⟨1235⟩ VACCINES FOR HUMAN USE—GENERAL CONSIDERATIONS

Add the following:

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- Regulations and Standards

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- Manufacturing Facilities and Systems
- Manufacturing Process
- Manufacturing Surveillance

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MATERIALS OF ANIMAL ORIGIN
SEED LOT SYSTEMS AND CELL BANKING
PROPAGATION AND HARVEST
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GLOSSARY
APPENDIX▲ (USP 1-May-2021)

Change to read:

INTRODUCTION

Vaccines have been used for centuries to immunize individuals against pathogenic organisms with the goal of preventing the associated diseases. Vaccines are biological products that contain antigens capable of inducing a specific and active acquired immune response in the body. Antigens present in vaccines are processed by specialized cells in the body's immune system, resulting in the development of blood proteins known as antibodies (i.e., humoral immunity), specialized lymphocytes (i.e., cell-mediated immunity), or both. Therefore, immune responses may be antibody mediated, cell mediated, or both. Antigens are ▲the components responsible▲ (USP 1-May-2021) for vaccine function and generally consist of a portion of the pathogenic organism, ▲such as an inactivated or▲ (USP 1-May-2021) attenuated form of the whole microorganism, ▲or are produced recombinantly from a non-infectious organism engineered to express antigens from a pathogenic species or recombinant protein expressed in genetically engineered cell line. In the case of vaccines derived from recombinant technology, the antigen is expressed in a host cell of an unrelated microorganism and then used for vaccine manufacture.▲ (USP 1-May-2021) In the case of

DNA-based vaccines (currently under development), the vaccine would contain nucleotide sequences (genetic material) that encode microbial antigens.

▲▲ (USP 1-May-2021) A list of vaccines currently licensed in the US is posted ▲on the FDA website (see *Appendix*). There are▲ (USP 1-May-2021) various types ▲of vaccines, which can be classified▲ (USP 1-May-2021) depending on their design and the processes involved in their manufacture ▲(see *Table 1*).▲ (USP 1-May-2021) Vaccines for human use may contain whole killed or attenuated organisms (e.g., bacteria or viruses) or antigens derived from portions of a pathogen (either by partitioning and purification or derived using recombinant technology, ▲or they may be engineered to be expressed by non-infectious viruses.▲ (USP 1-May-2021) Some polysaccharide vaccines are conjugated to a carrier in order to enhance their immune response.

▲Table 1. Bacterial and Viral Vaccines

Bacterial Vaccines	Viral Vaccines
Carbohydrate subunit vaccines[a]	Recombinant virus-like particles (VLPs)[b]
Purified polysaccharide vaccines	Isolated (virus-derived) viral proteins[a]
Glycoconjugate vaccines	Recombinant (monomeric) viral proteins[b]
Protein subunit vaccines	—
Toxoid vaccines	—
Isolated bacterial proteins	—
Recombinant bacterial proteins[b]	—
Bacterially derived protein-containing lipid vesicles[c]	—
Inactivated bacterial vaccines[d]	Inactivated viral vaccines[d]
Live attenuated bacterial vaccines	Recombinant chimeric viral vaccines
—	Live attenuated viral vaccines[e]▲ (USP 1-May-2021)

[a] Subunit vaccines are extracts from inactivated/killed viruses or bacteria and generally undergo some degree of purification.
[b] Recombinant protein viral and bacterial vaccines are derived from host cells that have been transformed with expression vectors that carry genes that encode antigenic material from infectious agents. The expression cells are grown in bioreactors to produce the recombinant antigenic material.
[c] Protein-containing vesicles are semi-purified native bacterial membrane fragments or artificial liposomes containing specific semi-purified native bacterial proteins or recombinantly expressed antigenic bacterial proteins. Processing and semi-purification renders the preparations non-viable while retaining their immunogenicity.
[d] Inactivated bacterial and viral vaccines are produced by growing cells of disease-causing bacteria or viruses in cell substrates and subsequently inactivating them to prevent replication in the recipient. These preparations may or may not be partially or completely purified.
[e] Live attenuated bacterial or viral vaccines are weakened (attenuated) forms of a pathogen. They contain antigens that are similar to disease-causing microbes. They may be derived from the pathogen itself, or from a different organism that contains antigens that cross-react with the virulent microbe (e.g., vaccinia and variola).

In addition to antigen(s), vaccines may contain ▲▲ (USP 1-May-2021) other components, such as adjuvants that enhance the immune response to the vaccine antigen, preservatives to prevent bacterial or fungal contamination of multiple-dose vials, or other excipients needed for pharmaceutical manufacturing or vaccine stabilization. Residual components from the manufacturing process also may be present in vaccine preparations. Examples of these categories are listed in *Table 2*.

Table 2. Vaccine Components

Antigens	Whole organisms
	Components/subunits
	Recombinant proteins
Adjuvants▲, CpG oligonucleotides▲ (USP 1-May-2021)	Aluminum salts
	▲Monophosphoryl Lipid A, saponins▲ (USP 1-May-2021)
	Squalene▲, CpG oligonucleotides▲ (USP 1-May-2021)
Antimicrobial preservatives	Thimerosal
	2-Phenoxyethanol
	Benzethonium chloride
	Phenol
Stabilizers	Salts
	Amino acids
	Sugars
	Proteins
	▲Surfactants▲ (USP 1-May-2021)

Table 2. Vaccine Components *(continued)*

Manufacturing residuals	Cell-derived residuals
	Materials of animal origin
	▲Residual conjugation reagents▲ (USP 1-May-2021)
	Antibiotic residuals
	Inactivating chemical agents
▲Containers	Vial and stopper
	Syringe and plunger▲ (USP 1-May-2021)

Different vaccine antigens are often combined in one final formulation in order to elicit immunity against multiple diseases and to reduce the number of separate administrations needed to achieve immunity to the various vaccine antigens.

Despite the multiple forms vaccines may take, several common features characterize the manufacture and testing of vaccines. This chapter focuses on commonalities throughout the manufacturing process, from raw material qualification to final release tests.

Regulations and Standards

Vaccines are regulated by the US FDA as biological products. The general requirements are listed in national laws and international guidances. For the US, national requirements are codified in 21 Code of Federal Regulations (CFR) §200 and 21 CFR §600, with additional recommendations available in the FDA's *Points to Consider* and guidance documents ▲(see *Appendix*).▲ (USP 1-May-2021) [International guidances are available from the International Council for Harmonisation (ICH) ▲(see *Appendix*)▲ (USP 1-May-2021) and the World Health Organization.] New methodologies are continually being developed and validated and will be included in the *USP* as they become available. Reference standards are available from USP and the FDA.

Change to read:

OVERALL MANUFACTURING PLAN

When considering the overall plan for manufacturing a vaccine, manufacturers need to consider the following factors:

- Physical facilities ▲and design, qualification, and maintenance of utilities and equipment▲ (USP 1-May-2021)
- Raw materials and process aids
- ▲▲ (USP 1-May-2021)Manufacturing process▲▲ (USP 1-May-2021)
  - Initial process (production of virus/bacteria and recombinant materials)
  - Downstream processes (purification or chemical modification, if applicable)
- Antigen modifications such as conjugation or toxoiding
- Storage of process intermediates and final bulk
- In-process and final product testing regimens and control schemes
- Addition of adjuvants, if applicable
- Formulation and filling
- Container–closure system
- Stability program that supports the ▲expiry▲ (USP 1-May-2021) dating ▲▲ (USP 1-May-2021) of the product

Quality systems are needed to support the following manufacturing process development: specifications for raw materials, process ▲controls and process▲ (USP 1-May-2021) intermediates, and final product ▲quality, stability, and shelf-life studies; analytical development and validation;▲ (USP 1-May-2021) change control; ▲facility, utility, and equipment qualification; cleaning, maintenance, and potential change-over protocols;▲ (USP 1-May-2021) and failure investigations and complaints. All of these elements are important in the life cycle of the vaccine product.

