drying or the date of filling. The maximum length of the storage period should always be based upon the expiry date of the least stable component.

I.1 Stability Protocol

A stability study protocol should be provided which includes, but is not limited to, testing for:

- potency;
- physicochemical measurements which are potency-indicating;
- moisture, if lyophilized;
- pH, if appropriate;
- sterility or control of bioburden;
- viability of cells, if frozen and thawed;
- pyrogenicity; and
- general safety.

I.2 Stability Data

The summary results which support the proposed expiration dating period, under recommended conditions, in the final container and closure system, should be provided. The stability of each dosage form should be separately documented. For lyophilized products the data supporting the shelf-life of the product following reconstitution should be included. If the drug product is frozen, data supporting the stability of the product through a stated number of freeze-thaw cycles should be provided.

I.3 Stability Program

A plan for an on-going stability program should be provided. This should include the protocol to be used, number of final lots to be entered into the stability protocol each year and how such lots will be selected.

Generally, the stability testing of vaccines should be complied with all requirements mentioned in WHO Guidelines titled by "Draft Guidelines on Stability Evaluation of Vaccines".

I. Labeling requirements

The label states:
- the name of the preparation,
- a reference identifying the final lot,
- the recommended human dose and route of administration,
- the storage conditions,
- the expiry date,
- the name and amount of any antimicrobial preservative,
- the name of any antibiotic, adjuvant, flavour or stabiliser present in the vaccine,
- the name of any constituent that may cause adverse reactions and any contra-indications to the use of the vaccine,

❖ for freeze-dried vaccines:
  - the name or composition and the volume of the reconstituting liquid to be added,
  - the time within which the vaccine is to be used after reconstitution.

❖ In addition to above requirements Specific labeling requirements for each vaccine mentioned under individual monograph should be followed.

Part 2 - Preclinical Testing of Vaccines

It is recognised that suitable animal models are not always available and responses in such models are not always predictive of human responses. Therefore, selection of animal species should be made on a case by case basis.

Potential safety concerns associated with vaccines include general systemic toxicity, (paradoxical) enhancement of the intended disease, induction of local toxicity, pyrogenicity, adverse immunologic effects such as autoimmunity or sensitization, and in some cases teratogenic/reproductive effects.

Historically, serious neurological events have been associated with the use of some vaccines. The availability of animal models to address these issues should be considered in the development of a new vaccine.

For new vaccine products, preclinical safety tests should always be part of the testing programme, even though it is recognized that full testing may not be necessary to the extent requested for conventional medicinal products. However, in the case of combined vaccines containing known antigens, preclinical toxicity testing may not always be necessary. Immunogenicity testing is still recommended (see D.1).

The route of administration should be as close as possible to the proposed clinical route. If this is for practical reasons not possible, another route of administration may be acceptable but this should be justified. Studies to address specific safety concerns (e.g. neurovirulence, testing for complete detoxification of toxins) will often need other routes of administration. It should be considered if methods described in relevant pharmacopoeial monographs are applicable.

Attention should be paid to additives including adjuvants, preservatives, and excipients (see H).

When a major change in the manufacture of a vaccine is being made, the need for preclinical testing should be reconsidered.

When preparing the preclinical documentation, the following issues have to be taken into account:

A. Toxicity

A.1 Single dose toxicity

Single dose toxicity data from at least one animal species should be performed with a dose providing an adequate safety margin in relation to the human dose. However, if toxic findings are seen in this study, the dose response relationship should be further characterized. These data may be part of animal immunogenicity studies (see D.1) or of safety pharmacology studies (see D.2), provided that histopathology of important organs is included.

A.2 Repeated dose toxicity

A study on repeated dose toxicity in one animal species is normally requested for vaccines that will require multiple doses in the clinical setting. Even in cases where only single doses will be administered in clinical use, a repeated dose toxicity study may nevertheless be appropriate. The selection of an appropriate animal species should be carefully evaluated on a case by case basis.

The route and dosing regimen should reflect the intended clinical use. Its design should take into account potential differences in response time between animals and humans (e.g. repeated doses at monthly intervals in humans may not give the same response as repeated doses at monthly intervals in animals).

Valuable information can be obtained by expanding multiple-dose immunogenicity studies to include measurements normally conducted in preclinical toxicity studies (i.e. body weight, food consumption, clinical pathology, gross necropsy and histopathology). The value of the protocol would normally be enhanced by concurrent measurement of the antibody response to all important components of the vaccine (immunogenicity studies).

Incorporation of safety pharmacology endpoints in the design of these studies should be considered (see also D.2).

The applicant should consider the following points on a case by case basis:
- Where appropriate, specific consideration should be given to immunological aspects of toxicity, such as production of complexes with host immunoglobulins (e.g. antibody-dependent enhancement of disease) or release of immunofunctional molecules, (e.g. cytokines) affecting functions of the immune system.

- Hypersensitivity reactions, induced by the antigen itself, by antigens (toxins) modified in new ways (new detoxification procedure, by antigen-carrier complex or presence of minute amounts of impurities) or by additives (adjuvants/excipients/preservatives) may be increased (especially for vaccines proposed to be injected more than once).
- In some rare cases, antigenic substances can induce antibodies that can cross-react with human tissue resulting in possible adverse effects and the availability of an animal model to address these issues should be considered.

B. Examination of reproductive function

Data on reproductive function (fertility) are usually not necessary. Histopathology in toxicity studies may provide sufficient information concerning the integrity of reproductive organs.

C. Embryo/foetal and perinatal toxicity

In most cases, vaccination of humans occurs during childhood. Therefore, embryo/foetal and perinatal toxicity studies are usually not necessary. Only if a vaccine is intended for use in women of child bearing age or during pregnancy may such studies become necessary.

Some existing vaccines, although safe for use in women who are not pregnant, may cause foetal infection resulting in malformations or abortions in women who are pregnant. Documentation on clinical and/or epidemiological data on exposure to the infectious agent or related vaccines during pregnancy should be provided, and may be sufficient to evaluate the risk. In other cases, the availability of appropriate animal models should be considered.

D. Pharmacodynamics

D.1 Primary pharmacodynamics (Immunogenicity and protection)

Primary pharmacodynamic studies with respect to the 'antigen-protective response' should be carried out in a relevant species. The endpoint in these kind of studies should preferably be the protection against a challenge from the pathogenic organism where there is an animal model reflecting the infections in humans. Therefore, quantification of the immunological response only, is in most cases not a sufficient indication of protection.

Studies that evaluate immune function should involve the evaluation of expected immunogenicity (level of antibody production, class and subclass of the antibody produced, cell-mediated immunity, and duration of immune response). In addition, the formation of neutralising antibodies, immune complex formation, interactions with immune cells to cause dysfunction, and release of other molecules that affect the immune system should also be investigated. It is

preferable to study new combined vaccines in comparison with the individual antigens in animals to determine if any augmentation or diminution of response occurs.

