Chapter 4
Vaccine Additives and Excipients

Joseph Domachowske

Introduction

Inactive substances included in vaccines are referred to as excipients. The microbiologic and biochemical methods needed to harvest, purify, and formulate vaccine immunogens for the end user are different for each vaccine. Specially formulated culture media containing various supplements are needed to support the growth of bacteria, yeast, and a variety of cell types. Chemical manipulations are performed to extract desired proteins, polysaccharides, or lipids. Treatment with inactivating agents may be necessary to neutralize viruses, bacteria, or toxins. During the final stages of vaccine production and formulation other substances, such as adjuvants, stabilizing solutions, buffers, and/or preservatives may be added. The goals of this chapter are to review the main categories of vaccine excipients, to list and define each of the specific excipients that are present in vaccines, and to describe why excipients are necessary and important for vaccine production.

Two Main Categories of Excipients

The two main categories of vaccine excipients are (1) residual concentrations of substances used during manufacturing and (2) substances added to the vaccine for a specific purpose.

J. Domachowske (✉)
Department of Pediatrics, SUNY Upstate Medical University, Syracuse, NY, USA
e-mail: domachoj@upstate.edu

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Residual Concentrations of Substances Used During Manufacturing

The vast majority of excipients listed as ingredients in vaccines belong to this category. These substances represent residual or trace amounts of every reagent used during the manufacturing process. A key concept, often overlooked, is that any substance not needed in the final product is reduced to microgram (mcg) or picogram (pcg) amounts, during subsequent purification steps. These excipients are present in such low amounts in the final vaccine product that many are listed by manufacturer's as present in "trace" or "residual" amounts (Table 4.1). The terms "trace" or "residual" may also be used to indicate that final concentrations are below the limit of detection using available assays. Any reagent that is used during vaccine production is listed as a vaccine excipient whether it is present in detectable amounts or not. Vaccines are complex products derived from biologic material grown in laboratories, involving large-scale cultures of bacteria, viruses, yeast, fertilized chicken eggs, insect cells, chicken fibroblasts, and a variety of different mammalian cell lines. Each culture type has different growth requirements. Carefully defined formulations of growth media are used to optimize the recovery of the desired immunogens from cultures of bacteria and yeasts. Viruses are obligate intracellular organisms that can only be cultured under laboratory conditions if target cells permissive to their infection are used. For example, embryonated chicken eggs have proven to be efficient incubation chambers for influenza viruses used to produce seasonal influenza vaccine. Other viruses, such as polio and hepatitis A, replicate very efficiently in cultures of eukaryotic cell lines specifically developed and propagated for this purpose. A variety of different cell lines are used to manufacture vaccines, each one supported by specially formulated culture medium specific to its growth requirements. Supplements, such as vitamins, amino acids, and fetal bovine serum, are added when necessary. Every component of culture medium used for vaccine manufacturing is considered an excipient.

Several different recombinant expression systems are also used in the mass production of vaccine immunogens. Recombinant technology has been used to introduce coding sequences for the immunogens of interest into bacteria, yeast, African green monkey kidney cells, and Chinese hamster ovary (CHO) cells. Each of these recombinant cell types has unique culture conditions and medium requirements that have been optimized for the recovery of the expressed protein(s).

Every ingredient used to manufacture a vaccine is listed in the product's prescribing information (package insert). Reading these long lists of ingredients can be disconcerting to those who do not understand the concept of an excipient, or the meaning of micro- or picogram amounts. Most of the chemicals and reagents listed have unfamiliar names (e.g., cetyl trimethylammonium bromide [CTAB], hexadecyltrimethylammonium bromide); some names are familiar, yet seem dreadfully out of context (e.g., yeast, gelatin, aluminum), some are clearly derived from animals (e.g., fetal bovine albumin, chick embryo protein), some are widely considered poisonous when present in much higher concentrations in other contexts, (e.g.,

Table 4.1 Vaccine excipients

Excipient	Category	Vaccine(s)[a]	Amount per dose[b]
2-Phenoxyethanol	Stabilizer	DTaP-HIB-IPV	3.3 mg
		DTaP-IPV	3.3 mg
		DTaP	3.3 mg
		TdaP	3.3 mg
	Preservative	IPV	0.5%
Albumin, bovine	Stabilizer	DTaP-HepB-IPV	trace
		DTaP-HIB-IPV	≤50 ng
		DTaP-IPV	trace
		Hepatitis A	<0.1 ng
		IPV	<50 ng
		JE	≤100 ng
		Rabies	trace
		Zoster	trace
Albumin, fetal bovine	Stabilizer	Pentavalent rotavirus	trace
		Varicella	trace
Albumin, human	Stabilizer and Diluent	MMR	≤0.3 mg
		MMR-V	0.31 mg
		Rabies	<100 mg
Albumin, egg (ovalbumin)	Medium ingredient	Influenza inactivated	≤1 mcg
		Influenza live attenuated	≤0.24 mcg
		Rabies	≤3 ng
Aluminum	Adjuvant	Anthrax	1.2 mg
		DTaP-HepB-IPV	≤0.85 mg
		DTaP-HIB-IPV	0.33 mg
		DTaP-IPV	0.33–0.6 mg
		DTaP	0.33–0.625 mg
		DT	1.5 mg
		HepA-HepB	0.45 mg
		Hepatitis A	0.45–0.5 mg
		Hepatitis B	0.5 mg
		HPV nonavalent	500 mcg
		JE	250 mcg
		Mening B	0.25–0.519 mg
		Pneumococcal 13-valent	0.125 mcg
		TD	0.33–0.53 mg
		TdaP	0.33–0.39 mg
Amino acid	Medium ingredient	Anthrax	Unspecified
		HepA-HepB	Unspecified

(continued)

Table 4.1 (continued)

