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4 Vaccine manufacturing

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The vast majority of the more than 1 billion doses of vaccines manufactured worldwide each year are given to perfectly healthy people.[1–4] It is this fact that drives the requirements for vaccines to be among the most rigorously designed, monitored, and compliant products manufactured today. The ability to manufacture these vaccines safely and consistently is built on four competencies:

1. the manufacturing process that defines how the product is made;
2. the compliance of the organization to successfully complete that process;
3. the testing of the product and supporting operations; and
4. the regulatory authorization to release and distribute the product.

This chapter examines how each of these components is established during the development of a new vaccine and how the field of vaccine manufacturing is responding to emerging challenges for increased capacity (eg, pandemic influenza), increased safety assurance (eg, barrier isolator filling), and increasing complexities of manufacture (eg, conjugate vaccines). All of this must be accomplished while consistently delivering more than 1 billion doses annually at the relatively low cost of similar therapeutic products.

In the United States, vaccines are regulated as biologic products. The Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER) is responsible for regulating vaccines in the United States. Current authority for the regulation of vaccines resides primarily in Section 351 of the Public Health Service Act and specific sections of the Federal Food, Drug and Cosmetic Act.[5,6] Section 351 of the Public Health Service Act gives the federal government the authority to license biologic products and the establishments where they are produced.[7] Vaccines undergo a rigorous review of laboratory, nonclinical, and clinical data to ensure safety, efficacy, purity, and potency. Vaccines approved for marketing may also be required to undergo additional studies to further evaluate the vaccine and often to address specific questions about the vaccine's safety, effectiveness, or possible side effects.[8]

In the European Union, animal and human vaccines are regulated by the European Medicines Agency (EMA), whose main responsibility is the promotion of public and animal health. The EMA's Committee on Medicinal Products for Human Use through its Vaccine Working Party has oversight for human vaccines. Vaccines are licensed through a centralized procedure that allows for simultaneous licensure within all countries within the European Union. Human vaccines manufacturing is regulated under a Good Manufacturing Practices (GMP) Directive 200/94/EEC, Annex 16, and Annex 2.

Harmonization of licensing and regulating procedures for vaccines worldwide has obvious benefits in rapidly delivering safe and effective vaccines to the market. Impediments to harmonization include lack of standardized regulatory procedures and mutual recognition of licenses and inspections between countries and worldwide regulatory agencies. Harmonization of regulation continues to progress as joint FDA-EMA establishment inspections programs have become reality and adherence to harmonized International Conference on Harmonisation (ICH) guidance expected.

New vaccines are subjected to a well-defined regulatory process for approval. The approval process consists of four principal elements:

- Preparation of preclinical materials for proof-of-concept testing in animal models, manufacture of clinical materials according to current GMP (cGMP), and toxicology analysis in an appropriate animal system
- Submission of an investigational new drug application (IND) for submission to FDA for review
- Testing for safety and effectiveness through clinical and further nonclinical studies (phase 1 to 3 clinical studies).
- Submission of all clinical, nonclinical, and manufacturing data to the FDA and EMA in the form of a Biologics License Application (BLA) for final review and licensure.

This chapter outlines the basics of manufacturing a vaccine and a description of some examples of currently licensed products. It then moves to the regulatory requirements for vaccine manufacturing including cGMP compliance and then discusses the development of new vaccines. The final section examines the great challenges in the field to deliver a product held to an ever-increasing standard of safety while providing sufficient doses at reasonable costs for an ever-increasing number of diseases.

Manufacturing basics

The manufacture of vaccines is composed of several basic steps that result in the finished product. A summary of these steps with examples for pathogens that have a licensed vaccine is given in Table 4-1. The first step is the generation of the antigen used to induce an immune response. This step includes the generation of the pathogen itself (for subsequent inactivation or isolation of a subunit) or generation of a recombinant protein derived from the pathogen. Vaccines under development use additional methods that will be discussed later. Viruses are grown on cells, primary cells such as chicken fibroblasts (yellow fever), or they are grown on continuous cell lines such as MRC-5

Table 4-1 Examples of Licensed Vaccine Manufacturing Processes

Disease	Trade name	Generic name	Cell culture/fermentation	Isolation	Purification	Formulation	Preservative
Anthrax	BIOTHRAX	Anthrax Vaccine Adsorbed	Chemically defined protein-free media growing a microaerophilic culture of avirulent, nonencapsulated *Bacillus anthracis*	ND	Sterile filtrate of culture medium	Aluminum hydroxide	Benzethonium and formaldehyde
Haemophilus influenzae	ActHIB	*Haemophilus* b Conjugate Vaccine (Tetanus Toxoid Conjugate)	Grown of *Haemophilus influenzae* type b strain 1482 grown in a semisynthetic medium	Centrifugation	Phenol extraction and alcohol precipitation; Hib polysaccharide conjugated to tetanus toxoid	Lyophilized	None
Hepatitis A	HAVRIX	Hepatitis A Vaccine, Inactivated	Hepatitis A (strain HM175) propagated in MRC-5 human diploid cells	Cells lysed to form a suspension	Purification by ultrafiltration and gel permeation chromatography followed by formalin inactivation	Adsorbed onto aluminum hydroxide	2-phenoxy-ethanol
Hepatitis B	Recombivax HB	Hepatitis B Vaccine (recombinant)	Recombinant hepatitis B surface antigen (HBsAg) produced in yeast cells grown in a complex medium of extract of yeast, soy peptone, dextrose, amino acids, and mineral salts	Released from yeast by cell disruption	Series of chemical and physical methods (ND) followed by treatment with formaldehyde	Coprecipitation of HBsAg with amorphous aluminum hydroxy-phosphate sulfate	None
Influenza	Fluzone	Inactivated Influenza Virus Vaccine	Propagation on embryonated chicken eggs	Low-speed centrifugation and filtration	Purification/concentration on linear sucrose density gradient using continuous flow centrifugation followed by additional purification by chemical means	Phosphate-buffered saline with gelatin as stabilizer	Thimerosal in some package configurations
Japanese encephalitis	JE-VAX	Japanese Encephalitis Virus Vaccine Inactivated	Intracerebral inoculation of mice	Harvest of brain tissue/ homogenization	Centrifugation, supernatant collection followed by formaldehyde inactivation; further purification by ultracentrifugation through 40% sucrose	Lyophilized	Thimerosal
Measles, mumps, rubella, and varicella	ProQuad	Measles, Mumps, Rubella and Varicella (Oka/ Merck) Virus Vaccine Live	Measles virus propagated in chick embryo cell culture; mumps virus in chick embryo cell culture; rubella virus propagated in WI-38 human diploid lung fibroblasts; varicella virus propagated on MRC-5 cells	ND	ND	Lyophilized	None

