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VACCINES FOR CONTAGIOUS DISEASES

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Abstract—Vaccination against contagious diseases which greatly decreases the mortality rate and promotes population health is a valuable invention in medical biotechnology. With the improvements made in biotechnology, the production and effectiveness of vaccines also experience an advancement from the commonly applied recombinant DNA technology to the reverse genetics' techniques and newly DNA vaccines which is still not common in the applications for humans. The application of biotechnologies in the vaccine preparation process greatly helps to save the time and expenses spent and many possible preventive measures with higher efficiency or new vaccines are discovered with the help of biotechnology applied. Breakthrough in prevention against antibiotic resistance bacteria or challenging diseases contribute to the development of the medical field. However, the rapid growth of biotechnology and their application in vaccination also raise the awareness of the public regarding the ethical and safety issues such as biological weapons and vaccine scares. Authorities should put in more efforts to overcome these challenges to maximize the benefits of vaccination to humans especially in preventing the deadly contagious diseases outbreaks in the world.

Keywords – Vaccines, biotechnology, population health, contagious diseases, economic growth, health, biological weapons, vaccine scare, immunity

5.1 Introduction

Biotechnology, a combination phase of two words, which is biology and technology. According to the Fifth edition of Webster's New World College Dictionary, the use of data and techniques of engineering and technology for the purpose of study and resolving problems concerning living organisms is known as biotechnology [1]. Meanwhile, for the contribution of biotechnology towards sustainable industrial development, biotechnology is acting as an important role to upgrade or modify the industrial in a biological aspect. For example, the application of modified biological organisms in the manufacturing process to improve production and increase the effectiveness or profit of the product.

In the years of 1919s, the word "biotechnology" was introduced by a Hungarian engineer Karoly Ereky with the description of biotechnology as a technology based on converting raw materials into a useful product [2]. Before this, people already used the biological techniques in their work but did not name their work as biotechnology. One of the

most primitive biotechnologies used by our ancestors back in 10000 years is the cultivation of plants and domestication of animals. At that time, wild animals were controlled to produce meat and milk. By using the fermentation process from microorganisms, various foods and drinks such as cheese, yogurt, bread, beer, and wine were also produced [3]. In the years of 1920s to 1930s, when the time of World War I and II, people started to make use of chemicals through biological processes. For example, during WWI, the manufacture of penicillin was introduced after Fleming discovered the use of microorganisms in the production of antibodies. As a result, during WWII, penicillin was produced on a large scale for the treatment of wounded soldiers [3].

There are a few major areas of biotechnology, as seen in Figure 1, which are medical, industrial and agricultural biotechnology denoted as red, white and green biotechnology respectively. Some of the classic examples for red biotechnology include vaccines, antibiotics and other new treatments, for the white biotechnology consists of utilization of enzymes or microorganisms to produce useful products and also genetically modified organisms (GMO) or transgenic plants for the green biotechnology.

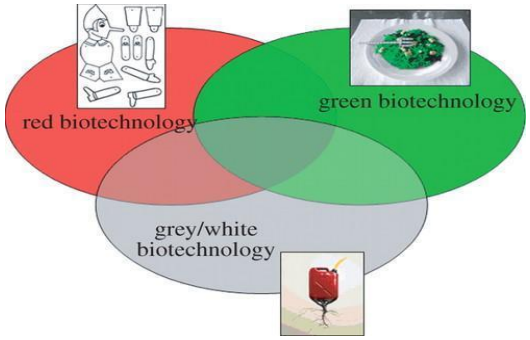

Figure 1: The division of biotechnology [4].

The main highlighted area is medical biotechnology in this report which involves the utilisation and researching of living organisms and different cell materials research to produce pharmaceutical and diagnostic products. Medical biotechnology aims to prevent, diagnose, and treat any human diseases. Thus, the investigation and research on the mechanisms of a particular disease are vital. Genetic investigation, drug treatments, and artificial tissue growth are the most recent applications in medical biotechnology [5].