Excipient	Category	Vaccine(s)[a]	Amount per dose[b]
		Hepatitis A	0.3% w/v
		Hepatitis B	Unspecified
		HPV nonavalent	Unspecified
		Influenza live attenuated	Unspecified
		Influenza recombinant quadrivalent	2.42 mg of arginine
		Mening B	10 mM or 0.776 mg of histidine
		MMR	Unspecified
		Rotavirus monovalent	Unspecified
		Typhoid, live attenuated	0.3–3.0 mg per capsule
		Zoster	Unspecified
Ammonium sulfate	Protein purifier	DTaP-HIB-IPV	Unspecified
		DTaP-IPV	Unspecified
		DTaP	Unspecified
		HIB	Unspecified
		Influenza live attenuated	Unspecified
		Meningococcal quadrivalent	Unspecified
		Pneumococcal 13-valent	Unspecified
		TdaP	Unspecified
		Td	Unspecified
Amphotericin	Antimicrobial	Rabies	<2 ng
$AS01_B$ suspension	Adjuvant	Recombinant zoster vaccine	50 mcg each of MPL and QS-21
Benzethonium chloride	Preservative	Anthrax	25 mcg
Beta-propiolactone	Virus inactivation	Influenza inactivated	≤0.5 mcg
		Influenza inactivated from cell culture	<0.5 mcg
		Rabies	<50 ppm
Calcium carbonate	Buffer	Rotavirus monovalent	Unspecified
Cetyl trimethylammonium bromide (CTAB)	Protein purifier	Influenza inactivated	≤12 mcg
		Influenza inactivated from cell culture	≤18 mcg

Table 4.1 (continued)

Excipient	Category	Vaccine(s)[a]	Amount per dose[b]
Chick embryo protein	Medium ingredient	Influenza inactivated	Trace residual
		MMR-V	Trace residual
		Varicella	Trace residual
		Yellow fever	Trace residual
Chicken fibroblasts	Medium ingredient	MMR	Trace residual
Chlortetracycline	Antimicrobial	Rabies	≤200 ng
Chinese hamster ovary cells	Medium ingredient	Recombinant Zoster vaccine	Trace residual
CpG 1018	Adjuvant	Hepatitis B Adjuvanted	3 mg
DOPC or dioleoyl phosphatidylcholine	Adjuvant carrier	Recombinant zoster vaccine	1 mg
Ethylenediaminetetraacetic acid (EDTA)	Medium ingredient	Influenza live attenuated	< 0.37 mcg
		Rabies	0.3 mg
		Varicella	Trace
Formaldehyde	Inactivating agent	Anthrax	<100 mcg
		DTaP-HIB-IPV	≤5 mcg
		DTaP-HepB-IPV	≤100 mcg
		DTaP-IPV	≤100 mcg
		DTaP	≤100 mcg
		DT	≤100 mcg
		HepA-HepB	≤100 mcg
		Hepatitis A	<0.8 mcg
		Hepatitis B	≤15 mcg
		HIB	<0.5 mcg
		Influenza inactivated	≤25 mcg
		JE	≤100 mcg
		Meningococcal quadrivalent	<2.66 mcg
		IPV	≤0.02%
		TdaP	≤100 mcg
		Td	≤100 mcg
		Typhoid inactivated	≤100 mcg
Gelatin	Stabilizer	Influenza live attenuated	2 mg
		JE	500 mcg
		MMR	14.5 mg
		MMR-V	11 mg
		Rabies	≤12 mg

(continued)

Table 4.1 (continued)

Excipient	Category	Vaccine(s)[a]	Amount per dose[b]
		Typhoid, live attenuated	Gelatin capsules
		Varicella	12.5 mg
		Yellow fever	Unspecified
		Zoster	15.58 mg
Gentamicin sulfate	Antimicrobial	Influenza inactivated	≤0.15 mcg
		Influenza live attenuated	<0.015 mcg/ml
Glutaraldehyde	Inactivating agent	DTaP-HIB-IPV	<50 ng
		DTaP-HepB-IPV	Unspecified
		DTaP-IPV	<50 ng
		DTaP	<50 ng
		TdaP	<50 ng
Hexadecyltrimethylammonium bromide	Protein purifier	Typhoid inactivated	Unspecified
Kanamycin	Antimicrobial	Influenza inactivated	≤0.03 mcg
		Mening B	0.01 mcg
MDCK cell residual	Medium ingredient	Influenza inactivated from cell culture	Unspecified
MF59C.1	Adjuvant	Influenza Adjuvanted	9.75 mg squalene in polysorbate 80
MPL or 3-O-desacyl-4′- monophosphoryl lipid A	Adjuvant	Recombinant zoster vaccine	50 mcg
MRC-5 cell residual	Medium ingredient	DTaP-HIB-IPV	Unspecified
		DTaP-IPV	Unspecified
		HepA-HepB	≤2.5 mcg
		Hepatitis A	≤5 mcg
		MMR-V	Residual
		Rabies	Unspecified
		Varicella	Residual
		Zoster	Residual
Neomycin	Antimicrobial	DTaP-HepB-IPV	≤0.05 ng
		DTaP-HIB-IPV	<4 pg
		DTaP-IPV	≤0.05 ng
		Hepatitis A	≤40 ng
		HepA-HepB	≤20 ng
		Influenza inactivated	≤81.8 ng
		MMR	25 mcg
		MMR-V	<16 mcg
		IPV	<5 ng

Table 4.1 (continued)

Excipient	Category	Vaccine(s)[a]	Amount per dose[b]
		Rabies	<150 mcg
		Varicella	trace
		Zoster	Trace
Phenol	Preservative	Pneumococcal 23-valent	0.25%
		Typhoid inactivated	0.25%
		Smallpox	0.25%
Polymyxin B	Antimicrobial	DTaP-HepB-IPV	≤0.01 ng
		DTaP-HIB-IPV	<4 pg
		DTaP-IPV	≤0.01 ng
		Influenza inactivated	≤3.75 mcg
		IPV	25 ng
QS-21	Adjuvant	Recombinant zoster vaccine	50 mcg
Sodium taurodeoxycholate	Protein purifier	Influenza inactivated	≤10 ppm
	Vesicle purifier	Meningococcal serotype B	Unspecified
Spodoptera frugiperda 9 (Sf9) cells	Medium ingredient	Influenza recombinant	Unspecified
Streptomycin	Antimicrobial	IPV	200 ng
Thimerosal	Preservative	DT	≤0.3 mcg
		Influenza inactivated	≤25 mcg from multidose vials only
		Td	≤0.3 mcg
		JE	0.007%
Vero cell residual	Medium ingredient	DTaP-HepB-IPV	Unspecified
		DTaP-IPV	Unspecified
		IPV	Unspecified
		JE	Unspecified
		Rotavirus monovalent	Unspecified
		Rotavirus pentavalent	Unspecified
WI-38 human fibroblast cell residual	Medium ingredient	Inactivated polio	Unspecified
		Rubella	Unspecified
Yeast	Medium ingredient	DTaP-HepB-IPV	≤5% yeast protein
		HepA-HepB	≤5% yeast protein
		Hepatitis B	≤5% yeast protein
		HPV nonavalent	<7 mcg

