Freeze-Drying of Vaccines for Eradication of Smallpox

Abstract

Smallpox, a virus, is one of the most deadly human diseases. Many years ago, vaccines were developed from weaker, related viruses, such as cowpox and vaccinia. Modern preservation techniques, such as freeze-drying, allowed for the widespread use of smallpox vaccine, leading to the eradication of naturally-occurring smallpox by 1980.

Introduction

Smallpox is a highly contagious viral infection caused by the *variola* virus. It is a disease that only infects humans and is considered to be one of the most deadly and persistent human pathogens (disease-inducing agent). According to the World Health Organization, fatalities due to smallpox accounted for 300-500 million deaths in the 20th century. Smallpox is most notorious for its rapid and vast depopulation of the Americas in the past five hundred years by infecting Native Americans who were not previously exposed to the virus. Brought by Spanish Conquistadors, smallpox was the main reason for the defeat of the Aztec and Incan Empires rather than swords and firearms, enabling the Spanish conquest of most of the New World (the Americas) between the 16th and 17th centuries. (Diamond 112-118) In 18th century Europe, one in three persons infected by smallpox died and the survivors were often disfigured by blister scars. (Smallpox, Smallpox Vaccine, Wikipedia)

The first recorded attempt at smallpox immunization was "variolation," used for several centuries in China, the Middle East and India. To immunize through variolation, an individual had to be healthy and the infectious material must come from a mild case of smallpox, which was later identified as a weaker variant of the smallpox *variola* virus, *variola minor*. The other, more lethal variant, *variola major*, killed 20-40% of its victims. Variolation proceeded by rubbing the infectious material through a scratch in the hands. This would induce mild symptoms, such as fever, soreness and blisters. However,

variolation is hazardous and could often cause death due to a weak immune system or due to the mistaken use of *variola major* materials. It was not until 1796 that an Englishman, Edward Jenner, first tested a smallpox vaccine prototype. (Variolation, Wikipedia)

Jenner observed that milkmaids were never infected by smallpox, even in the presence or contact with individuals infected with smallpox or with smallpox materials. Jenner theorized that a previous infection from cowpox, with symptoms similar to smallpox but much weaker, was the source of immunity for the milkmaids. He tested his hypothesis by infecting a boy with cowpox materials obtained from an infected milkmaid. Later, after his symptoms healed, the boy was exposed to variolation but did not show any symptoms. Further smallpox exposures led to no sickness. Jenner continued his experiments with positive results using cowpox. Later, the King and Parliament of England fully supported Jenner in his efforts. The vaccinated were either immune or suffered very weak symptoms when infected by smallpox. (Edward Jenner, Wikipedia)

The modern smallpox vaccine is derived from the *vaccinia* virus, which is closely related to the cowpox virus but produces fewer symptoms. It is the only commercially approved smallpox vaccine in the U.S. and is manufactured under the brand "Dryvax." All smallpox vaccines contain live virus, not just viral particles. Smallpox vaccines produced from vaccinia strains in England (Lister Strain) were responsible for the worldwide eradication of naturally occurring smallpox by May 1980 as declared by the World Health Organization. Recent terrorist threats of biological attacks have alerted authorities in the U.S. and Europe to stockpile new smallpox vaccines and to restart vaccine production. (CDC)

Vaccine Manufacture

Apart from Jenner's rudimentary vaccine preparation in the early 19th century, there have been several other smallpox-vaccine production methods. Replacing Jenner's vaccine method in late 19th century England was a type of smallpox vaccine prepared in glycerol, an alcohol. The mixture contained the vaccinia virus instead of cowpox and was

suspended in calf lymph (white blood cells in water, blood proteins and electrolytes) containing 40-50% glycerol. Because this vaccine was stable only below 0 deg. Celsius, it could not be used in tropical regions. In the 1920's, Leslie Collier, a researcher at the Lister Institute (the first modern medical research institution in the U.K.) devised a method to preserve the efficacy of the smallpox vaccine in hot and humid conditions. After numerous trials, the final Collier vaccine consisted of vaccinia virus suspended in calf lymph, water and 5% concentration amino acids. The mixture is transferred into a glass bulb and sealed under nitrogen gas. Collier's vaccine was stable for up to 2 years at 45° C and for 2 hours at 100° C. (Cambridge)