With the fast-paced growth of medical biotechnology, many new concerns have arisen and are regulated to ensure the well going of the biotechnology industry from the aspect of funding to ethics. Some concerns or safety issues brought by the development of medical biotechnology include high risk on human safety during the clinical tests, individual privacy issue and the bioterrorism in worldwide [5]. In order to ensure the safety of vaccination against contagious diseases to the public, a wide range of research and clinical trials need to be done before formally introducing a new vaccine into society. A new vaccine means it has a high potential of exposing the people in clinical tests to a certain extent or even death since it is a totally new technology. The participants in the clinical trials need to completely know all the possibilities and are willing to take the risk for the invention of vaccines. Besides, the advancement in genetic technology that allows researchers to obtain full information about the genetic sequences of someone may expose some private information of the patients. A worse situation comes when the terrorists are even planning to genetically modify the microorganisms or virus to become their bioweapons and initiate attacks towards others. This situation puts all the people in the world in jeopardy and thus more efforts need to be done on the preparation of vaccines to increase the immunity coverage and control the spread of contagious diseases if any outbreak happens.

Human health has always been a primary concern of the world. Various tools and products of medical biotechnology have been introduced to overcome the health issues. For example, prevention, diagnostics at the nucleic acid level, treatment such as application of recombinant DNA technology for drug development and treatment at the nucleic acid level. At different stages of contagious diseases, different prevention measures can be implemented to control the development of diseases [6]. The main four stages of contagious diseases:

1. Primary stage

The earliest stage of prevention. It is promoted before the diseases occur. It promotes the priority of human health. For example, increase the immunity of the body. Activities such as health promotion campaigns held by the government also increase the awareness of society to the prevention of diseases.

2. Secondary stage

Detection of diseases at the asymptomatic (early) stage. For example, screening case finding. The purpose of the screening case finding is to detect the indicator of the potential disease. It is also a strategy to target the

suspected individual or group who are at risk rather than waiting for the symptoms to occur.

3. Tertiary stage

Reversion and delay the development of diseases. It is to prevent the complication of chronic diseases such as diabetes. Medication and rehabilitation are involved in slowing and stopping the progression of diseases.

4. Quaternary stage

Prevention of consequence of over medication, over diagnosis, or incidental findings. For example, medical imaging. The purpose of imaging is to reveal the internal structures hidden by skin and bones to treat and diagnose diseases accurately.

With the new-found medical biotechnology knowledge, the application of health technologies and the value-adding outcomes has been created to upgrade the curing of diseases and illnesses. Nowadays, the global pandemic of contagious diseases has become a serious issue. With biotechnology, the research and investigation of contagious diseases can be carried out quickly. Meanwhile, vaccines can be invented to control the spread of contagious disease.

In general, vaccines can be defined as a preventive measure through biological preparation that helps to stimulate active immunity in humans towards some specific contagious diseases. In the early stage, vaccines normally consist of weakened or killed disease-causing microorganisms. The viral microorganisms are attenuated in the laboratory before being injected to the human body through heating or chemical treatments. The human body's immune system will recognize the injected antigens or microorganisms as a threat or foreign enemy that invades into the body and try to initiate a series of immune mechanisms to destroy the injected substances. There are several traditional ways of vaccination to humans including injection, intranasal, oral or percutaneous methodology. Meanwhile, the efficacy of every vaccine varies depending on various factors such as the vaccination schedule, the transformation of contagious disease itself, the vaccine strain, the various immunity responses of different people towards the disease, the age or health status of people who receive the vaccines and many other factors. Those factors are required to come into consideration when preparing a certain vaccine against a particular contagious disease to ensure the function of the vaccine from time to time.

The vaccines produced through the conventional approach which insert the attenuated or killed microorganisms expose a higher risk in developing contagious diseases in the community and more other methods are being investigated with the help of new technologies to produce vaccines while ensuring the public safety. Better understanding and wider knowledge in the mechanisms of pathogens infections or the complete genome sequence obtained through the new biotechnologies had greatly improved the new strategies to research and prepare the vaccines against various kinds of contagious diseases. According to World Health Organization (WHO) bulletin in 2008, one of the main branches of medical biotechnology vaccination greatly reduces diseases, disabilities, deaths, and inequities worldwide while the life expectancy of humans also increased [7]. Table 1 shows some of the popular vaccines against different types of diseases for humans that had been commercially marketed all around the world for years.

Table 1: Common vaccines for humans in the market [8].

Product	Recombivax HB®, Engerix B®, Elovac B®, Genevac B® Shanvac B®	Rotarix® RotaTeq®	Gardasil® Cervarix®	Dengvaxia®	Bexsero® Trumba®
Preventive infection	HBV	Rotavirus	HPV	Dengue virus	Neisseria meningtidis group B strain
Indication	Hepatitis B	Gastroenteritis	Cervical cancer	Dengue	Meningitis

Vaccine type	Subunit vaccine	Live attenuated vaccine	Subunit vaccine	Live attenuated vaccine	Subunit vaccine
Administration	IM	Oral	IM	IM	IM
Human papillomavirus (HPV): hepatitis V virus (HBV) and intramuscular injection (IM).					