The overall goal of a comprehensive manufacturing program is to consistently ▲and continuously▲ (USP 1-May-2021) produce a vaccine that is safe and effective. Concurrently with clinical development of the vaccine, the manufacturing process is refined, ▲the process is validated for consistency,▲ (USP 1-May-2021) and the ▲test▲ (USP 1-May-2021) methods are validated for ▲their intended use.▲ (USP 1-May-2021) This includes systems to control changes to the process or inputs. Manufacturers should expect that changes will be required during the vaccine's manufacturing life cycle, and manufacturers necessarily will use data from development and routine manufacturing to assess the process as well as proposed changes. The manufacturers should adopt systems that continually evaluate all aspects of manufacturing to identify unanticipated changes in vaccine quality and to assess them as quickly as possible.

Manufacturing Facilities and Systems

Manufacturers should ▲document the▲ (USP 1-May-2021) general layout of the manufacturing facilities, including diagrams that show the following: flow of raw materials and process inputs; movement of product, intermediates, waste streams, and personnel; and air flows and pressurization levels. These diagrams assist in minimizing the risk of potential product contamination from various sources. These sources can include cross-contamination from other products, contamination from different batches of the same product, and extraneous contamination from microorganisms ▲or foreign material.▲ (USP 1-May-2021)

Evaluation of the flow diagrams can assist with strategies for the development of engineering controls, personnel procedures, and monitoring systems to enable compliance with ▲current▲ (USP 1-May-2021) good manufacturing practices (▲cGMPs).▲ (USP 1-May-2021) Analysis of potential risks may also provide insights about what information should be recorded in batch documentation to facilitate consistent manufacture and failure investigations. Together, physical facilities, ▲utilities, equipment,▲ (USP 1-May-2021) procedures, personnel, training, and quality systems make up the GMP environment in which a vaccine will be produced.

Manufacturing Process

The manufacturing process includes process inputs such as raw materials and processing aids and unit operations comprising both the initial and downstream processing steps. A process flow map for the manufacturing process is useful and assists in validation of the manufacturing process. This map shows all unit operations, the inputs to each operation, and the outputs to subsequent manufacturing steps. Analytical testing done at relevant steps and the specifications required to proceed to the next stage of processing may be added to the map. A process map also supports a processing space to facilitate a rugged process (i.e., one based on suitable characterization studies to establish boundaries within which manufacturing can occur to promote unchanged safety and efficacy outcomes).

The process flow map should include all steps from making the seed/cell bank ▲▲ (USP 1-May-2021) to formulation, filling, ▲and packaging▲ (USP 1-May-2021) of the final product. The validation strategy should include the steps that require validation, along with identification of the process space, associated critical process parameters (CPPs), and critical quality attributes (CQAs). The CPPs are those that directly affect the core quality attributes needed to successfully manufacture a batch of product. Some manufacturers identify other processing parameters that are important for processing but do not affect CQAs. These important but noncritical factors help identify the process development space, can contribute to the development of a rugged process, ▲and▲ (USP 1-May-2021) can be useful when the company assesses processing deviations. The concepts of quality by design and exploration of the process space are relatively new to the biologics/vaccine industry but are becoming considerations for the overall development-planning process).

Manufacturing Surveillance

Manufacturing surveillance is the continual observation of how the process and the resulting product are performing. This section is not exhaustive; rather, the points raised here outline the types of considerations recommended for a manufacturer during the development of a vaccine. Manufacturing surveillance includes the following:

- Periodic review of the performance of the manufacturing process
- Analytical assays
- Stability programs
- ▲Facility evaluation through environmental monitoring▲ (USP 1-May-2021)
- Product complaints
- Adverse event reports
- Product failure investigations
- Atypical or deviation events

Taken as a whole, these activities allow a manufacturer to assess the state of the process and product and to evaluate which, if any, operations need to be modified. These same systems also provide a surveillance matrix to evaluate changes. In any of these programs, it is also valuable to develop additional characterization assays that are not used for process intermediate or product release purposes but may be used for further evaluation when additional information is needed or desired. These additional assays for characterization are often based on different underlying analytical procedures to provide different ways to evaluate materials.

Routine surveillance processes are increasingly implemented to attempt to detect changes in processes before any CQAs are adversely affected. Not all vaccine processes can be characterized to the same extent or level (e.g., a live virus vaccine vs. a recombinant protein vaccine), and statistical tools are often used to determine alert or action levels in surveillance programs. Exceeding these levels requires the manufacturer to evaluate the situation but does not necessarily signal product failure.

GMP manufacturing entails facility, ▲utilities, and equipment▲ (USP 1-May-2021) design; process development; quality systems; and manufacturing surveillance. Together, these systems help the manufacturer to control the production of a vaccine. As noted, many types of vaccines are marketed, and each has its unique features and therefore requires different plans for each of the steps mentioned in this section.

Delete the following:

▲SEED LOT SYSTEMS

Seed lots are the stocks of specific strains of bacteria, viruses, or biotechnology-engineered cells used to express vaccine antigens. All seed lots should be documented in terms of their isolation, derivation (or construction, in the case of recombinant vector or engineered cells), and passage history. The purpose of a seed-lot system, which typically includes master and working stock seeds, and associated master and working cell banks, is to help ensure the consistency of vaccine manufacturing. The use of master and working seed lots provides a method to limit the replication of the seed and to minimize the possibility of genetic variation.

A master seed lot is a physically homogeneous preparation derived from an original seed processed at one time and passaged for a limited number of times. The master seed lot is characterized for its biological, biochemical and genetic characteristics, and to ensure its purity, its freedom from adventitious agents, and its clinical ability to produce an effective vaccine.

Cultures from the working seed lot should have the same characteristics as the master seed lot from which they are derived. For influenza vaccines, which may be reformulated with new virus antigens each year, certified seed lots can be obtained from national regulatory agencies.

A working seed lot is derived from the master seed within a limited number of passages. The working seed is tested to ensure its purity, freedom from adventitious agents, and biochemical properties. The working seed is used for production of vaccine without intervening passages.

Bacterial Vaccine Seed Lot System

In the bacterial seed lot system, a master seed is subcultured to produce a working seed one passage beyond the master seed. An aliquot of the working seed is then expanded to produce a vaccine lot. The strain(s) used for the master seed lots are identified by historical records that include information about their origin. Information about the bacterial seed lot system should include source, passage history, and raw materials to which it was exposed, with specific emphasis on raw materials of ruminant origin. Seeds should be stored at an appropriate temperature in more than one location within a facility or at a distant site in order to decrease catastrophic risk.

Identity tests may include inoculation onto suitable biochemical media, Gram stains, genotype, and serological identification with suitable specific antisera. Special tests may be added, for example, to show culture viability but also lack of virulence.

Purity of the bacterial strains used for seed lots is verified by methods of suitable sensitivity to ensure that no adventitious agents are present. These purity tests often are performed in the presence of the seed under conditions where growth is inhibited by the presence or the absence of specific nutrients. Streaking can also be used to show that the cultured seed is a pure culture.

Viral Vaccine Seed Lot System

The derivation and passage history of viral seeds should be recorded in detail. Any manipulation of the viral phenotype (e.g., cold adaptation, development of temperature sensitivity, or attenuation of virulence) or intentional genetic manipulations (e.g., reassortment or recombination) should be documented.

These viral seeds are commonly differentiated into a master viral seed and working viral seeds or working viral stock. Viral seeds should be stored at cryogenic temperatures to promote stability and in more than one location within a facility or at a distant site to decrease catastrophic risk. Manufacturers should assess the following characteristics of the viral seed stock:

- Growth characteristics on the intended production cell substrate,
- Tissue tropism;
- Genetic markers;
- Identity (for recombinant vectors);
- Viability during storage,
- Genetic stability through production;
- Attenuation properties;
- Purity;
- Absence of adventitious agents. If attenuation or derivation is achieved by passage through different species, the viral seed should be assessed for absence of adventitious agents common to those species.

The master viral seed should be extensively characterized to demonstrate the stability of genotype and phenotype for a number of passages beyond the level used in production. Generally, during assessment of genetic stability, a master seed undergoes a minimum of five passes beyond the passage that will produce the final vaccine.