In America, modern vaccine development and production began in the 1950's, when the pharmaceutical company Wyeth manufactured Dryvax, a freeze-dried smallpox vaccine. Dryvax is manufactured by freeze-drying vaccinia-infected calf lymph. The infected lymph is produced by first infecting calves up to 22-28 times to obtain sufficient viral concentrations in the calf blisters that develop and are collected. The fluids from the blisters are extracted, separated, and purified from the blister skin and slightly diluted with calf lymph obtained from the calf's lymph nodes (lymph-containing organs in the calf). The viral mixture is then freeze-dried and sealed in vacuum containers or pouches. Upon application, the vaccine must be reconstituted in an aqueous solution (50% glycerol, 0.25% phenol and 0.005% malachite green, an antiseptic chemical). Wyeth discontinued most Dryvax production in 1983 because naturally-occurring smallpox was officially eradicated in 1980. The Dryvax and Collier vaccines are the two most important vaccines for smallpox. Both played significant roles in eradicating smallpox. (CDC)

Freeze-Drying

The key to good preservation and widespread use of smallpox vaccine is freeze-drying. Removing water from perishable products prevents degradation by molds and bacteria. A freeze-drying machine is often utilized for mass-production of freeze-dried

materials. At the end of the freeze-drying process, the dried product is sealed in a plastic or acrylic vacuum package that is resistant to wear and easy to store and transport. When needed, the vaccine can be reconstituted in the previously mentioned chemical solution.

The process is comprised of three steps: freezing, primary drying and secondary drying. Figure 1 shows the process with a phase diagram. During the first step, the vaccine (virus-containing lymph in liquid form) must be frozen at a temperature below its eutectic point (the lowest temperature where the liquid in the vaccine can transition between the solid and liquid states due to pressure changes). At this low temperature, sublimation (the transition of a substance from solid to gas states) can occur when the pressure is lowered with some added heat. Sublimation allows for faster removal of the liquid rather than evaporation.

During the primary drying stage, the pressure above the frozen vaccine is decreased below the triple point pressure (where all three phase boundaries meet). A temperature increase would lead to the gas phase, which results in sublimation of the solid (water in this case). The rate of pressure decrease and rate of heating must be carefully controlled. If the vaccine is subjected to too much heat, proteins will denature and vaccinia viruses will deactivate, rendering the vaccine useless. Sublimated water is condensed from the vaccine sample and collected for disposal. About 98% of the total water in the sample is removed. In the final step, the temperature is raised and the pressure decreased slightly to allow sublimation of any remaining water bound to the vaccine solids. The vaccine is then isolated under an inert gas, such as nitrogen or argon, to reestablish normal atmospheric pressure. The vaccine is then ready for packaging. (Freeze-Drying, Wikipedia)

