Chapter 3
Active Immunization

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Introduction

The goal of active immunization is to provide protective immunity if or when the individual is exposed to a specific pathogen. While the concept is straightforward, the process requires that the disease-specific immunogen(s) be delivered to the patient's immune system in a manner that has been optimized to achieve that goal. The immunogen(s) must be carefully selected as those capable of stimulating a protective response. They must be formulated in a manner that optimizes the quality of the protective responses. They must remain stable and sterile during any period of storage between manufacturing and use. Vaccine immunogens that are administered by the oral route must withstand passage through the low pH of the stomach, and those administered intranasally need to be formulated using a volume and consistency easily tolerated by the vaccine. Vaccine immunogens that are delivered by injection (intradermal, subcutaneous, or intramuscular) need to be prepared using volumes and viscosity that can be administered using a needle and syringe. Some require the addition of a substance called an adjuvant to enhance and modulate the immune response to the immunogen. The biologic and chemical processes needed to manufacture and purify active vaccine components are complex. Each vaccine immunogen must be produced in a highly controlled and regulated manner to ensure safety and consistency from lot to lot of the final product, year after year. The system, known as *Good Manufacturing Practice*, relies heavily on adherence to a series of detailed standard operating procedures and quality control measures. Active immunization programs, with the consistent and appropriate delivery of vaccines in our current armamentarium, are among the safest and most effective medical interventions available. Rigorous public health immunization efforts have led to the

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J. Domachowske, M. Suryadevara (eds.), *Vaccines*,
https://doi.org/10.1007/978-3-030-58414-6_3

elimination of polio and measles from the Western hemisphere, and the complete global eradication of smallpox and 2 of 3 poliovirus types that cause paralytic polio. Today, more than 30 infectious diseases are vaccine-preventable. This chapter offers a review of the basic concepts of active immunization followed by detailed descriptions for the active components included in each of the available, US-approved vaccines.

The World's First Vaccine

Smallpox was a highly contagious airborne infection once endemic to nearly all regions of the world. Outbreaks and epidemics, once impossible to control, were associated with 30% mortality. High rates of complications were seen among survivors including disfiguring facial scars, vision loss, neurologic sequelae, infertility, and pregnancy loss. Long before the discovery of vaccines, it was well recognized that survivors of smallpox would not become reinfected during subsequent community outbreaks or epidemics, even when occurring many years later. A related, but more subtle, observation was instrumental in driving the development of the smallpox vaccine. Milkmaids, especially those who had previously developed a relatively mild occupational infection from cowpox, were routinely unaffected during community outbreaks of smallpox. The British physician Edward Jenner was among those who suspected that infection with, or exposure to, the related cowpox virus was the key to this protection. In 1796, a local milkmaid named Sarah Nelmes sought treatment from Jenner for cowpox lesions that she developed on her hand. Jenner collected material from the cowpox lesions and used it to inoculate his gardener's 8-year-old son, James Phipps. James, as anticipated, developed a cowpox lesion at the inoculation site on his arm. The lesion and associated low-grade fever both resolved without treatment after a few days. Two months later, Jenner challenged James with material he had collected from the smallpox lesions of another patient. The boy remained completely healthy after the challenge. He was immune to smallpox. In describing his work, Jenner first coined the terms "vaccine" and "vaccination," from the Latin word *vacca*, meaning cow.

Beyond Smallpox Vaccine: The Jennerian Approach

The Jennerian approach of using a replication competent agent to induce protective immunity to a related pathogen has led to the development of at least 17 live and live attenuated viral and bacterial vaccines that remain in widespread use today. As such, more than half of the vaccines that are currently available in the USA were born from vaccine theory in alignment with the original and highly successful Jennerian approach.

An almost equal number of vaccines currently available are based on alternative, non-Jennerian approaches. Inactivated whole virus vaccines have proven to be a highly effective strategy for the prevention of polio, hepatitis A, influenza, rabies, tick borne encephalitis, and Japanese encephalitis. Inactivated whole cell pertussis vaccines were instrumental in reducing morbidity and mortality from whooping cough starting in the 1940s and are still used in much of the developing world. Acellular pertussis vaccines that include as many as five immunogens extracted and purified from whole bacteria have replaced whole cell vaccines in most developed countries. Several other vaccines use pathogen-specific proteins or polysaccharides that have been purified from cultures of the pathogen or generated using recombinant DNA technology as the active vaccine ingredient. In each case, the active ingredient can only perform its function as the vaccine immunogen if it is manufactured and formulated correctly.

Active Vaccine Components

This section includes descriptions for how the active ingredients in available vaccines are manufactured. Headings identify the targeted disease(s), the pathogen that causes the disease, and the immunogen(s) included in the final vaccine product. An overview of the methods used to produce each immunogen follows. Additional detail regarding the role(s) of each of the inactive vaccine ingredients used during manufacturing is discussed in the next chapter. The classification of vaccines according to the nature of the immunogen(s) used to produce them is also summarized in Table 3.1.

Disease: Respiratory infections caused by adenovirus

Pathogen(s) causing human disease: Adenoviruses types 4 and 7

Immunogen(s) used in the vaccine: Selective, live strains of adenoviruses types 4 and 7 (not attenuated)

Selected strains of adenovirus types 4 and 7 are amplified individually in cell culture using WI-38 human-diploid fibroblasts. The fibroblasts are supported in culture using medium containing glucose, mineral salts, amino acids, and vitamins that has been supplemented with fetal bovine serum. When ready for harvest, the viruses are purified using filtration, and dried by lyophilization. Replication-competent, lyophilized virus is used as the immunogen in the final vaccine product. The vaccine is provided to the end user as two tablets, one containing adenovirus type 4, the other type 7 formulated with an enteric-coating to allow passage through the stomach so that the live virus in released in the intestine.