Interactions between a particular vaccine and other vaccines may result in reciprocal antagonism. This is seen in certain cases following co-administration of two or more vaccines e.g. between cholera and yellow fever vaccines, and between measles vaccine and meningococcal A&C vaccine.

The study should be conducted with statistically and scientifically valid procedures for verifying the results and these should be described. It is advisable to perform such studies early in the product development cycle.

D.2 Secondary Pharmacodynamics (Safety pharmacology)

The potential for undesirable pharmacological activities e.g. on the circulatory and respiratory systems should be considered for new vaccines and investigated in appropriate animal models. Where necessary, particular monitoring of these activities might be incorporated in the design of toxicity and/or clinical studies. Effects on CNS parameters as well as on those organs associated with wild type organism pathology may also be included.

Repeated dose studies bearing in mind the proposed administration schedules (see A.2) may reveal significant effects better than a single administration.

E. Pharmacokinetics

Pharmacokinetic studies (e.g. determining serum concentrations of antigens) are normally not needed. The need for specific studies should be considered on a case by case basis and may include considerations such as local deposition studies which would assess the retention at the site of injection and its further distribution; histopathological studies of the draining lymph nodes (near the injection site) which might illustrate depot characteristics of the vaccine; and viral shedding of live vaccines. Distribution studies should be considered in case of new formulations, novel adjuvants or when alternative routes of administration are intended to be used (e.g. oral or intranasal).

F. Local tolerance

As vaccines will in most cases be administered intramuscularly, subcutaneously or intracutaneously, local tolerance should be evaluated. Ideally, the formulation intended for clinical use should be tested. In some cases, the potential local effects of the product can be

evaluated in single or repeated dose toxicity studies thus obviating the need for separate local tolerance studies.

G. Other aspects

Vaccines (either single or combined) may contain substances causing pyrogenic effects, e.g. lipopolysaccharides, endotoxins and other cellular components like glycoproteins, teichoic acid. Therefore, generally pyrogenicity or endotoxin tests should be performed for each product on a batch-to-batch basis to detect these potential contaminations or to confirm that levels are acceptable.

H. Additives (Adjuvants/Excipients/Preservatives)

In formulated vaccines, different additives such as preservatives (substances used in parental medicinal products), excipients (inactive components including stabilizers) and/or adjuvants (substances aimed at increasing the immunogenic response) can be added to the immunogenic entity. If use of a preservative is appropriate, the safety of the preservative has to be documented and discussed. When a new preservative is to be used, documentation should be provided to support the safety and it should be treated as a new pharmaceutical excipient. Safety of new additives can be appraised by using 'mock' vaccines (i.e. total vaccine formulation without antigen and having followed established production processes).

If a given additive has not caused important systemic or local reactions in an existing vaccine, this does not exclude the possibility that the same additive may cause serious side effects when used with other antigens.

Vaccines may be adsorbed to different compounds as described in the European pharmacopoeia. Although local reactions occur with vaccines containing adsorbents, the safety of vaccines containing adsorbents has in general been demonstrated with extensive preclinical and clinical use. Several adjuvants that are not currently components of licensed vaccines have recently been investigated in preclinical studies with the goal of developing more effective immune stimulants. In this case, appropriate preclinical studies should be developed on a case by case basis. The following points should be considered:

- Potential safety concerns for investigational adjuvants include injection site reactions (e.g., pain, induration, erythema, granuloma formation, sterile abscess formation), fever, other systemic adverse effects (e.g., nausea, malaise, headache), immune mediated events (e.g.

anaphylaxis, uveitis, or arthritis), systemic chemical toxicity to tissues or organs, teratogenicity, and carcinogenicity;

- In addition, preclinical studies to evaluate the safety profile of the additive/combined vaccine should be performed. It is recommended that the intended additive/antigen combination and the intended clinical route of administration be used where possible and to compare with adjuvant alone or a vaccine formulation without adjuvant;

- If there are limited or no toxicology data for the additive being considered for inclusion in a new combined vaccine, it is advisable to perform toxicity studies on the additive alone; and

- It is recommended that these preclinical studies are also designed to evaluate the adjuvant effects on the immune response when a relevant animal model is available. These studies should optimally utilize the exact adjuvant/antigen combination planned for human use, and include a control group that receives the antigen(s) alone or the antigen adjuvanted with an aluminum compound to provide evidence that the investigational adjuvant augments the immune response to the antigen(s).

In addition to the above information, all requirements mentioned in WHO Guidelines titled by "Guidelines for nonclinical evaluation of vaccines" should be recommended.

Part 3 - Clinical Evaluation of Vaccines

This section will discuss the design and statistical considerations for clinical studies to demonstrate the safety, immunogenicity and efficacy of vaccines. Generally, such studies should:

1) be randomized and controlled, and
2) demonstrate the safety and immunogenicity, which include comparisons of the separate but simultaneously administered individual vaccines with the combination.

Each new product should be supported by product specific data. Additional data obtained in uncontrolled studies may provide useful supplemental information.

A. Safety Studies

This section provides guidance on the essential data on vaccine safety needed to support a marketing authorization for a new vaccine. A brief overview of the special considerations for vaccine safety surveillance is also given.

A.1 Safety evaluation in pre-authorization studies

Pre-authorization studies are usually primarily designed to provide data on the immunogenicity and/or protective efficacy of a vaccine. However, it may be necessary to conduct pre-authorization studies that are primarily designed to address specific safety issues that may have been identified during preclinical testing or in the early clinical studies.

A.1.1 Extent of the database

As a minimum, the total data from pre-authorization studies should be sufficient to reliably determine the nature and frequency of local and systemic adverse events occurring at a frequency >1/1000. If the marketing authorization is based solely on immunogenicity studies, it is unlikely that the database would be sufficiently large to identify rare events. However, this may be possible if a large study of protective efficacy is performed.

Any cases of rare and/or unusual adverse events that are observed in pre-authorization studies trials should be subjected to a thorough causality assessment, taking into account biological plausibility. Depending on the nature of these events and their possible relatedness to vaccination, it may be necessary to expand the safety database in order to better evaluate a putative safety signal before initial authorization and/or to incorporate a prospective post-authorization evaluation of such events in the Pharmacovigilance Plan.

Studies might also indicate that the safety profile may be very different in various subsets of the target population or may be very different between doses (e.g. much higher rates of adverse events after boosting compared to the primary series). In these cases, it may be necessary to obtain sufficient data to detect at least uncommon adverse events in various subsets before a marketing authorization could be granted.

A.1.2 Methodological considerations

In each study performed during a clinical development programme, whatever the primary objective, every effort should be made to record safety information at appropriate protocol pre-specified intervals and for a sufficient period of time after each dose of vaccine in all vaccines. However, if a large study of protective efficacy is to be performed, it may sometimes be acceptable that all adverse events are actively collected from only a defined subset of vaccines. The size of this subset requires careful justification. Also, the nature of the subset should be appropriate to support extrapolation to the total target population. In such cases, all serious adverse events must still be collected on the entire study population.