(continued)

Table 4.1 (continued)

Excipient	Category	Vaccine(s)[a]	Amount per dose[b]
		Pneumococcal 13-valent	trace
		Typhoid	Not specified

[a]Vaccines containing the same immunogens, but different excipients, are available because manufacturers use different processes to produce their final products. The excipients listed are included in one or more of the available formulations

[b]Amounts listed are collected from package inserts for vaccine formulations approved for use by the US Food and Drug Administration

formaldehyde, phenol), and some are derived from cells originating from fetal lung tissue (MRC-5 and WI-38 cells), African green monkey kidney (Vero) cells, insect (Sf9) cells, or other unusual sounding sources. Learning about the role that each of the excipients plays in vaccine manufacturing and understanding that remnants of these substances are present in vaccines at very low concentrations, if detectable at all, can help resolve questions from those expressing concerns about vaccine ingredients.

Substances Added to the Vaccine for a Specific Purpose

The second main category of excipients are those added near the end of the manufacturing process. These excipients are added in precise amounts. They are not diluted or removed subsequently because they are necessary for the formulation of the final vaccine product. For example, many vaccine immunogens are formulated with a stabilizing solution or buffer to maintain their integrity prior to use. Other vaccines require the addition of a substance called an adjuvant to improve vaccine immunogenicity. Finally, vaccines that are provided to the end user in multidose vials require the addition of a preservative to prevent bacterial contamination during use.

Adjuvants

Ingredients referred to as adjuvants are added to some vaccines near the end of the manufacturing process. An adjuvant is any substance that enhances or modulates the immune responses to an antigen. Historically, inorganic adjuvants, in the form of aluminum salts, were the first to be added to vaccines. Aluminum phosphate, aluminum hydroxide, and alum remain the most common vaccine adjuvants in use today. While their precise mechanisms of action are incompletely understood, aluminum salts are known to trigger the activation of dendritic cells. Activated dendritic cells upregulate and release interleukin-1β, which provides an important signal to B-cells to initiate antibody production.

Several new organic adjuvants were recently approved for use in vaccines. Organic adjuvants are a group of substances that are capable of mimicking

molecular patterns recognized by the innate immune system as pathogen-derived foreign material. Examples of molecules that are easily identified by the innate immune system as pathogen-derived include various components of bacterial cell walls, the outer bacterial membrane lipopolysaccharide of Gram-negative bacteria (also known as endotoxin), liposomes, double-stranded RNA, and unmethylated-CpG-dinucleotide-containing DNA. The innate immune system recognizes and responds to these and other pathogen-specific molecules. When a specific vaccine immunogen is presented to the immune system together with an organic adjuvant that mimics one of these pathogen-specific molecular patterns, the ensuing innate immune responses are robust. Dendritic cells, macrophages, and natural killer cells are engaged and activated, thereby augmenting and modulating the adaptive immune response triggered simultaneously by the vaccine immunogen. Organic adjuvants currently used in US-approved vaccines include the cytosine phosphoguanine motifs in CpG1018, MF59c.1, a squalene-based substance, and liposomes containing equal amounts of 3-O-desacyl-4′-*m*onophos*p*horyl *l*ipid A (*MPL*) and the purified *Quillaja saponaria* plant extract QS-21.

Stabilizers

The stabilizers added to many vaccine immunogens near the end of the manufacturing process serve to maintain the integrity of the formulation prior to use. Stabilizers that are commonly added include protein in the form recombinant human albumin, bovine albumin, bovine casamino acid, or porcine-derived gelatin; sugars in the form of lactose, sorbitol, sucrose, or xanthan; and/or nonionic surfactants and emulsifiers such as polysorbate 20 and polysorbate 80.

Preservatives

In the USA, most vaccines that are prepared for delivery as unit doses do not contain a preservative. Several vaccines are, however, available in multidose vials, most commonly containing 10 doses, although some contain enough vaccine to immunize 50 individuals. By design, multidose vials require repeated access by inserting a sterile needle through a stopper to withdraw each dose. Careful aseptic technique alone is insufficient to prevent contamination when the integrity of the stopper is disrupted repeatedly, so a preservative is added to all multidose vaccine vial preparations. Preservatives that are licensed by the US Food and Drug Administration for use in vaccines include thimerosal, 2-phenoxyethanol, benzethonium chloride, and phenol.

A list of excipients included in the package inserts of US Food and Drug Administration -approved vaccines is provided in Table 4.1. The category for each of the listed excipients identifies substances that are present in residual amounts as antibiotics, protein purifiers, chemicals used to inactivate pathogens or toxins, or ingredients used in culture medium. Excipients that are added to serve the specific

purpose of adjuvant, stabilizer, or preservative are likewise identified. The amount of excipient per unit dose of vaccine is also listed.

Vaccine Excipients: What Is It? Why Is It Listed as an Ingredient in Some Vaccines?

This section includes definitions of the excipients listed in the package inserts of all vaccines approved for use in the USA. A description of each substance is provided, including its specific role in vaccine manufacturing. Mineral salts and simple nutrients used in various formulations of culture media that are not described separately in this section or listed in Table 4.1 include calcium chloride, carbohydrates, dextrose, glucose, ferric (III) nitrate, galactose, magnesium sulfate, phosphate buffer, potassium chloride, sodium bicarbonate, sodium borate, sodium citrate, soy peptones, mineral salts, and vitamins.

2-Phenoxyethanol

2-Phenoxyethanol, $C_6H_5OCH_2CH_2OH$, is a phenol-derived aromatic ether substituted on oxygen by a 2-hydroxyethyl group. It is widely used in manufacturing of inks, dyes, insect repellents, and antiseptics. It is used as a preservative in cosmetics, perfumes, and a variety of pharmaceutical products including antibiotic creams, ear drops, and multidose vials of trivalent inactivated polio vaccine.

Albumin (Bovine, Fetal Bovine, Human)

Albumin is the most abundant protein found in plasma. It is widely used in vaccine manufacturing as a stabilizer to protect the integrity of the active ingredients during manufacture, storage, and transport. Bovine serum albumin is used simply because it is plentiful and inexpensive. Recombinant human serum albumin is also used as a stabilizer in some vaccines.

