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Development of Vaccines

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In this thesis the vaccine types, adverse events and the development of vaccines were discussed.

Traditional vaccine types manufactured are live-attenuated, inactivated, subunit and toxoid vaccines. Also, combinations of these vaccine types are common. New vaccines that are manufactured include DNA technology. There are DNA vaccines and recombinant protein subunit vaccines.

Adverse events are the possible side effects that immunization can cause. They do happen in a certain frequency and can range from mild reaction to severe. Adverse events are monitored from the beginning of the development of vaccines.

Development of a vaccine is challenging and difficult. It includes many phases, high costs and can take several years, with no guarantee of success. Development starts with finding the suitable antibodies and antigens that provide the immunity against wanted disease. Then a prototype of vaccine is made and tested with animals. If the tests are showing good results, the vaccine is going to clinical trial phases, where it is tested with humans. If everything goes well, it is possible to apply marketing license. When the vaccine is in the market, it is still under surveillance for the adverse events.

Keywords	Vaccine, DNA Technology, Immunity, Upstream, Downstream

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Tässä opinnäytetyössä käsitellään rokote tyyppejä, haittavaikutuksia ja rokotteiden kehittämistä.

Perinteiset rokotetyypit, joita valmistetaan, ovat elävät heikennetyt, inaktivoidut, subunit ja toksoidi rokotteet. Näiden rokote tyyppien kombinaatiot ovat myös yleisiä. Uusien rokotteiden valmistuksessa käytetään DNA teknologiaa. On olemassa DNA- ja rekombinantti proteiini subunit rokotteita.

Haittavaikutukset ovat mahdollisia sivuvaikutuksia, joita immunisaatio voi aiheuttaa. Näitä tapahtuu tietyllä frekvenssillä ja ne voivat vaihdella miedoista reaktioista vaarallisiin. Haittavaikutuksia seurataan rokotteiden kehittämisen alkamisesta asti.

Rokotteen kehittäminen on haastavaa ja vaikeaa. Se sisältää monia vaiheita, se on kallista ja voi viedä useita vuosia, ilman mitään varmuutta onnistumisesta. Kehittäminen alkaa sopivien vasta-aineiden ja antigeenien löytämisestä, jotka tarjoavat immuniteetin haluttua tautia vastaan. Sen jälkeen valmistetaan rokotteelle prototyyppi ja sitä testataan eläimillä. Jos testit näyttävät hyviä tuloksia, voi rokote edetä kliinisiin kokeisiin, missä sitä testataan ihmisillä. Jos kaikki menee hyvin, on mahdollista hakea markkinointi lupaa. Kun rokote on markkinoilla, sen mahdollisia haittavaikutuksia seurataan vielä.

Avainsanat	rokotteet, DNA teknologia, immuniteetti, upstream, downstream

Contents

List of Abbreviations

AEFI	Adverse Event Following Immunization
DNA	Deoxyribonucleic Acid
EMA	European Medicines Agency
EPI	Expanded Programme on Immunization
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
HIV	Human Immunodeficiency Virus
MIT	Massachusetts Institute of Technology
NIP	National Immunization Programme
NRA	National Regulatory Authority
THL	Terveyden- ja hyvinvoinnin laitos (National Institution for Health and Welfare)
UNICEF	United Nations International's Children Emergency Fund
WHO	World Health Organization

1 Introduction

World Health Organization (WHO) defines a vaccine as follows:

> A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters. [WHO, 2018.]

Vaccines are proteins, polysaccharides, or pathogenic nucleic acids, that are used to stimulate immunity, to eliminate or weaken pathogens or pathogenic products. In other words, vaccines are biological preparations that help to acquire immunity against diseases. Vaccines are usually virus or bacterium-based preparations, that fight against diseases, caused by viruses or bacteria. There are different kind of vaccines researched and developed. Pediatric vaccines are the most researched kind. There is also influenza, adult, cancer, therapeutic, hepatitis and other vaccines.

The aim of this thesis is to discuss vaccines; how they function, how the production processes are developed, manufacturing of vaccines and what the future looks like. The production process is similar to the manufacturing of other biopharmaceutical preparations because the use of bacteria, viruses and even genome. Researchers continue to fight against diseases, infections and the variant strains of influenza.

Vaccines have had a huge impact in the world, especially in low-income countries, where there are lots of people in small areas and poor hygiene. Different organizations try to help these countries by offering vaccination programmes. Vaccination can reduce health-care costs for many countries, give longer life expectancy and lower mortality, which then again helps the economy. Vaccines can even effect in the injustice between rich and poor, by being available for both. Travelling is also safer, when it is possible to be protected against different diseases. [Andre et al., 2007.]

For the last half century, vaccines have eliminated contagious diseases and prevented millions of deaths and potential disabilities. Even so, vaccinating is not always ethically unproblematic. Vaccines are given to healthy people and small children that cannot make the decision on their own to be vaccinated. Ethics of vaccination is around three medical

principle: beneficence, autonomy and justice. Everyone in the vaccination programme must follow these principles according to their role and decisions. [Hedman et al., 2011: 776.]

2 Vaccines and How They Work

In this chapter, the vaccine mechanisms are discussed through immunization and immunity. Explanations are also given for antibodies and antigens. The traditional vaccine types are introduced in section 2.2.

2.1 Mechanisms of Vaccines

To understand how vaccines function, one needs to have knowledge about antibodies, antigens and immunization. Antibody is a protein that immune system has created in the presence of specific antigen (ANTIbody GENeration). Antibodies help to counter the effects of antigens, by attaching to them. Antigens are substances that stimulates the immune system to create antibodies. [Glossary of Terms, 2018.]

Immunization means making the body resistance against germs (germ = pathogenic microorganism). This happens with the help of antigens that stimulates the antibody production and cell-mediated immunity. When the immunity has been acquired, the body is resistant against specific antigens. [Hedman et al., 2011:770.]

Active and passive immunity are part of adaptive immune system. If the immunity is based on body's own cell activity (antibodies are made in the body), it is called active immunity. Active immunity can be achieved by natural infection or artificially with vaccination. Resistance is acquired with passive immunization, if antibodies are created somewhere else. Passive immunity happens naturally through maternal antibodies or through artificially made monoclonal antibodies. Active immunizations, in other words, vaccinations biggest advantage is that once acquired protection can last years. Protection trough passive immunization usually lasts only few months. [Hedman et al., 2011:770.] Sometimes adjuvants are needed to help enhance the immunity.

Immunity can be humoral or cell-mediated and these are often working together. [Hedman et al., 2011:770]. Humoral immunity is mediated by B-lymphocytes, when cell-mediated immunity by T-lymphocytes (lymphocyte = type of white blood cell). B-lymphocytes can divide to memory cells and cells that form antibodies. T-lymphocytes can recognize and destroy cells infected by a virus and help B-lymphocytes to create specific antibodies. Both lymphocytes can detect antigens. [Cruse & Lewis, 2009: 69, 432, 682, 686, 706.]

The function of vaccines is built on immunity. Immunity system is a collection of organs, cells, and molecules inside the body that identify germs as foreign invaders and reacts by producing proteins called antibodies. Antibodies destroy the germs and memorizes the invaders. They remain in the bloodstream and can detect if the same germs enter the body again to infect it. Since antibodies have already once destroyed them earlier, they can annihilate them before infecting the body. [How Vaccines Work, 2016.]

Vaccines work the same way, but without the body getting ill. They are made of pathogens disease causing properties that are weakened or killed first, by biological, chemical or physical means, while retaining its ability to induce an effective immune response [Ho & Gibaldi, 2003:315.] Immunity system reacts to the weakened or killed germs, the same way they do, when being invaded by the disease. New antibodies are made and germs have been memorized. [How Vaccines Work, 2016.]

2.2 Main Types of Vaccines

Vaccines improve immunity against a specific disease. Vaccination is one of the most effective ways to prevent infectious diseases from spreading. [Route of Administration, 2018.] Vaccine can be given orally or as an injection to a muscle, under the skin, inside the skin, or as a nasal spray (e.g. influenza vaccine). Vaccines that are given under the skin are usually injected in the shoulder. The ones that are given to the muscle are given to the thigh or to the shoulder muscle. Vaccine is absorbed better from muscle than from fatty tissue. [Hedman et al. 2011: 835-6.]

Each vaccine type is designed to instruct antibodies to destroy certain germs. The main four vaccine types that scientist create are live-attenuated, inactivated, subunit and toxoid vaccines. These are categorized by the antigen that is used in their manufacturing. Formulation of these vaccine types affects their use, storing and how they are controlled. [Types of Vaccine, 2018.]

Besides the traditional vaccines, there are also combination vaccines. They are defined as immunizing preparations which have antigens from several pathogenic microorganisms. This type of vaccine can create immunity against more than one disease. For example, MMR is a typical combination vaccine, which has live-attenuated viruses from measles, mumps and rubella. MMR vaccine protects against these diseases and is a part of childhood vaccination programme. [Cruse & Lewis, 2009:185.]

2.2.1 Live-attenuated vaccines

Live-attenuated vaccines resemble the natural infection, but in the weakened form. It is made of attenuated form of the germ that causes a disease, but retains the ability to infect and replicate in the vaccinated host. The weakened form is achieved by passaging the pathogen into tissue cultures repeatedly. The weakest variants of the pathogen are then selected from each repetition. [Ho & Gibaldi, 2003:315.] Live-attenuated vaccine can give a lifetime protection against germs that are responsible for the disease, only from one or two doses to the body. These vaccines can be used against for example measles, smallpox and yellow fever, which are caused by a virus. Tuberculosis vaccine is a bacterium based live-attenuated vaccine. Unfortunately, this vaccine type has some restrictions. It needs to be kept in cool, so delivery and storing might be an issue. Also, people with weakened immune systems might not be able to receive this type of vaccine because it contains a small amount of the attenuated live virus. [Vaccine Types, 2017.] Some viruses cannot be attenuated enough to be given to humans without a risk of getting the disease, for example HIV virus (Human Immunodeficiency Virus).