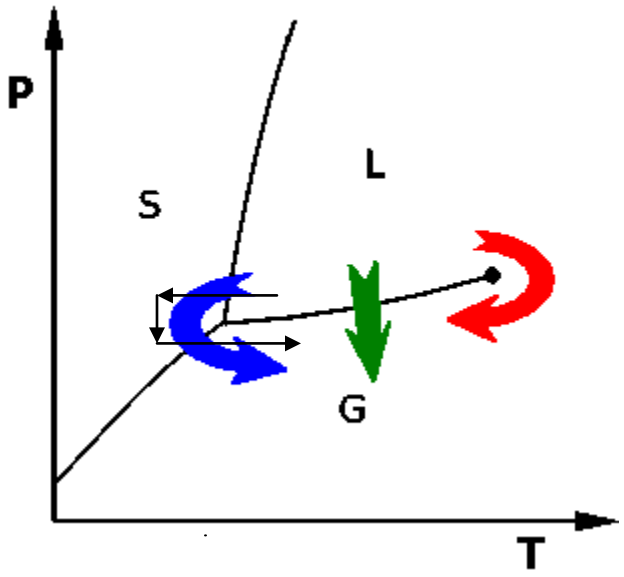
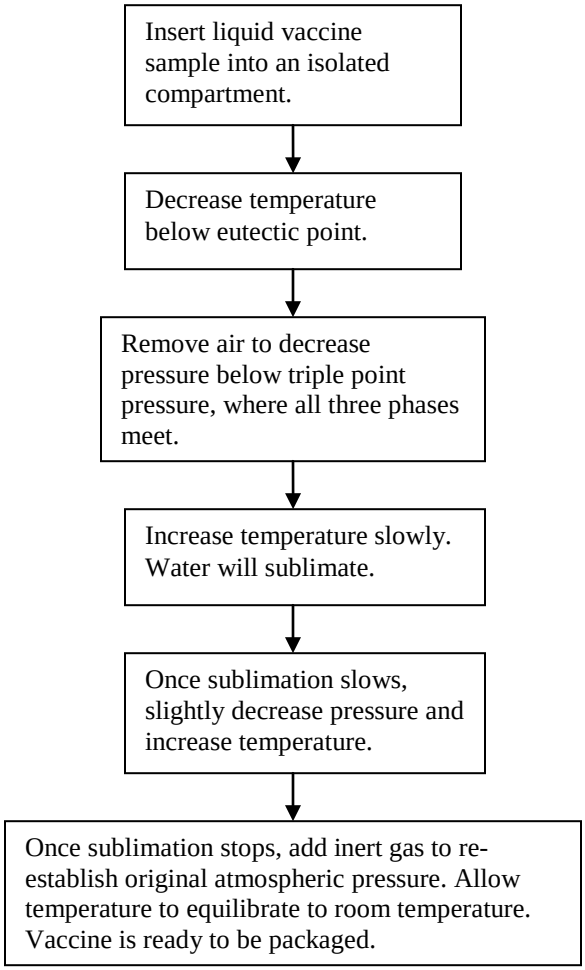


Figure 1: The three connected arrows (superimposed over the thick arrow to the left) represent the phase changes due to the freeze-drying process to remove water from the vaccine.

Flow Chart:



Consequences

In 1967, the World Health Organization (WHO) initiated a program completely to eradicate smallpox as a naturally occurring disease. At the time, most smallpox-vaccine laboratories used either the freeze-dried method of the Dryvax vaccine or the Collier vaccine. Between 1967 and 1984, a total of over 465 million doses of smallpox vaccine were donated to the WHO as part of the global smallpox eradication program. The former Soviet Union contributed over half (298 million) the total amount of smallpox vaccines to the WHO. Between 1967 and 1979, the WHO distributed over 360 million doses of smallpox vaccine around the world. The approach to eradication was precise identification of high-risk areas where smallpox was spreading, using a precise amount of vaccine doses, so the vaccine would not be wasted. Despite the WHO's desire to

conserve vaccine supplies, the ease of vaccine production is exemplified by India, where almost 800 million doses of vaccine were produced between 1967 and 1978 for a population of 600 million. Vaccine manufacture did not require a highly advanced infrastructure nor expensive materials. Smallpox cases dropped quickly after 1970, and only 1000 cases were reported worldwide in 1974. Eventually, the disease was essentially eliminated by 1978. (Smallpox and its Eradication, Chps. 7-10)

By the late 1960's and early 1970's, most smallpox vaccines adopted the freeze-drying approach of Dryvax because of its much longer shelf life than the nitrogen sealed vaccine of Collier. Freeze-dried vaccines are easier to produce and last for decades. Currently in the US, there are around 16 million doses of smallpox vaccine (produced in the mid-1970's) stockpiled and ready to use. The ease of production and efficacy of the smallpox vaccines based on Dryvax and Collier made smallpox vaccines the most produced anti-pathogenic product from 1950 to 1980. It also led to quick and extensive eradication of global smallpox, one of the most widespread and deadly diseases in human history. Several million people were dying from smallpox in the mid-1960's before the WHO smallpox eradication program began. After 1979, no cases were reported.

Conclusion

Smallpox was one of the most deadly and widespread diseases before the WHO eradicated naturally-occurring smallpox in the late 1970's. With the observations and discoveries of Edward Jenner, smallpox vaccines developed into a potent protection against the disease. Smallpox vaccines based on the weaker, but related cowpox and vaccinia viruses provided keys to effective immunization. While recipients suffered little or few symptoms, they were fully protected against smallpox. Preservation techniques based on the use of freeze-drying and nitrogen sealed containers allowed widespread use of vaccines. The simple techniques used to make vaccines facilitated local vaccine manufacture that helped to accelerate eradication of the disease.

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