Protocols should clearly define the method for collecting data on adverse events (e.g. diary cards, questionnaires), who will fill out the forms (e.g. investigators, nurses, vaccines, or parents/guardians), duration of follow-up and intervals for collecting safety data. Since most adverse reactions to vaccines occur within the first few days after each dose, it is common and generally acceptable practice that special attention is paid to collecting information on any adverse event that occurs within approximately 5-7 days (perhaps longer for live vaccines), whereas later events are elicited by telephone contact or when vaccines attend for the next dose.

Data collection should be sufficiently detailed so that, for example, any differences in adverse events according to the site and/or route of injection (e.g. intramuscular versus subcutaneous) could be assessed. Documentation of the batch number of the candidate and any co-administered licensed vaccines is essential and it may sometimes be appropriate to examine adverse events according to batch.

In order to facilitate and standardize the collection of all possibly relevant data in the immediate post- vaccination period, patient diaries may be very useful. These should usually provide a check list of local (e.g. injection site redness, swelling and induration) and systemic events (e.g. fever, abnormal crying or irritability in infants) that have the potential to be due to the vaccine and should determine when these should be recorded. To assist the use of diary cards, provision of graded rulers may help parents/guardians and vaccines to gauge the size of local redness or swelling. Digital reading thermometers make it easier to measure temperature by the chosen route and there should be clear guidance given on the prophylactic and/or therapeutic use of antipyretics. However, if patient diaries are employed, it is important that vaccines or their care-givers appreciate that all other adverse events regardless of perception of relatedness to vaccination should be reported. There should be an appropriate mechanism in place to collect this information, including attention to timing so that serious adverse events can be picked up and reported in accordance with regulatory requirements.

Clear guidance should be provided to investigators regarding the assessment of all adverse events according to causality, seriousness, expectedness and severity. For each serious adverse event that occurs, information is required on medical history (including any underlying diseases), concomitant medications and/or vaccinations, the course of the event, any interventions required, the outcome and the investigator's and sponsor's assessment of causality. Analysis of the possible vaccine-relatedness of the adverse event should use standardized categories for causality assignment. In addition, adverse events following immunization should also be categorized according to whether they are:

- due to intrinsic characteristics of the vaccine preparation and/or the individual response;
- vaccine precipitated i.e. triggered due to the receipt of the vaccine but probably would have occurred at a later time; and
- due to administrative and other errors, including GMP errors, dosing errors; and
- co-incidental i.e. temporally related but not due to immunization.

Applicants should always give consideration to the need to institute a Data Safety Monitoring Board during the clinical development programme. This may be particularly important if the candidate vaccine is of a very novel type and/or is to be given to a large population in pre-authorization studies.

A.2 Post Marketing Surveillance

In the post-marketing period a much larger and likely much more diverse population will be exposed to the vaccine compared to clinical studies. The general considerations for pharmacovigilance and for development of a pharmacovigilance plan are the same as for all other types of medicinal products. However, vaccines are almost always administered to healthy persons. This fact has implications for the continued re-assessment of the overall risk-benefit relationship for the vaccine.

B. Immunogenicity

This section provides guidance regarding the essential data on immunogenicity that should be assembled during the clinical development programme to support a marketing authorization regardless of whether or not studies of protective efficacy will be feasible or necessary (see section C). Further guidance on some specific types of immunogenicity studies that might be performed is given in section D.

B.1. General methodological considerations

Immunogenicity data are usually generated in all phases of a clinical development programme. If an appropriate animal disease model is available, primary pharmacodynamic studies to evaluate immunogenicity (and protection) of a new vaccine should be undertaken to indicate the doses, schedules and route(s) of administration to be evaluated in clinical studies.

Early clinical studies should provide sufficient information on the safety and immunogenicity of the antigenic components in a candidate vaccine in the target population to identify the optimal dose and primary immunization schedule to be evaluated in subsequent confirmatory studies of safety and immunogenicity and, where feasible and necessary, protective efficacy. If studies of

protective efficacy are performed, the immunological response should be characterized in a subset of the vaccinated population and the data should be used to attempt to identify an immunological correlate with protection if none is already established (see sections B.2.3 and section C.)

B.2. Characterization of the immune response
B.2.1 Minimum requirements for immunological testing

Biological specimens (e.g. serum, cellular fractions, mucus) should be collected from all participants at appropriate and pre-defined intervals throughout each study for the assessment of the immune response. The rationale for the timing of samples should be provided in the protocol and should take into account any data available on the kinetic of the immune response.

Protocols should specify and give details of the methodologies to be used to evaluate immune responses to vaccination. These should be consistent across studies, externally validated (including the use of international standards such as those of WHO if available) and demonstrated to be reproducible. If changes to methodologies are unavoidable during the clinical development programme, adequate cross-validation data should be provided.

Information should be provided on the quality and quantity of the immune response (humoral and cell-mediated) according to the known or presumed properties of each antigen in the candidate vaccine. Whenever feasible, immune responses to vaccination should be compared to those seen as a result of natural infection.

For antigens for which a widely accepted immunological correlate of protection already exists (e.g. diphtheria and tetanus toxoids and hepatitis B surface antigen), evaluation of the immune response to these antigens in a candidate vaccine may be limited to the usual parameters used to assess immunogenicity (and, thus, predict protective efficacy). For well known antigens for which no immunological correlate of protection exists (e.g. pertussis toxin), evaluation of the immune response should at least employ a comparison with results obtained with other vaccines containing the same or similar antigens.

For novel antigens, characterization of the humoral immune response should include:

- Determination of the amount, class, sub-class and function (e.g. neutralizing, bactericidal or opsonising ability) of specific antibody that is elicited by each antigen;

- Exploration of the relationship between functional (e.g. measured in neutralization assays) and non-functional antibody assays (e.g. measured in enzyme-linked immuno-assays);
- Description of the kinetic of the immune response such as the lag-time for onset, antibody persistence, seroconversion rate (which should be adequately defined) and induction of immune memory;
- Depending on the delivery route, monitoring of certain components of the immune response might be indicated, such as antigen specific secretory IgA responses after mucosal administration; and
- Assessment of the quality of the antibody response, which may include parameters such as specificity and/or epitope recognition and avidity. Changes in these parameters over time and/or with subsequent doses should be evaluated.
- Evaluation of the potential for formation of cross-reactive antibodies or immune complexes.
- Exploration of immunological factors that might affect the humoral immune response, such as pre-existing antibodies (including maternal antibodies).

An assessment of the cell-mediated immunity (CMI) component of the immune response to each novel antigen is considered to be important and, for some types of antigen, would be essential. It is recommended that studies should monitor quantity and quality of T-cell responses (for example antigen specific T-cell frequencies with methods of verifiable validity, Th1, Th2, T regulator cells, memory T cells and relevant cytokines). The range of tests performed, with an explanation of the rationale for each investigation, should be justified in the application dossier.