Albumin, Egg

Albumin is the most abundant protein found in eggs. The first step in manufacturing most influenza vaccines involves inoculating embryonated chicken eggs with vaccine strain influenza viruses. The eggs serve as the culture medium for the viruses, where they replicate until ready for harvest. Prior to 2016, chicken-egg-based

technology was the only process approved by the US Food and Drug administration for the production of influenza vaccines. In 2016, the first cell-culture-based influenza vaccine was introduced to the market.

Aluminum

Aluminum is a chemical element with the symbol Al, and atomic number 13. It is the most abundant metal, and third most abundant element on earth, accounting for 8% of the Earth's crust. Aluminum salts, such as aluminum hydroxide, aluminum phosphate, or potassium aluminum sulfate, are added to some vaccines to serve as adjuvants. Each of these aluminum salts occurs naturally in the environment. They are found in small amounts in food and drinking water. Aluminum salts are also one of the active ingredients in some antacids, and are used as stabilizers in many processed foods. Vaccines that contain aluminum are more painful when injected and more likely to cause self-limiting injection site redness and swelling compared with other vaccines.

Amino Acids

Amino acids are 20 related organic compounds that are used as the unit building blocks of protein. Humans can synthesize 11 amino acids de novo. The other nine, commonly referred to as "essential amino acids," are derived from digested dietary proteins. During vaccine manufacturing, amino acids are added to culture medium to support the replication of the bacteria, virus, or yeast cells being grown for the purpose of harvesting the desired immunogen.

Ammonium Sulfate

Ammonium sulfate is an inorganic salt $(NH_4)_2SO_4$ best recognized for its use as a soil fertilizer and other commercial agricultural purposes. In the chemistry laboratory, ammonium sulfate is used to purify proteins from complex mixtures. The technique, called ammonium sulfate precipitation, relies on a simple protein chemistry concept; protein solubility in a solution decreases as the ionic strength the solution increases. The method is called "salting out." After the ammonium sulfate is added to the solution containing the desired protein(s), centrifugation is used to separate the precipitate from the aqueous portion. The precipitated protein is then resolubilized using suspension buffers. Ammonium sulfate precipitation is a convenient, simple, and common technique used to fractionate complex protein mixtures during the early manufacturing steps for several vaccines.

Amphotericin

Amphotericin is a broad-spectrum antifungal agent. Several pharmaceutical formulations are available for intravenous use as medical treatment of invasive fungal infections in humans. It is also used in vaccine manufacturing as an additive to some culture media to prevent microbial cultures from becoming contaminated with mold.

AS01$_B$ Adjuvant Suspension

AS01$_B$ adjuvant suspension is a liposomal formulation comprised of equal amounts of 3-O-desacyl-4′-*m*onophos*p*horyl *l*ipid A (*MPL*) extracted from the bacterium *Salmonella minnesota* and QS-21, a plant extract purified from *Quillaja saponaria.* Liposomes are comprised of cholesterol and dioleoyl phosphatidylcholine (DOPC) suspended in phosphate-buffered saline.

Ascorbic Acid

Ascorbic acid is a synonym for vitamin C. It is found naturally in a variety of foods. It is used in vaccine manufacturing for its potent antioxidant properties.

Benzethonium Chloride

Benzethonium chloride is a synthetic quaternary ammonium salt with surfactant and antiseptic properties. Its broad-spectrum microbiocidal activity against bacteria, molds, and viruses makes it ideal for use in the restaurant industry as a hard surface disinfectant. It is an active ingredient found in many over-the-counter hand and body washes, mouthwash, topical first aid antiseptics, antibacterial wipes, and cosmetics. In the vaccine industry, it is used as a preservative in anthrax vaccine.

Beta-Propiolactone

β-Propiolactone ($C_3H_4O_2$) is an organic compound of the lactone family. It has excellent sterilizing activity against bacteria, fungi, and viruses. It has been used as a vapor-phase disinfectant for small, enclosed areas and to sterilize tissue grafts, surgical instruments, human plasma, water, and milk. In vaccine manufacturing, it is used to inactivate influenza virus during the production of some influenza vaccines.

Bovine Casamino Acids

Bovine casamino acids are a product derived from cow's milk casein. Bovine casein is digested using sulfuric or hydrochloric acid. The resulting hydrolysate consists chiefly of free amino acids. Bovine casamino acids are similar to tryptone. Tryptone is an incomplete casein hydrolysate that is used as a source of protein and amino acid in different formulations of bacterial culture media. In vaccine manufacturing, bovine casamino acids are used as an ingredient in bacterial culture medium early on in the production of some diphtheria vaccines. See also section "Amino Acids."

Calcium Carbonate

Calcium carbonate ($CaCO_3$) is a common, naturally occurring organic chemical compound found in limestone, coral reef structures, crustacean shells, and the eggs of reptiles and birds. It has a broad array of industrial applications. In the food industry, calcium carbonate is added to provide color, or as a buffer, stabilizer, or anticaking agent. It is approved as an additive to nondairy milk products (e.g., soy, almond) as a dietary calcium supplement, and it is the active ingredient in many over-the-counter antacids. The pharmaceutical industry relies on calcium carbonate as a filler "inactive ingredient" in the production of medications formulated as tablets. In vaccine manufacturing, calcium carbonate is used as a buffering agent for one of the oral formulations of rotavirus vaccine.

Cetyl Trimethylammonium Bromide (CTAB)

Cetyl trimethylammonium bromide $[(C_{16}H_{33})N(CH_3)_3]Br$ is a quaternary ammonium compound with surfactant properties. In the biochemistry laboratory, surfactants such as CTAB are used to extract proteins from cells and tissues. They do so by disrupting and disorganizing cell membrane lipid bilayers and solubilizing cellular proteins. Later biochemical steps are then used to purify the protein(s) of interest. Surfactants are used in industry for myriad practical applications that require dispersion, emulsification, foaming or antifoaming, and cleaning. While CTAB is most commonly used during protein purification steps in the biochemistry laboratory, a variety of other surfactants are used to manufacture soaps and laundry detergents, fabric softeners, inks, paints, waxes used for surfboards and skis, insecticides, spray and foaming sanitizers, and many others. The health and hygiene industry also depends on the use of surfactants for the manufacturing of cosmetics, liquid soaps and shampoos, hair conditioners, toothpaste, and spermicides. CTAB is used in the vaccine industry to extract

proteins from cultures of influenza virus during the manufacturing of some inactivated influenza vaccine formulations.