Disease: Anthrax

Pathogen(s) causing human disease: *Bacillus anthracis*

Immunogen(s) used in the vaccine: Sterile filtrate from cultures of *Bacillus anthracis*

The immunogen included in anthrax vaccine is produced from cell-free filtrates collected from cultures of *Bacillus anthracis.* A characterized and defined strain of

Table 3.1 Classification of vaccines according to the nature of the active component Shading indicates where vaccine formulations for the prevention of the same disease are available from more than one category

Vaccines to prevent viral infections				Vaccines to prevent bacterial infections							
Whole virus			Viral products	Whole bacteria			Bacterial products				
Live	Live attenuated	Inactivated	Recombinant	Live	Live attenuated	Inactivated	Native protein		Capsular polysaccharide		Recombinant protein
							Mixed	**Toxoid**	**Native**	**Conjugated**	
Adeno	Influenza	Influenza	Influenza	BCG	Cholera	Whole cell pertussis	Acellular pertussis	Diphtheria	PPSV	PCV	Men-B
RV5	RV1	Hepatitis A	Hepatitis B		Typhoid		Anthrax[a]	Tetanus	Typhoid	HIB	Plague
Smallpox	Polio	Polio	HPV					MCV4			
	MMR	Rabies									
	Yellow fever	JEV									
	Varicella	TBE									
	Zoster		Zoster								
	Dengue[b]										
	Ebola[b]										

Adeno Adenovirus, *RV5* pentavalent rotavirus vaccine, *RV1* monovalent rotavirus, *MMR* measles, mumps, rubella, *JEV* Japanese encephalitis virus, *TBE* tick-borne encephalitis virus, *HPV* human papillomavirus, *BCG* Bacille Calmette-Guérin, *PPSV* pneumococcal polysaccharide vaccine, *PCV* pneumococcal conjugate vaccine, *MCV4* meningococcal conjugate vaccine quadrivalent A, C, Y, W-135, *Men-B* meningococcal serotype B, *HIB Haemophilus influenzae* type b

[a]As culture filtrate

[b]Live chimeric virus vaccines

Bacillus anthracis bacteria is grown under microaerophilic conditions in a chemically defined, protein-free culture medium consisting of a mixture of amino acids, vitamins, inorganic salts, and sugars. At harvest, the culture medium containing the 83 kDa protective antigen (PA) and other proteins produced by the bacterium are separated by filtration. Sterile filtrate is used as the immunogen in the final vaccine product.

Disease: Cholera

Pathogen(s) causing human disease: *Vibrio cholerae*

Immunogen(s) used in the vaccine: Live attenuated *Vibrio cholerae*

The live attenuated bacteria used as the immunizing agent in oral cholera vaccine were generated by genetically modifying the *Vibrio cholerae* serogroup O1 Inaba strain 569B. The attenuating modifications prevent the bacteria from synthesizing active cholera toxin while retaining the ability to synthesize the immunogenic, but nontoxic subunit of the protein.

The vaccine strain bacteria are grown under carefully controlled conditions in culture medium containing casamino acids, yeast extract, mineral salts, and an antifoaming agent. At harvest, the bacteria to be used as the immunogen for the final vaccine product are concentrated using ultrafiltration.

Disease: Dengue

Pathogen(s) causing human disease: Dengue viruses types 1, 2, 3, and 4

Immunogen(s) used in the vaccine: Chimeric live attenuated yellow fever-dengue viruses encoding the premembrane and envelope proteins from dengue virus types 1, 2, 3, and 4.

Each of the four viruses used in the manufacturing of the quadrivalent live attenuated dengue vaccine was produced using recombinant DNA technology. The genes encoding the premembrane and envelope proteins in yellow fever vaccine strain virus 17D204 were removed and replaced with those encoding the homologous sequences of dengue virus serotypes 1, 2, 3, and 4. Each of the four chimeric yellow fever/dengue viruses is cultured separately in Vero cells. At harvest, each of the 4 culture supernatants is purified and concentrated to produce individual lots of each of the 4 immunogens to be used in the final vaccine product. The final vaccine product is prepared from a bulk lot after the 4 immunogens are combined using the correct ratio, and a stabilizer is added.

Disease: Diphtheria

Pathogen(s) causing human disease: *Corynebacterium diphtheriae*

Immunogen(s) used in the vaccine: diphtheria toxoid

Diphtheria toxin is produced from large bacterial cultures of toxigenic *Corynebacterium diphtheriae* grown under carefully defined conditions. At harvest, toxin is concentrated from the culture medium using ultrafiltration, then purified by ammonium chloride precipitation, and dialysis. Toxin is inactivated with formaldehyde to produce the bulk lot of diphtheria toxoid for use in several different combination vaccine products.

Disease: Ebola hemorrhagic fever

Pathogen(s) causing human disease: Ebola virus

Immunogen(s) used in the vaccine: live chimeric vesicular stomatitis virus expressing ebolavirus envelope glycoprotein

The Ebola virus vaccine currently approved by the US Food and Drug Administration is a live chimeric vesicular stomatitis virus expressing the envelope glycoprotein of the Kikwit strain of *Zaire ebolavirus*. The genetically engineered recombinant virus is amplified in cell culture using Vero cells. The Vero cells are maintained in serum-free cell culture medium. When ready, virus is harvested from the culture medium. Concentrated virus is purified, and then resuspended in stabilizer solution. The final product is used to fill unit dose vials, which are stored frozen. The vaccine is preservative-free.

Disease: Meningitis and other invasive infections caused by *Haemophilus influenzae type b*

Pathogen(s) causing human disease: *Haemophilus influenzae type b*

Immunogen(s) used in the vaccine: Capsular polysaccharide polyribosyl-ribitol-phosphate conjugated to a carrier protein

The capsular polysaccharide of *Haemophilus influenzae* type b is the high molecular weight polymer polyribosyl-ribitol-phosphate (PRP). PRP is prepared from large-scale cultures of a designated strain of encapsulated *H. influenzae* type b grown in a synthetic medium. Following heat inactivation and purification, PRP is covalently linked (conjugated) to either tetanus toxoid, to form PRP-T, or to the outer membrane protein complex (OMPC) of the B11 strain of *Neisseria meningitidis* serogroup B to form PRP-OMP. PRP conjugated to diphtheria toxoid (PRP-D) is used in some parts of the world.