B.2.2 Immunogenicity in various types of possible recipients for the vaccine

Potential effects on the vaccine immune response of various host factors (e.g. age, prematurity, maternal antibody, nutritional status, genetics, coexisting disease, immunosuppression, and prior exposure to an infectious agent) should be considered. Extrapolation of data from one population to another requires scientific justification that may not be possible without provision of specific data. For some types of vaccine it may be acceptable that some of these issues are explored after initial authorization. However, if the vaccine has potential to be useful in specific populations (e.g. the immunosuppressed) studies should be performed as early as possible in the clinical development programme.

B.2.3 Immunological correlates of protection

At present, widely accepted immunological correlates of protection exist for certain antigens only and consist of defined humoral antibody responses above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.

When there is no established immunological correlate for protection, every effort should be made to describe the correlation between the immune response to an antigen and the protective efficacy of the vaccine. Ultimately, it is desirable that one or more immunological correlate(s) of protection should be defined for short and long-term protection. In most cases it is anticipated that the immunological correlate will be based on measurement of functional antibody but a defined level of non-functional antibody (e.g. measured by enzyme-linked immunoassay) may be acceptable if the relationship with functional antibody is well described.

Ideally, confirmation of an immunological correlate for protection (at least in the short-term) should be based on exploration of immune responses in at least a subset of vaccines during clinical studies of protective efficacy. The protocols for protective efficacy studies should also pre-define when and how, in case of vaccine failure, the immunological evaluation of the patient and typing of the infecting micro-organism is performed (see section C).

However, efficacy studies will not always be feasible. For some antigens, a possible alternative may be to use estimates of effectiveness from prospective studies conducted during vaccination campaigns after authorization in order to establish at least putative correlates for short and/or long-term protection (see section C.4).

Established animal challenge models for infection could be used to support a putative immunological correlate for protection in man. Human challenge studies may also provide valuable information. However, such studies are appropriate only for selected diseases that have no serious complications or long-term sequelae and for which successful treatment is available. If applicable, data on the use of passive immunization may also assist in identifying threshold antibody levels for protection.

Although it would be expected, and in some cases has been demonstrated, that specific types of antigens elicit cellular immune responses, these have not been unequivocally correlated with protection against infection or disease progression. When it is expected that CMI constitutes an important or even essential component of the overall immune response to an antigen, clinical studies to evaluate some type of cell-mediated immune correlates are encouraged.

B.2.4 Clinically important differences in immune responses

In the pre-authorization period comparative immunogenicity studies are commonly performed to explore immune responses:

- to antigen(s) in a candidate vaccine *vs.* similar antigen(s) in licensed comparator(s);
- to antigens in a candidate vaccine when administered to different populations (e.g. age groups, ethnic groups, previous immunisation histories) or at different doses or schedules;
- to antigens when given separately *vs.* administration as components of a candidate combined vaccine;
- to antigens in a candidate vaccine when given alone or concomitantly with other vaccine(s); and
- to antigens in different formulations (including different antigen or adjuvant doses) or lots of a candidate vaccine.

In the post-authorization period, such studies may be used to support extensions of indications, modifications of dose schedules, changes to vaccine formulation and other modifications of the initial marketing authorization.

In most of the examples above, the primary aim of the study will be to demonstrate non-inferiority between treatment groups with respect to immune responses to each antigen of interest. However, in some cases (e.g. comparisons of formulations with and without an adjuvant) the aim will be to demonstrate superiority of the immune response to at least one antigen in the formulation. In both cases, criteria need to be established and laid out in the study protocol for the judgment of non-inferiority or superiority of immune responses to each antigen of interest.

The usual difficulty encountered in such studies is the selection of the most important primary criterion and the definition of what might constitute a clinically meaningful difference in immune responses to an antigen (whether the aim is to demonstrate non-inferiority or superiority) between vaccine groups. If there are established immunological correlates of protection relevant to one or more antigens in a vaccine, the primary focus should usually be on comparisons between seroprotection rates. If there is no established immunological correlate for protection with respect to an antigen, failure to achieve a certain seroconversion rate may be more important than differences between geometric mean antibody concentrations/titers (GMCs/GMTs).

Based on the criteria that are proposed with regard to clinically meaningful differences, the sample size should provide sufficient power to rule out and/or demonstrate such differences in one or more of seroconversion rates, seroprotection rates and GMCs/GMTs.

B.2.5 Analysis and presentation of immunological data

The immunological data obtained from each study should be presented in detail and using a standard approach in each study report. As a minimum:

- The percentage of "responders" should be presented. When there is an established immunological correlate of protection, "responders" should be defined as those vaccines that develop an immune response above a defined threshold level. Otherwise, "responders" might be defined as those reaching a certain minimum increment in antibody concentration/titer post-vaccination;
- "Non-responders" should be carefully characterised in order to attempt to provide specific recommendations (e.g. re-vaccination) for these individuals;
- GMCs/GMTs (with 95% confidence intervals) and pre-/post-vaccination ratios should be calculated;
- Reverse cumulative distribution curves should be provided; and
- When available, data on antigen specific T-cell responses including CD4+ T-cells and CD8+ cytotoxic T-lymphocytes (CTLs) and relevant cytokines should be presented.

It is important that protocols should select and justify the choice of the primary and secondary endpoints. All anticipated analyses should be described, including purely descriptive analyses. Any post-hoc analyses that might be performed require adequate justification.

Depending on the aim of the study, a *per protocol* (evaluable) population (e.g. defined as subjects completing vaccination with complete serological data and no major protocol violations) or a well defined intent to treat (ITT) population (e.g. as above but including those with protocol violations) may be chosen for the primary analysis. However, applicants should always provide analyses for both populations and any others (such as modified ITT) that may be defined in the protocol. Depending on the nature of the study population, it may be very important to plan for analyses in subsets according to factors such as age, ethnicity and pre-existing antibody status.

C. Efficacy

This section considers the design of pre-authorization studies that have the primary aim of evaluating the protective efficacy of a vaccine.

This section should be read in conjunction with:

- Sections B and D: These discuss the extent of the data to be provided when efficacy may be predicted or has to be otherwise inferred from information on immune responses to vaccination;
- Section B.2.3: This discusses the collection of serological data from subsets of vaccines during studies of protective efficacy in order to establish an immunological correlate of protection;
- Section D.6: This addresses the data that might form the basis for authorization when a study of protective efficacy cannot be performed and there are no known criteria on which to predict efficacy from data on immune responses; and
- Section A. This discusses the surveillance of vaccine failures during routine use and the monitoring for the possibility of strain replacement (i.e. emergence of types of an organisms not covered by the vaccine as important causative pathogens). Such information should appear in periodic safety update reports (PSURs) but may also be the subject of a more urgent report if a problem becomes apparent.

C.1 General Considerations

If a protective efficacy study is performed, the choice of study location(s) should be adequately justified.