First column is the vaccine against hepatitis B virus (HBV) and is being produced through recombinant DNA techniques with the help of yeast cells. The second column is the vaccine against rotavirus which normally results in severe vomiting or diarrhoea in young age babies or children. The vaccine consists of living weakened viruses which is highly risky and should not be vaccinated to children who are proven immunosuppressed. The vaccine in the third column is designed to fight against human papilloma virus (HPV) while the fourth column shows the vaccine against dengue. With the use of recombinant DNA technology, the genes responsible for dengue antigens are genetically modified to the virus to stimulate the body immune system towards four various serotypes dengue which are DENV-1,2,3, and 4. Lastly, the fifth column is the vaccine against *Neisseria meningitidis* group B strain (Men B) to prevent the invasive meningococcal diseases by initiating an active immune response.

The biotechnology applied in vaccine preparation provides us more vaccines against new contagious diseases. The advantages brought by the new vaccination programs are normally being underestimated as improvement in health only, but the improvement in health quality of the population actually also contributes to the advance of global economic growth at the same time.

5.2 BIOTECHNOLOGY ON VACCINES FOR CONTAGIOUS DISEASES

Basically, there are mainly three major methods for the application of biotechnology on the vaccines for contagious diseases including the isolation of pure antigen or protein molecules using a monoclonal antibody, antigen synthesizing through a cloned gene and arranging the peptides structure to produce vaccines [8]. Upon applying these methods for the application of biotechnology on vaccines, some of the vaccine features may experience minor or significant changes or be improved by the specific suitable biotechnology chosen. The invented vaccines are important for us to fight against some stronger microorganisms such as antibiotic resistance pathogens or severe contagious diseases for public health.

A) Recombinant Vaccines

The recombinant vaccines are known as the synthesized antigen or protein molecules from cloned DNA or a set of viral proteins that are used to stimulate a series of immunity mechanisms. The isolation and duplication of the viral DNA codes that produce particular antigen which initiates the immune response are carried out in the lab to make viral antigen and the viral antigen produced are then purified to be used as vaccines [9]. The viral DNA codes selected to produce specific antigen can be inserted and expressed in various vectors like bacteria or yeast. Other factors considered are the expression level gained using the expression vector, choice for the selection marker and the post-translational modification exhibited by the recombinant vector [10].

Even though in most of the applications of recombinant vaccines, bacteria serve as the most common expression system due to its high level of expression capacity and easy to handle, the utilization of mammalian or insect cells as vectors should come first if post-translational modification process such as glycosylation is required [10]. Antigen glycosylation is important in activating the adaptive immune system. For example, class I and class II glycoproteins major histocompatibility complex (MHC) present the antigenic peptides at cell surface forming complex, recognized by the circulating T cells and resulting in glycan-dependent TH cell and cytotoxic T-cell immune responses [11]. The main advantage of this approach to producing vaccines is that many different types of protein-based vaccines can be prepared through the recombinant techniques.

Highly purified recombinant proteins are widely used as the basics for vaccines and the

vaccine against hepatitis B is the most common example of recombinant protein vaccines for humans among many different types of vaccines in the market nowadays [10]. Hepatitis B virus (HBV) is a worldwide virus that is usually transmitted from human to human through blood, saliva or pregnancy that leads to liver infection and may cause both acute and chronic diseases. The expression of HBV surface antigen (HBVsAg) with yeast cells, as illustrated in Figure 2, is the most common vaccine against the virus which forms intensely immunogenic virus-like particles (VLPs) that makes the vaccine highly effective towards the virus HBV [10]. Yeast is chosen due the purifying ability by releasing the antigen into the culture supernatant and its eukaryotic post translational machinery supporting the fundamental glycosylation process [10]. Meanwhile, another new invented vaccine against hepatitis C virus (HCV) is also being investigated with the same technique [8]. However, the recombinant proteins vaccines still exhibit some disadvantages in the aspect of poor immunogenicity level even though it is safer and lower in cost to prepare compared to conventional types of vaccines. The utilization of adjuvants such as aluminium salt in the HBV vaccines are required to enhance long-term immunity in humans [10]. Thus, it is also vital to study the field of adjuvants to ensure the effectiveness of recombinant vaccines in the future.