Tests should be performed for identity (e.g., sequencing the entire virus or a portion of it), adventitious agents, viral phenotype, genetic stability, and, if applicable, agents that might be present in the seed as a result of its passage history. Viral phenotype can be assessed further for tissue tropism, attenuation properties, and temperature sensitivity. Not all of these tests may be necessary for every viral seed strain.

In some cases the viral seeds may have a broad host range and therefore may require neutralization of the vaccine virus before they are tested for adventitious agent(s). If possible, testing for adventitious agents should be done without neutralization in order to avoid an antiserum that may inadvertently neutralize an adventitious agent present in the seed. Sometimes it is not possible to effectively neutralize a viral seed, and in such cases alternative strategies can be used. For example, the test can be performed in a cell substrate that does not permit replication by the vaccine virus. However, such a substitution of the substrate cell may compromise the test's sensitivity for detection of other adventitious agents. Therefore, the tests may be supplemented with use of polymerase chain reaction (PCR) assays.

Assessment of neurovirulence may be appropriate if the virus is known to be neurotropic. Manufacturers should consult with regulators about appropriate animal models, methods, and scoring systems for this assessment before they initiate such studies. For viruses that are neurovirulent or may revert to neurovirulence (e.g., polioviruses), it may be necessary to assess neurovirulence beyond the master seed.

If the master viral seed is well characterized, the working viral seed may not require extensive characterization. For example, it may not be necessary to repeat testing for all the relevant viruses from the derivation history.

Systems for Biotechnology-Engineered Vaccines

For a vaccine produced via a biotechnology-engineered cell-expression system, a master seed lot or a master cell bank will be established during product development. The seed lot or cell banks should be homogenous, which is often accomplished

by limiting dilutions. The seed lot or cell bank system should be characterized in a manner analogous to that used for the cell substrate discussed in the next section, and additional tests can be used to demonstrate the genetic stability of the expression system.▲ (USP 1-May-2021)

Change to read:

▲RAW MATERIALS,▲ (USP 1-MAY-2021) FERMENTATION, AND CELL CULTURE MEDIA

▲Raw materials can directly affect the identity, strength, purity, and quality of vaccines. A consistent manufacturing process critically depends on the use of consistent raw materials (e.g., during seed banking, propagation, harvest, purification, and formulation). Accurate records of the composition and source of the culture medium used in seed banking and routine fermentation should be maintained, and the release criteria for raw materials or components should also be documented.▲ (USP 1-May-2021)

A medium is the material in which an organism is grown and amplified in quantity to produce mass material for vaccine production. Its composition is diverse and depends on the cell types that the medium supports, ranging from well-defined chemical media to chemically undefined media that contain natural components such as sera from animal origin (see *Bovine Serum* ⟨1024⟩). ▲Raw materials for bacterial growth media typically consist of both well-defined chemical entities (e.g., amino acids, carbohydrates, vitamins, minerals) and more complex components (e.g., protein hydrolysates, yeast extracts, peptones). Manufacturers should consider the source of each of these raw materials to ensure that they come from reliable vendors who adhere to cGMP quality standards and can assure a long-term supply. Manufacturers should communicate with raw material vendors in order to avoid any changes in the sourcing or manufacture of components and to avoid supply shortages. Without such communications, the consistency of the propagation processes and the supply of the vaccine can be adversely affected. Consistent raw materials are particularly critical for more complex fermentation components such as fetal calf serum, yeast extracts, or peptones, for which changes may be difficult to detect but are likely to have a direct effect on fermentation. In general, the use of animal-derived raw materials should be minimized because they pose additional risks and are more prone to variability. The section below, *Materials of Animal Origin*, describes additional considerations specific to animal-origin raw materials.

The use of antibiotics should be minimal or should be avoided to ensure that no unwanted antibiotics are included in the drug product unless they are intentionally used in manufacturing (e.g., as selective markers).

As manufacturers scale up fermentation to pilot production (i.e., within 10-fold of final manufacturing scale), they should also ensure, to the extent possible, the availability of multiple sources for all raw materials. This will ensure that supply or business instabilities at one vendor do not become the limiting factor in vaccine manufacture.

Specific requirements relating to bacterial vaccines are covered in *Vaccines for Human Use—Bacterial Vaccines* ⟨1238⟩, and aspects specific to virus-derived and recombinant viral vaccines are covered in *Vaccines for Human Use—Viral Vaccines* ⟨1239⟩.▲ (USP 1-May-2021)

Add the following:

▲MATERIALS OF ANIMAL ORIGIN

Some raw materials and reagents, such as gelatin, calf serum (see ⟨1024⟩), or trypsin for vaccine manufacturing raise concerns regarding the potential presence of adventitious agents. Gelatin or processed gelatin also is used as a vaccine stabilizer. The gelatin source may be either bovine or porcine. Although the conditions of manufacturing gelatin are harsh (i.e., the product is subjected to extremes of heat and pH), there remains a concern with bovine sources about the presence of the transmissible spongiform encephalopathy (TSE) agent, because this agent is known to resist such conditions. Therefore, if gelatin added to a vaccine or used in manufacturing is from a bovine source, the material should have the appropriate documentation certifying that it comes from a country or region that is in compliance with TSE guidance for industry. In case such a certification cannot be provided, tests should be performed on the raw material to demonstrate that it is TSE-free. If additives from animal sources are added to the culture medium, they should be certified to be free from contaminants and adventitious agents, or raw materials should be sourced from countries acceptable to the FDA. Vendors/manufacturers should provide information about the identity and source of additives and should test for adventitious agents. Additionally, manufacturers should test these materials when possible to minimize the risks of contamination with adventitious agents, such as those that cause bovine spongiform encephalopathy or TSE. Reduction of serum components [e.g., bovine serum albumin (BSA)] should be considered in processing.▲ (USP 1-May-2021)

Add the following:

▲SEED LOT SYSTEMS AND CELL BANKING

Cell banking is described in detail in *Cell Banking* ⟨1042⟩. Aspects specific to bacterial vaccines are included in ⟨1238⟩, and aspects related to viral vaccines (such as virus seed stocks) are described in ⟨1239⟩.▲ (USP 1-May-2021)

Change to read:

PROPAGATION AND HARVEST

The propagation and harvest phases follow the manufacturing process from the initiation of cell growth in the working cell bank to the separation of the crude drug substance. In addition, in these manufacturing process steps, raw materials, media, and solutions should be qualified for their intended use. Batch numbers should be clearly assigned as needed, and the relationship between component harvests and batches of individual drug substances should be recorded clearly.

▲Propagation and harvest for bacterial vaccines are described in detail in ⟨1238⟩ and in specific product class chapters. Propagation and harvest for viral vaccines are described in detail in ⟨1239⟩.▲ (USP 1-May-2021)

Add the following:

▲VACCINES PRODUCED BY RECOMBINANT TECHNOLOGY

The production of antigen, either monomeric or in self-assembling aggregates such as virus-like particles, is increasingly being performed using recombinant technology in a heterologous production cell line. In general, the requirements for the production of these products follow the requirements for other recombinant protein products.▲ (USP 1-May-2021)

Change to read:

PURIFICATION

The objective of the purification steps is to remove as much as possible of the impurities in the initial harvest and to maximize the purity of the final vaccine product. Process residuals may consist of materials from the culture medium and/or cellular components. Purification procedures should be optimized and validated. When applicable, viral clearance steps (viral removal or inactivation) should be included and validated using relevant model viruses. Special considerations are observed depending on the types of vaccines and production system used.

▲Information specific to the purification of bacterial vaccines is presented in ⟨1238⟩, material specific to bacterial polysaccharide and glycoconjugate vaccines is presented in *Vaccines for Human Use—Polysaccharide and Glycoconjugate Vaccines* ⟨1234⟩, and information specific to viral vaccines is presented in ⟨1239⟩.▲ (USP 1-May-2021)

Biotechnology-Engineered Cells

Of ▲particular▲ (USP 1-May-2021) concern in the purification of recombinant-derived vaccine components, ▲including virus-like protein vaccines,▲ (USP 1-May-2021) is the issue of residual host cell components that could produce an adverse immunogenic response in patients. This response could be exacerbated by the presence of vaccine adjuvants.