Chick Embryo Protein

Fertilized chicken eggs are used in the production of varicella, yellow fever, and most influenza vaccines. Vaccine strain viruses replicate efficiently in this system. When ready for harvest, the eggs, now containing amplified virus, are collected, combined, and subjected to a series of purification steps. The final preparations are highly purified, but do contain trace amounts of chick embryo proteins, including ovalbumin.

Chicken Fibroblasts

Chicken fibroblasts, or chick embryo fibroblasts (seen abbreviated as CEFs), are connective tissue cells derived from chicken embryos that grow and replicate in laboratory cultures containing cell culture medium. Harvested cells adhere to the culture dish, replicating to fill available space. Primary cells that are grown in laboratory cultures have a finite lifespan, but generally tolerate expansion by serial passage up to five times. CEFs are one of the many cell culture types used in laboratories to propagate viruses. In vaccine manufacturing, laboratory cultures of CEFs are used to support the manufacturing of measles and mumps vaccines.

Chinese Hamster Ovary Cells

Chinese hamster ovary (CHO) cells are a continuous epithelial cell line derived from the ovary of the Chinese hamster. Cell lines refer to well-characterized cells that are easily cultured and propagated in the laboratory, over time, using serial passage. Continuous cell lines, such as CHO cells, have been immortalized, allowing them to be passaged indefinitely. “Cell culture” refers to the process of growing animal (including human) cells in a suitable receptacle under laboratory-defined conditions. Culture conditions vary for each cell type, but generally include the use of a properly prepared liquid medium with careful regulation of temperature and pH. The culture medium for each cell line must be formulated to include all nutrients, growth factors, and hormones that are essential for its growth. As a laboratory tool, CHO cells are particularly efficient at expressing recombinant proteins. In vaccine manufacturing, genetically engineered CHO cells are used in the production of recombinant zoster vaccine.

Chlortetracycline

Chlortetracycline is a tetracycline class antibiotic used in veterinary medicine to treat bacterial conjunctivitis in dogs and cats, and a variety of bacterial infections in farm animals. In the vaccine industry, it is included in the cell culture media to prevent bacterial contamination during the manufacturing of rabies vaccine.

Citric Acid Monohydrate

Citric acid monohydrate $C_6H_8O_7{\cdot}H2O$ is a naturally occurring tricarboxylic acid present in citrus fruits. It is used as an excipient in some vaccine preparations for its antioxidant and pH-stabilizing properties.

Ethylenediaminetetraacetic Acid (EDTA)

Ethylenediaminetetraacetic acid is a chemical used for a broad array of industrial and medical applications. EDTA has a ring-like center that reacts with metal ions to form stable, water-soluble complexes. Chemicals that bind and sequester metal ions are called chelating agents. Solutions of EDTA are commonly used in biomedical laboratories during manipulation of cell cultures. The Ca^{2+} present in cell culture medium is necessary for cells to adhere to the culture flask and to adhere to one another. When EDTA is added, it chelates (sequesters, functionally removes) the Ca^{2+} from the culture media, causing the cells to detach from the flask for passaging or harvesting. During vaccine manufacturing, EDTA is often used during steps that require cell culture manipulation.

Formaldehyde

Formaldehyde CH_2O is a colorless, flammable, strong smelling organic compound that occurs naturally in the environment. When formed by the action of sunlight and oxygen on atmospheric methane and other hydrocarbons, it becomes part of smog. It is also formed as an intermediate during the combustion of fossil fuels. Formaldehyde is one of the many chemicals released and inhaled during cigarette smoking. It is a well-recognized component of air pollution and is classified as a known human carcinogen. Formaldehyde is used commercially in the manufacturing of industrial and household products such as particleboard, plywood, glues and adhesives, resins, plastics, paints and industrial fungicides, germicides, and disinfectants. It is, perhaps, best known for its use as a preservative in biology

laboratories and as a main chemical ingredient in embalming fluid. Perhaps unexpectedly, formaldehyde is also produced endogenously by most living organisms as part of normal metabolic processes. Biologically, it is essential for normal cellular metabolism. In humans, formaldehyde produced in the liver is used for the biosynthesis of purines, pyrimidines, and amino acids. Due to its rapid metabolic turnover, it does not accumulate in the body. Normal, healthy, endogenous production of formaldehyde in the human body results in stable blood concentrations of approximately 0.1 millimolar at all times. At this concentration, a healthy 6-month-old infant has approximately 2 mg, and an adult has approximately 15 mg of formaldehyde in their blood at all times.

Formaldehyde is used in the vaccine industry for the purpose of inactivating viruses and converting toxins into toxoids by altering their tertiary protein structure sufficiently to render them nontoxic, while retaining immunogenicity. Residual amounts of formaldehyde in amounts no greater than 100 micrograms (0.1 mg) may be present in the final vaccine products. Exposure to formaldehyde at the exceptionally low concentrations found in some vaccines is well below the physiologic range occurring from endogenous production.

Gelatin

Gelatin is a translucent, colorless, flavorless substance derived from animal byproducts of the meat industry, including skin, bones, and connective tissue. Commercially, it has a prominent role in the food industry as a gelling agent for flavored gelatin snacks, gummy candies and multivitamins, ice cream, and yogurt. It is also used to manufacture edible capsules that can be filled with medications or vitamin supplements. Outside of the food industry, gelatin is used in the manufacturing of cosmetics and in the production of film used for photography. Porcine gelatin is used as a stabilizer in several vaccines, and to manufacture the gelatin capsules used to formulate live attenuated typhoid vaccine. A bovine-gelatin-like product is used as a stabilizer in one of the available rabies vaccines. While true allergic reactions are uncommon events following receipt of vaccines, when they do occur, animal gelatin should be considered as the possible allergen.

Gentamicin Sulfate

Gentamicin sulfate is an aminoglycoside class antibiotic. Various pharmaceutical formulations are available to treat infections in humans including products for intravenous and intramuscular injection and topical treatment of infections localized to the eye, ear, or skin. Gentamicin sulfate is used in vaccine manufacturing as an additive to some growth media to prevent cultures from becoming contaminated with bacteria.