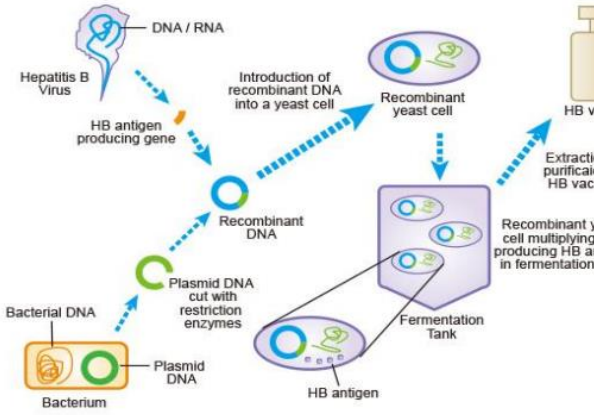

Figure 2: Recombinant hepatitis B vaccine production [10].

After the discovery of HBV vaccines through recombinant DNA technology, the method and process to produce the recombinant HBV vaccine is distributed to several vaccine manufacturers. Comparing the recombinant hepatitis B vaccines with the traditionally plasma-derived HBV vaccines, both are safe to humans and share similar effectiveness in fighting against the contagious diseases. However, since the cost for both of the vaccines are almost the same due to competition among the same type of vaccine producers, the plasma-derived HBV vaccine is gradually substituted by the recombinant HBV vaccine. However, as time passes, the cost for HBV vaccines also reduces as shown in Figure 3. This makes the vaccine more affordable by people who live in poverty in most developing countries.

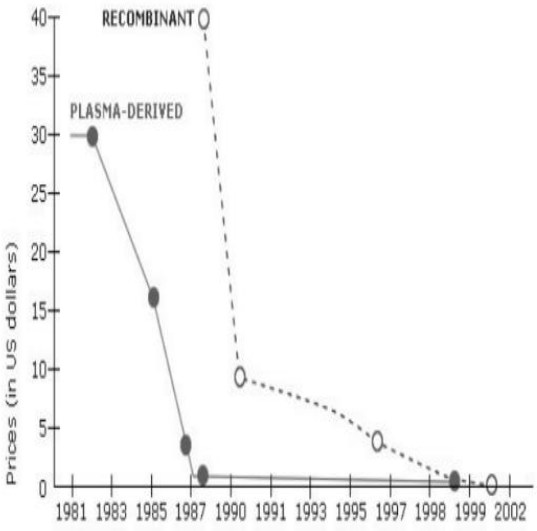

Figure 3: Decreasing prices of plasma-derived and recombinant HBV vaccines [10].

On the other hand, the vaccine invented against human papillomaviruses (HPVs) is another successful example of recombinant vaccines injected to young women [10]. The dangers of the HPVs infection are that various mucocutaneous diseases in humans will occur including cancers that are well known in females such as cervical or vaginal cancers that lead to high rates of mortality. There are generally two types of HPV vaccine in the market known as Gardasil ® and Cervarix ® while both are having virus-like particles (VLPs) that contribute to the vaccine efficiency [12]. In Gardasil TM, the major capsid protein molecule L1 protein that form the VLPs is obtained from HPV type 6, 11, 16 and 18 while type 16 and 18 for Cervarix TM since L1 proteins of the HPV type 16 and18 are showing the highest potential of causing cervical cancer

[12]. Gene-fusion technology and the exploration of better adjuvant systems are new technologies given attention to incorporate with the recombinant DNA technique to improve production and efficiency of recombinant subunit vaccines.

B) Reverse Vaccinology by Reverse Genetics Technique

The design and preparation of vaccines for various types of contagious diseases is hard as there are too many different types of microorganisms or pathogens that harm the human body with their own special mechanisms or characteristics. Thus, scientists or researchers have to break the usual and conventional methods to prepare a vaccine if the previous approaches failed to reach the target. Innovation in preparing vaccines allows us to discover more preventive measures to avoid particular diseases. As biotechnology develops from time to time, the complete genome sequences discovered have given researchers another possibility to design new vaccines. In general, the preparation of vaccines through the application of genomic information using computational analysis without undergoing the culturing of microorganisms is generally known as reverse vaccinology (RV) [13]. RV has modified the normal concepts to select and design a suitable vaccine. The reverse genetics (RG) technique used in reverse vaccinology utilizes the reversing routine of Crick's Central Dogma research from the deoxyribonucleic acid (DNA) to the formation of ribonucleic acid (RNA) and the last synthesizing of protein molecules [14].