2.2.2 Inactivated vaccines

In the inactivated vaccines the pathogen is inactivated by chemical or physical means or combination of both. Unfortunately, inactivation process could destroy the antigen conformation required to elicit protective immunity. [Ho & Gibaldi, 2003:316-317.] Inactivated vaccines differ from live-attenuated vaccines by using the killed version of the germ that

causes illness. It is not as effective as live-attenuated vaccine and does not provide protection of a lifetime, but because it uses the killed version of the germ, it can be received by the people who have weakened immunity systems. It needs to be taken periodically over time to get permanent immunity. This type of vaccine can protect against for example hepatitis A and rabies, which are caused by a virus. Typhoid vaccine is a bacterium based inactivated vaccine. [Vaccine Types, 2017.]

2.2.3 Subunit vaccines

Subunit vaccine is a type of vaccine that uses only the protein part of the germ and does not contain its genetic material. To prepare subunit vaccines, one must have identified the key antigenic target to include in the vaccine. Immunogens of these vaccines are composed of bacterial proteins, polysaccharides or conjugates of both. [Ho & Gibaldi, 2003:318-319.] This type of vaccine gives a strong immune response because it targets the key parts of the germ. There are similarities with inactivated vaccines. Subunit vaccine can also be given to infants and people with weakened immunity system. It needs to be taken several times over period to keep the protection against diseases. [Vaccine Types, 2017.] This type of vaccine is used to protect against hepatitis B and pneumococcal disease [Types of Vaccine, 2018].

2.2.4 Toxoid vaccines

Toxoid is a chemically inactivated toxin. Toxoid vaccines are little different from the vaccines discussed previously. They use the germs toxin that causes a disease. The immunity is created to the parts that causes the disease, so instead of targeting the whole germ, the immune response targets only the toxin. A toxoid vaccine does not give a lifetime protection, thus booster shots might be needed. This type of vaccine protects against diphtheria and tetanus. These diseases are caused by a bacterium. [Vaccine Types, 2017.]

Benefits of using a vaccine that contain live organisms is that they are natural and easy to store to refrigerators and coolers. They also often provide a lifetime protection against diseases. Problems that occur with vaccines that contains live organisms are that they are hard to produce, they might spread to the environment or change to pathogenic. Preservation might become problem with very warm countries because these vaccines need to be kept cool, usually between +2℃ to +8℃. Some vaccines need to be stored

below zero degrees, -25℃ is the minimum. Other vaccines, like inactivated or toxoid vaccines, have benefits of easy use, they cannot induce an infection and they have only little adverse events. Problems of these are that they are much weaker than the ones that use live organisms, so they provide a weaker immunity or one that lasts less than a lifetime. Sometimes the antibodies will not protect as they are meant to and they can be harmful. [Hedman et al. 2011: 773.]

3 History of Vaccines

The use of vaccination to immunize people against infectious diseases has influenced the world for the past 200 years. It is one of the most remarkable achievements in the history of medicine. Early form of vaccination was developed in 1100's China. This method is called variolation, and it arrived at Europe around 1700's. Variolation is discussed more specifically in section 3.1.

Most vaccines invented before 20th century, were often created by accident. Scientists tested different techniques and substances very freely because there were not much restrictions or regulations concerning it. Most of the experiments failed and possibly caused injuries, disabilities or let to the death of the test subjects, but sometimes the experiments succeeded. After the realization that diseases were caused by microorganisms, the first created vaccines were usually bacterium based because viruses were too small to be detected with microscopes of that time.

3.1 Variolation

Smallpox is a disease caused by a variola virus. Smallpox cause vesicular and pustular skin injuries that are painful. Risk of death was very high before the vaccine was invented. [Cruse & Lewis, 2009.]

Variolation was the early form of vaccination. It was based on inoculating the smallpox virus in to person’s skin to produce immunity against the disease. A lancet or a needle was used to put pulverized dried smallpox scabs or pustule fluid into the slightly cut skin of the person. Drying made the form of the virus milder, which was shown as a lower fatality rate. Variolation was developed in China as early as 1100’s. It came in Europe 600 years later. Nowadays it is not used anymore as a medical practice. It was not that

safe because variolated persons could still transmit smallpox to others and the inoculation method spread diseases, for example syphilis and hepatitis. [Louten J., 2016.]

3.2 Remarkable moments

Edward Jenner, born in 1744, is often referred to as the founder of immunology, based on his contribution of the first reliable method of giving lasting immunity to a major contagious disease [Cruse & Lewis, 2009:424]. He figured out that the inoculation of cowpox could create immunity against smallpox. This procedure was called vaccination and it came from the word "vacca", which means cow in Latin. First smallpox vaccine was developed in 1798. This discovery lead to eradication of smallpox in 1979. [All Timelines Overview, 2018.]

Louis Pasteur followed in Edward Jenner's footsteps and successfully created the first live-attenuated cholera vaccine in 1879. Few years later he also created vaccine against rabies. Development of bacteriology led to new discoveries in bacterial vaccine development, for example the creation of plague vaccine in the 1897. [All Timelines Overview, 2018.] In the late 19th century Robert Koch proved that infectious diseases are caused by microorganisms and that each one of them are responsible for a particular disease. Now there are four categories recognized as disease causing microorganisms or pathogens: viruses, bacteria, fungi and parasites. [Murphy Etc. 2008:1.] This information made a major impact in the research of vaccines.

Alexander Glenny created a method to inactivate tetanus toxin with formaldehyde in the 1923. This method was applied to create other vaccines. Between the 1950 and 1985 viral tissue culture methods developed, for example growing viruses in the laboratories. It meant prompt discoveries and innovations, which lead to polio vaccine in the 1955. Since then, polio has been 99% eliminated from the world. [All Timelines Overview, 2018.]

Researchers concentrated in the mid-20th century to the creation of vaccines for the childhood diseases, such as measles and rubella. Childhood diseases were especially targeted because children have much weaker immune systems compared to adults. After the founding of the United Nations International Children's Emergency Fund (UNICEF) in the 1946 and the World Health Organization (WHO) in the 1948, children's

immunization programmes were introduced to the world. [Stern & Markel, June 2005.] In 1974 WHO launched Expanded Programme on Immunization (EPI), which targeted diphtheria, whooping cough, tetanus, measles, poliomyelitis and tuberculosis. The goal was to establish immunization for all children by 1990. [The Expanded Programme on Immunization, 2018.]

Figure 1 describes a few remarkable moments in the history of vaccines. It starts as early as 12th century China, in the form of variolation. The important evolving steps of vaccination are focused on the late 18th to 20th centuries.

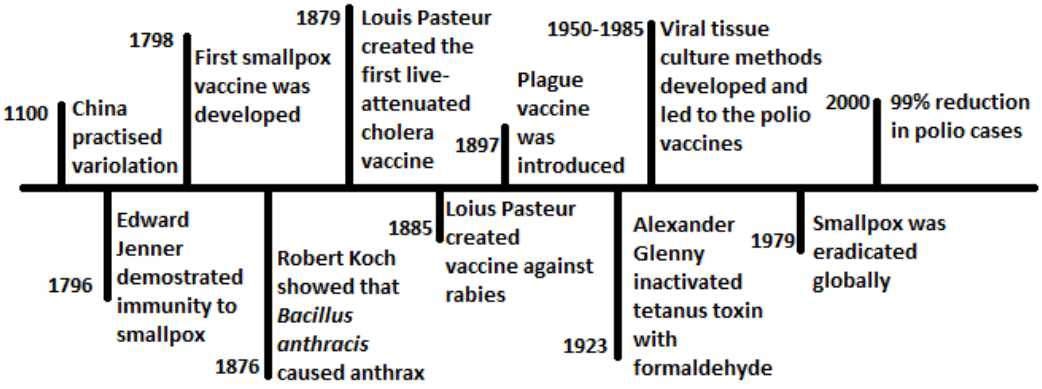

Figure 1. Brief timeline of vaccine history [All Timelines Overview, 2018].

3.3 Eradication and Elimination of Diseases

This section is divided to two categories and it is about eradication and elimination of diseases. Eradication is discussed in section 3.3.1 and elimination in section 3.3.2.

3.3.1 Eradication

When talking about vaccines, eradication means that the disease is destroyed with the cause of the disease. Elimination then again means that the disease is eliminated but the disease-causing factors are remaining. Eradication is the main target for an immunization programme, but only smallpox has been eradicated completely (1979) from everywhere. After that the second-best goal is to eliminate diseases from different parts of the world, and hope that it would eventually lead to the eradication from the whole world. Polio is hopefully the next disease to be eradicated. At the moment, type 2 poliovirus has been eliminated globally, but types 1 and 3 are still met in some countries. Eradication

of a disease is possible with an effective vaccine, but it also needs majority of people in the world, to have immunity in all regions, for constant period of time and a good surveillance. [Andre etc., 27.11.2007.] Eradication can be confirmed after there has not been any reported disease cases in a couple of years.