Pre-authorization studies of protective efficacy are not always necessary or feasible, as in the following situations:

- A study of protective efficacy is not necessary if the applicant can justify the use of immunological data to predict protection against infection. For example, when there is a well established immunological correlate for protection against a specific infection (e.g. diphtheria, tetanus) the candidate vaccine should elicit satisfactory responses based on the relevant correlate(s);
- Estimating protective efficacy is not feasible if the potentially preventable infectious disease does not occur (e.g. smallpox) or occurs at too low a rate for a study to be performed in a reasonable period of time (e.g. brucellosis, Q fever). Also, such studies may not be feasible if the disease tends to occur in unpredictable and short-lived outbreaks that would not allow for an assessment of vaccine efficacy (e.g. some viral haemorrhagic fevers);
- If there is no immunological correlate of protection and it is not feasible to perform a study of protective efficacy, it may sometimes be justifiable to gauge the likely efficacy of a vaccine by comparison of immunological responses with those seen

in past studies of protective efficacy with similar vaccines (e.g. acellular pertussis vaccines); and

- There will be instances in which an efficacy study is not feasible and there is no established immunological correlate of protection or previous efficacy studies that might provide immunological data for comparison (e.g. anthrax).

The applicant should always provide a sound justification for the lack of data on protective efficacy in an application dossier.

C.2 Endpoints in studies of protective efficacy
C.2.1 Possible clinical endpoints

When efficacy studies are feasible and are deemed to be necessary:

- In most instances, the evaluation of protective efficacy will focus on the ability of the vaccine to prevent clinically apparent infections (e.g. past studies that have looked at the prevention of invasive disease due to *Haemophilus influenzae* type b, invasive pneumococcal infections and rotavirus infections). If an organism is able to cause a range of infections (e.g. from life threatening meningitis to otitis media), it may be appropriate that the primary analysis should focus only on specified manifestations of infection while secondary analyses might consider all infections. Occasionally, the primary endpoint will be based on clinical relapse of infection (e.g. vaccines intended to prevent herpes zoster);
- It may sometimes be appropriate to base the estimation of efficacy on prevention of infection that may or may not be clinically apparent at the time because it is known that this will prevent an infection-related disease later in life (e.g. this situation might apply to candidate vaccines against hepatitis C infection);
- Less commonly, the primary endpoint may be some other marker that predicts progression to clinically apparent disease (e.g. vaccines against specific types of human papilloma virus may focus on histological changes in the cervix); and
- A candidate vaccine may contain antigens derived from one or several types of the same species for which there is a potential for cross-protection against types not included in the vaccine (e.g. as may be postulated for pneumococcal vaccines, rotavirus vaccines and human papilloma virus vaccines). While the primary endpoint will usually be defined as protective efficacy against any vaccine type, it may sometimes be justifiable to base the primary analysis on all infections due to the species (i.e. vaccine type and non-vaccine type) while a secondary analysis focuses on infections due to vaccine types. In any case, studies with candidate vaccines with a potential to confer cross protection should plan for secondary analyses of rates of infection due to nonvaccine types (see also section D.1.2).

In all the possible scenarios that may arise, the applicant must provide a clear and adequate justification for the primary and secondary endpoints. In turn, the choice of primary endpoint may have a major influence on the selection of the most appropriate study design (see section C.3).

C.2.2 Case definition and detection

Case definition

Whatever the chosen endpoint(s), well-validated methods should be used for diagnosis (e.g. clinically apparent and/or non-apparent infections) or for other evaluation (e.g. histology) and should be predefined in the protocol. However, there may be instances when it is necessary or even desirable that the applicant employs experimental laboratory methods for establishing infection and/or progression of infection because no well-validated methods exist. In such cases, every effort should be made during the clinical development programme to evaluate the sensitivity, specificity and reproducibility of the methods used. See also section B.2.1.

Ideally, all clinical staff involved in case ascertainment should be kept unaware of the treatment group. If possible, a centralized laboratory should be used or should or at least confirm the findings of local laboratories and laboratory staff should always be blinded as to treatment assignment.

- When clinically apparent disease is the primary endpoint, immunological confirmation of an acute infection would usually be expected whenever relevant tests exist. When such data are not relevant (e.g. in the diagnosis of tuberculosis), the diagnosis may rest on clinical features that may include radiological studies and other investigations and/or laboratory confirmation and characterization of the organism;
- If clinically non-apparent infections are to be monitored, the diagnosis may be immunological and/or may involve isolation and characterization of the causative pathogen; and
- If other endpoints are proposed, it is critical that the criteria for staging and progression are pre-defined in protocols as appropriate to the nature of the investigation.

Once a case of infection (or appropriate alternative marker of progression) is confirmed in a vaccinated subject, it is necessary to consider whether the case represents a true vaccine failure. For example, depending on knowledge of the kinetic of the immune response, it may be appropriate that true vaccine failures are limited to subjects that have completed the primary immunization series and have a failure-defining event more than a specified number of days after

the final dose. However, the applicant should always provide an analysis of all cases of infection or progression (i.e. breakthrough cases) regardless of time in relation to vaccine doses and it may also be informative to look at numbers of cases that occur after sequential doses in a schedule. All vaccine failures (as defined) and any other breakthrough cases should be investigated in detail to determine whether they might have failed to mount a response due to host-related factors.

Case detection

Whatever the chosen study design (see C.3), accurate and comprehensive case detection is essential. When the study aims to compare rates of specified endpoints between vaccinated and unvaccinated groups or between groups that receive a candidate vaccine or a licensed vaccine, it is critical that the same methodology for case detection is applied in all treatment groups and throughout the duration of the study.

If the primary endpoint is clinically apparent disease, the possible range of clinical presentations will determine the mode of case ascertainment. For example, this may be hospital-based for cases of life- threatening infections or community based for less severe infections. If community based, case detection may depend on family practitioners and on first suspicion of infection by vaccinated subjects themselves or their parents/guardians. In each case, it is critically important that the individuals who are most likely to initiate detection of a possible case should have clear instructions. These may need to cover issues such as criteria for stimulating contact with designated healthcare professionals, telephone contacts, initial investigations and further investigations once a case is confirmed.

When the endpoint is other than clinically apparent disease, it becomes critical that subjects are monitored at regular intervals to detect clinically non-apparent infections or changes in other selected markers. The frequency of visits, and acceptable windows around the visits, should be laid down in the study protocol and must be carefully justified.

The appropriate period of pro-active case ascertainment during a study requires special attention and will be determined mainly by the characteristics of the disease to be prevented and the claim for protection that is sought at the time of initial authorization. Anticipating that in most instances such studies will cover periods of perhaps 1-5 years (at most), plans should be in place to determine the duration of protection and need for boosting or for additional booster doses. This follow-up will likely have to be performed in an unblinded fashion.

C.3 Possible study designs

The following sections discuss some general statistical considerations and the most common study designs, including the selection of appropriate controls. Other study designs may be applicable under specific circumstances and applicants are encouraged to discuss these with the SFDA. The determination of vaccine effectiveness in the post-authorization period is discussed in section C.4.