Table 4-1 Examples of Licensed Vaccine Manufacturing Processes—cont'd

Disease	Trade name	Generic name	Cell culture/fermentation	Isolation	Purification	Formulation	Preservative
Meningococcal	Menactra	Meningococcal (groups A, C, Y, and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine	Meningococcal strains are cultured individually on Mueller-Hinton agar and grown in Watson Scherp media; *Corynebacterium diphtheriae* grown on modified Mueller and Miller medium	Extraction of polysaccharide from cell	Polysaccharide purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction, and diafiltration; diphtheria purified by ammonium sulfate fractionation and diafiltration; conjugate purified by serial diafiltration	Sodium phosphate–buffered isotonic sodium chloride	None
Pneumococcal	Prevnar	Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein)	*Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually grown on soy peptone broth; *Corynebacterium diphtheriae* strain containing CRM197 grown in casamino acids and yeast extract–based medium	Polysaccharides isolated by centrifugation; CRM197 ND	Polysaccharides purified by precipitation, ultrafiltration, and column chromatography; CRM197 purified by ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography; conjugation done by reductive amination and the conjugate purified by ultrafiltration and column chromatography	Aluminum hydroxide suspension	None
Polio	IPOL	Poliovirus Vaccine Inactivated	Types 1, 2, and 3 poliovirus individually grown in Vero cells on microcarriers using Eagle MEM modified medium supplemented with newborn calf serum	Clarification (method ND) and concentration	Purification by three chromatography steps: anion exchange, gel filtration, and anion exchange; inactivation by formalin	Medium M-199	2-phenoxy-ethanol
Rabies	RabAvert	Rabies Vaccine	Rabies virus grown in primary culture of chicken fibroblasts in synthetic cell culture medium with the addition of human albumin, polygeline, and antibiotics	Inactivated with beta-propiolactone	Purification by zonal centrifugation in a sucrose density gradient	Stabilized with buffered polygeline and potassium glutamate; lyophilized	None
Streptococcus pneumoniae	PNEUMO-VAX	Pneumococcal vaccine polyvalent	ND	ND	ND	Isotonic saline	Phenol
Typhoid fever	Vivotif	Typhoid Vaccine Live Oral Ty21a	Fermentation using medium containing a digest of yeast extract, an acid digest of casein, dextrose, and galactose	Centrifugation	ND	Enteric-coated capsule containing lyophilized product	None
Yellow fever	YF-VAX	Yellow Fever Vaccine	Strain 17D-204 of yellow fever is cultured on living avian leukosis virus-free chicken embryos	Homogenization	Centrifugation	Lyophilized product containing gelatin and sorbitol as stabilizer	None

ND, not disclosed.
Source: package inserts.

(hepatitis A). Bacterial pathogens are grown in bioreactors using medium developed to optimize the yield of the antigen while maintaining its integrity. Recombinant proteins can be manufactured in bacteria, yeast, or cell culture. The viral and bacterial seed cultures and the cell lines used for viral production are carefully controlled, stored, characterized, and, often, protected. The first step in manufacture is the establishment of a "master cell bank". This is a collection of vialed cells which form the starting material for all future production. It is extensively characterized for performance and the absence of any adventitious agents. From this bank, working cell banks are prepared which are used as the routine starting culture for production lots. The final vaccine is a direct function of its starting materials, and a change in this seed can be as complicated as initiating a new product development altogether.

The next step is to release the antigen from the substrate and isolate it from the bulk of the environment used in its growth. This can be isolation of free virus or of secreted proteins from cells or of cells containing the antigen from the spent medium. The next step is purification of the antigen. For vaccines that are composed of recombinant proteins, this step may involve many unit operations of column chromatography and ultrafiltration. For an inactivated viral vaccine, there may simply be inactivation of isolated virus with no further purification. The formulation of the vaccine is designed to maximize the stability of the vaccine while delivering it in a format that allows efficient distribution and preferred clinical delivery of the product. The formulated vaccine may include an adjuvant to enhance the immune response, stabilizers to prolong shelf life, and/or preservatives to allow multidose vials to be delivered.

Formulation consists of combining all components that constitute the final vaccine and uniformly mixing them in a single vessel (Figure 4-1). Operations are conducted in a highly controlled environment with employees wearing special protective clothing to avoid adventitious contamination of the critical work area. Control monitoring of the environment and critical surfaces is conducted during operations. Quality control (QC) testing at this stage usually consists of safety, potency, purity, sterility, and other assays specific to the product.

During this phase, individual, scrupulously cleaned, depyrogenated, single or multiple-dose containers are filled with vaccine and sealed with sterile stoppers or plungers. If the vaccine is to be lyophilized, the vial stoppers are inserted only partially to allow moisture to escape during the lyophilization process, and the vials are moved to a lyophilization chamber. All vials receive outer caps over the stopper to secure them. To preclude the introduction of extraneous viable and nonviable contamination, all filling operations must take place in a highly controlled environment where people, equipment, and components are introduced into the critical area in a controlled manner. After filling, all containers are inspected using semiautomated or automated equipment designed to detect any minute cosmetic and physical defects. As with the formulation phase of the vaccine manufacturing operation, extensive control and monitoring of the environment and critical surfaces are conducted during operations. QC testing at this stage also consists of safety, potency, purity, sterility, and other assays that may be specific to the product.

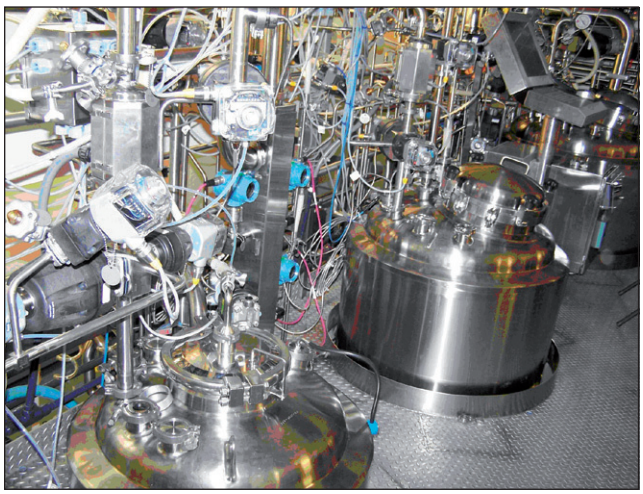

Figure 4-1 Automated vaccine formulation vessels.

Vaccine efficacy can be adversely affected by improper distribution and storage conditions. The sensitivity of vaccines to adverse environmental conditions, particularly temperature extremes, varies depending on their composition. Live attenuated vaccines tend to be more susceptible than killed vaccines and toxoids.[1] The addition of stabilizers or lyophilization, when feasible, tends to improve the thermal resistance of vaccines.

Although recommended storage conditions for many vaccines have been detailed,[9] the vaccine manufacturers are responsible for developing data before and after licensing that demonstrate the stability of their vaccines under recommended storage conditions for the claimed shelf life. Generally, these programs provide data in excess of the claimed shelf life (up to 3 years) to support the development of new products intended for clinical use, routine support of currently marketed products, expiration date extension, and supporting distribution conditions.[10,11] Accelerated studies conducted at elevated temperatures are commonly applied to better understand the impact of transient temperature excursions on the vaccine. Manufacturers are required to assure that products under their control are maintained under appropriate conditions so that the identity, strength, quality, and purity of the products are not affected.[12]

Currently, only a limited number of vaccines are required by federal regulation to have specified shipping temperatures.[10] Although most vaccine manufacturers use insulated containers and other precautions for the brief (usually 24-72 hours) shipping time, occasional, unanticipated temperature excursions may occur that could have a detrimental impact on the shipped product. Before accepting any vaccine shipment, users should look for any evidence of improper transportation conditions, including excessive transport time and possible adverse ambient temperature conditions.[1]

Examples of vaccine production

Inactivated virus (influenza)

Influenza Virus Vaccine, USP, for intramuscular use is a sterile suspension prepared from influenza viruses propagated in chicken embryos. This vaccine is the primary method for preventing influenza and its more severe complications.[13]

This vaccine contains two strains of influenza A viruses (H1N1 and H3N2) and a single influenza B virus. An additional strain of the influenza B virus has recently been added, with the first four antigen containing vaccine licensed in 2012.[13a] The two type A viruses are identified by their subtypes of hemagglutinin (HA) and neuraminidase (NA). The HA and NA glycoproteins of influenza A virus comprise the major surface proteins and the principal immunizing antigens of the virus. These proteins are inserted into the viral envelopes as spike-line projections in a ratio of about 4:1.[14]

The trivalent subunit vaccine is the predominant influenza vaccine used today. This vaccine is produced from viral strains that are identified early each year by the World Health Organization, the Centers for Disease Control and Prevention (CDC), and the CBER. For US-licensed manufacturers, the viral strains are normally acquired from the CBER or CDC. European strains are typically provided by National Institute for Biological Standards and Control and Southern Hemisphere

strains by the Therapeutic Goods Administration of Australia. These viral strains are used to prepare the inoculums for vaccine production.

The substrate most commonly used by producers of influenza vaccine is the 11-day-old embryonated chicken egg. A monovalent virus (suspension) is received from the CBER or the CDC. The monovalent virus suspension is passed in eggs. The inoculated eggs are incubated for a specific time and temperature regimen under controlled relative humidity and then harvested. The harvested allantoic fluids, which contain the live virus, are tested for infectivity, titer, specificity, and sterility. These fluids are then stored wet frozen at extremely low temperatures to maintain the stability of the monovalent seed virus (MSV).[15] This MSV is also certified by the CBER.