Glutaraldehyde

Glutaraldehyde ($C_5H_8O_2$) is a clear oily, strong smelling organic liquid chemical used for a variety of industrial, agricultural, and medical purposes. It plays a role in waste water treatment, disinfecting sugar mills, and fogging and disinfecting enclosures used to house poultry. It is used to disinfect hard surfaces, sterilize medical instruments that cannot be autoclaved, process radiographic film, and fix tissue for electron microscopy. Combined with methanol and formaldehyde, glutaraldehyde is one of the primary chemical ingredients used in embalming fluid. Glutaraldehyde is used in the vaccine industry for the purpose of inactivating bacterial toxins by converting them to toxoids. See also section "Formaldehyde."

Hexadecyltrimethylammonium Bromide

Hexadecyltrimethylammonium bromide is a chemical synonym for cetyl trimethylammonium bromide $[(C_{16}H_{33})N(CH_3)_3]Br$, a quaternary ammonium compound with surfactant properties. See also section "Cetyl Trimethylammonium Bromide."

Kanamycin

Kanamycin is an aminoglycoside class antibiotic. Pharmaceutical formulations are available for intravenous and intramuscular injection as medical treatment of serious bacterial infections in humans. It is used in vaccine manufacturing as an additive to some culture media to prevent cultures from becoming contaminated with bacteria.

Madin-Darby Canine Kidney (MDCK) Cells

Madin-Darby canine kidney cells were derived from the kidney of a normal adult cocker spaniel in 1958 for use as a cell-culture-based model of virus infection. This continuous "immortal" cell line has been propagated under laboratory conditions for more than 60 years. Cell biologists now use them to study cellular mechanisms necessary to establish polarity, signaling pathways important in cell-to-cell adhesion, and a variety of other epithelial cell functions.

In biomedical terms, "cell culture" refers to the process of growing animal (including human) cells in a suitable receptacle under laboratory-defined conditions. These conditions vary for each cell type, but generally include the use of a properly prepared liquid culture medium and careful regulation of temperature and pH. The culture medium for each cell line must be formulated to include all

nutrients, growth factors, and hormones that are essential for its growth. Cell lines refer to well-characterized cells that can be cultured and propagated in the laboratory, over time, using serial passage. Continuous cell lines, such as MDCKs, are cells that have been immortalized, allowing them to be passaged indefinitely. Growth of semicontinuous cell lines, like MRC-5 fibroblasts, can be supported across 30 or more serial passages, while the propagation of primary cells is typically limited to 3 passages or less.

The discovery and development of cell culture methods proved invaluable to the field of virology. Viruses are obligate intracellular pathogens. Viral replication is completely dependent on host cell machinery. Traditional diagnostic virology involves inoculating cell cultures with biologic samples collected from patients, and then monitoring the cells microscopically for visual evidence of infection such as virus-associated cytotoxicity and/or cytopathic effects. Because the process requires a high level of technical expertise and is time consuming, the use of viral cultures for diagnostic testing has been largely replaced by high-throughput molecular diagnostic tests. Viral cultures, however, remain fundamental and essential to the manufacturing of several vaccines. Large-scale cell cultures seeded with polio, influenza, live attenuated measles, live attenuated rubella virus, and live attenuated varicella virus serve as factories to amplify the viruses needed to produce the vaccines.

MDCK cells support the growth of influenza viruses, making them suitable for virology research, diagnostics, and vaccine production. Currently, it is the only cell line approved for use in the manufacturing of inactivated influenza vaccine. To distinguish MDCK-derived inactivated influenza vaccine from the long list of available chicken-egg-based inactivated influenza vaccines, the abbreviations ccIIV (cell culture inactivated influenza vaccine) and IIV (inactivated influenza vaccine) are used. See also sections "Chicken Fibroblasts," "MRC-5 Cells," "Vero Cells," and "WI-38 Cells."

MPL or 3-O-Desacyl-4′-Monophosphoryl Lipid A

The organic vaccine adjuvant MPL, or 3-O-desacyl-4′-monophosphoryl lipid A, is a detoxified form of endotoxin from the Gram-negative bacterium *Salmonella minnesota*. The adjuvant properties of MPL are explained by its proinflammatory interactions with toll-like receptor 4, triggering and enhancing the innate immune response. MPL is used as an adjuvant in the manufacturing of recombinant zoster vaccine.

MRC-5 Cells

MRC-5 (Medical Research Council cell strain 5) cells are a human diploid fibroblast cell line originally developed from the lung of a human fetus that was aborted at 14 weeks gestational age. This cell line has been propagated under

laboratory conditions since 1966 using established cell culture techniques. MRC-5 cells are currently used in the production of varicella and polio vaccines. See also sections "Chicken Fibroblasts," "MDCK Cells," "Vero Cells," and "WI-38."

Neomycin

Neomycin is an aminoglycoside class antibiotic. Pharmaceutical formulations are available for topical treatment of human eye, ear, and skin infections. It is used in vaccine manufacturing as an additive to some culture media to prevent cultures from becoming contaminated with bacteria.

Octoxynol-10

Octoxynol-10 is a nonionic chemical surfactant used primarily in the cosmetics and personal hygiene industry to aid in the formation of emulsions. It is found in hair dyes, hair conditioners, permanent wave products, and spermicides. In the biochemistry laboratory, surfactants such as octoxynol-10 are commonly used to extract proteins from cells and tissues. They do so by disrupting and disorganizing cell membrane lipid bilayers and solubilizing cellular proteins. Later biochemical steps are then used to purify the protein(s) of interest. In the vaccine industry, octoxynol-10 is used in the manufacturing of influenza vaccines, but subsequently removed during protein purification steps. Residual amounts may be detected in the final products.

Phenol

Phenol C_6H_5OH is a weakly acidic aromatic organic compound with a variety of industrial applications. It plays an important role in the synthesis of plastics, polycarbonates, epoxies, nylon, and some herbicides. In the molecular biology laboratory, phenol-chloroform extraction techniques are used to isolate DNA or RNA from cells and tissues. In the pharmaceutical industry, phenol is used as a precursor in the synthesis of a long list of medications, including acetylsalicylic acid (aspirin). Some over-the-counter oral analgesic sprays contain 1.4% phenol as an active ingredient. Phenol was also once widely used as an antiseptic. In the vaccine industry, phenol is added as a preservative to a final concentration of 0.25% in the 23-valent pneumococcal vaccine, the inactivated typhoid vaccine, and in smallpox vaccine.