When selecting the most appropriate study design, it should be borne in mind that protective efficacy may be evaluated in various settings that may influence the perceived overall benefit of vaccination. For example, depending on the infectious disease to be prevented and so the likely mode of use of the vaccine once licensed, it may be appropriate to conduct a study in which large sectors of the population are vaccinated. This has the potential not only to protect individuals but also to confer a degree of herd immunity. In contrast, when the intent is to protect travellers against specific infections much smaller studies may be appropriate that will usually provide results that reflect only the benefit to recipients.

C.3.1 Statistical considerations

Applicants should consult all relevant ICH guidance that would be appropriate to the selected study design and objectives. The following constitutes only some of the most important issues that should be addressed.

Whatever the study design and objectives, the protocol should state the hypothesis (es) to be tested and clearly describe the primary and secondary endpoint(s). The study populations of interest (e.g. per protocol, intent to treat and any others to be analyzed) should be defined and the primary analyses should be listed in accordance with the main study objectives. While the primary population for analysis will depend on whether the study is intended to demonstrate superiority or non-inferiority, it is expected that sensitivity analyses of efficacy will be provided (i.e. analyses of efficacy in other defined study populations). Exclusions from each defined population must be justified and described in detail. The primary analysis should focus on cases that meet the definition of vaccine failures although the applicant should also provide an analysis based on all confirmed cases of the disease to be prevented.

The sample size calculation will inevitably reflect the study design and planned analysis. The underlying assumptions (e.g. unit of randomization, Type I error) should be stated in the protocol and there should be sufficient power to address the study objectives. Special attention should be paid to defining the criteria on which judgments of superiority or non-inferiority are to be made.

C.3.2 Randomized controlled studies

The absolute protective efficacy of a vaccine for a specific disease is usually defined as the reduction in the chance of developing the disease after vaccination relative to the chance when unvaccinated as determined in a prospective randomized controlled study. Depending on the disease to be prevented and the acceptability of withholding a potentially efficacious vaccine from some study participants, the control group might be given a placebo or an alternative vaccine that does not protect against the disease under study but provides some other potential benefit to vaccines. In both these instances, a double blind study design would be possible. The alternative is that the control group receives no treatment but this means that a double blind design is not possible.

If it is not appropriate that a potentially efficacious vaccine might be withheld from some study participants it may be possible to use a randomized controlled study design to estimate the relative protective efficacy of a candidate vaccine by comparing it with a licensed vaccine that protects against the same infection. However, the fact that at least one vaccine is already approved for prevention of the disease may make it difficult to identify a study population that still has a sufficient incidence of disease before the study commences to allow for reliable estimates of efficacy to be made.

If an active comparator is to be used, the choice of vaccine should take into account the strength of the evidence to support its efficacy. If it is well-recognized that the protective efficacy of the licensed comparator(s) is sub-optimal and the candidate vaccine has been developed to improve on available products (e.g. as might be the case for new vaccines against tuberculosis), the study should demonstrate that the candidate vaccine is superior to the licensed product(s).

C.3.3 Secondary attack rate studies

In the context of determining protective efficacy, the commonest alternatives to prospective randomized controlled studies are secondary attack rate studies. These may be appropriate when the infection to be prevented is associated with a relatively high incidence of secondary cases and are based on an assumption of equal chance of vaccines and non-vaccines catching the infection from the index case. However, such an assumption requires justification and may need to be investigated prior to starting the study. Units of randomization to vaccination may include the individual, the household or the cluster under study (e.g. a school population). Possible biases include the need to use an open label or single blinded study design and the fact that such studies may be partly retrospective. In addition, estimates of vaccine efficacy from such studies should

be viewed with some caution because of the select nature of the study population compared to the target population.

C.4 Vaccine effectiveness

Vaccine effectiveness reflects direct (vaccine induced) and indirect (population related) protection during routine use. Thus, the assessment of vaccine effectiveness can provide useful information in addition to any pre-authorization estimates of protective efficacy. Even if it was not feasible to estimate the protective efficacy of a vaccine pre-authorization it may be possible and highly desirable to assess vaccine effectiveness during the post-authorization period.

Vaccine effectiveness may be estimated from observational cohort studies that describe the occurrence of the disease to be prevented in the target population over time. However, there is no randomization step and there is the potential for considerable biases to be introduced. Alternatively, vaccine effectiveness may be estimated during a phased (e.g. in sequential age or risk groups) introduction of the vaccine into the target population in which the groups might form the units of randomization.

It may not be possible or appropriate for applicants to conduct studies to estimate vaccine effectiveness since co-ordinated regional or national networks may be necessary to ensure that cases are reliably detected. However, applicants should discuss arrangements for ongoing disease surveillance and the potential for estimating effectiveness with appropriate public health authorities in countries where the product is to be marketed. It may be that reliable estimates of effectiveness can only be obtained in certain countries in which appropriate vaccine campaigns are initiated and where there is already a suitable infrastructure in place to identify cases. Therefore, it would likely be inappropriate to extrapolate any estimates of effectiveness that are obtained to other modes of use (such as introducing the same vaccine to different or only to highly selected sectors of the population).

Even if it is not possible or necessary to make formal estimates of effectiveness. it is considered very important that countries that have an appropriate infrastructure should conduct surveillance to monitor for any sign of waning protection of a vaccine or type of vaccine within a population. In addition, for vaccine that may protect against only some types of organism within a species, appropriate surveillance should be in place to detect strain replacement phenomena. It is understood that these issues would usually fall to public health authorities rather than to marketing authorization holders.

Also, in conjunction with the Ministry of Health, applicants should try to ensure that emerging data that might throw light on the duration of protection, need for boosting, immune interference and the description or further confirmation of putative immunological correlates of protection are provided to SFDA, and that the prescribing information is updated accordingly. As appropriate to the vaccine and its anticipated mode of use, the potential long-term impact of vaccination on the epidemiology of the vaccine preventable infection(s) should also be addressed in the post-authorization period.

In addition to the above information, all requirements mentioned in WHO Guidelines titled by "Guidelines for nonclinical evaluation of vaccines" should be recommended.

D. Special Consideration for Vaccine Development

D.1 Vaccines that contain more than one antigen
D.1.1 Immune interference
There is a potential for each antigen in a vaccine to interfere with immune responses to one or more other antigens in the same product. Immune interference may be due to chemical interactions and/or immunological interactions and may result in enhancement or depression of responses to one or more antigens and/or may alter the nature of the immune response. Responses to antigens that are conjugated to protein carrier molecules may be especially unpredictable when more than one is included in the same vaccine. Also, inclusion of a conjugated antigen in a vaccine may affect responses to certain other antigens that are the same as (e.g. tetanus toxoid) or similar to (e.g. diphtheria toxoid and CRM197) the carrier protein. If notable enhancement or interference is detected, the amount of antigen(s) in the product may need adjustment and/or other formulation changes might be needed and/or a change in dosing regimen might need to be explored. In association with these phenomena, there could be effects on the local and systemic tolerability of vaccination.