At the beginning, the idea came from genetic engineering knowledge by identifying and culturing the pathogenic part of organisms in the laboratory but it was not very successful [13]. Later, genomic technology that is able to determine the whole complete genome sequence of an organism has allowed us to investigate the appropriate potential protein coding sequences to be an antigen that can stimulate the host organism's immune response [14]. The researchers utilize various types of genomics methodologies or technologies including DNA microarrays, proteomics or comparison between the genome analysis to recognize the virulence factors and select the suitable potential antigens to produce vaccines [8]. Then, the researchers will try to prepare the vaccines based on the genomic analysis aided by the computer without the need to culture the microorganisms. After applying these approaches in reverse techniques, the researchers discover a significant decline in the time required and cost using this new strategy to produce vaccines instead of culturing the pathogenic microorganisms to diagnose the potential vaccine candidates (PVCs) [15]. However, there are some limitations in reverse vaccinology since some of the active molecules that exist in vaccines are not in antigen or protein form, they can exist as polysaccharides or lipids which cannot be predicted by the reverse techniques [14]. More investigations and trials need to be carried out by researchers to prove the effectiveness and wider applications of reverse vaccinology. Figure 4 and Figure 5 below compare the differences between the vaccine produced through traditional approach and the reverse vaccinology.

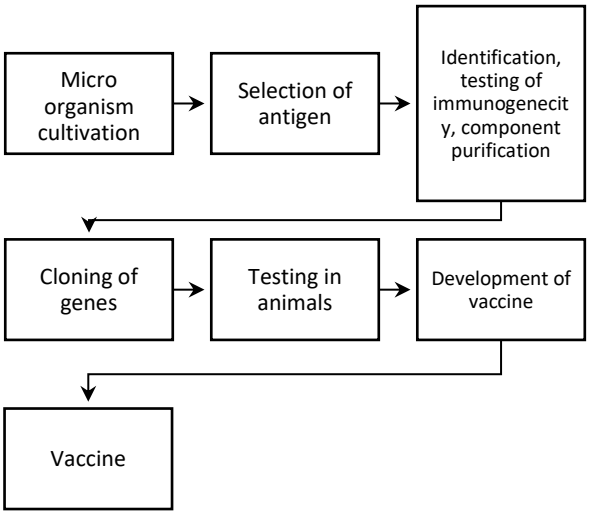

Figure 4: General traditional process to design and produce vaccines [13].

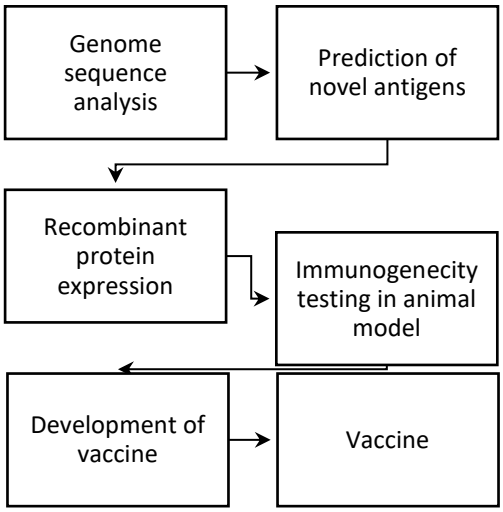

Figure 5: General procedure of reverse vaccinology to develop vaccines [13].

For example, the development of a vaccine against *Neisseria meningitidis* serogroup B (MenB) pathogen which may lead to meningitis and other meningococcal diseases is the first milestone of RV [15]. Meningitis is the swelling or inflammation that occurs at the spinal cord membrane and brain that leads to symptoms such as fever, confusion or coma and is associated with a high mortality rate. The discovery of vaccine against MenB pathogen is a breakthrough in vaccine development as many trials using the conventional technology to produce the vaccine had failed and the possibility is very limited due to the identical capsular polysaccharide structure of MenB pathogen with the human-self antigen and extremely variable feature of the pathogen [12]. Then, the certain selected protein sequence through computational analysis from 600 potential antigens tested for antigenicity is expressed in *Escherichia coli* and is confirmed through enzyme-linked immunosorbent assay (ELISA) and fluorescence-activated cell sorting (FACS) [13]. In an experiment, 29 out of 90 undiscovered surface proteins from the sera extracted from immunized mice are identified for their abilities to produce antibodies to kill pathogens *in vitro* [12]. Preclinical studies of MenB vaccines on different ages of volunteers including infants and adults are then continued to be carried out to ensure the safety and immunogenicity of the vaccine. A phase III clinical trial had been carried out for the future development of MenB vaccines and also served as a basic requirement for European license application [12].