Polymyxin B

Polymyxin B is an antibiotic with activity against most Gram-negative bacteria. Pharmaceutical formulations are available for intravenous and intramuscular injection to treat serious human infections caused by Gram-negative pathogens and for topical treatment of skin infections in combination with bacitracin and/or neomycin (e.g., triple antibiotic cream). It is used in vaccine manufacturing as an additive to some culture media to prevent cultures from becoming contaminated with bacteria.

Polysorbate 20, Polysorbate 80

Polysorbate 20 (Tween 20) and polysorbate 80 (Tween 80) are chemical surfactants and emulsifiers consisting of 20 and 80 repeat units of polyethylene glycol, respectively. Their stability and safety profiles identify them as excellent candidates for use in the health and hygiene industry for the manufacturing of cosmetics, liquid soaps and shampoos, hair conditioners, mouthwash, and toothpaste. They are also found as listed ingredients in soaps and laundry detergents, fabric softeners, inks, paints, waxes used for surfboards and skis, insecticides, spray and foaming sanitizers, and many other common household products. Stamp collectors use polysorbate 20 to remove adhesive and other residues from their collectables without damaging their quality or value. Industrial applications for polysorbates include a variety of manufacturing processes that require dispersion, emulsification, foaming, or cleansing. In the food industry, polysorbate 20 is used as a wetting agent in flavored mouth drops; polysorbate 80 is used as an emulsifier in ice cream. In the biochemistry laboratory, these agents are added to immunoassay wash buffers used to eliminate unbound proteins, and to lysing buffers used to extract proteins from cells and tissues. They do so by disrupting and disorganizing cell membrane lipid bilayers and solubilizing cellular proteins. In the pharmaceutical industry, polysorbates are used to stabilize emulsions and suspensions, including medications formulated for intravenous injection and more than a dozen different vaccine formulations.

QS-21

QS-21 is a purified plant extract from the soap bark tree *Quillaja saponaria.* In vaccine manufacturing, QS-21 is used as an adjuvant in the production of recombinant zoster vaccine in combination with MPL.

Sodium Taurodeoxycholate

Sodium taurodeoxycholate is a naturally occurring bile salt formed in the liver by conjugation of deoxycholate with taurine. Bile salts aid in the digestion of dietary fats by acting as anionic detergents and surfactants. The emulsification of dietary lipids in the small intestine leads to the formation of micelles, thereby facilitating intestinal absorption. In the biochemistry laboratory, sodium taurodeoxycholate and other surfactants (see also section "CTAB") are used to extract and purify proteins from cells and tissues. They do so by disrupting and disorganizing cell membrane lipid bilayers and solubilizing cellular proteins. In the vaccine industry, sodium taurodeoxycholate is used in the manufacturing of some inactivated influenza vaccines and in the production of the outer membrane vesicles used to produce one of the meningococcal serotype B vaccines.

Sorbitol

Sorbitol is a naturally occurring, sweet tasting, sugar alcohol found in berries, peaches, apples, and other fruits. Most of the sorbitol used commercially, however, is made from potato starch. Sorbitol is best known for its use in the food industry as a sweetener for sugar-free drinks, syrups, ice cream, and candies. Beyond its use as a sweetener, sorbitol is also used to reduce the loss of moisture over time from foods like peanut butter and fruit preserves, and to slow the staling process of baked goods. In the pharmaceutical industry, sorbitol is used to manufacture softgel capsules used to deliver single doses of liquid medicines. In the vaccine industry, sorbitol is added to several different products as a stabilizer.

Streptomycin

Streptomycin is an aminoglycoside class antibiotic. Pharmaceutical formulations are available for intramuscular injection as medical treatment of serious bacterial infections in humans. It is used in vaccine manufacturing as an additive to some growth media to prevent bacterial contamination.

Thimerosal

Thimerosal is an organic chemical that is approximately 50% ethylmercury by weight. It has played an important role as a preservative in vaccines and other pharmaceutical products since the 1930s by preventing bacterial and fungal

contamination during storage and use. Preservatives are particularly important as excipients in multidose vials of medications because of the need to enter and withdraw doses from the vial on more than one occasion. The history of thimerosal use as a vaccine preservative shows it to be both safe and effective. Mercury, however, is a heavy metal with no known physiologic role. Moreover, mercury is known to be toxic to humans when ingested as methylmercury in environmentally contaminated food. Organic methylmercury is present in many types of seafood, with the highest amounts accumulating in fish at the top of the food chain (e.g., sharks, swordfish, tuna). In contrast, thimerosal is metabolized to ethylmercury. Unlike methylmercury, ethylmercury is rapidly cleared from the body via the gastrointestinal tract. The amount of ethylmercury (as thimerosal) once included in childhood vaccines was not associated with toxicity. To offer added perspective on the amount of mercury-containing preservative once included in vaccine formulations administered to infants, consider the following: A single 0.5 mL dose of vaccine containing 0.01% thimerosal as a preservative contains 50 micrograms of thimerosal. This is equivalent to approximately 25 micrograms of mercury per vaccine dose. By comparison, this is roughly the same amount of elemental mercury contained in 1 ounce of swordfish or 3 ounces of canned albacore tuna.

Acknowledging that mercury has no physiologic function, and that methylmercury is known to be toxic, the FDA Modernization Act of 1997 recommended that, wherever feasible, mercury-containing preservatives must be removed from vaccines and other pharmaceutical products. The recommendation was not made because of any recognized toxicity of thimerosal or ethylmercury. In fact, the FDA noted its long safety history as a vaccine preservative stating that the recommendation was simply part of an ongoing effort to modernize vaccine formulations. The recommendation was made out of abundance of caution. Subsequently, thimerosal use was rapidly phased out as most vaccines were reformulated as single unit doses. Thimerosal remains FDA-approved for use as a preservative and continues to be used in the manufacturing of multidose vials of inactivated influenza vaccines, DT (diphtheria and tetanus), Td (tetanus and diphtheria), and Japanese encephalitis virus vaccine. Outside of the USA and most European countries, thimerosal is still used routinely. The World Health Organization has concluded that thimerosal is safe and that there is no need to change to the more expensive single-dose delivery via their Expanded Program on Immunization.

Thimerosal is also still used as a preservative in the manufacturing of the antivenins used to treat pit viper, coral snake, and black widow bites, and as a preservative in some immunoglobulin preparations.