Disease: Viral hepatitis A

Pathogen(s) causing human disease: hepatitis A virus

Immunogen(s) used in the vaccine: inactivated hepatitis A virus

A known strain of hepatitis A virus (HM175) is amplified in MRC-5 human diploid cells. At harvest, the virus-infected cells are lysed, and then purified by ultrafiltration and chromatography. Purified virus is then inactivated with formaldehyde to generate the vaccine immunogen.

Disease: Viral hepatitis B

Pathogen(s) causing human disease: hepatitis B virus

Immunogen(s) used in the vaccine: recombinant hepatitis B surface antigen

The immunogen used in hepatitis B vaccines is hepatitis B surface antigen (HBsAg). Molecular techniques were used to clone the DNA coding sequence for HBsAg into *Saccharomyces cerevisiae* yeast. The recombinant HBsAg-expressing yeast strain is grown in large fermentation vats using a culture medium containing yeast extract, peptones, dextrose, amino acids, and mineral salts. When the culture is ready for harvest, the yeast cells are disrupted, releasing the recombinant HBsAg protein. The immunogen is purified by a series of physical and chemical methods, treated with formaldehyde, and then coprecipitated with aluminum sulfate. The lot is then used to complete production of monovalent and combination hepatitis B vaccine containing vaccine doses.

Diseases: Cancers of the anus, cervix, oropharynx, penis, vagina, and vulva; genital warts; laryngeal papillomatosis

Pathogen(s) causing human disease: High-risk oncogenic human papillomavirus types 16, 18, 31, 33, 45, 52, 58, and others. Low-risk types, including 6 and 11, cause genital warts and laryngeal papillomatosis.

Immunogen(s) used in the vaccine: Recombinant virus-like particles comprised of HPV type- 6, 11, 16, 18, 31, 33, 45, 52, and 58-specific L1 capsid proteins.

The immunogens used in HPV vaccines are recombinant major capsid (L1) proteins that self- assemble into virus-like particles as they are being produced in vitro. The 9-valent HPV vaccine currently used in the USA contains virus-like particles that express L1 proteins from HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58. Molecular techniques were used to clone each of the 9 L1 DNA-coding sequences into *Saccharomyces cerevisiae* yeast. Each of the 9 recombinant L1-expressing yeast strains is grown in large fermentation vats using a culture medium containing vitamins, amino acids, mineral salts, and carbohydrates. Each culture is harvested separately. The yeast cells are disrupted to release the recombinant viral-like particles, which are then purified by a series of chemical and physical techniques. Each of the purified immunogens is adsorbed onto amorphous aluminum hydroxyphosphate sulfate, and then combined with each of the others in defined ratios to produce the final bulk suspension product.

Disease: Seasonal influenza

Pathogen(s) causing human disease: Many different influenza A and B viruses

Immunogen(s) used in the vaccine: Standardized by quantity of measured hemagglutinin from 2 subtype strains of influenza A [A(H1N1) and A(H3N2)] and 1 or 2 lineage strains of influenza B [B(Victoria) and B(Yamagata)] viruses presented in one of the following formulations: (1) split, inactivated viruses, (2) live attenuated viruses, or (3) purified, recombinant protein.

Influenza vaccines are currently produced in trivalent and quadrivalent formulations. The immunogens used in quadrivalent influenza vaccines include 2 subtype strains of influenza A [A(H1N1) and A(H3N2)] and 2 lineage strains of influenza B [B(Victoria) and B(Yamagata)] viruses. Trivalent influenza vaccines include the same 2 subtype strains of influenza A [A(H1N1) and A(H3N2)] and 1 of the same 2 lineage strains of influenza B [B(Victoria) and B(Yamagata)] viruses. Each year, influenza vaccine formulations undergo strain modifications based on recommendations from the World Health Organization, and the US Centers for Disease Control and Prevention.

Inactivated Influenza Vaccines

Egg-based and cell-culture-based technologies are used to produce inactivated influenza vaccines. The process used to generate egg-based influenza vaccines starts with inoculating embryonated chicken eggs with each of the selected strains of influenza virus. When ready for harvest, allantoic fluid containing high

concentrations of the virus is collected. Next, the virus is inactivated by treatment with formaldehyde, then concentrated, and purified using gradient centrifugation. Virus is then disrupted with a nonionic surfactant to produce a split virus preparation. The split virus is further purified, and then resuspended in phosphate-buffered saline. Split virus preparations of appropriate strains are then combined to produce the final trivalent or quadrivalent vaccine products. Standard inactivated influenza vaccines contain 15 mcg of hemagglutinin from each of the virus strains included as immunogens. High-dose inactivated influenza vaccines contain 60 mcg of hemagglutinin from each strain.

Cell culture technology has emerged as an alternative to egg-based technology for the manufacturing of inactivated influenza vaccines. The production of cell-culture-based influenza vaccine starts with inoculating suspension cultures of Madin Darby canine kidney cells with each of the selected strains of influenza virus. At harvest, cell culture supernatant containing high concentrations of the virus is collected. Next, the virus is inactivated with ß-propiolactone, disrupted using a detergent, and then purified using chemical and mechanical techniques. As with egg-based production technology, each strain is produced and purified separately before being pooled to formulate the final trivalent or quadrivalent influenza vaccine product. Like other standard inactivated influenza vaccines, cell culture produced influenza vaccine contains 15 mcg of hemagglutinin from each of the included virus strains.