3.3.2 Elimination

Elimination of disease can be done locally, without the need to destroy the causative microorganism, globally. Measles has been eliminated in most of the developed countries and completely from the United States of America. This has been achieved through a two-dose vaccination programme. Combination vaccine of measles, mumps and rubella has high hopes of eliminating, and someday eradicating, rubella and mumps. Unfortunately, even though some disease might have been eliminated locally, it does not mean that the danger of infection is over because the disease-causing factor has not been destroyed. It can only need one person who carries the pathogen, to visit disease eliminated country, to reintroduce the disease all over again. [Andre etc., 27.11.2007.]

Proof that eliminating measles, mumps and rubella is possible in the future is that it has already happened in Finland. Figure 2 shows how vaccination programme effected in Finland and eliminated these diseases. Elimination happened through a two-dose vaccination programme that begun in the 1982. As can be seen from the Figure 2, the cases of these diseases have started to decrease clearly from 1982. The rate has been downward since 1990. Measles cases have had the most significant difference between the years 1960 to 1994. Cases have dropped from about 80 000 cases in a year, to about 10 cases in a year. [Peltola et al., 1994.] Measles was completely eliminated from Finland in 1996. Since then it has not been met in Finland. [Heinonen et al., 1998].

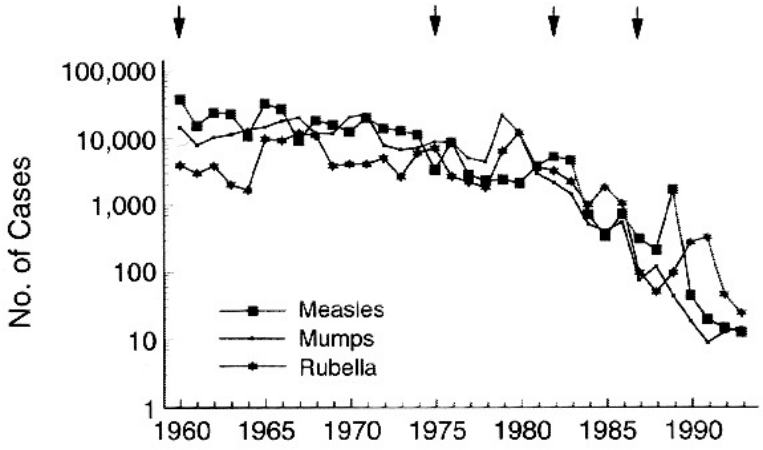

Figure 2. Cases of measles, mumps and rubella in Finland from 1960 to 1994 [Peltola et al., 1994].

Even though eradication and elimination of diseases has happened after vaccines were introduced to the world, some studies argue that vaccines did not have actually that huge impact on the eradication or elimination. It might have made the elimination just happen faster. These studies are based on the thought that availability of clean water, improvement of hygiene, sanitation and development of modern health made the impact for elimination of diseases. Figure 3 shows that the polio, smallpox and diphtheria cases had been reduced already, even before the vaccines were introduced. Typhoid fever disappeared with no vaccination programme.

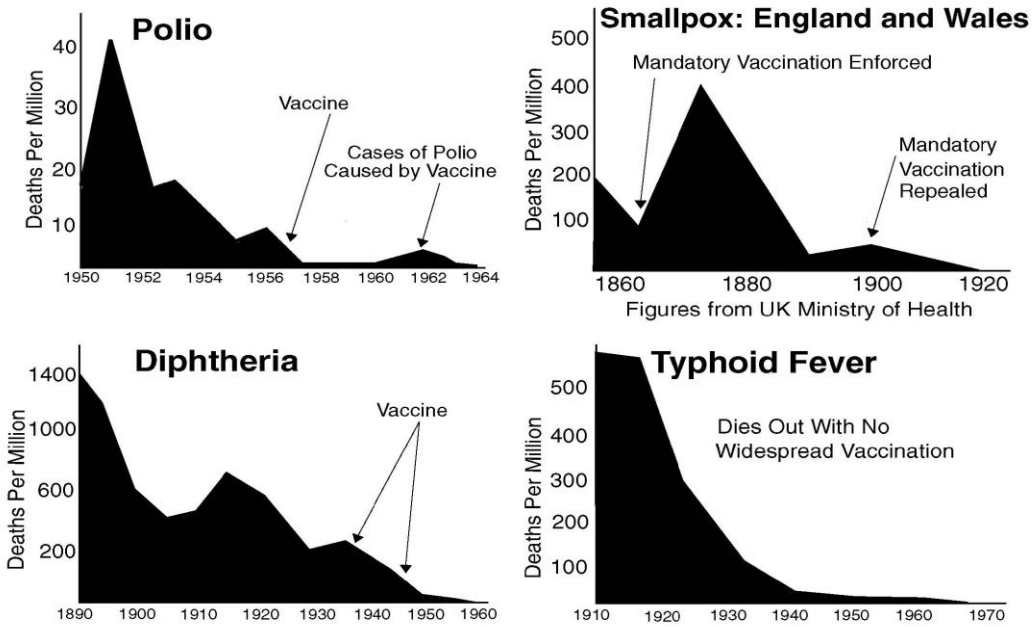

Figure 3. Vaccines effect on diseases [Borne, 2005].

4 Side Effects of Vaccination

Adverse events are reactions that are undesired. They could be referred as a side effects. Vaccines have lots of components and each component can add risk. It must be assured that vaccine components do not cause any danger to vaccine safety, used separately and in a combination. Some vaccines can have components to strengthen immune response with adjuvants and conjugated proteins. They can also include components to lessen contamination during the production process. The used components can be antibiotics and/or stabilizers. [Adverse Events: Causes, 2018.]

Adverse Event Following Immunization (AEFI) can be any adverse medical hazard, which happens after immunization, but does not necessarily happen because of the vaccine. These events can be compartmentalized to five categories by the cause of the event. These events can be related to vaccine products, vaccine quality defects, immunization errors, immunization anxiety or coincidental events, which are discussed in sections 4.1 - 4.5.

4.1 Vaccine Product-Related Reaction

Vaccine product-related reaction is the first category of the AEFI's. This reaction is caused by one or more of the vaccine components. In this case, the contents of the vaccine are causing the side effects. Swelling of limbs, after vaccination, is one example of this reaction. [Adverse Events: Classification, 2018.]

4.2 Vaccine Quality Defect-Related Reaction

Second category is *vaccine quality defect-related reaction*. It is caused by vaccine quality defects. These defects could occur, for example, if polio virus has not been completely inactivated in the vaccine. In this case, the quality defect could lead to paralytic polio, which paralyses the spinal cord. [Adverse Events: Classification, 2018.]

4.3 Immunization Error-Related Reaction

Third category is *immunization error-related reaction.* This error could be caused by the improper handling of vaccine, mistakes in the description or in the administration. This could happen, for example, because of contaminated vial. [Adverse Events: Classification, 2018.]

4.4 Immunization Anxiety-Related Reaction

Fourth category is *immunization anxiety-related reaction,* which arises from the anxiety concerning the immunization [Adverse Events: Classification, 2018]. This anxiety could be caused by the lack of knowledge in the vaccine or because of the reportage of the side effects that people have had from the vaccine. The more people hear news about the side effects that the vaccine might have caused, the more they start to question its safety.

4.5 Coincidental Event

Fifth category is called *coincidental event,* which is caused by something else than the error or anxiety of the immunization or the vaccine [Adverse Events: Classification, 2018]. For example, nausea occurs at the same time as the vaccine is given, but it is caused by something else, like food poisoning.

Ideal situation would be that vaccines would not cause adverse events and would completely prevent the infection they target, but unfortunately, it is impossible with the current technology. Currently, the only way is to minimize adverse events and make sure that the vaccines are used safely. Adverse events of vaccines occur with a certain frequency. Common and usually minor reactions can be fever and malaise. Rare and more severe reactions can be allergic reactions to vaccine antigen or its component. Frequency differs from very rare (<0, 01%) to very common (≥ 10%). [Adverse Events: Frequency and Severity, 2018.]

4.6 AEFI surveillance system

It is important to survey adverse events because vaccines are meant to prevent diseases and are given to healthy people. The tolerance for risks is lower compared to drugs that are made for curing people. That is why there is a major need to detect and investigate any adverse event following immunization. Surveilling adverse events helps the vaccination programmes to become successful.

AEFI surveillance system is meant to help the National Regulatory Authority (NRA) by observing and reporting the adverse events. NRA is the agency which makes sure that vaccines are safe, efficient and their quality hits the standards. Safety of the vaccines is mostly measured by the adverse events. AEFI surveillance system oversees that. For a strong AEFI surveillance system, it is important that the communication between NRA and National Immunization Programme (NIP) is good, to ensure the use of worldwide data. [Interactions Between AEFI and ADR Surveillance Systems, 2018.]

AEFI surveillance system has many objectives like detecting, correcting and preventing immunization errors, caused by mistakes in preparation, handling, preserving, or in administration. It is crucial to identify vaccine reaction problems and prevent false blames on adverse events, when there is unrelated cause to the immunization. AEFI surveillance system is also reducing the incidence of injection reaction by educating the public. These reactions can be caused by anxiety about the vaccine or the fear of pain of the injection. Teaching about the vaccines, their risks and responding to concerns, helps to maintain the public's confidence. One important task that AEFI surveillance system has, is the estimation AEFI's in the population, for a new vaccine, by comparing the trial and the international data about adverse events. [AEFI Surveillance Components, 2018.]