▲▲ (USP 1-May-2021)

Change to read:

INTERMEDIATES

Intermediates are defined here as the unformulated active (immunogenic) drug substances that are ▲further▲ (USP 1-May-2021) processed before final formulation and can be stored ▲▲ (USP 1-May-2021) before further processing. Those intermediates ▲that will▲ (USP 1-May-2021) be stored should be included in a formal stability program ▲to assess maximum storage times.▲ (USP 1-May-2021) Examples of intermediates include bulk polysaccharides, purified recombinant proteins (concentrates), ▲toxoids, purified and inactivated virus harvests, and polysaccharide activation and carrier-protein conjugation intermediates.▲ (USP 1-May-2021)

Production of Intermediates

Intermediates are manufactured from starting materials by one or a combination of different processes (e.g., fermentation, cultivation, isolation, or synthesis). Subsequent steps of the procedure involve preparation, characterization, and purification, eventually resulting in the drug substance ▲intermediate.▲ (USP 1-May-2021) Quality systems documents are adopted for ▲process development, in-process controls, and▲ (USP 1-May-2021) production and all applicable information should be recorded in a controlled document (i.e., a batch record). When applicable, stability studies ▲should be available▲ (USP 1-May-2021) and release tests should be performed before proceeding to the next ▲processing▲ (USP 1-May-2021) steps (see below).

Tests for Intermediates

The quality attributes of the intermediate are commonly tested in conjunction with further ▲batch▲ (USP 1-May-2021) processing. Characterization beyond release testing should be considered. Characterization methods can use appropriately qualified procedures. Depending on the individual vaccine, some tests are routinely performed before the intermediates are converted to the final bulk.

If intermediates need to be stored and/or subsequently shipped to a different location for further processing, the stability of these materials should be demonstrated. Stability tests can be a combination of both physicochemical analysis and biological assays.

Add the following:

▲FILTERED BULK OR DRUG SUBSTANCE

The final step in the manufacturing of antigen intermediates is the preparation of filtered bulk or drug substance. It is acceptable to have a low-bioburden drug substance, provided that sterile filtration is performed prior to use of this material to prepare formulated bulk. Preparation of low-bioburden drug substance involves filtration using 0.22-µm sterilizing grade filter membranes. For some antigens, which are not amenable to filtration, the process of manufacturing should be an aspectic process demonstrated through the use of media-fill tests. According to the FDA guideline (see *Appendix*) and industry standards, filters used for the final filtration to prepare sterile filtered bulk should be validated to reproducibly remove microorganisms from a carrier solution containing bioburden of a high concentration of at least 10^7 colony forming units (cfu)/cm^2 of effective filter area (EFA). Thus, the retention capacity of a validated sterilizing-grade filter with an EFA of A (cm^2) is at least A × 10^7 cfu. However, the currently used 0.22-µm sterile filter membranes can withhold much higher microbial challenges (unpublished company results for 0.22-µm polyvinylidene fluoride membranes demonstrated that a bacterial challenge concentration as high as 10^9 cfu/cm^2 can be validated).▲ (USP 1-May-2021)

Change to read:

FINAL BULK ▲(FORMULATED BULK)▲ (USP 1-MAY-2021)

Final bulk is the ▲formulated▲ (USP 1-May-2021) drug product that contains the drug substance(s), excipients, and other ingredients at the desired concentrations and is ready for filling into individual ▲dosage containers from which the vaccine would be administered.▲ (USP 1-May-2021)

Production of Final Bulk

Appropriately controlled amounts of all ingredients are blended to uniformity to produce the final bulk. The processing may include one or more steps such as buffer exchange and addition of diluents, bulking agents, stabilizing excipients, adjuvants, and preservatives. Final bulk may be prepared aseptically or processing may include a sterilization step.

Tests for Final Bulk

The quality attributes of the final bulk should be tested. Appropriate testing should be performed with respect to identity, purity, potency, sterility (see *Sterility Tests* ⟨71⟩), and antimicrobial effectiveness (see *Antimicrobial Effectiveness Testing* ⟨51⟩). Tests demonstrating safety ▲and potency,▲ (USP 1-May-2021) if applicable, are performed.▲▲ (USP 1-May-2021)

Testing is required for specific process-related and product-related impurities, depending on the vaccines being manufactured. In addition, tests are required for the bulking agent, stabilizing excipients, adjuvants, and/or preservatives, if used. All the testing should be done according to respective standard operating procedures (SOPs), and all tests should have specifications (or provisional specifications, where applicable).

Stability Test for Final Bulk

If final bulks are stored and/or subsequently shipped to a different location for further processing, the stability of these materials should be demonstrated. Stability tests can be a combination of both physicochemical analysis and biological assays. Implementation of a stability program is required for formal stability studies, and the studies should be executed according to a protocol that contains detailed information about the types of tests, including specifications, ▲storage condition,▲ (USP 1-May-2021) testing intervals, and data and analysis.

Change to read:

FINAL CONTAINER

A final container of vaccine contains the active ingredient(s) [i.e., antigen(s)] as well as additional components, such as stabilizers, adjuvants, ▲and/▲ (USP 1-May-2021)or antimicrobial preservatives. They also may include residual materials from the manufacturing process.

Excipients and Other Additives

In addition to specific antigens, vaccines often include excipients and other additives that are intentionally added to the vaccine by the manufacturer for a specific purpose. These include adjuvants, antimicrobial preservatives, and stabilizers. Vaccines also contain manufacturing residuals, which are trace amounts of various components used during manufacturing. Thus, the

combinations of these components comprise and define the complete vaccine product. Manufacturers must adhere to regulations governing permissible limits of such components, as indicated in the product's license.

ADJUVANTS

Adjuvants are agents incorporated into vaccine formulations to enhance and increase the immune responses generated by the vaccine antigens. Specifically, they can increase the amount of antibody produced, direct the immune response (Th1 or Th2), increase the duration of antibody presence (persistence), or produce a combination of these effects.

Aluminum compounds have long been the most widely used adjuvants worldwide. Two methods traditionally have been used for combining aluminum adjuvant to antigen to form aluminum-adsorbed vaccines. The first involves the addition of the antigen solution to preformed aluminum precipitate. The second involves the addition of an antigen to aluminum in solution and the addition of a compound that will coprecipitate the aluminum salt and the antigen in situ. Solutions of aluminum potassium sulfate, known as alum or aluminum chloride, have been used together with phosphate salts as precipitating agents. A number of aluminum adjuvant formulations are used in vaccines.

Tests for aluminum are based on metal detection tests described in *Aluminum* ⟨206⟩. Regulations ▲[see 21 CFR §610.15(a), *Ingredients, preservatives, diluents, adjuvants*]▲ (USP 1-May-2021) limit the amount of aluminum permitted in a dose of vaccine.

▲Other adjuvants have recently been used in vaccine products licensed in the US, including squalene and monophosphoryl lipid A (MPLA) (in combination with an aluminum-based adjuvant). MPL is quantified either through degradation to fatty acid methyl esters and quantification by gas chromatography, or through quantification of the glucosamine content. Squalene is quantitated by HPLC. Squalene adjuvants should be characterized for both the mean particle size and the number of large particles. The surfactants (e.g., sorbitan trioleate, Tween 80) used to formulate the squalene adjuvant can also be quantitated using HPLC. Specific tests to monitor the stability of bulk adjuvant should be developed.▲ (USP 1-May-2021)

Note that adjuvants are not licensed by themselves; they do not constitute a product. Rather, a vaccine consisting of specific antigen(s) and an adjuvant are licensed together as a drug product.

ANTIMICROBIAL PRESERVATIVES

In the case of multiple-dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced from repeated puncture of multidose vials. With certain exceptions, a preservative is required to be present in vaccines marketed in multidose containers [21 CFR §610.15(a)]. Exceptions include yellow-fever vaccine; measles, mumps, and rubella ▲vaccine;▲ (USP 1-May-2021) and dried vaccines when the accompanying diluent contains a preservative.

The microbial preservatives currently used in vaccines are thimerosal, 2-phenoxyethanol, benzethonium chloride, and phenol. These agents must pass the appropriate antimicrobial effectiveness test, as described in ⟨51⟩. Antimicrobial test challenges should be conducted as part of the normal formal stability program, including at the expiration date. Various tests for preservatives can be found in *Antimicrobial Agents—Content* ⟨341⟩.