Live Attenuated Influenza Vaccine

The immunogens included in the live attenuated influenza vaccine are adapted to replicate well at 25 °C while being restricted in replication at or above human core body temperature. Each year, four reassortant influenza strains are developed for use based on vaccine strain selection for the upcoming seasonal quadrivalent influenza vaccine. Reassortant strain production starts with a master donor influenza virus that has already been engineered and characterized as cold adapted, temperature sensitive and attenuated. Gene segments that encode for the hemagglutinin and neuraminidase glycoproteins are derived from the selected, antigenically relevant pool of influenza viruses. Accordingly, each of the four viruses used as immunogens in the quadrivalent live attenuated influenza vaccine maintains the replication characteristics and phenotypic properties of the master donor virus while also expressing the hemagglutinin and neuraminidase of the wild-type viruses related to strains expected to circulate during the coming influenza season.

Embryonated chicken eggs are inoculated with each of the four reassortant influenza vaccine strains and then incubated to allow vaccine virus amplification. To harvest, the allantoic fluid is collected and purified using filtration. Next, the virus is concentrated using ultracentrifugation, and then diluted to a working concentration with a stabilizing phosphate buffer to obtain the final sucrose and potassium phosphate concentrations. The viral harvests of each of the four reassortants are then filter-sterilized. Each of the monovalent bulk preparations is tested and verified

to retain cold adaptation, temperature sensitivity, and attenuating phenotypes before being combined at the desired potency. The bulk lot of combined quadrivalent vaccine is then used to fill individual sprayers for nasal administration.

Recombinant Influenza Vaccine

The coding sequences for the hemagglutinin gene products of interest are cloned into baculovirus vectors. Each of the recombinant baculoviruses is then used to transfect Sf9 insect cells growing in a defined serum-free culture medium containing lipids, amino acids, vitamins, and mineral salts. When cultures are ready to harvest, the baculovirus-encoded hemagglutinin proteins are extracted from the insect cells with a surfactant, and then further purified using column chromatography. Each of the recombinant hemagglutinins is produced and purified separately before being pooled to formulate the final trivalent or quadrivalent influenza vaccine product. Recombinant influenza vaccine is formulated to contain 45 mcg of each hemagglutinin.

Disease: Viral encephalitis

Pathogen(s) causing human disease: Japanese encephalitis virus

Immunogen(s) used in the vaccine: Inactivated Japanese encephalitis virus

Production of inactivated Japanese encephalitis vaccine begins with inoculating Vero cell cultures with a seed stock of a known strain of Japanese encephalitis virus. Cells are incubated, allowing for virus replication. When ready for harvest, cell culture supernatants are pooled, filtered, and then concentrated. The suspension is then fractionated using sucrose density gradient centrifugation. Fractions containing the highest yield are pooled, and then treated with formaldehyde to inactivate the virus. Once the lot is brought to the specified antigen concentration used in the final product, and formulated with aluminum hydroxide, it is ready to be used to fill unit dose syringes.

Disease: Measles

Pathogen(s) causing human disease: Measles virus

Immunogen(s) used in the vaccine: live attenuated measles virus

In the USA, measles vaccine is currently only available in combination with mumps and rubella vaccines. The immunogen in measles vaccine is a live, attenuated measles virus derived from the Enders' attenuated Edmonston strain. The vaccine strain virus is propagated in cultures of primary chick embryo fibroblasts grown in a buffered salt solution supplemented with vitamins, amino acids, and fetal bovine serum and stabilized with sucrose, phosphate, glutamate, and recombinant human albumin. Neomycin is added to prevent bacterial contamination during manipulation. Harvested virus is purified, concentrated, and then brought to the concentration of virus desired in the final vaccine product. Sorbitol and hydrolyzed gelatin are added as stabilizers to complete the production of the bulk lot of monovalent measles vaccine.

Disease: Meningococcal meningitis, and other invasive infections including meningococcemia.

Pathogen(s) causing human disease: *Neisseria meningitidis*

Immunogen(s) used in the vaccine(s): Type-specific capsular polysaccharides conjugated to a carrier protein are used in serotype A, C, Y, and W135 vaccines. One serotype B vaccine uses outer membrane vesicles containing porin A in combination with recombinant factor H binding protein, *Neisseria* adhesin A, and *Neisseria* heparin-binding protein; another used four different recombinant-factor-H-binding proteins.

Worldwide, a variety of monovalent and combination meningococcal vaccines are available for protection against invasive meningococcal infection caused by different capsular serotypes of the pathogen. Currently, in the USA, quadrivalent vaccines are used to immunize against *Neisseria meningitidis* capsular types A, C, Y, and W135, while multicomponent, monovalent vaccines are used to immunize against capsular serotype B disease.

The immunogens used to manufacture quadrivalent A, C, Y, W135 vaccines are derived from the polysaccharides that make up the capsule for each of the four serotypes covalently linked (conjugated) to either diphtheria toxoid or CRM_{197} (cross-reacting material 197) protein, a nontoxic mutant of diphtheria toxin.

Known strains of *N. meningitidis* A, C, Y, and W-135 are each grown separately in defined bacterial culture media. When ready for harvest, the capsular polysaccharides are extracted using detergents and alcohols, and then separated from the bacterial cells using centrifugation.

After the polysaccharides undergo depolymerization by hydrolysis and reductive amination, they are filter-purified. The purified products are then covalently linked to either diphtheria toxoid or CRM197 protein (manufacturer dependent). The serogroup-specific glycoconjugates produced from each of the four bacterial cultures are combined in the correct ratios to produce the final quadrivalent conjugated meningococcal vaccine products.

Like *N. meningitidis* serotypes A, C, Y, and W-135, *N. meningitidis* serotype B produces a capsule that is rich in polysaccharides, but its specific biochemical structure is quite similar to human polysialic acid. The result of this molecular mimicry is that serogroup B capsular polysaccharide is poorly immunogenic because it is recognized as self. As a result, the currently available monovalent serotype B vaccines do not contain type-specific capsular polysaccharide. The two formulations of *N. meningitidis* serogroup B vaccines that are currently available are very different multicomponent products with only minimal overlap. Despite their differences in immunogens, they have been shown to be safe and effective in preventing invasive serogroup B disease. One combines four immunogens, all produced using recombinant technology. The other combines bacterial outer membrane vesicles (OMVs) with three recombinant proteins as immunogens.