Once the MSV is introduced to the egg by automated inoculators, the virus is grown at incubated temperatures, and then the allantoic fluid is harvested and purified by high-speed centrifugation on a sucrose gradient or by chromatography. The purified virus is often split using a detergent before final filtration. The virus is inactivated using formaldehyde before or after the primary purification step, depending on manufacturer. This is repeated for three strains of virus, and the individually tested and released inactivated viral concentrates are combined and diluted to final vaccine strength. The overall process is outlined in Figure 4-2.

Recombinant protein (hepatitis B)

In July 1986, a recombinant hepatitis B vaccine was licensed in the United States. This vaccine built on the knowledge that heat-inactivated serum containing hepatitis B virus (HBV) and hepatitis B surface antigen (HBsAg) was not infectious, but was immunogenic and partially protective against subsequent exposure to HBV.[16] It was determined that the HBsAg was the component that conferred protection to HBV on immunization.[17] To produce this vaccine, the HBsAg or "S" gene was inserted into an expression vector that was capable of directing the synthesis of large quantities of HbsAg in *Saccharomyces cerevisiae*. The HbsAg particles expressed by and purified from the yeast cells have been demonstrated to be equivalent to the HBsAg derived from the plasma of the blood of hepatitis B chronic carriers.[16,18,19]

The recombinant *S. cerevisiae* cells expressing HBsAg are grown in stirred tank fermenters. The medium used in this process is a complex fermentation medium that consists of an extract of yeast, soy peptone, dextrose, amino acids, and mineral salts. In-process testing is conducted on the fermentation product to determine the percentage of host cells with the expression construct.[7] At the end of the fermentation process, the HBsAg is harvested by lysing the yeast cells. It is separated by hydrophobic interaction and size-exclusion chromatography. The resulting HBsAg is assembled into 22-nm-diameter lipoprotein particles. The HBsAg is purified to greater than 99% for protein by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde, sterile filtered, and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate. The vaccine contains no detectable yeast DNA but may contain not more than 1% yeast protein.[7,18,20] In a second recombinant hepatitis B vaccine, the surface antigen expressed in *S. cerevisiae* cells is purified by several physiochemical steps and formulated as a suspension of the antigen absorbed on aluminum hydroxide. The procedures used in its manufacturing result in a product that contains no more than 5% yeast protein. No substances of human origin are used in its manufacture.[19] Vaccines against hepatitis B prepared from recombinant yeast cultures are noninfectious[19] and are free of association with human blood and blood products.[18]

Each lot of hepatitis B vaccine is tested for safety, in mice and guinea pigs, and for sterility.[18] QC product testing for purity and identity includes numerous chemical, biochemical, and physical assays on the final product to assure thorough characterization and lot-to-lot consistency. Quantitative immunoassays using monoclonal antibodies can be used to measure the presence of high levels of key epitopes on the yeast-derived HbsAg. A mouse potency assay is also used to measure the immunogenicity of hepatitis B vaccines. The effective dose capable of seroconverting 50% of the mice (ED_{50}) is calculated.[20]

Hepatitis B vaccines are sterile suspensions for intramuscular injection. The vaccine is supplied in four formulations: pediatric, adolescent/high-risk infant, adult, and dialysis.

All formulations contain approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate) per milliliter of vaccine.[18]

The QC testing requirements for the release of recombinant hepatitis B vaccine are summarized in Table 4-2.

Most vaccines are still released by the CBER on a lot-by-lot basis, but for several extensively characterized vaccines, this requirement has been eliminated. They include hepatitis B vaccines and human papillomavirus (HPV) vaccines, which are manufactured using recombinant DNA processes. Their manufacturing process includes significant purification, and they are extensively characterized by their analytical methods. In addition, hepatitis B vaccine had to demonstrate a "track record" of continued safety, purity, and potency to qualify for this exemption.[7,21]

Conjugate vaccine (*Haemophilus influenzae* type b)

The production of *Haemophilus* type b conjugate includes the separate production of capsular polysaccharide from *Haemophilus influenzae* type b and a carrier protein such as tetanus protein from *Clostridium tetani* (ie, purified tetanus toxoid), CRM protein from *Corynebacterium diphtheriae*, or outer membrane protein complex of *Neisseria meningitidis*.

The capsular polysaccharide is produced in industrial bioreactors using approved seeds of *H influenzae* type b. A crude intermediate is recovered from fermentation supernatant, using a cationic detergent. The resulting material is harvested by continuous-flow centrifugation. The paste is then resuspended in buffer, and the polysaccharide is selectively dissociated from disrupted paste by increasing the ionic strength.

The polysaccharide is then further purified by phenol extraction, ultrafiltration, and ethanol precipitation. The final material is precipitated with alcohol, dried under vacuum, and stored at – 35°C for further processing.

Tetanus protein is prepared in bioreactors using approved seeds of *C tetani*. The crude toxin is recovered from the culture supernatant by continuous-flow centrifugation and diafiltration. Crude toxin is then purified by a combination of fractional ammonium sulfate precipitation and ultrafiltration.

Table 4-2 Testing Requirements for the Release of Recombinant Hepatitis B Vaccine

Type of test	Stage of production
Plasmid retention	Fermentation production
Purity and identity	Bulk-adsorbed product or nonadsorbed bulk product
Sterility	Final bulk product
Sterility	Final container
General safety	Final container
Pyrogen	Final container
Purity	Final container
Potency	Final container