4.7 Evolution of an Immunization Programme

Immunization programme is a programme that targets to give health benefit, by providing immunization against diseases. The aim is to eradicate them. Many of the programmes are targeting vaccination of children, to ensure that they will also benefit from the life-saving inventions. One good example of an immunization programme is EPI that was established in the 1974 by the 27th World Health Assembly. It recommended that all chil-

dren from all countries should receive a protection against tuberculosis, tetanus, diphtheria, measles, whooping cough and poliomyelitis. [National Programmes and Systems, 2018] Because the immunization programmes have been beneficial and many of the ones feared diseases are only rarely seen nowadays, the concentration of people has moved to the adverse events, instead of the severity of the diseases. However, there is a problem how to prove the causal relation between the vaccine and the adverse event. [Hedman etc. 2011: 841.]

Figure 4 shows potential stages in the evolution of an immunization programme. Stage 1 is the phase, where there are lots of cases of a disease and the vaccine has not been introduced yet to the market. Stage 2 is after effective vaccine has been introduced to prevent a disease. Disease rate will decrease, but number of adverse events will follow. The threat of the disease vanishes and attention moves to adverse events. Stage 3 is the loss of confidence in the vaccine. When more adverse events are introduced, people will start to question the safety of the vaccine and vaccination rate decreases. With less vaccination, the outbreak of the disease occurs. The loss of confidence is always a problem for the succession of the vaccination programme. Stage 4 is resumption of confidence. Confidence is gained through the outbreak of the disease or by an alternative vaccine for the disease. Vaccination levels increase again and disease cases decrease. Stage 5 is the eradication of the disease (if the disease is vaccine-preventable), for example smallpox was eradicated. Vaccination is stopped when eradication is confirmed and adverse events do not happen. [Vaccine Safety in Immunization Programmes, 2018.]

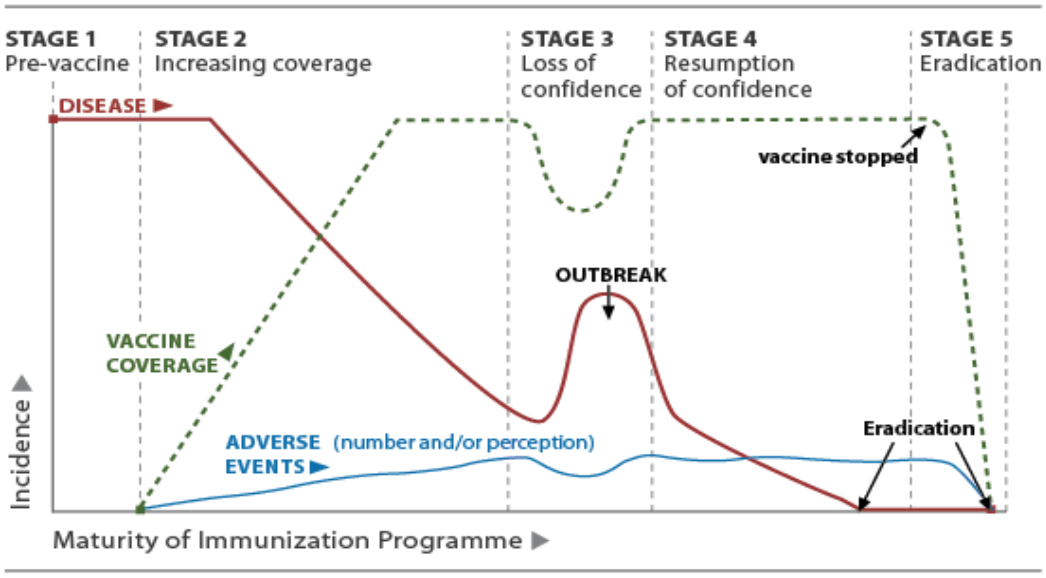

Figure 4. Potential Stages in the Evolution of an Immunization Programme [Vaccine Safety in Immunization Programmes, 2018].

4.7.1 Pandemrix vaccine in Finland

One example of adverse event is the swine flu pandemic in Finland 2009. Vaccine against the swine flu was called Pandemrix. It was questioned to cause increase in narcolepsy cases in kids and young people. [THL 2018, Narkolepsia ja sikainfluenssarokote.] Narcolepsy is a sleep disorder, which appears as excessive sleepiness, sleep paralysis, hallucinations and sometimes as episodes of cataplexy [National Sleep Foundation, 2018].

The Pandemrix vaccine was taken away from use, as a precaution. Pandemrix vaccine and the model vaccine before it had gone through examinations, demanded by EU's medical legislation and had gotten the license to sell. In the examination, it did not occur, that there might be side effects relating to narcolepsy. [THL 2018, Narkolepsia ja sikainfluenssarokote.] Since the vaccine was new and created in a short period of time, there was not any recordings of the long-term side effects.

THL (The National Institute for Health and Welfare) started research connections between narcolepsy, swine flu and Pandemrix vaccine in 2010. In the final report of this research, THL admitted that Pandemrix vaccine had influenced in the increase of the narcolepsy cases in people between ages 4 to 19. Most likely, the vaccine had increased narcolepsy synergy between inheritances and other environment factors. [THL 2018, Narkolepsia ja sikainfluenssarokote.]

In May 2013 THL published a new report, where they stated that according to surveillance information of the Pandemrix vaccine, it can be said that the vaccine increased the possibility to have narcolepsy in people under 65 years old, who had had the vaccine compared to people, whom were unvaccinated. In the year 2015, THL stated that the rate of new narcolepsy diagnoses had returned to the same level as it was before the Pandemrix vaccine. [THL 2018, Narkolepsia ja sikainfluenssarokote.]

5 Development and Manufacturing of Vaccines

Vaccines are made the same way as other biological preparations, but with even more higher standards, to ensure that the final product is as pure as possible. Every step from the beginning must be validated and the whole process must happen in controlled and safe environment. Vaccines must be safe from contaminants, otherwise rate of adverse

events will rise. Safety of the new vaccine is tested in the clinical trials. Vaccine development has a lot of challenges, and besides finding safe and effective adjuvants, antigens and delivery systems, it is important to be in balance with cost, risks and benefits [Nascimento & Leite, 2012.]

Validation is a crucial part when manufacturing a biological preparation. It is often said that what is not validated, is not done. Validation is a regulation requirement and gives assurance of quality, provides economic value and helps to understand and control the process. Possible mistakes are easy to find when every step is validated. It also works as a proof that everything has been done according to regulations. [Lane, 2016.]

Vaccines are prepared following Good Manufacturing Practices (GMP). A cleanroom is a part of GMP regulation for vaccine development. It is a room where the environment is controlled. Humidity, air and temperature are controlled alongside with the number of people entering the room at the same time. Contaminants in the air are controlled by High Efficiency Particulate Air (HEPA) filters. Humans are the biggest source of contamination. Cleanrooms are categorized by permissible number of particles inside a volume of one cubic meter (m^3) of air. There are three different standards for this: ISO, EU and US-FDA. Cleanroom standards in the vaccine manufacturing depends on the vaccine type. Different parts of manufacturing have different standards, usually going from low purity (upstream) to highest purity (formulation). The EU GMP standards are categorized from A to D, A as the lowest amount of particles allowed in the m^3 of air. Upstream process is often executed in cleanroom's categorized to D and C. Formulation and filling are often executed in cleanroom's categorized to B and A. [Lane, 2016.] The size of the particles and operation modes (*at rest* or *in operation*), affects inside the standards to the allowed number of particles in the m^3 of air. More particles in the air are allowed in the "in operation" mode, compared to "at rest" mode.

5.1 Phases of Vaccine Development

The development, testing and regulation of vaccines, evolved to the current system in the 1900's. Development can take many years and if it succeeds, it could end up to a large-scale vaccination programme, for example EPI. Many of the vaccines fail at the beginning because they are unsuccessful to produce the desired immune response. Only a few of the possible vaccines make it to the licensing. [Stages of Vaccine Development,

2017.] Vaccine development is very expensive, but it is financially supported by many organizations. Phases of the development are shown in the Figure 5.

Development of every vaccine starts with the exploratory stage. It consists of laboratory research to identify antigens that might be able to treat or prevent a disease. [Vaccine Development, Testing, and Regulation, 2018.] The found microorganisms are weakened or killed so that they cannot replicate and cause a disease anymore. The wanted part of the microorganism is used to manufacture a vaccine.

In the EU, development of vaccines can be divided into two stages, pre-clinical and clinical development [Stages in Vaccine Development, 2017]. Pre-clinical studies include laboratory and animal studies, once the wanted microorganism has been found and antigens isolated. The purpose of pretesting is to assess safety, biological activity and formulations. Animal studies can also help to see what kind of cellular responses is expected in humans. GMP standards are used when the cell line is constructed and cell banking is done. This stage can take already 6.5 years and cost about 350M dollars. [Lane, 2016.]

In the stage of clinical development, the vaccine is tested in humans. The testing happens in four phases and can take many years. First three phases are the clinical trials. The stage of clinical development is based on strict ethical principles. Volunteers are well informed of the vaccine safety and efficacy. [Stages in Vaccine Development, 2017.]

Phase 1 is done in small scale with 20 to 100 healthy volunteers. Its purpose is to determinate safety and dosage [Lane, 2016.] In Europe, there is phase 1a and 1b. These are for testing vaccine for the diseases of poverty. Phase 1a is for European volunteers and phase 1b for volunteers in developing countries. [Stages in Vaccine Development, 2017.] Vaccines that are targeted to children, are first tested with adults. Volunteers in these studies are attentively monitored and the conditions are controlled. [Vaccine Development, Testing, and Regulation, 2018.]