The immunogens used for the production of four-protein recombinant meningococcal serogroup B vaccine include different variants of lipidated-factor-H-binding protein (fHBP). The genes for each of the four proteins were introduced into *E. coli* expression systems. Each of the four *E. coli* recombinants is grown in liquid media

under defined conditions to a specific density. The cultures are harvested, and the recombinant proteins are extracted and purified using chromatography. The four purified recombinant fHBP proteins are combined in the desired ratios in the presence of polysorbate 80 as an emulsifier, and loaded into syringes for use as individual doses.

The other available meningococcal serogroup B vaccine is produced using a single recombinant fHBP in combination with recombinant *Neisseria* adhesin A (NadA), and recombinant *Neisseria* heparin-binding antigen. The three recombinant proteins are produced from cultures of recombinant *E. coli,* and then extracted and purified using chromatography. Outer membrane vesicles are produced by culturing a characterized strain of *N. meningitidis* that expresses a known outer membrane porin protein, called PorA. At harvest, bacteria are inactivated with deoxycholate, a surfactant that also mediates the formation of OMVs. The primary immunogens included in the final vaccine include fHBP, NadA, *Neisseria* heparin-binding antigen, and PorA. The antigens are adsorbed onto aluminum hydroxide as an adjuvant.

The PorA- containing OMVs included in the final product also carry a number of lesser protein immunogens that are not clearly defined. The 3 purified recombinant immunogens are combined in the required rations with 25 mcg of the OMV preparation per dose to formulate the final vaccine product.

Disease: Mumps

Pathogen(s) causing human disease: Mumps virus

Immunogen(s) used in the vaccine: Live attenuated mumps virus

The immunogen used in mumps vaccine is the live attenuated Jeryl Lynn™ strain of mumps virus. The vaccine strain virus is propagated in cultures of primary chick embryo fibroblasts grown in a buffered salt solution supplemented with vitamins, amino acids, and fetal bovine serum and stabilized with sucrose, phosphate, glutamate, and recombinant human albumin. Neomycin is added to prevent bacterial contamination during manipulation. Harvested virus is purified, concentrated, and then brought to the concentration of virus desired in the final vaccine product. Sorbitol and hydrolyzed gelatin are added as stabilizers to complete the production of the bulk lot of monovalent mumps vaccine. In the USA, mumps vaccine is currently only available in combination with measles and rubella vaccines.

Disease: Pertussis, or whooping cough

Pathogen(s) causing human disease: *Bordetella pertussis*

Immunogen(s) used in the vaccine: Whole cell pertussis vaccines contain inactivated *Bordetella pertussis*. Acellular vaccines include inactivated pertussis toxin alone or together with one or more of the following purified native bacterial proteins: pertactin, filamentous hemagglutinin, and a mixture of fimbria agglutinogen types 2 and 3

Whole cell inactivated pertussis vaccines were introduced in the 1930s, and are still used throughout the developing world today. Acellular pertussis vaccine formulations became widely available in the early 1990s, gradually replacing the use of whole cell inactivated vaccines in most developed countries.

The manufacturing of whole cell inactivated pertussis vaccine begins with growing a characterized strain of *Bordetella pertussis* in a defined bacterial culture medium. The timing of harvest is dictated by the turbidity (opacity) of the liquid culture medium. Bacterial lots that meet quality control indices for purity and opacity are killed and detoxified using a method approved by the country's national regulatory authority, such as heat inactivation or chemical treatment with glutaraldehyde. Sterility and lack of toxicity are verified by culture and bioassay, respectively, and the final product is prepared by adjusting its opacity to a predefined optical density known to contain the desired concentration of killed bacterial cells. Whole cell pertussis vaccines are estimated to contain approximately 3000 different antigens. The number of these antigens that serve as immunogens when the vaccine is administered is unknown, but likely number in the hundreds.

Acellular pertussis vaccines used throughout the world have included between 1 and 5 of the following immunogens: pertussis toxin (inactivated), filamentous hemagglutinin, pertactin, fimbria type 2, and fimbria type 3. The manufacturing process starts with growing a characterized strain of *Bordetella pertussis* in defined bacterial culture media. Subsequent steps are carried out, as needed, depending on which of the 5 immunogens are to be included in the final vaccine product. Pertussis toxin (inactivated) and filamentous hemagglutinin are produced and released by the bacterium into the culture supernatant. Supernatant is collected and processed to concentrate them. Fimbrial agglutinogens and pertactin are extracted directly from the bacterial cells using heat and flocculation. Each of the pertussis antigens is then precipitated using ammonium chloride, and ultrafilter-purified. Filamentous hemagglutinin is treated with formaldehyde, and pertussis toxin is inactivated with glutaraldehyde. Residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate as an adjuvant, and then combined for use as a bulk stock for the production of acellular pertussis containing combinations vaccines (e.g., DTaP-HepB-IPV, DTaP-HIB-IPV) Monovalent pertussis vaccine is no longer available for use in the USA.

Disease: Plague

Pathogen(s) causing human disease: *Yersinia pestis*

Immunogen(s) used in the vaccine: Recombinant fusion product of *Yersinia pestis* F1 capsular and virulence (V) proteins expressed in *E. coli*

A vaccine for the prevention of plague, an infection caused by the bacterium *Yersinia pestis* was approved by the US Food and Drug Administration as an "orphan drug." The antigen used in the vaccine is a fused recombinant protein referred to as rF1V. The coding sequences for *Y. pestis* F1 capsular and virulence (V) proteins were cloned into *E. coli*. Recombinant *E. coli,* expressing the fused rF1V protein, are grown in a defined bacterial liquid culture medium. rF1V that is isolated and purified from the cultures is formulated with a 2% aluminum hydroxide wet gel suspension as an adjuvant to produce the immunogen for a final vaccine product.