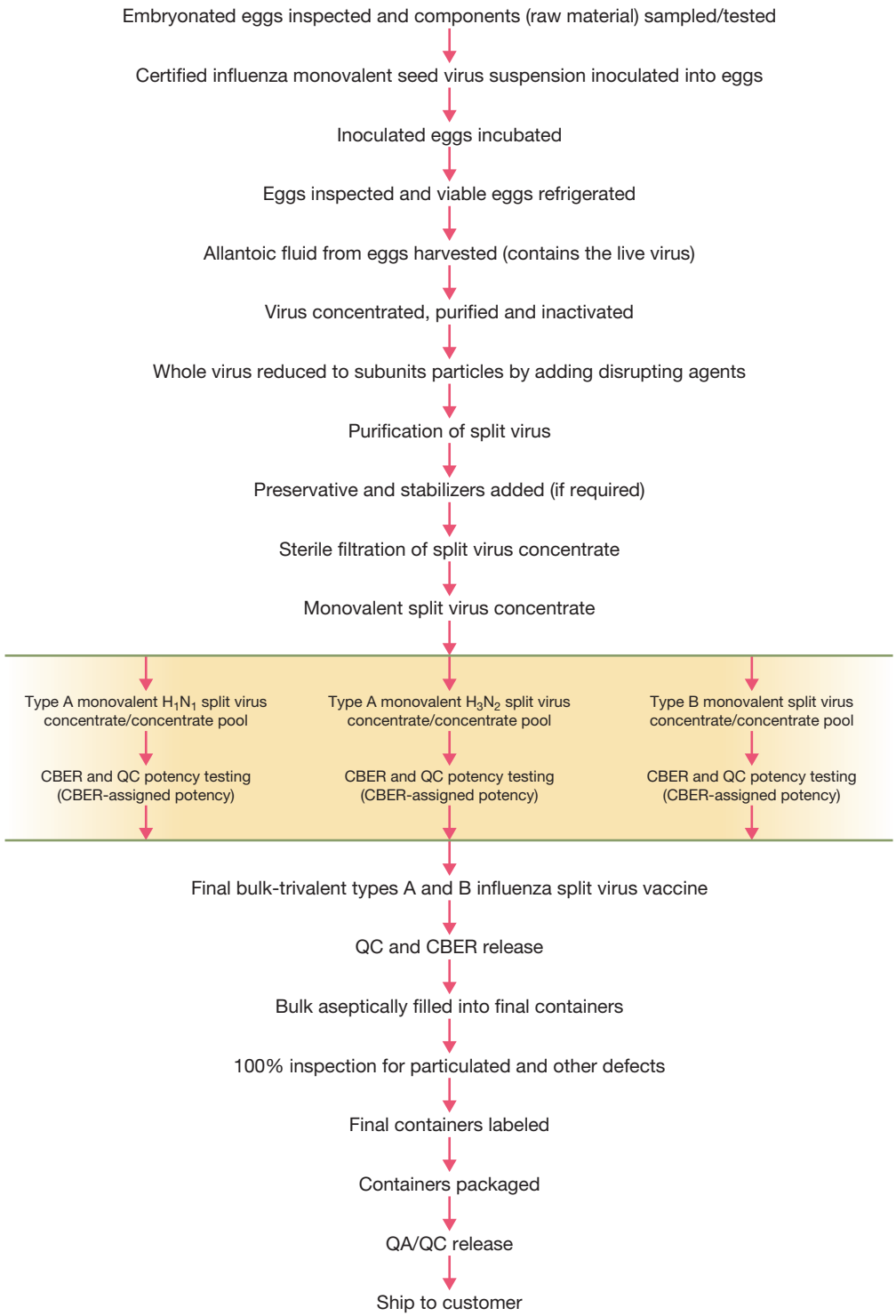

Figure 4-2 The egg-based influenza vaccine manufacturing process flow. CBER, Center for Biologics Evaluation and Research (of the US Food and Drug Administration); QA, quality assurance; QC, quality control.

The resulting purified toxin is detoxified using formaldehyde, concentrated by ultrafiltration, and stored at more than 2°C up to 8°C for further processing.

The industrial conjugation process was initially developed using tetanus toxoid by the J.B. Robbins team at the National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland.[22] Conjugate preparation is a two-step process that involves:

- activation of the Hib capsular polysaccharide and
- conjugation of activated polysaccharide to tetanus protein through a spacer.

Activation includes chemical fragmentation of the native polysaccharide to a specified molecular weight target and covalent linkage of adipic acid dihydrazide. The activated polysaccharide is then covalently linked to the purified tetanus protein by carbodiimide-mediated condensation using 1-ethyl-3(3-dimethylaminopropyl) carbodiimide. Purification of the conjugated material is performed to obtain high-molecular-weight conjugate molecules devoid of chemical residues and free protein and polysaccharide.

Conjugate bulk is then diluted in an appropriate buffer, filled into unit-dose and/or multidose vials, and lyophilized.

Live attenuated vaccine (measles)

The measles virus, isolated in 1954, is part of the genus Morbillivirus in the family Paramyxoviridae. Current vaccines are derived from Edmonston, Moraten, or Schwarz strains. Such vaccines have been on the market since the 1960s and in combination (MMR) since the 1970s. The final vaccine is a live attenuated viral vaccine inducing immunity in more than 90% of recipients.

The manufacture of measles starts with specific pathogen-free (SPF) embryonated chicken eggs that are incubated several days. The embryos are collected and treated with trypsin to prepare the chick embryo fibroblast for cell culture. All of the operations are done under strict aseptic conditions, performed by well-trained operators.

The cell culture is grown in roller bottles using fetal calf sera and M199 Hanks media for optimal cell growth. The chick embryo fibroblast cells are further infected by the viral working seed and incubated several days for viral culture. At the end of the viral culture, the cells are disrupted by mechanical lysis to release the virus. The virus is purified by centrifugation and filtration and stored frozen.

After release of all QC tests, the vaccine is formulated alone or with mumps and rubella vaccines and lyophilized to obtain the stable product. The vaccine is reconstituted just before use.

Virus-like particle–based vaccines

Traditional viral vaccines rely on attenuated virus strains or inactivation of infectious virus. Subunit vaccines based on viral proteins expressed in heterologous systems have been effective for some pathogens but have often had poor immunogenicity due to incorrect folding or modification.[23] Virus-like particles (VLPs) are designed to mimic the overall structure of virus particles and, thus, preserve the native antigenic conformation of the immunogenic proteins. VLPs have been produced for a wide range of taxonomically and structurally distinct viruses and have unique *potential* advantages in terms of safety and immunogenicity over previous approaches.[1] Attenuation or inactivation of the VLP is not required; this is particularly important as epitopes are commonly modified by inactivation treatments.[24] However, if a viral vector (eg, baculovirus) is used as the expression system, inactivation may be required if the purification process cannot eliminate residual viral activity.

For a VLP to be a realistic vaccine candidate, it needs to be produced in a safe expression system that is easy to scale up to large-scale production[1] and by an accompanying purification (and inactivation) process that will maintain native structure and immunogenicity and that will meet the requirements of today's global regulatory authorities. A number of expression systems have been demonstrated to manufacture multimeric VLPs, including the baculovirus expression system (BVES) in Sf9 and High Five cells, *Escherichia coli, Aspergillus niger*, Chinese hamster ovary, human function liver cells 4, baby hamster kidney, transgenic plants (potato, tobacco, soybean), *S. cerevisiae*, *Pichia pastoris*, human embryonic kidney 293 (HEK293), and lupin callus with yields ranging from 0.3 to 10 μg/mL or as high as 300 to 500 μg/mL with *E. coli* and HEK293 (purified).[2] The BVES has proven quite versatile, demonstrating the capability of preparing vaccine candidates for papillomavirus, feline calicivirus, hepatitis E virus, porcine parvovirus, chicken anemia virus, porcine circovirus, SV40, poliovirus, bluetongue virus, rotavirus, hepatitis C virus, human immunodeficiency virus (HIV), simian immunodeficiency virus, feline immunodeficiency virus, Newcastle disease virus, SARS coronavirus, Hantaan virus, influenza A virus, and infectious bursal disease virus.[1]

Many pathogenic viruses such as influenza, HIV, and hepatitis C are surrounded by an envelope, a membrane that consists of a lipid bilayer derived from the host cell, inserted with virus glycoprotein spikes. These proteins are targets of neutralizing antibodies and are essential components of vaccine. Owing to inherent properties of the lipid envelope, assembly of VLPs in insect cells for these viruses is a different type of technical challenge to those produced viruses with multiple capsids.[1] For these targets, production of VLPs is a challenging task because the synthesis and assembly of one or more recombinant proteins may be required. This is the case for VLPs of rotavirus (RLP), which is an RNA virus with capsids formed by 1860 monomers of four different proteins. In addition, the production of most VLPs requires the simultaneous expression and assembly of several recombinant proteins, which, for the case of RLP, needs to occur in a single host cell.[25] Purification of VLPs also constitutes a particularly challenging task. VLPs are structures of several nanometers in diameter and of molecular weights in the range of 10^6 Da. Also, for guaranteeing the quality of the product, it is not sufficient to demonstrate the absence of contaminant proteins; it is also necessary to show that proteins are correctly assembled into VLPs.

The HPV type 16 major 55-kDa capsids protein, L1, when produced in certain recombinant expression systems such as *S. cerevisiae,* can form irregularly shaped VLPs with a broad size distribution. These HPV VLPs are inherently unstable and tend to aggregate in solution. The primary challenge of HPV vaccine formulation development was the preparation of aqueous HPV VLP solutions that are stable under a variety of purification, processing, and storage conditions. By treating the HPV VLPs through a process of disassembly and reassembly, the stability and in vitro potency of the vaccine are enhanced significantly. In addition, the in vivo immunogenicity of the vaccine was also improved by as much as approximately 10-fold, as shown in mouse potency studies.[26] The disassembly and reassembly of particles may also be important to remove residual proteins from the expression system/host cell used in the production and is a serious processing challenge, particularly for enveloped VLPs.