An adequate exploration of the effects, if any, of combining the antigens in any one vaccine on the immune responses to each component is usually required. Nevertheless, there may be circumstances in which it might be considered unnecessary to give all or even some antigens in a novel combination separately and together if the ultimate product can be shown to be satisfactorily immunogenic, safe and efficacious. Therefore, consideration of the need for and extent of immune interference studies should be on a case by case basis.

In most cases, the assessment of immune interference will be based on serological data. Special difficulties in assessing immune interference occur when there are no immunological correlates of protection for some or all of the antigens of interest. In these circumstances, the assessment of immune interference can only be based on simple comparisons and it is recommended that, whenever possible, the focus should be on parameters most likely to reflect clinical protection, such as functional antibody levels.

The design of studies to evaluate interference will depend on the nature of the antigens that are to be combined. For example, if two antigens have never been formulated together before, the immune response to each antigen when given alone should be compared with administration in a combined product. However, it may not be necessary or feasible to compare the separate and combined administration of every antigen in a product if several of these have already been formulated together in licensed products or if there are very many antigens involved. In such cases, the effects of adding antigen(s) to an established combination product can be evaluated by comparing responses to the novel combination and separate administrations of the additional antigen(s) plus the licensed combination. All such studies should also provide a careful comparison of safety data.

D.1.2 Cross-reacting immune responses

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

A beneficial cross-reaction might occur when antibody to an antigen from a particular micro-organism (species or type within a species) shows considerable affinity to antigen(s) of one or more other species or types within a species. In some cases, it may be possible to accumulate sufficient evidence from studies of protective efficacy and/or from studies of functional immune responses to support a claim for protection against species or subtypes not included within the vaccine.

In contrast, antibody elicited by a vaccine that shows cross-reactivity to human antigens may trigger a harmful effect. It may not be possible to frilly explore the potential for this to happen before initial authorization. If there are grounds to anticipate such problems, very special consideration is needed for post-marketing safety studies.

D.2 Concomitant administration of vaccines

The potential for immune interference and effects on overall safety are also important considerations for the concomitant but separate administration (by whatever route) of two or more vaccines. While there are general principles that may be applied in the absence of specific data, several examples of unexpected immune interference have come to light in recent years. These have included the effects of acellular pertussis vaccines on responses to conjugated saccharides and variable enhancement or depression of immune responses to the conjugated saccharides when the carrier proteins are the same or different. In assessing the potential for immune interference to occur, it is very important to justify the criteria applied to judge whether concomitant administration exerts potentially clinically significant effects on immune responses to individual antigens (see section B.2.4). If any studies identify important immune interference or an unacceptable increase in unwanted effects, applicants should explore the minimum interval that might be allowed between administrations to avoid these problems.

At the time of initial authorization of a novel vaccine, it would be desirable that there should be safety and immunogenicity data on concomitant administration with at least one type of licensed vaccine that would very likely be given at the same time. In many circumstances, satisfactory results would likely suffice to make a general statement about co-administration with particular types of antigens without referring to brand names. However, there may be occasions when product-specific problems could be anticipated or may come to light that might necessitate distinguishing between brands in the prescribing information.

For some vaccines, such as those intended for the primary series in infants, the clinical trials will inevitably involve co-administration with certain products at one or more schedules since protocols must allow for the usual recommended antigens to be given on time. Therefore, it is likely that information on the safety of co-administration and some data on immune responses to all antigens before and after completion of the primary series would be available. A formal assessment of immune interference might not be necessary if it can be established that the antigens satisfactorily prime infants and elicit acceptable antibody responses for at least short-term protection. However, studies might need to involve omission of the new vaccine from one group may compare concomitant administration with administrations made in a staggered fashion (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).

The data on immune interference based on one schedule cannot necessarily be extrapolated to other schedules. For example, potentially clinically important interference may be detected at an accelerated schedule but may not be apparent at less concentrated schedules. Therefore, if only the latter is studied, immune interference that could occur might not be identified.

For routine vaccinations administered later in life or administered for travel purposes, studies that evaluate immune interference should usually compare concomitant with separate administrations of products. As for studies in the primary series, it may be acceptable that the data are derived from co-administration with only one brand of a particular type of vaccine that is likely to be co-administered.

D.3 Interchange of vaccines within a schedule.

For most inactivated vaccines it is necessary to give more than one dose of an antigen to obtain adequate priming and to maintain protection against infection. Therefore, for primary series and for booster doses, the question arises as to whether the first and all sequential doses must be administered with the same product or whether other products that contain similar antigens can be used interchangeably.

If active endorsements in the prescribing information for switching are sought, these need to be supported by appropriate data. The design of studies intended to support claims for inter-changeability should be tailored to reflect the exact claim required and should provide safety and immunogenicity data. 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.

D.4 Vaccine lots and lot-to-lot consistency studies

Ideally, vaccine from several lots of the exact formulation intended for marketing should be adequately tested during the clinical development programme, especially during the confirmatory studies of immunogenicity and, if feasible, in protective efficacy studies. In addition, the manufacturers should ascertain that the lots used in the clinical trials, especially those in the later stages of development, are adequately representative of the formulation intended for marketing throughout its shelf life. See also sections B.2.4.

The need for a formal lot-to-lot consistency study should be considered on a case by case basis. Such a study might be important when there is an inherent and unavoidable variability in the final formulation of the vaccine in one or more respects. However, for vaccines with a very

reproducible method of manufacture such studies may not provide useful information in addition to that generated during the rest of the clinical programme.

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

The pre-defined criteria for concluding comparability between lots will usually be based on one or more immunological parameters although a comparison of safety data is also important in these cases. Very careful consideration needs to be given to which immunological parameters are the most valid and clinically relevant and how large a difference between lots might be potentially clinically significant.

D.5 Bridging studies

Classically, clinical bridging studies generate immunogenicity data to support the extrapolation of data on safety and protective efficacy obtained under specific circumstances of use to other situations (e.g. different formulations, additional schedules and/or populations). In designing such studies, it is important to consider the critical immunological parameters for determining comparability of immune responses (see B.2.4). When there is an established immunological correlate for protection, the proportions reaching this level should not only be similar between treatment groups but should also be acceptably high in the light of all previous experience with responses to the antigen in question. When there is no known correlate or this is questionable (for example, with respect to predicting long-term efficacy), it may be more relevant to compare proportions reaching a pre-defined cut-off for functional antibody than to compare GMCs.

On occasion, the term may be more loosely applied to simple comparisons between immunogenicity data sets. For example, between data from premature infants compared to full term infants, immunosuppressed compared to healthy individuals or between different formulations of the same vaccine. The same considerations as outlined above apply to the assessment of the findings. Special caution may be needed if comparisons are made between studies rather than within a single study.