Disease: Pneumococcal meningitis, pneumonia, bacteremia, and other forms of invasive disease.

Pathogen(s) causing human disease: *Streptococcus pneumoniae*

Immunogen(s) used in the vaccine: 13 purified serotype-specific pneumococcal polysaccharides conjugated to a carrier protein (conjugate vaccine) or 23 serotype-specific pneumococcal polysaccharides (pure polysaccharide vaccine)

Vaccines currently manufactured for the prevention of invasive pneumococcal disease are available as two different formulations. The immunogens included in the 23-valent pneumococcal polysaccharide vaccine are pure capsular polysaccharides. The immunogens included in the 13-valent conjugate pneumococcal vaccine are capsular polysaccharides that have been individually, covalently linked (conjugated) to CRM_{197} protein. For both vaccine formulations, the manufacturing process begins with culturing each of the desired *S. pneumoniae* serotypes in a defined soy peptone broth. When ready for harvest, the individual polysaccharides from each of the cultures are purified using physical and chemical means. From this point, the manufacturing steps differ between the 23-valent pneumococcal polysaccharide vaccine and the 13-valent conjugate pneumococcal vaccine.

To complete production of the 23-valent pneumococcal polysaccharide vaccine, purified polysaccharide immunogens representing pneumococcal serotypes, 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F, are combined in the correct ratio using saline. Phenol is added as a preservative to a final concentration of 0.25%.

To complete production of the 13-valent conjugate pneumococcal vaccine, each of the individual 13 purified polysaccharides is chemically depolymerized, and then conjugated to CRM_{197} protein using reductive amination. Each of the 13 resulting glycoconjugates is then purified using ultrafiltration and column chromatography before being combined in the desired saccharide to protein ratios, to formulate the final vaccine product containing pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F conjugated to CRM_{197}.

Disease: Paralytic poliomyelitis

Pathogen(s) causing human disease: Poliovirus type 1. Poliovirus type 2 was globally eradicated in 2015; poliovirus type 3 was globally eradicated in 2019

Immunogen(s) used in the vaccine: Live attenuated polio virus types 1, 2, and/or 3 or inactivated polioviruses types 1, 2, and/or 3

Inactivated trivalent polio vaccine remains the standard for polio prevention in the USA; however, live attenuated bivalent and trivalent vaccines continue to be used in other parts of the world.

The immunogens included in the manufacturing of inactivated trivalent polio vaccines are derived from well-characterized strains of the three poliovirus types 1, 2, and 3. Each virus is propagated individually in cell culture. The eukaryotic cells (e.g., Vero cells, WI-38 fibroblasts) used to support growth of the virus are grown in carefully formulated culture medium. When ready for harvest, cell culture supernatants containing high concentrations of each of the amplified polioviruses are clarified and concentrated using filtration, and then purified using liquid chromatography. Each of the monovalent viral suspensions is then inactivated using chemical exposure to formalin for a minimum of 12 days. Inactivated viral suspensions are then

adjusted to the desired immunogen concentration before being combined in ratios required in the final inactive polio vaccine formulation for injection.

Live-attenuated poliovirus vaccines are manufactured using cell culture to amplify specific Sabin strains of attenuated poliovirus types 1, 2, and/or 3 that are known to be derived from original seed stocks. The manufacturing processes used to amplify, harvest, clarify, and concentrate each strain of virus are similar to that used to produce inactivated polio vaccines. Instead of proceeding next to virus inactivation steps, the monovalent viral suspensions of Sabin strain viruses are used directly to produce doses of live attenuated mono-, bi-, and trivalent polio vaccines in formulations suitable for oral administration.

Disease: Rabies encephalitis

Pathogen(s) causing human disease: Rabies virus

Immunogen(s) used in the vaccine: Inactivated rabies virus

Both formulations of rabies vaccine that are currently used in the USA use inactivated rabies virus as the immunogen. The two vaccines are derived from separate and distinct strains of virus amplified using cell culture techniques. One manufacturer amplifies virus in MRC-5 fibroblasts, and the other uses primary chicken embryo fibroblasts. The fibroblasts are grown in synthetic cell culture medium containing human albumin. Antibiotics are included to prevent contamination. When ready for harvest, virus is concentrated and purified using physical techniques (ultrafiltration or centrifugation in a sucrose density gradient). Both manufacturers use β-propiolactone to inactivate the virus. A stabilizing solution is added and the final product is freeze-dried in unit dose vials without preservatives. The lyophilized vials are provided to the end user along with a 1 mL vial of sterile water for injection to be used for reconstitution.

Disease: Rotavirus gastroenteritis

Pathogen(s) causing human disease: Rotaviruses

Immunogen(s) used in the vaccine: Either live attenuated rotavirus serotype G1P[8] or five different live human-bovine reassortant viruses

The two oral formulations of live rotavirus vaccine currently available in the USA are formulated quite differently: one as a monovalent live attenuated human rotavirus and the other as five live human-bovine reassortant rotaviruses.

The immunogen included in the monovalent, live attenuated rotavirus vaccine is derived from a pathogenic G1P[8]-type human rotavirus. The attenuated vaccine strain is amplified in cell culture using Vero cells. The Vero cells are supported using liquid culture medium containing glucose, sodium pyruvate, L-cystine, L-tyrosine, L-glutamine, other amino acids, vitamins, and mineral salts. When ready, virus is harvested, concentrated, and purified. Sorbitol and sucrose are added as stabilizers prior to freeze-drying the final product. Lyophilized vaccine is provided to the end user along with a liquid diluent containing sterile water, calcium carbonate, and xanthan. The vaccine is preservative-free.