Product development

Vaccine development involves the process of taking a new antigen or immunogen identified in the research process and developing this substance into a final vaccine that can be evaluated through preclinical and clinical studies to determine the safety and efficacy of the resultant vaccine. During this process, the product's components, in-process materials, final product specifications, and manufacturing process are defined. The manufacturing scale used during development is usually significantly smaller than that used in the final manufacturing process. Phase 1 and, sometimes, phase 2 clinical trial vaccines are typically produced in product development, but it is usually anticipated that at least one of the three or more consistency lots used for phase 3 clinical trials will be manufactured at full-scale production volume. The product manufactured during the development phase is manufactured according to cGMP.[27]

Current GMP considerations

Historically, US manufacturers were bound to the cGMP as detailed in Sections 210 through 226 and Section 600 of the US Code of Federal Regulations (CFR),[28,29] which apply specifically to approved drug and biological products. Federal regulations set forth detailed cGMP that provide principles and methods to ensure that the product meets safety requirements and the manufacturing process will consistently produce a product

that meets the specified identity, strength, quality, and purity specifications set forth for licensed vaccine products.

During the 1960s and 1970s, domestic and international drug and biologic manufacturers saw a rapid increase in laws, regulations, and guidelines for reporting and evaluating the data on the safety, quality, and efficacy of new products. The industry, at the time, was becoming more international and seeking new global markets, but the registration of vaccines remained a national responsibility. Although different regulatory systems were based on the same fundamental obligations to evaluate quality, safety, and efficacy, the detailed technical requirements had diverged to an extent that industry found it necessary to duplicate many time-consuming and expensive test procedures to market new products internationally.

The need to harmonize regulation was also fueled by concerns about rising costs of health care, escalation of the cost of research and development, and a public expectation that there be a minimum of delay in making safe and efficacious new treatments available to patients. To this end, the International Conference on Harmonization (ICH) was born in 1990.[30] This committee includes regulatory and scientific representation from the United States, Europe, and Asia and has been providing regulatory and scientific guidance and requirements for the manufacture of pharmaceuticals and biologics. These guidelines and requirements do not take the place of the codified US cGMP; however, manufacturers applying the ICH guidance will by default be in compliance with the US cGMP and international cGMP requirements. The harmonization of currently licensed and new vaccine specifications and testing requirements remains a challenge to industry and regulatory authorities.

The application of cGMP to materials produced for human clinical trials is not codified or well defined in domestic and international regulations. Although not specifically noted in the current CFR, the introduction of the cGMP in 1978 included a preamble stating that cGMP requirements are applicable to investigational materials. It is intended that manufacturers apply the cGMP where applicable and practical, taking into account the intent of the clinical trial phase and manufacturing process development. The application of the cGMP is summarized in initial IND and subsequent supplements that further build on the manufacturer's control and assurance of the safety and efficacy of the product. The European Union has issued a directive (EMEA 2003) specific to the manufacture of investigational materials, which though lacking specific detail on application of the cGMP, provides further focus on the quality of investigation supplies.[31] The directive has gone as far as to require inspection and certification of manufacturers preparing investigational materials intended for use in European clinical trials.

Clinical materials produced for phase 1 clinical trials are used to demonstrate candidate product safety in a relatively small number of healthy human patients (tens of volunteers) and to verify the ability to manufacture the product duplicating the theoretical process used to manufacture preclinical materials used in animal toxicology studies. The application of appropriate written controls, accurate and consistent data recording, and controlled equipment in the preparation and testing of even early-stage candidate vaccine materials is critical to ensuring the desired outcome and to set the foundation for subsequent development of the potential candidate vaccine.

The expectations for phase 1 cGMP applications include the following[32]:

- Personnel with adequate education, experience, and training to perform function, including cGMP training.
- Written quality unit responsibilities with appropriate training to disposition components, procedures, assays, batches, and investigation of deviations.
- Adequately designed facilities and utilities, including HVAC design to minimize contamination; appropriate water source; and quality and basic procedural controls to prevent contamination and mix-ups.
- Appropriate equipment for intended function that is properly designed, maintained, calibrated, and operated per written instructions.
- Appropriate qualification and controls to assure safety of product (eg, viral clearance, toxin inactivation).
- Written component and raw material controls including established acceptance criteria, appropriate disposition, and traceability.
- Written production records and procedures that include records of components and equipment, changes to procedures and processes, and microbial control records where appropriate.
- Aseptic processing performed under adequate conditions by trained personnel.
- Written test procedures performed under controlled conditions using scientifically sound analytical methodology with specified acceptance criteria. Laboratory instrumentation should be operated per written procedure, maintained, and calibrated.
- Qualified safety testing (eg, sterility, endotoxin).
- Packaging to protect product from contamination and controlled labeling operations designed to prevent mix-ups.
- Adequate and documented storage and shipping controls to ensure the integrity of the product.

Phase 2 clinical trials are intended to demonstrate safety and dose response in a larger target population than would be expected to receive the vaccine (hundreds of volunteers). Manufacture of phase 2 clinical trial materials will be used to develop initial consistency of product manufacture, incorporating modifications and improvements based on the phase 1 production and testing experience. Identification of key candidate process control points for monitoring and trending and evaluation of equipment and materials to assure applicability of GMP conformance is considered at this stage.

Phase 3 clinical trials are intended to demonstrate safety and efficacy in a statistically significant target population (up to tens of thousands of volunteers) and to demonstrate the ability to consistently manufacture materials meeting predescribed quality attributes. Modifications and improvements based on phase 1 and 2 production and testing experience are incorporated into the manufacturing process, and specifications for process and control points are defined. All processes and systems necessary for the manufacture and testing of late-stage clinical materials should be validated according to cGMP, essentially mimicking the requirements applied to approved products.

A risk-based approach to cGMP application consistent with the phase of development will provide for assurance and evidence of product safety and assurance that the desired target molecule has been derived from the process. These controls at minimum should include the following:

- removing potential variability surrounding the manufacturing process through validation and/or monitoring potential contributors such as the environment, utilities, and equipment;
- validating finished product safety testing, including general safety, sterility, and endotoxin;
- ensuring that process design is capable of removing undesirable contaminants from process streams through validation or testing per process step; and
- comprehensive documentation of manufacturing and testing experience.

The cGMP are detailed in Sections 210 through 226 and Section 600 of the CFR.[28,29]

Analytical testing

The analytical testing of vaccines provides evidence that the vaccine and any of its intermediates meet the specifications defined within the BLA. Safety, efficacy, and potency tests associated with a licensed vaccine are maintained within the approved filing and published in 21 CFR Part 610. In addition, the USP has prepared monographs for all approved vaccines to provide standardized requirements and continues to add monographs as new vaccines proceed to commercialization.[33] Most bulk vaccines must be tested for safety and efficacy by the manufacturer and CBER before release for final formulation and packaging. In the mid-1990s, CBER developed the concept of a *well-characterized biologic,* which was defined as a chemical entity whose identity, purity, impurities, potency, and quantity can be determined and controlled through analytical testing and control of the manufacturing process.[34] The advantage of well-characterized products is that the quantifiable analytical measurements can relate molecule structure to function. The application of this definition allowed manufacturers of biologics to eliminate the need for CBER release of biologics before distribution into the market and also allowed for process changes to take place after the product had been licensed. Current analytical and process technologies allow the application of this definition for most recombinant DNA proteins and monoclonal antibody products; however, with the exception of one currently licensed vaccine, vaccines in general do not meet the criteria for "well-characterized" owing to their complexity, size, and structure and the inability to fully characterize and quantify all analytical and biological parameters.[35] Recent vaccine development has focused on molecules that can meet criteria for well-characterized products. Chemical, microbial, and physical assays for vaccines are developed in concurrence with product and process development. Process step outputs are tested using a variety of analytical techniques designed to understand and characterize the structure of the target molecule and any associated impurities. Many of these tests are used during process validation activities to demonstrate the capability and robustness of the process.

Identity testing of biologicals includes a wide variety of tests designed to be specific to the moiety based on unique characteristics of its molecular structure or other properties. Identity testing may include one or many complementary tests including physicochemical, biological, or immunological assays. Identity tests traditionally used were relatively simple tests, but advances in analytical technologies are providing for better specificity, which, in multivalent and combination vaccines, becomes critical to ensuring that all components are adequately distinguished. One example of an advanced technology is the replacement of multiple colorimetric assays by single NMR spectral analysis for multivalent polysaccharide identification.[36] Current methods are listed in Table 4-3.