Vero Cells

Vero cells are a lineage of African green monkey kidney cells initially established in 1962. Vero cells are interferon-deficient, so they do not secrete interferon alpha or beta when infected by viruses. They make excellent target cells for culturing viruses

needed for vaccine manufacturing and for a wide variety of experimental applications because they grow rapidly and continuously in cell culture. Vero cells are currently used in the production of some polio vaccines. See also sections "Chicken Fibroblasts," "MDCK Cells," "MRC-5 Cells," and "WI-38 Cells."

WI-38 Cells

WI-38 (Wistar Institute 38) cells are human diploid fibroblasts originally developed from the lung of a human fetus that was aborted at 12 weeks gestational age. This semicontinuous cell line has been propagated under laboratory conditions since the 1960s using established cell culture techniques. WI-38 cells are currently used in the production of rubella and polio vaccines. See also sections "Chicken Fibroblasts," "MDCK Cells," "MRC-5 Cells," and "Vero Cells."

Xanthan

Xanthan, or xanthan gum, is a complex polysaccharide used in the food industry as a thickening agent and as a stabilizer to prevent ingredients from separating. It is used in the vaccine industry to stabilize the active components in one of the available live, attenuated oral rotavirus vaccines.

Yeast

Yeast are single-cell, eukaryotic fungi that are widely used in the food industry and in the field of biotechnology. Fermentation of sugars by different types of yeasts is used to make bread and other baked goods, and in the fermentation steps needed to make beer and wine. Yeasts are one of the most widely used model organisms to study genetics and cell biology. A number of yeast species have been genetically engineered to efficiently produce large amounts of proteins used in the pharmaceutical industry including insulin and the immunogens used to produce hepatitis B and human papillomavirus vaccines.

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https://www.immunize.org/talking-about-vaccines/vaticandocument.htm.
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World Health Organization technical report series 980. Recommendations to assure the quality, safety and efficacy of tetanus vaccines (adsorbed). 2012;980:273–332.

Links to Package Inserts for U.S. 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

https://www.fda.gov/media/124379/download.

Diphtheria, Tetanus, Pertussis

https://www.fda.gov/media/75157/download.
https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/Package-Insert%2D%2D-DAPTACEL.pdf.
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https://www.vaccineshoppe.com/image.cfm?doc_id=12617&image_type=product_pdf.
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https://www.vaccineshoppe.com/image.cfm?doc_id=13791&image_type=product_pdf.
https://www.vaccineshoppe.com/image.cfm?doc_id=12609&image_type=product_pdf.
https://www.merck.com/product/usa/pi_circulars/v/vaxelis/vaxelis_pi.pdf.

Ebola

https://www.fda.gov/media/133748/download.

Haemophilus influenzae type B

https://www.vaccineshoppe.com/image.cfm?doc_id=13692&image_type=product_pdf.
https://gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Hiberix/pdf/HIBERIX.PDF.
https://www.merck.com/product/usa/pi_circulars/p/pedvax_hib/pedvax_pi.pdf.

Hepatitis A

https://gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Havrix/pdf/HAVRIX.PDF.
https://www.merck.com/product/usa/pi_circulars/v/vaqta/vaqta_pi.pdf.
https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Twinrix/pdf/TWINRIX.PDF.

Hepatitis B

https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Engerix-B/pdf/ENGERIX-B.PDF.
https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Twinrix/pdf/TWINRIX.PDF.
https://www.heplisavb.com/assets/pdfs/HEPLISAV-B-Prescribing-Information.pdf.
https://www.merck.com/product/usa/pi_circulars/r/recombivax_hb/recombivax_pi.pdf.

Human Papillomavirus

https://www.merck.com/product/usa/pi_circulars/g/gardasil_9/gardasil_9_pi.pdf.

Influenza

http://labeling.seqirus.com/PI/US/Afluria/EN/Afluria-Prescribing-Information-QIV.pdf.
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https://www.azpicentral.com/flumistquadrivalent/flumistquadrivalent.pdf#page=1.
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https://www.vaccineshoppe.com/image.cfm?doc_id=14671&image_type=product_pdf.
https://www.vaccineshoppe.com/image.cfm?doc_id=14674&image_type=product_pdf.

Japanese Encephalitis

https://www.fda.gov/media/75777/download.

Measles, Mumps, Rubella

https://www.merck.com/product/usa/pi_circulars/m/mmr_ii/mmr_ii_pi.pdf.
https://www.merck.com/product/usa/pi_circulars/p/proquad/proquad_pi_4171.pdf.

Meningococcus

https://www.vaccineshoppe.com/image.cfm?doc_id=12580&image_type=product_pdf.
https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Menveo/pdf/MENVEO.PDF.
https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Bexsero/pdf/BEXSERO.PDF.
http://labeling.pfizer.com/ShowLabeling.aspx?id=1796.

Pneumococcus

https://www.merck.com/product/usa/pi_circulars/p/pneumovax_23/pneumovax_pi.pdf.
http://labeling.pfizer.com/ShowLabeling.aspx?format=PDF&id=501.

Polio

https://www.vaccineshoppe.com/image.cfm?doc_id=5984&image_type=product_pdf.

Rabies

https://www.vaccineshoppe.com/index.cfm?fa=anon.catalog&category=1&section=4&family=24#category=1&section=4.
https://www.vaccineshoppe.com/image.cfm?doc_id=5983&image_type=product_pdf.
https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Rabavert/pdf/RABAVERT.PDF.

Rotavirus

https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Rotarix/pdf/ROTARIX-PI-PIL.PDF.
https://www.merck.com/product/usa/pi_circulars/r/rotateq/rotateq_pi.pdf.

Smallpox

https://www.fda.gov/media/75792/download.

Tickborne Encephalitis

http://mri.cts-mrp.eu/download/AT_H_0126_001_FinalPL.pdf.

Tuberculosis

https://www.merck.com/product/usa/pi_circulars/b/bcg_vaccine/bcg_pi.pdf.

Typhoid

https://www.vaccineshoppe.com/image.cfm?doc_id=9372&image_type=product_pdf.
https://www.fda.gov/media/75988/download.

Varicella and Shingles

https://www.merck.com/product/usa/pi_circulars/v/varivax/varivax_pi.pdf.
https://www.merck.com/product/usa/pi_circulars/p/proquad/proquad_pi_4171.pdf.
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https://www.merck.com/product/usa/pi_circulars/z/zostavax/zostavax_pi2.pdf.

Yellow Fever

https://www.vaccineshoppe.com/image.cfm?doc_id=13799&image_type=product_pdf.