In contrast, the immunogens included in the pentavalent vaccine are reassortants derived from viruses that were originally isolated from humans and cows. Four of the reassortants in the vaccine express one of the outer capsid proteins (G1, G2, G3,

or G4) from the human rotavirus parent strain and the attachment protein (type P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein type P[8], from the human rotavirus parent strain and the outer capsid protein of type G6 from the bovine rotavirus parent strain. The result is a vaccine containing 4 human-bovine reassortant rotaviruses G1P7, G2P7, G3P7, G4P7, and G6P8 where either the attachment protein or the outer capsid protein is bovine-derived. The 5 reassortant viruses are amplified in Vero cells using techniques similar to those described above. When ready, virus is harvested, concentrated, and purified. The reassortants are resuspended in a buffered solution containing sucrose and polysorbate 80 as stabilizers to produce the final product. The vaccine is preservative-free.

Disease: Rubella infection including congenital rubella syndrome

Pathogen(s) causing human disease: Rubella virus

Immunogen(s) used in the vaccine: Live attenuated rubella virus

The Wistar RA 27/3 strain of live attenuated rubella virus is used as the immunogen for rubella vaccine. The vaccine strain virus is propagated in cultures of WI-38 human diploid lung fibroblasts grown in a buffered salt solution supplemented with vitamins, amino acids, and fetal bovine serum and stabilized with recombinant human albumin. Neomycin is added to prevent bacterial contamination during manipulation. Harvested virus is purified, concentrated, and then brought to the concentration of virus desired in the final vaccine product. Sorbitol and hydrolyzed gelatin are added as stabilizers to complete the production of the bulk lot of monovalent rubella vaccine. In the USA, rubella vaccine is currently only available in combination with measles and mumps vaccines.

Disease: Smallpox

Pathogen(s) causing human disease: Variola virus

Immunogen(s) used in the vaccine: Vaccinia virus

The smallpox vaccine that is currently approved for use in the USA is live vaccinia virus. The vaccine virus currently used as the immunogen in smallpox vaccine is derived from an archived stock of the vaccine used in the USA until the 1970s. Virus is amplified in Vero cells that are supported in defined cell culture medium that includes human serum albumin and antibiotics. When ready for harvest, virus is concentrated and purified, and then freeze-dried in vials as unit doses. Lyophilized vaccine is provided to the end user along with a vial of sterile water containing 50% glycerin as stabilizer and 0.25% phenol as a preservative. To administer the vaccine, the lyophilized virus is reconstituted with the diluent provided. A stainless-steel bifurcated needle is dipped into the reconstituted vaccine, and used to jab the skin 15 times, a technique called scarification. Smallpox remains the only vaccine administered in this manner.

Disease: Tetanus

Pathogen(s) causing human disease: *Clostridium tetani*

Immunogen(s) used in the vaccine: Tetanus toxoid

The production of tetanus vaccine requires growing *Clostridium tetani* in liquid culture medium under conditions that encourage optimal toxin production. When

ready to be harvested, the culture medium containing the toxin is separated from the bacteria by filtration, and then purified by fractionation with ammonium sulfate, dialysis, gel filtration, ion exchange chromatography, or a combination of these biochemical techniques. Next, the concentrated, now purified tetanus toxin is inactivated. Exposure to the proper concentration of formaldehyde for the proper period of time partially denatures the toxin. These changes in the tertiary structure of the toxin covert the toxigenic protein to a toxoid protein. The tetanus toxoid retains immunogenicity but is rendered nontoxic. Amino acids, such as lysine or glycine, may be added to facilitate crosslinking and prevent reversion. After purification and sterilization, the product is tested for sterility, purity, toxicity, and reversion to toxicity. During the final step, tetanus toxoid is adsorbed onto an aluminum salt adjuvant. The final product can be used, as is, to fill unit dose syringes for administration as monovalent tetanus vaccine, or combined with other immunogens. Final preparation of all tetanus-toxoid-containing combination vaccines, including Td, Tdap, DT, DTaP, DTaP-IPV, DTaP-HepB-IPV, DTaP-HIB-IPV, and DTaP-HepB-HIB-IPV, requires that each of the necessary immunogens be prepared and quality tested separately.

Disease: Tickborne encephalitis

Pathogen(s) causing human disease: Tickborne encephalitis virus

Immunogen(s) used in the vaccine: Inactivated tickborne encephalitis virus

The immunogen in tickborne encephalitis vaccine is whole inactivated virus. Vaccine manufacturing begins with amplifying a well-characterized TBE virus using cell cultures of chick embryo fibroblasts. Fibroblasts are grown in a defined cell culture medium with antibiotics added to prevent bacterial contamination. Harvested virus is concentrated by ultracentrifugation and purified using chromatography. Purified virus is inactivated using formaldehyde, and then adsorbed to aluminum hydroxide as an adjuvant. Improvements in purification procedures, including the addition of continuous flow zonal density gradient centrifugation, have substantially reduced vaccine reactogenicity.

Disease: Tuberculosis

Pathogen(s) causing human disease: *Mycobacterium tuberculosis*

Immunogen(s) used in the vaccine: Live bacteria in the form of Bacille Calmette-Guérin (BCG)

Bacille Calmette-Guérin (BCG) is the only vaccine available worldwide for the prevention of tuberculosis

The immunogens included in BCG are live, attenuated bacteria derived from well-characterized strains of *Mycobacterium bovis*. The TICE® strain of *M. bovis* that is used for the vaccine manufactured in the USA was developed at the University of Illinois from a strain originating from the Pasteur Institute in Paris, France. The TICE® BCG are grown in broth culture medium containing glycerin, asparagine, citric acid, potassium phosphate, magnesium sulfate, iron ammonium citrate, and lactose. When grown to the necessary density, the bacteria are lyophilized. The final vaccine product is provided to the end user in unit dose vials of freeze-dried bacteria

standardized to contain 1×10^8 colony-forming units along with a separate vial of sterile water for injection to be used as the diluent for reconstitution. BCG vaccine is preservative-free.

Disease: Typhoid or typhoid fever (not to be confused with typhus)

Pathogen(s) causing human disease: *Salmonella enterica subsp. enterica* serovar Typhi; less formally, and more frequently referred to as *Salmonella typhi*

Immunogen(s) used in the vaccine: Two formulations are available. One contains purified bacterial surface Vi polysaccharide, the other is a mixture of live and nonviable attenuated *S. typhi* bacteria.