Purity tests are developed to identify potential process- or product-related impurities that may be traced to the process, equipment, or inherent product impurities. These tests may include chromatographic methods that allow for quantitation of trace amounts of impurities from raw materials or process-related impurities such as chromatographic resin leakage. Inherent or copurified protein contaminants can be detected using electrophoresis techniques that can quantify trace amounts of proteinaceous contaminants. Detection of residual DNA traditionally used spectral analysis for quantitation, although advances in polymerase chain reaction (PCR) assays are allowing even greater sensitivity.[37] Examples of current methods are listed in Table 4-4.

Microbial tests, including bioburden, sterility, endotoxin, and adventitious viral and mycoplasma agent testing, are also developed based on process capability to control contaminating microorganisms and inherent process impurities. Most vaccines are large molecules that depend on their biochemical makeup and physical configuration to provide the desired immunologic response. Unlike small molecules, which can be steam sterilized, most vaccines are prepared aseptically. Sterility testing is used for bulk vaccines and final dosage forms but still provides limited assurance of sterility because only a sample of the bulk and of a finished product lot can be tested. Current advances in sterility and bioburden testing include the use of process analysis technology and rapid micro testing sensitive down to one bacterial cell per sample. These are currently being validated and implemented to reduce current sterility testing time from 14 days down to hours. Traditionally, endotoxin or pyrogen testing has been performed using animal response to assess pyrogenicity of compounds. Reliance on the endotoxin limulus amebocyte lysate (LAL) test to assess pyrogenicity has become standard for testing new vaccines. Current testing has traditionally been performed according to the 1993 Points to Consider and ICH guidance regarding adventitious agent testing, including viral testing and mycoplasma testing. Advances in technology also involve PCR to detect the presence of contaminating adventitious agents in the process and in final formulation.

In addition to pyrogen testing, **general safety testing** (abnormal toxicity testing) is performed. This testing is required only for vaccines with product-specific safety tests and is performed in laboratory animals by injecting mice and guinea pigs and assessing the animals for distress. There is currently no alternative for abnormal toxicity testing. For example, specific

Table 4-3 Identity Test Methods

Identity tests	Current methods
Chromatographic	Gas chromatography High-performance liquid chromatography
Electrophoresis	Polyacrylamide gel electrophoresis Immunoelectrophoresis Capillary electrophoresis
Immunological	Immunodiffusion Immunoelectrophoresis Dot-blot Western blot Enzyme-linked immunosorbent assay Radioimmunoassay
Amino acid analysis Peptide mapping Enzyme activity Bioidentity/cell based N- or C-terminal sequencing	

Table 4-4 Product- and Process-Related Impurity Testing

Process-related impurity testing	Product-related impurity testing
Chromatography	Chromatography
Electrophoresis	Electrophoresis
Mass spectrometry	Mass spectrometry
Nuclear magnetic resonance	Nuclear magnetic resonance
Loss on drying	
Water determination	

safety testing in animals is used to ensure effective inactivation of exotoxins produced by host bacterial cells. Advances in replacing animal testing include use of Vero cell assays, which are extremely sensitive to toxins, and assays that measure enzymatic activity of toxins. Also, biochemical assays using fluorescent peptides mimicking natural substrate have been developed for tetanus and pertussis toxins[38] Live virus vaccines also use safety tests to assure that viruses have not reverted to wild virulent types. Many animal models are used to assess viral vaccine safety. Advances in PCR technologies provide for a sensitive alternative by assessing any mutations in the genome of the virus. Quantitation tests are performed to determine the content or mass of the target active moiety in vaccine formulations through physiochemical procedures and to detect changes in the molecule over time. Traditional tests include colorimetric and spectrophotometric assays that, in general, do not have the precision and sensitivity to detect changes in the target molecule. Separation assays, such as high-performance liquid chromatography, capillary electrophoresis, and sodium dodecyl sulfate–polyacrylamide gel electrophoresis, can indicate stability and lack of product degradation. Protein-based vaccines will routinely include a total protein test. Antigenicity provides a quantitative measure of antigen present by measuring antigen-antibody interaction. Antigen assays fall into four groups:

- radioimmunoassay,
- enzyme immunoassays,
- nonlabeled immunoassays, and
- biosensor analysis.[38]

Potency tests are used to assure that the vaccine produces the desired immunologic effect per specification. Most potency tests fall into one of four categories:

- traditional animal-based assays that measure biological response in an animal model;
- cell culture–based assays that measure biochemical and physiological response at the cellular level;
- biological activity or response induced by immunologic interactions, and
- ligand or receptor binding assays, which are based on in vivo attributes of the active molecule.[37]

Cell culture–based enzyme-linked immunosorbent assay testing using known antibody-antigen interaction continues to replace or accentuate classical animal-based models. It is desirable that the potency assay provide for the ability to detect changes in the activity of the molecule over time. The challenge for vaccine manufacturers is to develop the appropriate correlates between chemical and biological approaches to replace current animal models using a corresponding assay.[38]

Physical and chemical tests are performed on final formulations and dosage forms to characterize the material. These tests include pH, quantitation of preservatives, quantitation of adjuvants, uniformity, particulate matter, loss on drying, and residual moisture and dissolution for lyophilized final dosage forms. A subset of the release testing provides for assessment of stability to ensure that the vaccines continue to meet product specifications over time.

Industry's response to new challenges

New technologies for manufacturing and testing

As described earlier, currently licensed vaccines are manufactured as live attenuated, inactivated, purified subunit, conjugate, or recombinant protein antigens. The recent introduction of conjugate vaccines introduces greater complexity into the supply chain as many components must be manufactured separately, conjugated, purified, and then formulated into a single vaccine. Current trends in the field are aimed at enhancing the supply chain robustness (cell culture–derived flu vaccine) and developing the ability to manufacture new types of vaccine (plasmid DNA, viral vectors, peptides, live bacteria, irradiated sporozoites).

Cell culture–derived flu vaccine

The currently licensed flu vaccine is made in embryonated chicken eggs. This process requires extremely large quantities of pathogen-free eggs for the manufacturing process. New technologies are under development to use continuous cell lines for the production of influenza virus vaccine. A variety of cell lines are currently under development and promise to provide a more robust system for the manufacture of bulk influenza vaccine. Cell lines under development include MRC-5, Vero, and PER.C6.[39] These processes will eliminate the risk that an avian influenza virus could infect the flocks that produce the eggs for the current vaccine manufacturing. Such an infection would potentially eliminate the supply of the vaccine entirely. A number of cell culture–derived influenza vaccines have been approved in Europe, although not at a scale capable of providing sufficient vaccine to replace egg-derived vaccine or to respond to a pandemic event. Strong industry, academic, and government focus in recent years promises to advance the development of this technology. Manufacturers are developing processes to produce influenza vaccine using cell culture, which uses bioreactors similar to the 2,000 L model (Figure 4-3) at the Vaccine Pilot Plant of the Vaccine Research Center, NIAID, National Institutes of Health. Contrary to popular belief, there is no reduction in production cycle time for a batch of vaccine made by cell culture compared with the use of embryonated eggs, although availability of the latter can be a problem during a pandemic event. Furthermore, the limiting factor in timely supply of vaccine can be the capacity to perform the release testing, sterile filtration, and sterile filling of the vaccine.

Figure 4-3 Typical bioreactor used in vaccine manufacturing.

Plasmid DNA vaccines

Several vaccines are under development that use plasmid DNA as the delivery vehicle. Known as "genetic" vaccination, the protein antigen is delivered as DNA sequence, which is taken up by the host and expressed in vivo. This particularly provides an advantage for viral vaccines in which the host expression of the protein may provide protein in a more native conformation and, thus, a better immune response.