Phase 2 is tested with a larger group of 100 to 500 patient volunteers. The purpose of this phase is to evaluate effectiveness of the vaccine versus clinical diseases and artificial infections. Immune response, alongside side-effects, are also evaluated and the method of the delivery is decided. [Stages in Vaccine Development, 2017.] In this phase

the trials are controlled and randomized. A group of people is also having a placebo vaccine in the tests. [Vaccine Development, Testing, and Regulation, 2018.]

Phase 3 confirms effectiveness and monitors adverse events originating from long term use. This phase is tested with 1000-5000 patient volunteers to evaluate the effectiveness under natural disease conditions. [Lane, 2016.] The tests in this phase are randomized and the developed vaccine is tested against a placebo [Vaccine Development, Testing, and Regulation, 2018]. If the vaccine is safe and effective for a defined period, can the manufacturer apply for a license from the regulatory authorities. If the license is admitted, can the manufacturer start to market the vaccine for human use. [Stages in Vaccine Development, 2017.]

In phase 4, the vaccine has been licensed and is available for the users. The vaccine is still being under surveillance and the aim is to detect adverse events. The effectiveness of the vaccine is monitored throughout its long-term use. [Stages in Vaccine Development, 2017.] The vaccine development in US is approximately the same as in Europe, but different agencies are responsible for the surveillance and evaluating.

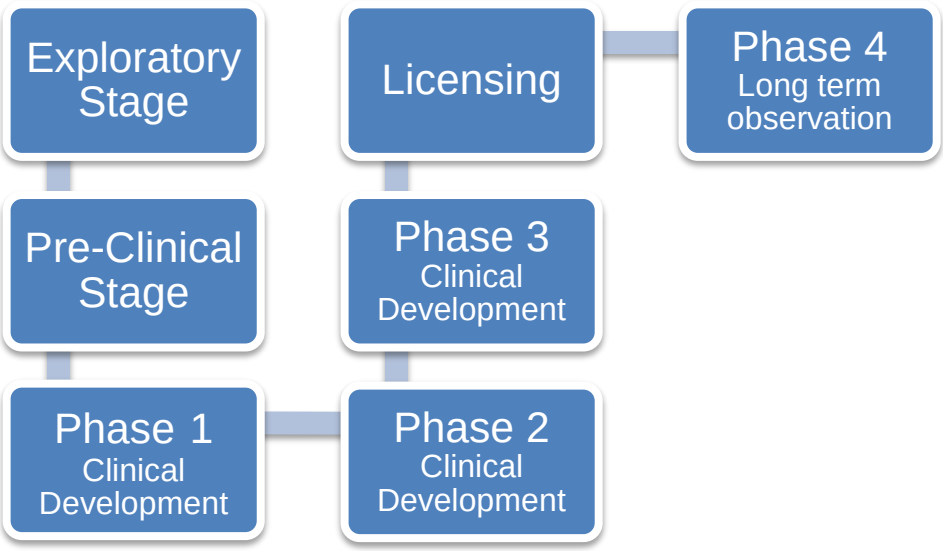

Figure 5. Phases of Vaccine Development.

5.2 Production of the Vaccine Agent

Process for manufacturing a biological preparation consists of upstream and downstream processes. There are different techniques and ways to produce a vaccine agent, but all the ways are based on upstream and downstream processes. These different techniques and ways are discussed in sections 5.2.2 and 5.2.3.

Upstream includes the entire process from early cell isolation and cultivation, to cell banking, to the culture expansion of the cells fermentation process and the final harvest. Basically, the first step is the cell bank storage. The wanted cell line is taken from a working cell bank. Then comes the inoculum, which involves thawing of cells, use of a shake flask of 100 to 500 milliliters and a bioreactor of 3 to 20,000 litters. The cell culture is scaled up. Then comes the primary recovery, cell and debris removal. This involves centrifugation, depth membrane filtration and harvest. [Lane, 2016.] Section 5.2.1 introduces a bioreactor more closely.

5.2.1 Bioreactor

Most bioreactors used in the vaccine production are stainless steel stirred-tank bioreactors (STB). Bioreactor is part of the upstream process and is used for the growing of the microorganisms. It provides an environment, sterile conditions and agitation. Figure 6 shows what STB consist of. Mixing is achieved by using the agitators and gas bubbles from the sparger. This type of bioreactor is typically used for microbial and mammalian cells. [Lane, 2016.]

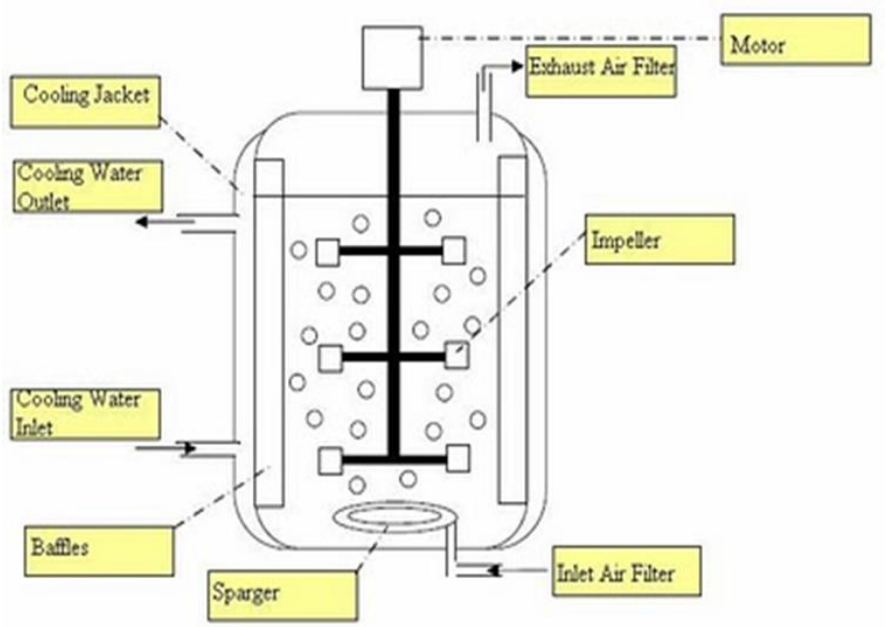

Figure 6. Stirred-Tank Bioreactor [Lane, 2016].

5.2.2 Egg and Cell Culture-Based Manufacturing of Influenza Vaccine

When producing influenza vaccine, egg and cell culture-based manufacturing methods are used, to create millions of vaccine doses. Every few years there is different kind of seasonal influenza virus infecting people and new vaccine must be made. Influenza effects globally so the manufacturing scale is huge.

The WHO officials are working together with national health agencies to identify the virus strains that might cause the next influenza pandemic. The production of a new seasonal influenza vaccine is based on the surveillance that the WHO and the health agencies do. Production of a seasonal influenza vaccine is difficult because the WHO announces the seasonal strains on February, which gives manufacturers six months to develop a vaccine for the people of Northern Hemisphere and create a supply. Same applies for the Southern Hemisphere with an offset. This six month period includes the announcement of seed strains, monovalent vaccine production, approvals, vaccine formulation and the clinical trials. Virus growth and fulfilling the regulatory aspects are the most crucial parts for success on the six month period. [Milián & Kamen, 2015.]

The egg-based manufacturing system is the most used method for production of an influenza vaccine. The method was developed in the 1940's, almost 80 years ago. It uses embryonated hens' eggs to replicate the virus. Advantages of this method are safety and

effectiveness that are well established. It has limitation as it needs constant supply of eggs. One or two eggs yield one vaccine dose. When the influenza pandemic outbreaks, the production of the vaccine might be problematic. Another disadvantage in the use of eggs is that it might leave some egg components that might cause an allergic reaction. Because the demand is increasing as the world population grows, scientist have introduced new ways to mass produce influenza vaccines faster and safer. [Milián & Kamen, 2015.]

Cell culture-based manufacturing is one of the new ways to produce an influenza vaccine effectively. It uses mammalian cells to culture the influenza virus for vaccine production. Unlike in the egg-based manufacturing, this way can respond to market needs quickly. The production has also better process control, higher safety level and shorter production cycle. So higher quantities are made in a shorter period of time and pathogenic viruses can be produced safely. It also reduces the possibility of mutation of the virus culture in the manufacturing process. Problems for cell-based manufacturing are that the costs are relatively high and the cells should be free from another virus. The volumetric virus yields are lower than in the egg-based process. Since it is a new production method, it does not have long term safety data. [Milián & Kamen, 2015; Health Sciences Authority, 2014.]

At the moment egg-based production is mainly used for civilian vaccines, like influenza vaccine and bacterial fermentation based vaccines. These products are made with old technologies and processing strategies. For these “old products” the manufacturing technology is not changing any time soon because of the work amount it would require. Regulatory process and obtaining the new licenses for the new processes would be too difficult and expensive to arrange. The manufactured products would also need a proof that the new technology can manufacture everything as effectively and safely as the old process. Fortunately, new technologies can be used for new products. Cell culture methods can be used for virus particle-based vaccines. [Smart, 2013.]

5.2.3 Batch Processing vs. Continuous Processing

Batch processing is the old way of producing pharmaceuticals compared to continuous process. The traditional batch processing is showed in Figure 7 on the left side of the Figure. The process has at least six steps and six pieces of equipment before it is completed. The production stops every time when the equipment is changed. Components of the drug are added on through the process and new batch cannot start before the

previous one is completed. Advantages of using a batch processing are that the setting up is little bit less expensive and the process is easily modified. Some products can be only made with batch processing. [Batch vs. Continuous Pharmaceutical Manufacturing, 2017.]