D.6 Circumstances in which approval might be based on very limited data

Special consideration is needed for the clinical development of vaccines when protective efficacy studies are not feasible and when there is no established immunological correlate of protection. For example, vaccines intended to prevent rare infections that carry considerable morbidity and

mortality including some pathogens that have the potential to cause widespread disruption to mankind in case of an epidemic or deliberate release.

In principle, there are several ways of approaching this scenario. In some cases, it may be possible to obtain some relevant data on protective efficacy from challenge studies in animal models. There may be immunological correlates of protection established for very similar but not identical antigens that might be used pro tem as a guide to likely efficacy. If possible, immunological studies should focus on the measurement of functional immune responses. Taking the results of these and any other relevant investigations together, it is possible that a reasonable case for likely efficacy could be put together. A presumptive risk-benefit relationship could be derived that might support authorization. However, the prescribing information should explain the basis for the opinion.

If authorization has had to be based on such limited data, it may not be possible to estimate vaccine effectiveness in the post-authorization period unless a substantial natural epidemic or deliberate release occurs. In any case it is likely that reliable data can only be obtained from national surveillance programmes operated by public health authorities. Therefore, applicants should work with public health authorities to develop plans that would allow for the collection of data on safety and efficacy if the opportunity (e.g. a significant outbreak or major epidemic) should arise.

E. Consideration for the Summary of Product Characteristics (SPC)

E.1 Therapeutic indications
The indication should routinely cover:
- The disease(s) to be prevented (including specific types of an organism if appropriate to the vaccine content);
- The minimum age for use (e.g. infants from the age of 2 months);
- Appropriate age categories (e.g. neonates, infants, children, adolescents, adults); and
- The maximum age for use if such a limit would be appropriate based on factors such as the disease epidemiology or antigen content of the vaccine.

It may also be necessary to mention:
- Particular populations for which the vaccine is suitable (e.g. naïve, primed, at risk); and
- Populations for which the vaccine is not suitable should usually be mentioned elsewhere.

E.2 Posology and method of administration

E.2.1 Posology

If appropriate, this section should clearly describe and separate doses and schedules for primary and booster vaccinations. In general, the recommendations should reflect the minimum age at the time of the first dose, minimum dose interval and minimum interval between the last dose of the primary series and first (and perhaps sequential) booster dose(s) that were evaluated in clinical studies.

For most vaccines intended for use in infancy, and for many intended to boost antigens routinely delivered in infancy, it will be necessary to include a general statement regarding the need to follow official guidance on the exact timing of these doses.

Advice on dose and schedule may need to be given separately for different age groups or other defined populations (e.g. the immunosuppressed).

It may be appropriate to state whether interchangeability of vaccines within a schedule can be recommended.

E.2.2 Method of administration

The route of injection should be specified, preferably with the place of first choice (e.g. deltoid muscle).

Important statements may include:

- Do not inject intravascularly; and
- Exceptional administration subcutaneously to patients with thrombocytopenia or bleeding disorders.

E.3 Contra-indications

The contra-indications should usually be limited to absolute contra-indications that should apply at the time of administration.

The following should usually appear as a minimum:

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

E.4 Special Warnings and precautions for use

Appropriate common statements might include:

- As with all injectable vaccines, appropriate medical treatment and supervision should always be readily available in case of a rare anaphylactic event following the administration of the vaccine;
- (Trade name) should under no circumstances be administered intravascularly;
- Thiomersal has been used in the manufacturing process of this medicinal product and residues of it are present in the final product. Therefore, sensitization reactions may occur; and
- As with any vaccine, a protective immune response may not be elicited in all vaccines.

This section may also describe:

- Lack of protection or limits of any cross protection there may be against strains or serotypes not in the vaccine;
- Situations (e.g. administration to persons already in the incubation phase) or populations (e.g. elderly) in which the efficacy of the vaccine has not been investigated or could not be anticipated;
- Factors that might be associated with an impaired immune response; and
- For live attenuated vaccines, the potential for transmission of vaccine strains should be described, as well as the possibility of reversion to virulence or of re-assortment with wild-type strains.

E.5 Interaction with other medicinal products and other forms of interaction

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.

Clinically important or potentially clinically important immune interference should be mentioned.

If there are no data regarding co-administration with a type of vaccine that is very likely to have to be co-administered, this should be stated.

Appropriate common statements may include:

- It may 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 given with TRADENAME; or
- If it is necessary to provide immediate protection, TRADENAME may be given at the same time as (normal/x-specific) immunoglobulin. Injections of TRADENAME and immunoglobulin should be made into separate limbs.

E.6 Pregnancy and lactation

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

- TRADENAME is not intended for use in adults. Human data on use in pregnancy or lactation and animal reproduction studies are not available.

For vaccines to be used in individuals of childbearing age, the section should describe the available preclinical and clinical experience.

For inactivated vaccines, it is usual to advise the following:

- As with other inactivated vaccines, harm to the fetus is not anticipated. However, TRADENAME should only be used during pregnancy when there is a clear risk of infection.

For live attenuated vaccines it is usual for use to be contra-indicated in pregnancy. However, if the vaccine is a well known product for which there is reported experience, it may be sufficient to discourage vaccination during pregnancy unless clearly necessary.

Regarding lactation, in the absence of data, it is usual to state for inactivated vaccines:

- The effect on breastfed infants of administration of TRADENAME to their mothers has not been studied.

Recommendations for live attenuated vaccines must be considered on a case by case basis.

E.7 Effects on ability to drive and use machines

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.

The usual considerations apply regarding statements to be made when the vaccine is intended for adults.

E.8 Undesirable effects

Some considerations specific to vaccines may include:

- Details of local and systemic reactions
- Special notes on certain ADRs such as fevers, febrile convulsions
- ADRs and ADR rates separated according to age group, number of doses, previous vaccination history, occurring in studies or reported from post-marketing surveillance
- Special notes on any increased rate of ADR(s) observed on concomitant administration with other vaccines.

E.9 Overdose

Any experience with overdose should be mentioned. It may be appropriate to mention that overdose is unlikely due to the mode of presentation (e.g. single dose pre-filled syringe).

E.10 Pharmacodynamic properties

This section should briefly summarize (tabulation may be appropriate) the most pertinent immunological data (using the most 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 data should be broken down by primary series and boosting, by age group or by other factors, such as immunosuppression.

The section may include details of the established or putative immunological correlate of protection.

The most recent WHO guidelines for particular vaccines should be followed.

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

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. 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 bank of a cell substrate from which all subsequent cell banks used for vaccine production will be derived. The MCB represents a characterized collection of cells derived from a single tissue or cell.

Master Virus Seed (MVS): A viral seed of a selected vaccine virus from which all future vaccine production will be derived, either directly, or via Working Virus 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.

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.

Virus Seed or Viral Seed: A live viral 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 Virus Seed (WVS): A viral seed derived by propagation of virus from the MVS under defined conditions and used to initiate production cell cultures lot-by-lot.

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