STABILIZERS

The primary purpose of stabilizers is to protect certain vaccines from adverse conditions such as heat or to serve as a cryopreservative during the lyophilization process, usually during the freezing step. The particular materials chosen for this purpose include sugars (e.g., sucrose or lactose), amino acids [e.g., glycine or glutamic acid (monosodium salt)], glycerol, and proteins [e.g., human serum albumin (HSA) or gelatin]. Materials should be customized to a specific vaccine formulation and selected with patient safety in mind.

When a protein is chosen as a stabilizer, two main safety concerns arise. One stems from the source of the protein: animal or human origin raises the possibility of the presence of an adventitious agent. The second concern is the possibility of an allergic reaction in persons sensitized to that protein. This should be evaluated as part of the clinical program during vaccine development. At present, two proteins are used as stabilizers for vaccines: HSA and gelatin. The FDA requires that any serum-derived albumin used in manufacturing be US-licensed HSA. FDA guidance further recommends that a statement indicating the source and related risks appear in the "Warnings" section of the labeling for HSA-containing products.

▲In case such a certification cannot be provided, tests should be performed on the raw material to demonstrate that it is TSE-free.▲ (USP 1-May-2021)

MANUFACTURING RESIDUALS

Vaccines may contain residual amounts of any of the materials used in the manufacturing process. These materials are termed manufacturing residuals. As a general principle, it is not possible to remove a particular substance completely, nor is it possible to conclusively demonstrate that a particular substance has been completely removed. Therefore the goal is to reduce these substances to an undetectable level, using a sensitive and validated analytical methodology. Some products are tested for pyrogenic substances as a manufacturing residual (see *Pyrogen Test* ⟨151⟩); and, if the product is freeze-dried, it should be tested for residual moisture (see *Loss on Drying* ⟨731⟩). Residual levels of manufacturing materials, including inactivating agents if applicable, should be justified. The release specifications of these components are required as part of the approved license ▲and can be validated out as manufacturing experience increases.▲ (USP 1-May-2021)

CELL-DERIVED RESIDUALS

Live attenuated bacterial vaccines are not usually subject to a high degree of post-expansion purification. However, killed bacterial component vaccines typically undergo significant purification to reduce cell-derived residuals. Common cellular components to be reduced are proteins, nucleic acids, and polysaccharides. Assays for these components are routinely conducted, if appropriate, to ensure purity. A common residual in bacterial vaccines made from gram-negative bacteria is

lipopolysaccharide (LPS), commonly known as endotoxin. Endotoxin testing is performed during the manufacturing process for any gram-negative bacterial vaccine. In the case of gram-positive bacterial vaccines, the endotoxin testing should be conducted to ensure that no contaminants from gram-negative bacterial growth are present. Also, there must be a release specification for this residual. Two tests are currently used to detect LPS in biological products, the *Limulus* amebocyte lysate (LAL) test (see *Bacterial Endotoxins Test* ⟨85⟩) and the rabbit pyrogen test (see ⟨151⟩). The *Limulus* lysate that is used to test for bacterial endotoxin in FDA-regulated products is itself a US-licensed product. The rabbit pyrogenicity test requires the use of animals and is more difficult to perform; therefore, it is not employed to the ▲same▲ (USP 1-May-2021) extent as the LAL test.▲▲ (USP 1-May-2021)

Viral vaccine manufacturing requires cell substrates to produce the viruses, which are then taken through purification processes. Generally, killed viral vaccines are more highly purified than live attenuated ones. Depending on the method used to manufacture the vaccine, manufacturers work with the FDA to develop prudent specifications for the final vaccine. Animal-derived host cells have been used extensively in vaccine manufacturing, particularly for viral vaccines. For example, influenza and yellow fever vaccines are produced in egg allantoic fluid and chicken embryos, respectively. Mumps, measles, and some rabies vaccines are produced in chick embryo cells. The labels of these products must state that residual chicken proteins may be present in the final vaccine, and the label may indicate how much is present. Further, the label also urges practitioner caution when vaccinating a person with a known hypersensitivity to eggs.

▲Some▲ (USP 1-May-2021) hepatitis B vaccines are based on recombinant DNA-derived proteins expressed in yeast cultures. In ▲these▲ (USP 1-May-2021) cases, the labels notify health care professionals that yeast protein may be present in the vaccine and recommend that suitable precautions be exercised. In the case of live viral vaccines, considerations may be given to the reduction of cellular residual materials (e.g., host DNA, proteins). ▲▲ (USP 1-May-2021)

ANTIBIOTIC RESIDUALS

Some antibiotics (but not penicillin) can be used in minimal amounts in the manufacturing process for viral vaccines, according to 21 CFR §610.15(c). Those that have been used include gentamicin, streptomycin, neomycin, and polymyxin B. There is no requirement for tests of residual levels of these antibiotics in the final vaccine. However, according to 21 CFR §610.61(m), the calculated amount expected to remain as a residual in the final vaccine, based on the amount added and the dilution factor in the manufacturing process, must be stated on the product label.

INACTIVATING CHEMICAL AGENTS

Several chemical agents have been used to inactivate bacteria and viruses or to detoxify toxins in vaccine production processes. Formaldehyde and β-propiolactone are the most commonly used inactivating agents. Other less often used inactivating agents include glutaraldehyde and hydrogen peroxide. As a manufacturing residual, the inactivating agent should be removed from the final product as thoroughly as possible. The upper limit for formaldehyde is generally 0.02%, equivalent to 0.1 mg per 0.5-mL vaccine dose. The limit for β-propiolactone should be below the limit of detection.

Change to read:

EVALUATING THE STABILITY OF VACCINES

The stability of vaccine products depends on the nature of the vaccine antigen, the product formulation, ▲the container–closure system,▲ (USP 1-May-2021) and the control of vaccine storage prior to use.

Vaccine products are evaluated using programs that include real-time long-term storage under prescribed conditions. The use of extreme temperatures ▲or other factors▲ (USP 1-May-2021) to potentially accelerate degradation may help manufacturers understand the stability of the product.

Vaccine products, like all pharmaceutical products, should be evaluated to define suitable conditions for storage (21 CFR §610.50 and §610.53). General principles of stability testing for biological products are described in *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* ⟨1049⟩. Typically, these concerns are focused on the final vaccine product, but evaluations are also needed for bulk intermediates to justify the conditions under which they are held. In both cases, manufacturers define in advance the conditions to which the product will be exposed (e.g., temperature, light, and humidity) and the time range during which the product will be exposed to those conditions. Stability studies should evaluate all storage conditions to which the product or intermediate is likely to be exposed during production, handling, shipping, and storage so that appropriate time limits can be placed on the exposure to those conditions.

The primary criteria for defining the storage conditions for these intermediates and the final products are generally focused on acceptable maintenance of potency; however, as discussed below, there often are other attributes that need to be considered.

Evaluation of the stability of vaccine products has three general purposes. First, the products are shown to maintain an acceptable analytical profile throughout manufacture and use to preserve safety and effectiveness. Second, stability studies across several product batches provide an effective way to characterize the inherent properties of the product. This in turn leads to the third use, demonstrating manufacturing consistency in the product. ▲Higher-temperature stability studies are usually performed to generate data in support of excursions and investigations and are not expected to be performed on every batch that is placed on stability.▲ (USP 1-May-2021)

Stability Protocols

The overall experimental plan for evaluating the stability profile of a given set of product or intermediate batches typically specifically defines the conditions under which the samples will be stored, why these conditions are relevant, the length of time

the samples will be stored at each condition, when samples will be tested during this time course, and the analytical measurements at each time point. Additionally, these stability protocols itemize the analytical procedures to be used. For stability studies that occur early in product development, the studies may be conducted to confirm the suitability of the product formulation and/or storage conditions. Later in development, stability studies are typically conducted to provide data supporting the product ▲expiry dating▲ (USP 1-May-2021) or intermediate hold time, to provide more elaborate product characterization, and to evaluate manufacturing consistency. These latter studies define the product end–expiry specifications that allow definitions of acceptable and unacceptable product. Unacceptable product is defined as product that is no longer acceptable for use in clinical studies or for commercial use (e.g., because of degradation or loss of potency). Stability studies should be conducted over a duration sufficient to determine the point of loss of acceptable potency or other relevant parameters.