Manufacture of plasmid DNA vaccines is done in *E. coli* grown in large bioreactors and a variety of downstream processing unit operations.[40] The isolation of the plasmid DNA from the *E. coli* after lysis (chemical or heat) is followed by solids removal and chromatographic or selective precipitation. The plasmid must be separated from genomic DNA, host cell proteins, host cell RNA, and endotoxins. Although plasmid productivity can be relatively high, human clinical studies for prophylactic vaccines have used doses up to 8 mg of plasmid DNA, making their potential manufacture a significant economic and engineering challenge.[41]

Viral vector vaccines

Another genetic immunization strategy uses viruses to deliver genetic sequences for pathogen protein. Viral vectors include recombinant adenoviruses, poxviruses, and alphaviruses.[42] Some of the vectors are developed to be replication-incompetent, like many of the adenoviral vectors. These vectors contain deletions in the native viruses to render them replication-incompetent for safety reasons. The deletion also allows for the insertion of the gene of interest into the vector. As the vectors cannot replicate on their own, they require complementary cell lines called "packaging cell lines" that provide the missing genetic information for replication. After the vectors are expanded on the packaging cell line, the vectors must be purified to separate them from host cell protein, DNA, and RNA. Other vectors like poxvirus vectors are replication-competent and can be grown on permissive cell lines without requiring genetic modifications. Production techniques usually include cell lysis, chromatography, and ultrafiltration to isolate the purified vectors.[43]

Other novel vaccine production technologies (sporozoites for malaria)

One of the most novel vaccine production technologies recently proposed is the production of irradiated *Plasmodium falciparum* sporozoites in mosquitoes for malaria. *P. falciparum* is the pathogen responsible for malaria, and it has been reported that a vaccine containing irradiated sporozoites conveys more than 90% efficacy.[44] Current estimates are that enough irradiated sporozoites could be harvested from a single mosquito to immunize a single human.[45] One company, Sanaria, is exploring whether sufficient quantities could be grown in mosquitoes and harvested in a consistent and quality manner that would allow for licensure of this type of vaccine. Initial clinical testing showed suboptimal immunogenicity and protection when given intravenously.[46] Although certainly unconventional, the fact that malaria currently causes approximately 300 million clinical cases and 1 million deaths each year warrants further development.

Increased capacity and responsiveness (avian influenza pandemic preparedness)

One of the most recent major shifts in the industry has been around the concern of an avian influenza pandemic and preparing a strong response to reduce the impact of such a pandemic. The pandemic would be triggered by the combination of an avian influenza strain with a human strain or mutation of an avian strain allowing it to infect and spread in the human population. As humans do not have natural immunity to avian strains, even healthy adults are not expected to have the background antibody levels to fight infection. Such an event could trigger a global epidemic taking millions of lives and also taking a major toll on global economics.

As a first line of defense against the possibility of a pandemic, the government and vaccine manufacturers have teamed up to secure the supply chain for influenza vaccine, prepare and clinically test avian influenza vaccine from circulating avian strains, stockpile vaccine from circulating avian strains, and expand manufacturing and distribution infrastructure for preparation and delivery of vaccine. In addition to the fear the thought of a pandemic brings to everyone who tries to understand it, a pandemic triggers many new issues for vaccine manufacturing.

Where vaccine is made

During previous threats of pandemic influenza (eg, swine flu in 1976), the borders of countries were closed to transport of vaccines, leaving countries without manufacturing infrastructure also without vaccine, at least for an initial response. The manufacturers are not able to produce global supplies from a single location if borders are blocked, and a more capital-intensive regional approach (ie, multiple small plants) would be required.

Protection of the supply chain

The production of influenza vaccine requires many components outside the manufacturing plant itself. All components of this supply chain—eggs, vials, stoppers, reagents, and the labor to prepare and deliver these components to the manufacturer—are at risk during a pandemic. The vendors supporting the vaccine manufacturers need to be protected and prioritized. One might expect major disruptions in supply of goods during a pandemic event; some firms could close or restrict operations owing to the risk of illness or to absenteeism. Noncritical workers may be restricted from the operating site or the critical workforce sequestered to protect them from the spread of disease while they support the production of vaccine to fight the disease. This applies not only to vaccine manufacturers, but also to the industries and agencies that support them.

Securing year-round egg supply

Today's vaccines are still made in embryonated chicken eggs. Traditionally, influenza vaccine was made January through July, and chickens were replaced each year to maintain productivity and egg quality. If a pandemic were to start during the time when chickens were not producing eggs for vaccines, the response would be rather slow. Manufacturers have now established year-round egg supplies allowing a strong and instant response to a pandemic at any point in the year. (In the United States, the Department of Health and Human Services supported and funded this contingency supply.) Also, avian strains are not abnormal in the bird market in the United States. (It is the threat of human infection that is new.) Protecting the flocks from disease is always a concern and focus of manufacturers and the vendors that support them. Biosecurity measures have been in place since 1983, when a major avian outbreak destroyed the majority of egg-producing flocks established by US manufacturers. These measures have secured the supply of eggs ever since. Regardless, contingency supplies have been added to secure production quantity of eggs even if some chicken flocks are lost to avian influenza.

Impact on interpandemic production

An outbreak of avian influenza would require termination of the interpandemic production (annual flu vaccine) for up to 2 years, as excess capacity does not exist, even in support of today's growing influenza vaccine needs. Even with routine

annual vaccination, more than 200,000 people are hospitalized annually due to influenza. This number is expected to increase significantly without annual vaccination. However, the lack of inherent protection against avian strains makes this vaccine still more important than the routine influenza vaccination.

Expansion of manufacturing

The current targeted vaccination response in the United States is to prepare up to 600 million doses of monovalent avian flu vaccine within 6 months. Approximately 166–173 million trivalent doses of influenza vaccine were available in the US during the 2011–2012 flu season.[46a] To react in a more urgent manner, facilities are being built and expanded globally to support a larger, faster responsiveness with vaccine to control a pandemic outbreak. The ideal situation is one in which the interpandemic production of influenza vaccine does not have to be interrupted for a pandemic event.

Fill and finish considerations

The bottleneck for production in a pandemic event is expected to be the supply of vaccine concentrate itself, but the need to fill and finish large quantities in a short time is also not to be overlooked. To meet the 600 million dose target in 6 months, 4 million doses will need to be filled each day in addition to other products that will continue to be supplied to maintain vaccination against all disease. Supplies (and suppliers) of filling components could be impacted by a pandemic event because absenteeism is expected to be high and critical supplies could be at risk.

Distribution

As transportation is an industry expecting significant impact in case of a pandemic, vaccine distribution is not an aspect of supply to be taken for granted. An added consideration is the scarcity of vaccine in the early response period, increasing the need for security of shipments from loss due to temperature excursion or interruption of shipments because of theft.

Overall, the planning and preparation for a pandemic event is an all-encompassing exercise of every part of the vaccine business and supporting agencies. The cooperation and assistance of the governments globally has benefited all parties and will benefit consumers in the end.

Growth in emerging market manufacturing

Many countries have long had domestic vaccine production capability. Countries like China, India, Brazil, and South Korea have more recently been developing new state-of-the art manufacturing capability for the introduction of novel vaccines to domestic populations and for exporting vaccines to the international market.

The expansion in emerging market manufacturing has been driven by several key enablers. The first is the establishment of central funds for the procurement and distribution of vaccines in emerging markets. These organizations allow manufacturers to work with single-point contact to procure and coordinate distribution of vaccines to the markets they serve. Examples of such organizations are the Pan American Health Organization, which procures vaccines for much of Central and South America, and the GAVI Alliance, formerly called the Global Alliance for Vaccines and Immunization. This eliminates the need to establish separate supply chain and sales channels into these diverse markets.

Most of the vaccines exported into emerging markets use the World Health Organization (WHO) qualification process. The process established by WHO examines the quality of the vaccine and manufacturing process and, in a separate evaluation, the technical and commercial ability of the organization to manufacture the vaccine.[47] This process provides a standard regulatory framework to enable manufacturers to gain approval that is recognized in many of the emerging markets and replaces the need to gain regulatory approval in individual countries.