Two formulations of vaccines are available in the USA for the prevention of typhoid, an infection caused by the bacterium *Salmonella enterica subsp. enterica* serovar Typhi. The immunogen used for the vaccine that is administered by injection is purified bacterial surface Vi polysaccharide. The alternative formulation is a live attenuated vaccine that is taken by mouth.

Manufacturing of the typhoid Vi polysaccharide vaccine starts with growing the well-characterized Ty2 strain of *S. typhi* in defined semisynthetic liquid culture medium supplemented early on with casein-derived proteins, carbohydrates, and amino acids. When the fermentation process is complete, the bacteria are inactivated by chemical treatment with formaldehyde. Capsular polysaccharide is extracted and precipitated from concentrated culture supernatant by adding hexadecyltrimethylammonium bromide. Extracted bacterial capsular polysaccharide is then purified using differential centrifugation. Phenol 0.25% is added as a preservative.

The live attenuated typhoid vaccine is manufactured using a bacterial strain known as *Salmonella typhi* Ty21a. This vaccine strain is grown in fermenters under controlled conditions using a broth culture medium supplemented with dextrose, galactose, yeast extract digest, and a digest of acid-treated casein. When fermentation is complete, the bacteria are separated from the culture medium using centrifugation, and then resuspended in a stabilizing solution containing sucrose, ascorbic acid, and amino acids to the desired concentration. The prepared live attenuated bacteria are freeze-dried, mixed with lactose and magnesium stearate, and then loaded into enteric-coated gelatin capsules.

Varicella and Shingles

Disease(s): Varicella, or chickenpox; zoster, or shingles

Pathogen(s) causing human disease: Varicella zoster virus

Immunogen(s) used in the vaccine: A live attenuated varicella virus vaccine is used to prevent chickenpox. Two formulations of vaccine are available for the prevention of shingles. One is a live attenuated varicella virus; the other contains recombinant varicella zoster virus surface glycoprotein E (gE)

The varicella vaccine used in the USA comprises the Oka/Merck strain of live attenuated varicella virus. The original, virulent wild-type varicella virus was isolated from a child with varicella infection. Attenuation was achieved using the classic method in virology of adapting the virus to grow in laboratory cell culture by

first growing it under ideal laboratory conditions using a human cell line. After the virus became well adapted to growing in a human embryonic cell line, it was passaged to embryonic guinea pig cell cultures. The adapted virus was then passaged to WI-38 cell cultures. Serial passage of the adapted, attenuated virus was completed in MRC-5 cells to prepare the seed stocks for manufacturing varicella vaccine. Manufacturing of varicella vaccine lots involves amplifying a seed stock of the live attenuated virus in cell cultures of MRC-5 cells maintained in culture media supplemented with bovine calf serum. Antibiotics are included to prevent bacterial contamination. When ready for harvest, the virus is concentrated and purified. Sucrose, processed porcine gelatin, and urea are added as stabilizers; then the virus is lyophilized and provided to the end user in unit dose vials containing a minimum of 1350 plaque-forming units (PFUs) of the live attenuated varicella virus. A vial of sterile diluent, to be used for reconstitution of the lyophilized vaccine virus, is provided with each dose. The vaccine is preservative-free.

The live attenuated zoster vaccine is manufactured using the same methodology used to produce varicella vaccine. The only major difference is that the unit doses of the final product contain a minimum of 19,400 PFUs of the Oka/Merck varicella virus (14 times more than varicella vaccine). Lyophilized vaccine is provided to the end user in unit dose vials paired with a vial of sterile diluent to be used for reconstitution. The vaccine is preservative-free.

The immunogen used for the recombinant zoster vaccine is a genetically engineered, varicella zoster virus surface glycoprotein E. A truncated form of the gene encoding the virus surface glycoprotein E was stably transfected into Chinese hamster ovary cells. Cells expressing the truncated surface glycoprotein E are grown in synthetic cell culture media supplemented with amino acids. When ready for harvest, the recombinant protein is concentrated and purified using column chromatography. Purified protein is formulated with sucrose as a stabilizing agent, and then lyophilized. Lyophilized vaccine is provided to the end user in unit dose vials paired with a vial of $AS01_B$ adjuvant suspension to be used for reconstitution. $AS01_B$ adjuvant suspension is a liposomal formulation of QS-21, a saponin purified from the plant *Quillaja Saponaria* and 3-O-desacyl-4′-monophosphoryl lipid A (MPL). The liposomes are composed of cholesterol and dioleoylphosphatidylcholine (DOPC) in phosphate-buffered saline. The vaccine is preservative-free.

Disease: Yellow fever

Pathogen(s) causing human disease: Yellow fever virus

Immunogen(s) used in the vaccine: Live attenuated yellow fever virus

Yellow fever vaccine is produced by amplifying the attenuated 17D-204 strain of yellow fever virus in embryonated chicken eggs. When ready for harvest, allantoic fluid containing the virus is collected and the virus is purified and concentrated, and then resuspended in a stabilizing solution containing sorbitol and gelatin. The product is lyophilized in unit dose vials containing a minimum of 4.74 log10 plaque-forming units of the live attenuated virus. Lyophilized vaccine is provided to the end user in unit dose vials paired with a vial of sterile saline for injection to be used for reconstitution. The vaccine is preservative-free.

References

Links to Package Inserts for US Food and Drug Administration Approved Vaccines

Adenovirus

https://www.fda.gov/media/80211/download.

Anthrax

https://www.fda.gov/vaccines-blood-biologics/vaccines/biothrax.

Cholera

https://www.fda.gov/media/128415/download.

Dengue

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Diphtheria, Tetanus, Pertussis

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Human Papillomavirus

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Measles, Mumps, Rubella

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Meningococcus

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Typhoid

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