Analytical Measurements

Manufacturers should consider the rigor of the analytical method(s) used to evaluate the stability of complex products and improve their understanding of the parameters that are critical to immunogenicity (including stability-indicator parameters). Selection of the stability-indicator parameters varies with the unique characteristics of each vaccine.

The primary parameter that reflects stability for most vaccines is the potency assay (see *Potency Tests* below). This assay can take many forms, depending on individual vaccines (e.g., an infectivity assay for a live virus vaccine or a measure of the ▲quantity▲ (USP 1-May-2021) of conjugated ▲saccharide in a glycoconjugate▲ (USP 1-May-2021) vaccine). The potency assay is generally the key analytical result predicting whether a vaccine will remain suitable for use and whether it will produce the expected clinical response. Other analytical measurements can provide important supplemental data, particularly those that have a clear link to the potency of the product. Examples include the degradation profile, dissociation of a carrier protein from conjugated vaccines, and dissociation of an adjuvant from an antigen complex. Additionally, other common assays typically are performed as part of the stability study and may address physical or chemical changes in the product that may or may not affect its potency (e.g., general safety, degree of aggregation, pH, moisture, container, preservative, and enclosure).

Formal Evaluation of Stability Data and Product Expiry Dating

Vaccines must remain within potency specifications at the expiration date, provided that the product was stored under the normal conditions specified. Manufacturers should conduct stability studies to determine those storage conditions and that ▲expiry dating▲ (USP 1-May-2021) to demonstrate that the product remains within the potency specifications. Manufacturers should conduct stability studies on a continuing basis. If a major manufacturing process changes, additional stability studies should be conducted to verify that there is no adverse impact on the stability profile. ▲▲ (USP 1-May-2021) An accelerated study involving temperatures both higher and lower than routine can evaluate the impact of temperature excursions on products. A similar evaluation should be done for product intermediates to establish how long a given intermediate can be held under defined conditions before it is processed further or discarded.

NOMENCLATURE

There are no uniform systems for naming new vaccines. 21 CFR §299 describes the cooperation of the FDA and the US Adopted Names Council (USAN) in naming drugs, including vaccines. USAN is a private organization sponsored by the American Medical Association, USP, and the American Pharmacists Association. Section 262 in Title 42 of the Public Health Service Act requires that each package of the biological product be plainly marked with the proper name (name designated in the license 21 CFR §600.3) of the biological product contained in the package.

Add the following:

▲CONTAINER

The final container for the drug product is typically a vial with a rubber stopper and seal, or a prefilled syringe with a glass barrel and rubber plunger. Adsorption of the drug product on the container is prevented by selecting appropriate liquid formulation components, which may include proteins or mild non-ionic detergents; they also prevent virus aggregation and promote a more consistent product. Plastic containers have been introduced recently. Stopper and plunger components are typically butyl rubber compounds, which can be polytetrafluoroethylene- or silicone-coated to minimize vaccine adsorption, leaching of extractable components, or both. Sterilization methods should be characterized to ensure that they do not alter the container material properties with respect to vaccine interactions; for example, moisture absorbed during steam sterilization may add moisture over time to lyophilized vaccines, and ionizing radiation may alter the surface chemistry of polymer compounds. All containers and product-contact components should meet USP Class VI extractables testing (see *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants* ⟨1031⟩) for the formulation chemistry and sterilization methods of interest and be compliant to relevant biocompatibility testing according to the risk of the intended use.▲ (USP 1-May-2021)

Change to read:

LABELING

Vaccine product labeling is regulated in compliance with 21 CFR §201 and §610. Requirements are set for container labeling and package labeling.

Container Label

Provisions are made for the following labels:

- Full label
- Partial label
- No label on the container itself when the containers cannot support a label that includes all required information; such products should be placed in a package that does include all required information

The label should be affixed to the container in a manner that allows visual inspection of the contents for the full length or circumference of the container. If no package exists, the container bears all of the information required for the package label.

The full container label normally contains the following:

- Proper name of the product
- Name, address, and license number of the manufacturer
- Lot number or other lot identification
- Expiration date
- Recommended individual dose, for multiple-dose containers
- The phrase "Rx only" for prescription biologicals
- ▲Storage conditions
- Instructions related to resuspension or shaking, if appropriate▲ (USP 1-May-2021)
- Any applicable cautionary statements.

Package Label

In addition to the information required on the container label, the package label should describe the following:

- Any preservative used and its concentration, or the words "no preservative" if no preservative is used and its absence is a safety factor
- Number of containers, if more than one; or amount of product in the container, expressed as number of doses, volume, units of potency, weight, and equivalent volume (for dried product to be reconstituted); or a combination of the above to provide an accurate description of the contents, as applicable
- Recommended storage temperature
- The words "shake well", "do not freeze", or the equivalent, as well as other instructions when indicated by the character of the product
- Recommended individual dose, for multiple-dose containers;
- Recommended route of administration, or reference to such directions in an enclosed circular
- Presence of known sensitizing substances;
- Type of antibiotics added during manufacture and the amount calculated to remain in the final product
- Inactive ingredients, when they constitute a safety factor or are referenced to an enclosed circular
- Adjuvant, if present
- Source of the product, when this may be a factor in safe administration
- Identity of each microorganism used in manufacture and, if applicable, the production medium and the method of inactivation or reference to an enclosed circular
- Minimum potency in terms of official standard of potency, or the words "no US standard of potency".

Prescribing Information

Detailed information about a vaccine appears in its prescribing information, commonly called the package insert. Increasingly, vaccines are distributed with patient package inserts written in lay language. Prescribing information (21 CFR §201.56 and §201.57) includes the following:

- Highlights of prescribing information
- Product names, other required information
- Boxed warning
- Recent major changes
    - Indications and usage
    - Dosage and administration
    - Dosage forms and strengths
    - Contraindications

- Warnings and precautions
- Adverse reactions
- Drug interactions
- Use in specific populations (e.g., pregnancy, nursing mothers, pediatric, geriatric)
- Drug abuse and dependence
- Overdosage
- Description
- Clinical pharmacology
- Nonclinical toxicology
- Clinical studies
- References
- How supplied/storage and handling
- Patient counseling information

Change to read:

LOT RELEASE TESTING

General Principles

▲Drug product is defined in 21 CFR §210.3(b)(4) as "a finished dosage form, for example, tablet, capsule, solution, etc., that contains an active drug ingredient generally, but not necessarily, in association with inactive ingredients. The term also includes a finished dosage form that does not contain an active ingredient but is intended to be used as placebo." In compliance with 21 CFR §600, *Biological products: general*, and in particular, 21 CFR §601.2, *Applications for biologics licenses; procedures for filing*, the manufacturer "shall submit data derived from nonclinical laboratory and clinical studies which demonstrate that the manufactured product meets prescribed requirements of safety, purity, and potency…" Documentation of the vaccine drug product includes the following: components used in manufacture; composition of the drug product; specification for each component; manufacturing and packaging procedure and in-process controls for the drug product; and specifications necessary to ensure identity, strength, quality, purity, and potency in accordance with 21 CFR §211, *Current good manufacturing practice for finished pharmaceuticals*.▲ (USP 1-May-2021)

Manufacturers perform all appropriate tests for the licensed specifications for the product, according to 21 CFR §610.1 and §610.2. ▲Some tests that are performed on the final bulk need not be repeated on filled vials (e.g., when one final bulk is used to fill multiple batches of filled vials).▲ (USP 1-May-2021) Samples of each licensed lot and protocols ▲for vaccines▲ (USP 1-May-2021) containing ▲all appropriate tests from▲ (USP 1-May-2021) the manufacturers' test results are submitted to the FDA ▲for review, testing, or both.▲ (USP 1-May-2021) After the FDA evaluates the protocol to ensure that the product specifications are met, and after satisfactory confirmatory testing, the FDA approves the release of the lot if all tests meet the standards of safety, purity, and potency established for the particular vaccine product. After approval is granted, the manufacturer distributes and markets ▲that lot of product. The lot release protocol for each vaccine includes the specific potency test, as well as common tests, such as the visual inspection of final vials, and safety, sterility, and purity testing for each lot.▲ (USP 1-May-2021)

Guidelines are available regarding alternatives to lot release and a surveillance system. All of these variations are subject to the regulations in 21 CFR §610.2 that allow the FDA to require that samples of any lot of licensed product (e.g., vaccine), together with the protocols showing results of applicable tests, be sent to the FDA.