In the continuous process, the product is done continuously, which means that there is no need to interrupt the process. The right side of the Figure 7 shows the continuous process. All the base ingredients are added at the beginning of the process. Advantage of using continuous processing is that it can speed up the process significantly compared to batch processing. It can also be more environment friendly option. Since the process is continuous and fewer people are involved in the process, the risk of human error is reduced. [Batch vs. Continuous Pharmaceutical Manufacturing, 2017.]

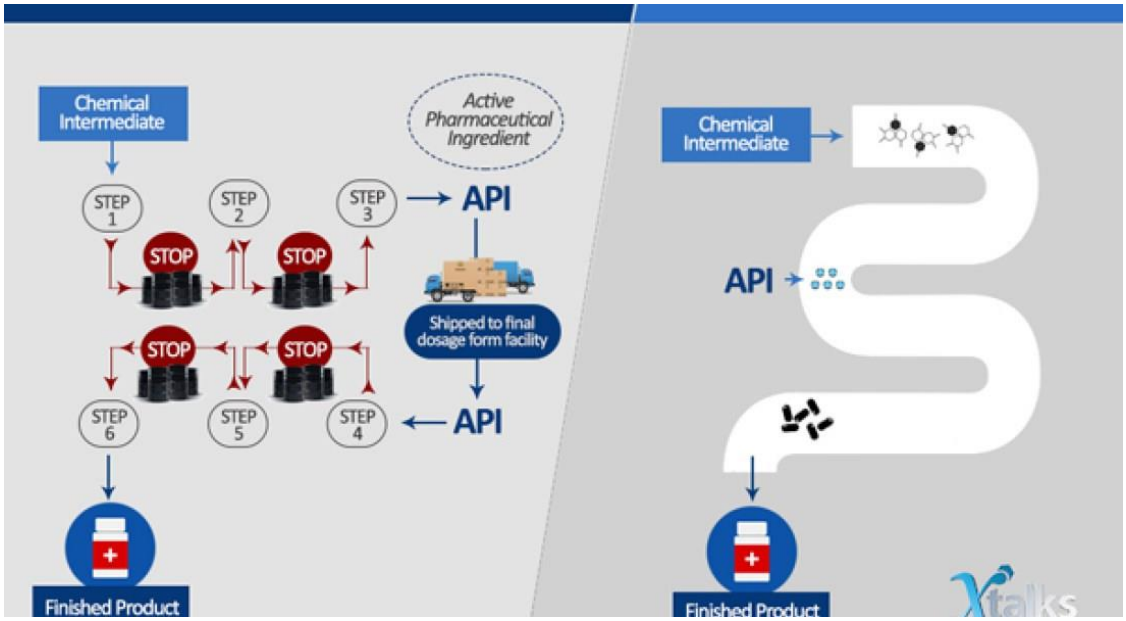

Figure 7. Batch Process vs. Continuous Process [Massey, 2016].

Batch processing has been used for a long time by biopharmaceuticals when manufacturing vaccines. Continuous processing has been mainly used, for example, in the energy generation industries and in the food processing. The use of continuous process, when manufacturing vaccines, has been rising as a new way. Massachusetts Institute of Technology (MIT) and Novartis have perfected a continuous process for manufacturing a pharmaceutical product. [Challenges in the Development of Continuous Processes for Vaccines, 2017.]

It is challenging to develop continuous process for a vaccine because the production has many phases. The process involves upstream and downstream processes, which include harvest, immunogen separation and purification, multiple filtrations and chromatography. It is expensive to maintain continuous substrate supply and sterilization. Subunit vaccines require higher purity, compared to live-attenuated vaccines, because of the different dose levels. Downstream processing is the most challenging part of continuous process because it is so complex. When manufacturing a new vaccine, cost analysis is done and evaluation must be made whether the continuous process is better compared to the traditional manufacturing methods. If the continuous process is successful, it could decrease the costs and production time. Also, it would lessen the quality control validation because it is safer from contaminants. Continuous process development might be the new modern way of manufacturing vaccines. [Challenges in the Development of Continuous Processes for Vaccines, 2017.]

5.3 Purification Methods of Downstream Processes

Downstream involves separation of the cells from the media, isolation of the product, purifying, polishing and packaging of the product. In short, it is where the capturing of the desired protein happens. Harvesting happens between upstream and downstream processes. There the particulate matter is removed, for example cell debris. Capturing is the first purification step aiming at a volume reduction and partial purification of the product. After that comes few more purification steps to remove the contaminating solutes. Polishing is the last purification step removing minor impurities, for example denatured protein and aggregates. The whole downstream process includes ion exchange, affinity and HPLC chromatography and micro / ultra-filtration. Chromatographic techniques are used in the purification. Filtration is used for the reduction of the volume and for removing impurities. Every step is done in cleanroom conditions. Downstream process uses the purest water called Water for Injection (WFI). [Lane, 2016.]

Making of toxoid vaccine describes well the upstream and downstream processes. (Upstream changes to downstream when the harvesting starts.) Toxins are produced by bacterial fermentation. Media preparation and the scale-up happens with fermentation process that takes about 42 hours. Harvest is done with centrifugation or microfiltration, using often a tangential-flow filtration. Particulate matter is removed by using 0.45 µm or 0.22 µm membrane filters. Toxoidation is done with formalin that weakens the toxin. This

operation can take up to 42 days and is intensively monitored with sterility and pH testing. The resulting toxoid is concentrated with ultra- or diafiltration and then ammonium sulfate is added for precipitation. After that follows more concentrations steps and formulation using ultra- or diafiltration, before the final filtration for the filling. [Adams et al. 2011.]

In the Figure 8 simplified upstream and downstream processes of influenza vaccine production are shown. Upstream process includes the selection of the strains used in the production of vaccine and the growing of the selected microorganisms in a bioreactor. Harvesting and purification of the microorganisms happens between the upstream and downstream processes. Downstream process includes inactivation and splitting of the organism, formulation, quality control and packaging the vaccine.

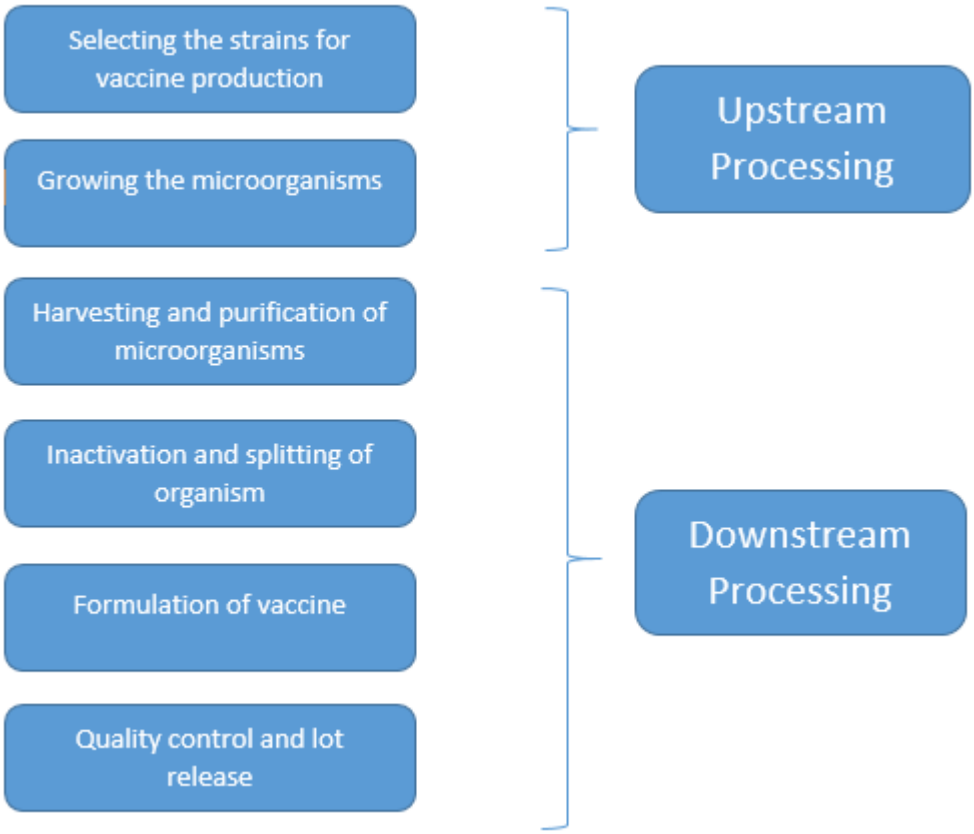

Figure 8. Upstream and Downstream Processes of Influenza Vaccine Production.

5.3.1 Equipment Used in Downstream Processes

In this section, examples of equipment used in the downstream process are introduced more closely. The examples picked for the thesis are affinity chromatography and tangential flow filtration.

Affinity chromatography is used in the downstream process for the purification of vaccine. Affinity chromatography is a separation method between immobilized ligand and its binding partner, for example antibody and antigen. To elute the target molecule from the affinity medium, the interaction can be reversed, either specifically using a competitive ligand or non-specifically, by changing the pH, ionic strength or polarity. [Lane, 2016.]

Figure 9 shows the basic principle of affinity chromatography. First, the mixture of proteins is poured to the column, as can be seen in the loading phase. In the separation phase, the target analyte binds with the immobilized receptor. Then, the wash is added to the column and the discard is flown through the column with the unwanted proteins. An elution buffer is added to the column and the target analyte flows through it. The target analyte is then purified from the other unwanted proteins and collected.