The global transition of manufacturing of many industrial products into lower cost geographic regions is also evident in vaccines. Most global manufacturers like GlaxoSmithKline, Sanofi Pasteur, and Merck have all established joint ventures or made acquisitions in India, China, or Brazil. These ventures have in some cases, however, run into challenges for the multinational firms. Sanofi, for example acquired Shanta Biotechnics in 2009.[48] Shanta was an India-based vaccine manufacturer supplying vaccines under the WHO prequalification program. Soon after the acquisition, however, Shanta was cited for quality problems by WHO and lost its prequalification status for one of its vaccines.[49]

Therapeutic vaccines

There has been substantial interest and development during the past decade to use vaccines to elicit the body's immune system to treat diseases after onset. These target diseases can be caused by infectious agents, cancer, or autoimmunity. Current efforts are being devoted to developing therapeutic vaccines against tumors, AIDS, hepatitis B, tuberculosis, malaria, and autoimmune diseases such as myasthenia gravis, systemic lupus erythematosus, and rheumatoid arthritis.[50]

In 2010, the FDA approved the first therapeutic cancer vaccine, sipuleucel-T (Provenge), which is developed and manufactured by Dendreon. The vaccine was approved for use in men with metastatic prostate cancer whose tumors are no longer responding to hormonal therapy and has been demonstrated to provide for more than a 4-month median improvement in overall survival compared with a placebo vaccine.[51]

The approval validates the concept of an active treatment approach such as immunotherapy, which is intended to train the immune system to attack cancer cells and potentially get a response with long-lasting effect. Though not a cure, the vaccine provides significant clinical benefit.[51]

Sipuleucel-T is customized to each patient. Before treatment, patients undergo a procedure called leukapheresis to isolate antigen-presenting cells (APCs) from their blood. These APCs include dendritic cells and macrophages, among other cells, that can "present" markers, or antigens, on their surfaces that are recognized by other immune cells, thereby sparking an immune response.[51]

The APCs are cultured with a proprietary manufactured protein. The end result is a vaccine with hundreds of millions of "activated" APCs loaded with an antigen commonly found on most prostate cancer cells, called prostatic acid phosphatase (PAP). The vaccine is returned to the patient's treating physician and infused into the patient, with the intent of spurring immune system cells, T cells, to neutralize tumor cells that express PAP.[51]

Single-use manufacturing technologies

Historically, vaccines were each manufactured in a dedicated facility to ensure segregation of each product from other products. The development of more sophisticated engineering controls for air handling, automated cleaning, and analytical testing of residual product resulted in an FDA guideline in 1994 on the use of pilot manufacturing facilities (which are multiproduct) for the launch of biologics.[52] This has resulted in a tremendous amount of innovation in technologies that can enable rapid conversion of facilities from one product to another. Typically, cells would be grown in bioreactors made of stainless steel, as shown in Figure 4-3. After use, these reactors have to be extensively cleaned

Figure 4-4 Single-use bioreactor for cell culture (courtesy GE Healthcare).

and tested to ensure they are cleaned adequately. Newer technologies use plastic bags, which are used once and then discarded. An example of this system is shown in Figure 4-4. The use of single-use technologies allows faster changeover from one batch to another and from one product to another and reduced cleaning and sterilization validation. Recent reports have shown substantial savings in the cost and lead time of new facilities and a reduction in overall operating expenses using the new technologies.[53–55]

Removal of preservatives

The elimination of preservatives is an extension of increased vaccine purity and a specific issue related to public perception of vaccine safety. Examples of preservatives used in vaccine formulation include thimerosal (a derivative of mercury), phenol, benzethonium and formaldehyde, and 2-phenoxyethanol. Benzethonium or 2-phenoxyethonol is often mixed with formaldehyde to improve effectiveness against a broader spectrum of potential contaminants. These compounds have bactericidal and/or bacteriostatic properties. Preservatives have been necessary to improve the safety of vaccines historically, as the ability to make a sterile product through the manufacturing, formulation, and filling process with legacy manufacturing processes and facilities is challenging, if not impossible, without preservatives. Furthermore, a preservative is required in a multidose container to prevent contamination of future doses during the extraction of the first doses from the vial. The risk of an infection or sepsis due to a dose from a contaminated, unpreserved vial is considered far greater than the risk of an adverse event from the preservative itself. To eliminate preservatives, the facilities and processes used to manufacture vaccines had to be overhauled.

In some cases, the preservatives are used as inactivation agents integrated in the manufacturing process and cannot be fully removed. In these cases, the levels have been significantly reduced by diafiltration but not fully eliminated.

Conversion to unit-dose presentations

A significant impact of the elimination of the preservatives was the consequential switch from multidose vials to single-dose presentations. The impact is three-fold: decreased production capacity, increased product consumption per unit, and higher consumption of storage space at the manufacturer, distributor, and physician's office.

The largest initial impact on the manufacturer of the removal of preservatives is the greater number of units that now need to be filled. As an example, Sanofi Pasteur has typically filled about 50 million doses of flu vaccine in 12 to 14 weeks in 10-dose vials on a single filling line. In all, 5 million units (10 doses each) needed to be filled, inspected, and packaged in this period. With the elimination of preservatives in influenza vaccine, 50 million units would now need to be filled in the same time, and all existing filling lines at the manufacturing site combined are not capable of filling that number of vials and/or syringes in the time required to meet the need for the influenza vaccine demand. A large investment in new lines is necessary before making the switch to unit-dose presentations. Because the same change to single dose is already underway for other products, the capacity is already consumed.

The effort necessary to produce the high volume of vaccines to satisfy the health need is increased 10-fold. Likewise, filling is not the only challenge. Every vial and syringe also needs to be inspected for product and container/closure defects, packaged with inserts into cartons, stored while awaiting regulatory release, and shipped to customers. The entire supply chain for this product is expanded 10-fold with this pending change.

A great benefit to multidose product presentations for manufacturers and consumers is the savings in product for filling. It is impossible to get the contents of a single dose vial into a syringe for administration. Therefore, manufacturers need to fill extra product that will never be administered to aid practitioners in delivering the intended dose. This is called "overfill". Overfill for a 10-dose vial is about 16% to 24% (or 0.58-0.62 mL fill volume for a 0.50-mL dose). (Some practitioners claim the ability to get 11 doses from a 10-dose vial.) When a single dose is put into a vial, a higher overfill is needed to ensure a full withdrawable dose. For unit-dose vials, overfill is 28% to 44% (or 0.64-0.72 mL per vial for a 0.5-mL dose). The conversion of a product from multidose vials to unit-dose vials results in an additional loss of product owing to overfill of 20%. (Therefore, Sanofi Pasteur's stated capacity of 50 million doses of flu vaccine is reduced to 40 million doses by this change alone.) This change was not recognized in the rush to remove preservatives from acellular pertussis vaccines and resulted in a nationwide shortage of DTaP vaccine, as manufacturers did not have time to increase capacity before the switch.

An alternative presentation, with less overfill for unit-dose unpreserved product, is the prefilled syringe. The fill volume for a prefilled syringe varies with design, but the fill volume for some commercially available designs is as low as 0.53 mL, equal to the lower milliliter per dose consumption range of 10-dose vials. Syringes are more complicated to handle on a filling line and also carry a higher per-unit cost of materials, but the savings of bulk make the change positive for manufacturers who are limited for total bulk capacity. The US vaccine market has been a largely vial market. Some products have been successfully moved to the syringe in the United States as unpreserved presentations have been developed, including tetanus-diphtheria vaccine (DECAVAC). However, total syringe filling capacity has been limited, and major expansions are underway globally for added syringe filling capacity to support the industry.

A negative impact from the conversion of products to unit-dose presentations has been one of space. The storage capacity of manufacturers, distributors, and practitioners will need to be expanded because the package for 10 unit-dose vials is more than two times larger than that for a 10-dose vial, and likewise, the space required for 10 syringes can be more than 5 times more than for a 10-dose vial. The industry is developing more compact package designs to minimize the impact of the change on consumers. The investment in new packaging equipment is large and urgent.

The issue of preservatives, and their removal has had a great cascading impact on the manufacturing and supply of vaccines and has consumed a great deal of technical and engineering effort and capital investment to resolve and to deliver existing products to consumers in new forms. The true added benefit to consumers may be difficult to measure, but the added costs and complexity to the consumer are obvious.

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