Common Tests

The tests common to all lots of all products include tests for potency, general safety, sterility, purity, identity, and constituent materials. The manufacturer completes these tests for conformity with standards applicable to each product. The results of all tests are considered, except when a test has been invalidated as a result of causes unrelated to the product (21 CFR §610.1).

POTENCY TESTS (VACCINE-SPECIFIC)

The basic definition and requirements for vaccine potency and potency assays are provided in 21 CFR §600.3 and §610.10. A vaccine potency assay should indicate the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data. Potency may be expressed in terms of units by reference to a standard ▲("relative potency").▲ (USP 1-May-2021) Product potency tests vary with vaccine product types (e.g., viral, bacterial, live attenuated, inactivated, or polysaccharide). As a result, potency assays for vaccines ▲are based on a wide range of analytical approaches.▲ (USP 1-May-2021) In vitro potency tests for live virus may include plaque formation assays, endpoint dilution assays [e.g., the tissue culture infective dose ($TCID_{50}$)], virus neutralization assays, or quantitative polymerase chain reaction (PCR) assays. Quantitative colony formation assays are used for live attenuated bacterial vaccines. Animal challenge tests for immunogenicity assays of potency, such as those for diphtheria and tetanus (US Department of Health, Education, and Welfare, 1953; see ▲*Appendix*),▲ (USP 1-May-2021) or rabies and anthrax show in vivo response. ▲Antigen quantification▲ (USP 1-May-2021) assays use enzyme-linked immunosorbent assays (ELISA; e.g., with hepatitis A) or rate nephelometry and rocket immunoelectrophoresis (e.g., with pneumococcal polysaccharides). The potency tests for bacterial vaccines, such as the meningococcal polysaccharides, pneumococcal polysaccharides, or *Haemophilus b* protein conjugate vaccines use chemical and physical chemical assays. In the case of pure polysaccharide vaccines, the concentration or quantity of the vaccine component (polysaccharide) and its quality (e.g., size) have been shown to be indicative of the human immune response.

Assay precision and reproducibility vary with the different methodologies that are used in potency assays, ranging from the high accuracy and precision of chemical tests at one end of the spectrum to bioassays at the other end. *Design and Analysis of Biological Assays* ⟨111⟩ provides guidance for bioassays and applies to vaccine potency assays. Other tests should be validated as described in *Validation of Compendial Procedures* ⟨1225⟩.

RELEASE TESTS

Official release of vaccines by the vaccine regulatory authority may be based on either the bulk or the final container. It is highly desirable to perform potency tests on the final container. However, under certain circumstances this may not be practical or even possible ▲(e.g., for some combination or multivalent vaccines);▲ (USP 1-May-2021) thus, a case-by-case approach ▲is sometimes▲ (USP 1-May-2021) required. The choice of whether to test the bulk or the final container derives from a number of considerations, such as the quantity of vaccine available for tests at the different manufacturing stages. For certain vaccines, both bulk and final container receive official release.▲▲ (USP 1-May-2021)

GENERAL SAFETY

For biological products that are intended for administration to humans, manufacturers perform a general safety test in order to detect any extraneous toxic contaminants. Procedures and exceptions are specified in 21 CFR §610.11.

STERILITY

A sterility test of each lot of each product is conducted according to procedures described in ⟨71⟩ and 21 CFR §610.12 for ▲▲ (USP 1-May-2021) bulk and▲/or▲ (USP 1-May-2021) final container material▲, as appropriate.▲ (USP 1-May-2021)

BACTERIAL ENDOTOXINS

Each lot of final containers of a vaccine intended for use by injection is tested for bacterial endotoxins ▲when appropriate,▲ (USP 1-May-2021) as indicated in ⟨85⟩.

PURITY

Vaccines need to be ▲as▲ (USP 1-May-2021) free of extraneous material ▲as possible.▲ (USP 1-May-2021) Approved vaccine license applications indicate extraneous materials that are unavoidable in the manufacturing process for a specific product. The application may indicate test results and allowable limits for such materials, according to procedures described in 21 CFR §610.13.

RESIDUAL MOISTURE

Each lot of dried product is tested for residual moisture [see 21 CFR §610.13 (a), ⟨731⟩, and FDA's *Guideline for the Determination of Residual Moisture in Dried Biological Products* (see ▲*Appendix*)].▲ (USP 1-May-2021)

PYROGENS

Each lot of final containers of a vaccine intended for use by injection is tested for pyrogenic substances, as indicated in ⟨151⟩ ▲as appropriate,▲ (USP 1-May-2021) and 21 CFR §610.13(b).

IDENTITY

The contents of a final container of each filling of each lot are tested for identity after labeling is completed. Identity is established by the physical or chemical characteristics of the vaccine, inspection by macroscopic or microscopic methods, specific cultural tests, or in vivo or in vitro immunological tests. In large part, identity tests are performed to distinguish the subject vaccine from other materials manufactured at the same site (21 CFR §610.14).

CONSTITUENT MATERIALS

Ingredients, preservatives, diluents, adjuvants, extraneous protein, cell culture-produced vaccines, and antibiotics are tested according to 21 CFR §610.15.

Permissible Combinations

Formulations that combine several vaccines must be licensed as combinations (21 CFR §610.17). The potency of each vaccine in the combination is individually tested and must meet the specifications in the context of the final combined product; other appropriate quality tests also apply. For vaccines that are physically combined in clinical locations just before administration to a patient, prescribing information should describe specific procedures to follow in those settings.

Quality

In general, quality control systems for vaccine manufacture are identical to those routinely employed for the production of other pharmaceuticals. These include raw material testing and release, manufacturing, process-control documentation, and

aseptic processing. Manufacturers formally assign responsibility to designated staff for maintaining the continued safety, purity, and potency of the product and for ensuring compliance with applicable product and establishment standards, along with compliance with cGMPs. Analysts use reference standards and validated methods to determine active ingredients, residuals, and impurities. Manufacturers determine product safety in a variety of ways that may include the use of experimental animals, procedures to demonstrate product sterility, and tests to ensure product potency. The complexity of the quality control systems for vaccines lies in the variety of methods used to produce and control production. Lot release testing proceeds according to 21 CFR §610.2 and involves evaluating lots for safety, purity, and potency before release. Manufacturers follow FDA and applicable international standards for testing and validation. The basic considerations for validation are included in ⟨1225⟩, in addition to guidance documents issued by the FDA and the ICH (see ▲*Appendix*).▲ (USP 1-May-2021)

Alternative Tests

Modification of test methods or manufacturing processes as licensed may be permitted if the regulatory authority can be assured that the modifications cause no reduction in the safety, purity, potency, and effectiveness of the biological product. It may be necessary for the manufacturer to file the proposed changes prior to implementation (21 CFR §601.12 and 21 CFR §610.9).

Add the following:

▲RETENTION SAMPLES AND OTHER REQUIREMENTS

Retention samples are held by the manufacturer for at least 6 months after the expiration date. An adequate quantity of material from each lot of each product is held for examination and testing for safety and potency (see 21 CFR §600.13).

Records are maintained concurrently with each step in the manufacture and distribution of the product such that at any time successive steps of manufacture and distribution may be traced (see 21 CFR §600.12).

For storage conditions, see 21 CFR §610.50 and §610.53.

For shelf life/expiry date, see 21 CFR §610.50 and §610.53.▲ (USP 1-May-2021)

Change to read:

GLOSSARY

Acceptance criteria: The product specifications and acceptance or rejection criteria, with an associated sampling plan, necessary for making a decision to accept or reject a lot or batch (or any other convenient subgroups of manufactured units).