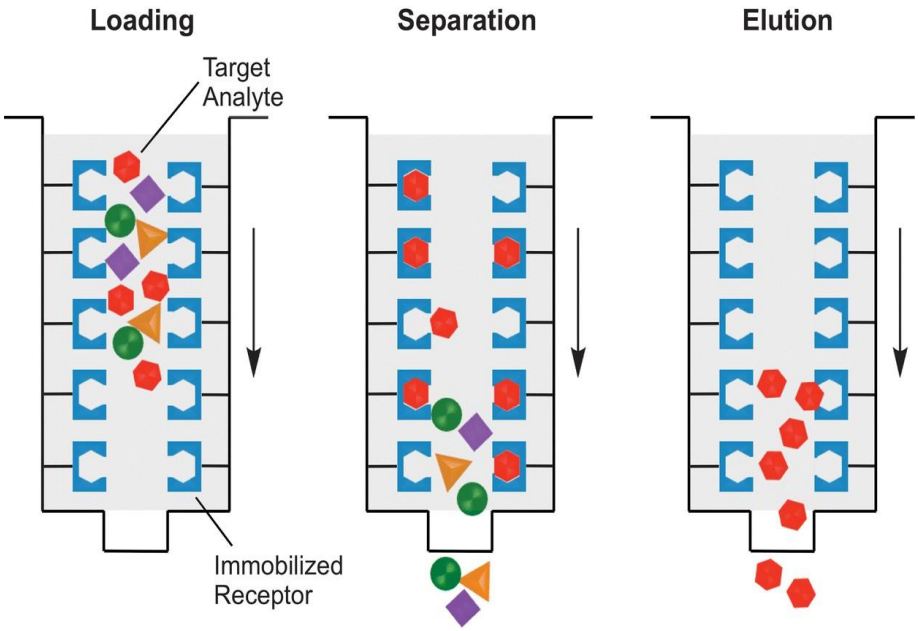

Figure 9. Principle of Affinity Chromatography [Creative Biostructure, 2018].

Tangential flow filtration (TFF) is filtration method used in downstream process of manufacturing for example influenza vaccines. TFF is usually used for the concentration of the solution. It removes the fluid from a solution while retaining the solute molecules. Halving the solution volume can double the concentration. TFF is a rapid and efficient method for separation and purification of biomolecules. The product flow is directed tangentially along the surface of a membrane, with most of the solution circulated back to the feed tank. Many processes in biopharma industry use this filtration method. It's easy to set up and operate. It can also be scaled-up or scaled-down. TFF is economical because it can be cleaned and the cassettes can be reused as well as other parts of the device. [Lane, 2016.]

In Figure 10 a simple TFF device is shown. Pump moves the sample from sample reservoir to the filter. The feed stream passes horizontally over the membrane. Ultrafiltration membranes filtrate the smallest particles and the retentate goes through the valve back to the sample reservoir. This continues until the wanted concentration is achieved.

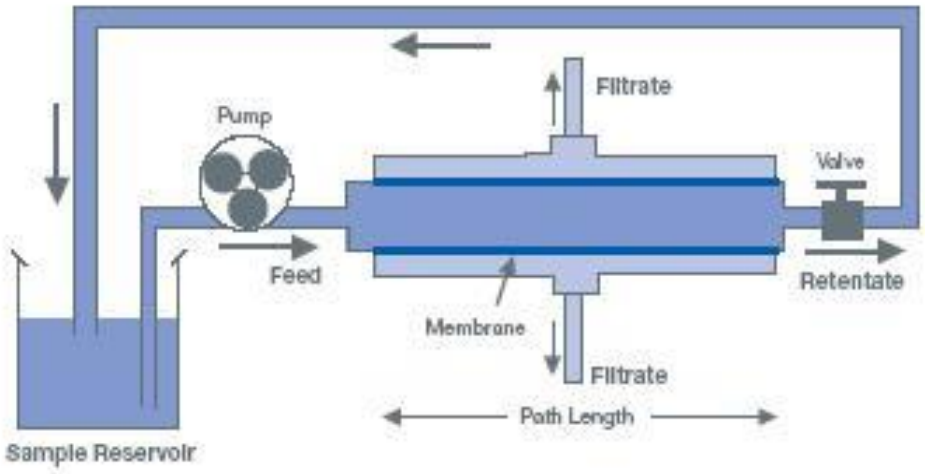

Figure 10. Simple Tangential Flow Filtration Device [Lane, 2016].

5.4 Regulation of Vaccines

The pharmaceutical industry is one of the most regulated industry known. Governments in all world regions have established regulatory agencies to ensure that every aspect of pharmaceutical activity is tightly controlled. The WHO does the recommendations for biological products that are used internationally. Many countries follow the WHO standards. [Lane, 2016.]

National Regulatory Authorities (NRAs) are agencies which ensure that the pharmaceuticals and biological products that goes to the market, meets the international expectations of quality and safety. All countries that produce vaccines, must follow six critical control functions. These functions are a published set of requirements for licensing, surveillance of vaccine field performance, system of lot release, use of laboratory when needed, regular inspections for GMP and evaluation of clinical performance. [National Regulatory Authorities, 2018.]

In the US there has been vaccine regulation for more than 100-years. The American regulatory authority is the Food and Drug Administration (FDA). FDA's mission is to protect human health. Inside the FDA there is Centre for Biologics Evaluation and Research (CBER), which is responsible for Biologics, for example vaccines. FDA's responsibilities include to decide if clinical trials can be allowed to begin, to protect rights of the volunteers participating in the clinical trials, to allow license for marketing and oversee the manufacturing process. [Lane, 2016.]

European regulations have similar philosophy for the regulation but differ in the implementation of the legislation. Every European country has a national regulatory authority appointed by the government. European Medicines Agency (EMA) was established in the 1995 in attempt to streamline drug approval for all European countries. It supervises regulation of vaccines and other drugs. Japan is also a major leader in the pharmaceutical markets. The ministry of health and welfare in Japan has overall responsibility to implement Japanese pharmaceutical law. [Lane, 2016.]

5.5 Single-use Technology

For a long time, biopharmaceutical industries have used large stainless-steel bioreactors for the scale-up phase of production. Single-use technology is starting to compete against the old equipment. Advantages of this technology are that it reduces set-up time and cleaning, it eliminates possibility of cross-contamination, it is easy to use and the technology can be ordered as needed, so it won't need a storage space. Downsides are that they can only be used once, they create waste and are limited in size. Usual single-use technology products are single-use bags (small bioreactors), mixers and filters. [Lane, 2016.]

At the moment the maximum size of single-use bag is 2000 liters. The sizes of single-use bags are suitable for vaccine production because vaccine doses are small (measured in micrograms) and 200-2000 liters is enough for making a batch. Single-use technology is mainly used for new vaccines because of the regulation and licensing processes. It would be expensive and slow to chance the production equipment of the old products.

One example of single-use technology is WAVE bioreactor system. It consists of a presterile disposable chamber that has cell culture medium inside. It is placed on top of a special rocking platform. Rocking creates waves that provides the mixing and oxygen transfer. WAVE bioreactor has 0.2 µm air filters, which allow gas to flow into and out of the bag. It also has sensors (e.g. pH), ports, tubes, connectors and clamps. Figure 11 is an example of a WAVE bioreactor system.

Figure 11. WAVE bioreactor system [Lane, 2016].

6 Future of Vaccines

The future of vaccines presents continues challenges for creating effective vaccine, for example for HIV virus and against parasitic diseases, like malaria. Production has to have lower costs, better delivery, and environmental endurance. Already existing vaccines are also developed further to be even more effective.

If researchers would find a vaccine for HIV virus, it could be possible that the cost would be so high that the poorer countries could not afford it. Developing countries usually need it the most. Also, the delivery might be very difficult to some areas. Vaccine materials for the tolerance of different temperatures and delivery methods should be improved, so that the vaccine could remain viable through the transportation and storing. Vaccination programmes should also be improved in a way that they would be available for everyone, especially to the places where vaccination is poor or non-existing at the moment. [The Future of Immunization, 2018.]

New delivery methods of vaccines are also researched. MIT has been investigating a new approach for the giving of booster shot. They are developing a new technique where multiple vaccine shots could be given as one shot by capsuling the shots within tiny biodegradable polymers. Depending on the polymer, they would break down at different times and release the contents to the body. It would be valuable invention for treatments of diabetes, allergies and other situations where multiple shots are needed. According to Ana Jaklenec from MIT, this invention would be important for developing countries where mothers rarely bring their children to get booster shots. [Davis Nicola, 2017.]

In the process tiny silicone molds are made. Biodegradable polymers are then added to the molds, to create box-like shape, around 400 µm across. Then, the molds are filled with the vaccine and dried. Biodegradable polymers have already been approved to human use and animal tests with the capsulated vaccines have showed to be successful. [Davis Nicola, 2017.]

The production of vaccines has already showed new ways to enhance the manufacturing and new procedures. New ideas are constantly invented to help to improve the immune system against various diseases. Manufacturing is evolving with new techniques, for example using single-use technologies. Molecular biology has evolved a lot in few decades and is now applied to the research and production with DNA technology.

6.1 Recombinant Vaccines

Recombinant vaccines are the new kind of vaccines developed by a new recombinant DNA technology (DNA = Deoxyribonucleic Acid). It is a technique where genes from one organism are isolated, purified and then reproduced in another organism and it is often performed through ligation [Cruse & Lewis, 2009:614.] Vaccines manufactured by using a recombinant DNA technology are safe and production costs are lower compared to traditional vaccines. Vaccines made by this technology can be divided to DNA vaccines and recombinant (protein subunit) vaccines. These vaccine types are discussed more in sections 6.2 and 6.3.