▲Adjuvant: A component added to a vaccine which, although not pharmacologically active in its own right, enhances or modifies the patient's response to the vaccine immunogens.▲ (USP 1-May-2021)

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

▲Ancillary raw material: Ancillary raw materials are components or reagents used during the manufacture of vaccines and biologicals that exert an effect on the product but are not intended to be part of the final product.▲ (USP 1-May-2021)

Batch: A specific quantity of a drug or other material intended to have uniform character and quality, within specified limits, and produced according to a single manufacturing order during the same cycle of manufacture.

Biological product: Any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man.

▲▲ (USP 1-May-2021)

Characterization: Determination of the properties of a substance.

Component: Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product.

Container (also final container)**:** The immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.

Control: Having responsibility for maintaining the continued safety, purity, and potency of the product and for compliance with applicable product and establishment standards, and for compliance with current GMPs.

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) to perform quality control tests on cells that have not been exposed to the vaccine virus (which may interfere with some tests).

▲Expiry dating:▲ (USP 1-May-2021) The period beyond which the product cannot be expected beyond reasonable doubt to yield its specific results.

▲▲ (USP 1-May-2021)

Drug product: A finished dosage form (e.g., solution, suspension) that contains an active drug ingredient generally in association with inactive ingredients ▲in the final container–closure.▲ (USP 1-May-2021)

▲Excipient: Excipients are substances other than the active pharmaceutical ingredients (API) that are appropriately evaluated for safety and enable the drug substance to be delivered to the patient in the right form and supports the way and place of action without being active themselves.▲ (USP 1-May-2021)

Expiration date: The calendar month and year, and where applicable, the day and hour, that the ▲expiry dating▲ (USP 1-May-2021) ends.

Filling: A group of final containers, identical in all respects, that have been filled with the same product from the same bulk lot without any change that will affect the integrity of the filling assembly.

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

Free of and freedom from: For a substance to be considered free of a contaminant, an assay must demonstrate that a defined quantity of the substance is negative for that contaminant to a defined level of sensitivity. The level of assay sensitivity is defined by the choice of assay and can be determined experimentally using standardized reagents. Alternatively, a validated process that is known to remove a contaminant to a defined level may be used to demonstrate freedom from that contaminant.

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

Inactive ingredient: Any component other than an active ingredient ▲or an adjuvant.▲ (USP 1-May-2021)

In-process material: Any material fabricated, compounded, blended, or derived by chemical reaction that is produced for, and used in, the preparation of the drug product.

Intermediates: Unformulated active ingredients that are processed before final formulation and can be stored ▲▲ (USP 1-May-2021) before further processing.

Label: Any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.

▲▲ (USP 1-May-2021)

Lot: A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.

Lot number, control number, or batch number: Any distinctive combination of letters, numbers, or symbols, or any combination thereof, from which the complete history of the manufacture, processing, packing, holding, and distribution of a batch or lot of drug product or other material can be determined.

Manufacture: All steps in the propagation or manufacture and preparation of products including, but not limited to, filling, testing, labeling, packaging, quality control, and storage by the manufacturer.

Manufacturer: Any legal person or entity engaged in the manufacture of a product subject to license under the Public Health Service (PHS) Act. Also includes any legal person or entity who is an applicant for a license where the applicant assumes responsibility for compliance with the applicable product and establishment standards.

▲▲ (USP 1-May-2021)

Master virus seed: A viral seed of a selected vaccine virus from which all future vaccine production will be derived, either directly or via working virus seeds.

▲▲ (USP 1-May-2021)

Package: The immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package is used, the container will be deemed to be the package.

▲▲ (USP 1-May-2021)

Potency: The therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data. Potency may be expressed in terms of units by reference to a standard.

▲▲ (USP 1-May-2021)

Process: A manufacturing step performed on the product itself that may affect its safety, purity, or potency, in contrast to manufacturing steps that do not intrinsically affect the safety, purity, or potency of the product.

Proper name: The name, designated in the license, to be used on each package of the product.

Purity: Relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity includes, but is not limited to, relative freedom from residual moisture or other volatile substances and pyrogenic substances.

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

▲Residual impurity: Residual impurities are unwanted chemicals or biologicals present in traces in the final product and arise from a normal manufacturing process. They have no therapeutic value and are potentially harmful. Therefore, they need to be controlled during the manufacturing process.▲ (USP 1-May-2021)

Safety: The relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.

Specification: The quality standard (i.e., tests, analytical procedures, acceptance criteria) provided in an approved application to confirm the quality of products, intermediates, raw materials, reagents, components, in-process materials, container–closure systems, and other materials used in the production of a product.

Standards: Specifications and procedures applicable to an establishment or to the manufacture or release of products, which are prescribed in this chapter or established in the biologics license application and designed to ensure the continued safety, purity, and potency of such products.

Sterility: Freedom from viable contaminating microorganisms, as determined by tests prescribed by the FDA.

▲▲ (USP 1-May-2021)

Unacceptable product: Product that is no longer acceptable for use in clinical studies or for commercial use (e.g., because of degradation or loss of potency).

Validation: 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 the extent to which a process meets the requirements for the various performance characteristics and the demonstration that the process uniformly performs to defined characteristics. Validation is generally performed in accordance with ⟨1225⟩ and the relevant ICH guidelines.

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 (not necessarily clonal) derived from a single culture process, aliquoted into appropriate storage containers, and stored under appropriate conditions.

▲▲ (USP 1-May-2021)

Working virus seed: A viral seed derived by propagation of virus from the master virus seed under defined conditions and used to initiate production cell cultures lot-by-lot.

Change to read:

▲APPENDIX▲ (USP 1-MAY-2021)

Selected Regulatory Documents

- ▲Food and Drug Administration. US Code of Federal Regulations.
  - 21 CFR §201 (Labeling)
  - 21 CFR §299 (Drugs; official names and established names)
  - 21 CFR §600 (Biological products: general)
  - 21 CFR §610 (General biological products standards)
  - 42 USC §262 (Regulation of biological products)▲ (USP 1-May-2021)
- Food and Drug Administration. Guidance for industry. Revised preventive measures to reduce the possible risk of transmission of Creutzfeldt-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (vCJD) by blood and blood products. Rockville, MD: Food and Drug Administration; January 2002. ▲https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM307137.pdf.▲ (USP 1-May-2021)
- Food and Drug Administration. Guideline for the determination of residual moisture in dried biological products; January 1990.▲▲ (USP 1-May-2021)
- Food and Drug Administration. Guidance on alternatives to lot release for licensed biological products. *Federal Register*. 1993;58(137):38771–38773. http://www.fda.gov.
- Food and Drug Administration. Guidance for industry. Characterization and qualification of cell substrates and other biological starting materials used in the production of viral vaccines for the prevention and treatment of infectious diseases. Rockville, MD: Food and Drug Administration; September 2006. ▲https://www.fda.gov/regulatory-information/search-fda-guidance-documents/characterization-and-qualification-cell-substrates-and-other-biological-materials-used-production.▲ (USP 1-May-2021)
- Department of Health, Education, and Welfare (now the National Institutes of Health). *Minimum Requirements for Immune Serum Globulin (Human).* 3rd rev. Bethesda, MD: Department of Health, Education, and Welfare, 1953.
- International Council for Harmonisation. Q2(R1) Validation of analytical procedures: text and methodology. ▲Geneva, Switzerland: ICH, 2005. http://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html.
- Food and Drug Administration. Guidance for industry. Pyrogen and endotoxins testing: questions and answers. Rockville, MD: Food and Drug Administration; June 2012.
- Food and Drug Administration. Guidance for industry: sterile drug products produced by aseptic processing—current good manufacturing practice. Rockville, MD: Food and Drug Administration; 2004. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM310098.pdf.
- Food and Drug Administration. Guidance, compliance & regulatory information (biologics). https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm.
- Food and Drug Administration. Vaccine and related biological product guidances. https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/vaccine-and-related-biological-product-guidances.
- Food and Drug Administration. Vaccines listed for use in the United States. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm.
- International Council for Harmonisation (ICH) website. http://www.ich.org.▲ (USP 1-May-2021)