6.1.1 Expression systems

Expression systems are important parts of recombinant DNA technology. Expression system is a combination of DNA to be cloned, expression vector (often a plasmid) and host cell. Expression system is manipulated to produce high levels of desired protein. [Lane, 2016.] Bacteria, yeast, insect and mammalian cells are used as protein expression systems. The features of target protein effects, which system is used. The features are sequencing, structure, activity, function, folding and post translational modification. Bacterium and yeast are the least expensive options, but the yield (mg/L) is not that good compared to mammalian cells. [Choosing a Protein Expression System, 2018.]

6.1.2 Adjuvants

Adjuvants that enhance the immunity in vaccines are main reason for the recombinant vaccines to be successful. These vaccines would not give a good protection against diseases if they would not include any adjuvants. The use of aluminium salt as adjuvant made the success of vaccines against hepatitis B virus and human papillomavirus. Investigation of new adjuvants has showed to be extremely important. [Nascimento & Leite, 2012.]

6.1.3 Prime-Boost

Some vaccines need a booster shot to maintain the protection against diseases. This kind of shot is called homologous prime-boost because it uses same formulation. Now scientists are testing a heterologous prime-boost immunization, where the antigens are the same but formulated in different ways. They can be either purified antigens or recombinant proteins with appropriate adjuvants. These adjuvants can be DNA vaccines or live recombinant viral or bacterial vector vaccines. This method appears to be able to induce a better immune response. It is more efficient because it combines both humoral and cell-mediated immunity. It has showed potential in the animal testing phase of HIV vaccine development. [Nascimento & Leite, 2012.]

6.2 DNA Vaccines

For over a hundred of years there have been two approaches to create immunity by vaccination: attenuation or inactivation of pathogens. Researchers have investigated new ways as the knowledge of molecular biology and technology has improved. As a result, DNA vaccines have been invented.

DNA vaccines are immunizing preparations, which include a gene that encodes the antigens, instead of having the antigens ready in the vaccine. Thus, it includes the "recipe" for making the wanted antigens. [Donnelly et al., 2005.] Gene that is responsible for encoding the antigen is inserted into a bacterial plasmid. This plasmid is then injected to the muscle cells of the host. For manufacturing and expressing the antigen, the gene uses the nuclear machinery of the host cell. DNA vaccines are able to induce both humoral and cell-mediated immune responses. [Cruse & Lewis, 2009:233.]

Plasmid in DNA vaccine contains one origin of the replication, *E. coli*, for the amplification of the plasmid. A strong promoter, multiple cloning sites and antibiotic selection marker are also included to the plasmid. The bacterium is isolated and purified for the vaccine. The idea of the DNA vaccine is based on what is occurring during a viral infection. Antigen is expressed directly by the host cells. As a result, it is possible for antigens to be processed as proteins that are synthesized in the cytoplasm. DNA vaccines have no risk of infection and strong and lasting immunity might be possible to achieve. [Nascimento & Leite, 2012.] DNA vaccine for HIV virus has been on development for 20 years and has entered the human trials phase. Unfortunately, DNA vaccine for HIV virus has

showed low immunogenicity in humans. It is extremely important to discover a suitable adjuvant to enhance the immunity.

The first DNA vaccines in the market will use a plasmid from bacterial cells. In the future, it might be possible to use RNA (Ribonucleic Acid) or other complexes of nucleic acid molecules. The development is evolving rapidly and new guidelines are needed for regulation. Control should be flexible for the modifying as the experience is gained in the production and use. [DNA Vaccines, 2018.]

6.3 Recombinant Protein Subunit Vaccines

Recombinant protein is a form of protein that has been manipulated. The gene has been isolated and cloned into an expression vector. A vector is a segment of DNA that can clone a foreign DNA fragment and autonomically reproduce in the host cell [Cruse & Lewis, 2009:739.] Recombinant proteins are often made for therapeutic use. The genes are usually coming from humans but they are expressed in other organisms. [Clark & Pazdernik, 2016.]

Recombinant protein subunit vaccine is a subunit vaccine that contains only a small part of the germ. These are often synthetic peptides that work as the protein components by inducing the immune response. Also, antigens that are expressed in a heterologous system, made by recombinant protein expression technologies, can be used. Vaccines that are made nowadays often use this technology to purify recombinant proteins or antigen subunits. Currently there is Hepatitis B virus vaccine made using this technology and it is in the market for humans. [Recombinant Vaccine, 2018.]

Figure 12 shows the production steps of Hepatitis B virus vaccine. Plasmid DNA is taken from bacterium and cut with restriction enzymes. The gene that produces Hepatitis B antigen is isolated from Hepatitis B virus and combined with the cut plasmid DNA. Recombinant DNA is made and introduced to a yeast cell. The recombinant yeast cell is moved to a fermentation tank to multiply. Hepatitis B antigen is produced in the fermentation tank. After the multiplying, the Hepatitis B antigen is extracted and purified. Finally the hepatitis B vaccine is formulated to its final form.

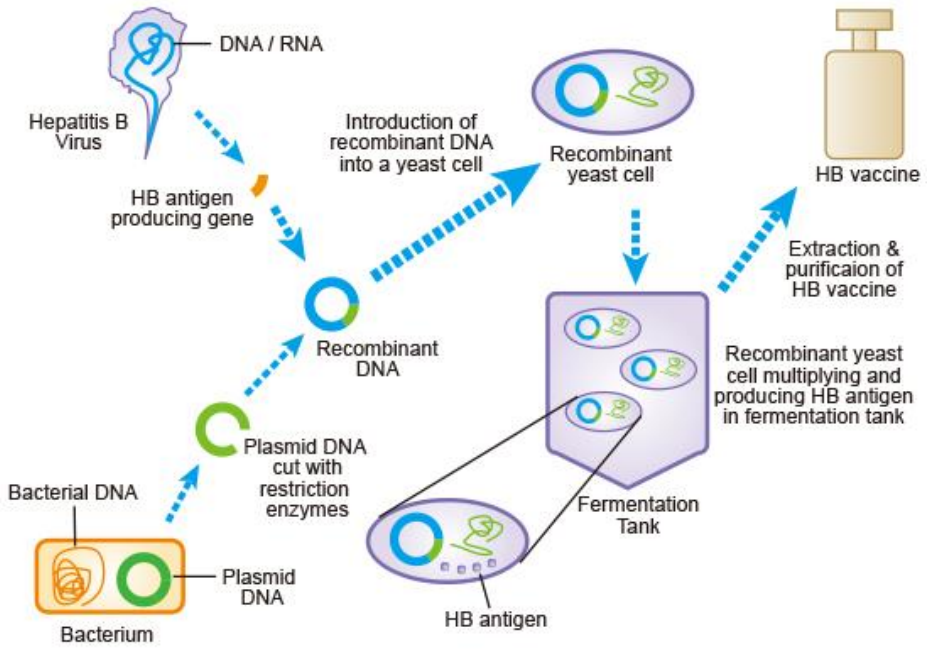

Figure 12. Production of Hepatitis B Vaccine by DNA technology [GenScript, 2018].

6.4 Mutation of Viruses

Mutations of viruses is a continuous problem for all RNA virus based vaccines. The evolution of a strain is hard to foretell. In RNA viruses the genetic material is encoded by RNA, instead of DNA. RNA is not as stable molecule as DNA and result from this is that if there are mistakes in the copying of RNA, there is no build-in proofreading that would repair the mistake. Virus mutations in RNA happen frequently. Therefore, it is very difficult to create one vaccine for influenza viruses or a working vaccine for HIV virus. [Viruses and Evolution, 2018.]

7 Conclusions

Vaccine manufacturing is a long and expensive process. Vaccines have proven to have important influence in the human health. The field of vaccines is evolving rapidly and new findings are constantly made. The future of vaccines depends on the successful medical research and new vaccine technologies for the development.

With good immunization programmes, eradication of vaccine preventable diseases is possible, but a working immunization programme with efficient vaccines is not enough. For a disease to be eradicated, the programme needs to include the whole world, it needs the vaccines to be given to everyone, everywhere, even to the areas that are hard to reach. Eradication is already close for measles and polio virus.

Vaccines need to be affordable to everyone, so the costs of vaccine development and manufacturing must be low. New ways are searched to get the costs as low as possible. Also new ingredients are researched to enhance the stability and endurance of the vaccine.

Knowledge about health and vaccination should be taught to everyone. Constantly growing population is a risk because diseases will spread more easily in the very crowded places. Basic hygiene, for example, hand washing, will already decrease possibility to the disease to spread. Childhood vaccination will provide good and lasting immunity to children and still effect in the adulthood. It will also help to prevent diseases from spreading in a population, in a long-term.

Adverse events of immunization are under surveillance and the information gathered, will help with the development of vaccines. Reporting adverse events is extremely important because there is not often any long-term information about possible undesired reactions that the vaccines might cause. Even though the development process is long and well established and regulated, it is possible that some of the adverse events will not appear in the trial phases. This can cause problems after the vaccine is provided to larger groups of people.

In conclusion vaccines are important preparations with proven health benefits. The development is difficult with its many time-consuming phases, high costs and regulations. Research and development of vaccines are financially supported by governments, foundations and by private people. Vaccines have saved millions of lives in the past 200 years and will continue to do so, and maybe eventually have even the ability to cure.

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