On the other hand, another application of reverse vaccinology is the invention of a vaccine against a food borne contagious disease listeriosis that is always a danger to the food industry and caused by *Listeria monocytogenes* [14]. The symptoms of the disease shown in humans are normally septicemia, pneumonia and encephalitis that may lead to deaths. Luckily, with the advanced genomic technology nowadays, the full genome sequence of *Listeria monocytogenes* is identified and able to be utilized by reverse vaccinology methods to produce vaccines [14]. The surface proteins and other protein molecules can be recognized with different types of tools and being applied as the antigenic epitopes against the vaccines designed.

There are several web-based tools with their own functions that can be applied to identify the various kinds of surface proteins. For example, Signal IP 3.0 is a tool that functions to check the availability of signal peptide positions that undergo cleavage while LipoP is another equipment to determine the total number of lipoproteins and other terminal membrane helices [14]. Both of these tools obtain the results by referring to the Gram-positive bacteria and the results are beneficial for genome analysis and the vaccines development. In addition, the total number of transmembrane helices existing proteins can be calculated with Transmembrane Helices Hidden Markov Models (TMHMM) through hydrophobic amino acid assistance while the determination of location of subcellular of protein molecules in genomic analysis can be accomplished with the help of the currently most accurate bacterial protein subcellular localization (SCL) technology PSORTb.

Other than the application of reverse vaccinology in developing MenB vaccines, the reverse genetics technology also has been implemented to design bacteria-based vaccines to initiate the human bodies' immune system against the antibiotic-resistant pathogens such as *Staphylococcus aureus* and *Streptococcus pneumonia* [8]. Reverse genetics technique is important in designing the vaccines as the Streptococcus type pathogens will stimulate antibodies that will cross-react with human self-antigens leading to infections [12]. Thus, it is necessary for the researchers to find out the antigens that are not similar to human self-antigen and continue to process and purify them into vaccines. Reverse vaccinology approach has also been tried to be implemented in the

progress of developing vaccines against contagious disease malaria. The disease is easily spread among the community through transmission of plasmodium into human blood with mosquitoes' bites. Moreover, the resistance of the pathogens *Plasmodium falciparum* towards the drugs even contributes to a high rate of fatality in the society. It is estimated around 1.2 million residents passed away due to this contagious disease in 2010 [14]. With the application of biotechnology in computational genomic analysis, 2 out of 14 chromosomes of the pathogen *Plasmodium falciparum* are recognized and being selected to be translated into proteins for the next vaccine preparation process [14].

C) *Deoxyribonucleic Acid (DNA) Vaccination*

In general, deoxyribonucleic acid vaccination is a direct injection of an engineered DNA plasmid into human muscle cells to improve body immunity to a certain disease [10]. With the aid of genetic engineering technology, the injected DNA plasmid is engineered to consist of 4 major components including *Escherichia coli*, cytomegalovirus promoter, cloning sites and an antibiotic [10]. The *Escherichia coli* allows the plasmid amplification while the antibiotic inserted serves as a selection marker. The main idea supporting the deoxyribonucleic acid (DNA) vaccination is that it mimics the reaction of the host cell presenting the antigen during the infection of contagious diseases and initiating the immune system response. The antigens will then be synthesized as protein molecules in the cytoplasm. Class I major histocompatibility complex (MHC) will process some of the fragmented protein molecules or peptides while the Class II MHC will be responsible for the processing of secreted protein molecules [10] and both mechanisms will eventually lead to the immune system response.

DNA vaccination is expected to provide a wider and longer range of immunity compared to other types of vaccines since it directly mimics the mechanism of viral infections. This type of vaccine performs better in stimulating long-term immunity and enhancing the response of cytotoxic T-cells while preventing problems arising in conventional vaccine producing methods such as the expensive cost in purifying the recombinant proteins used in recombinant subunit vaccines [10]. However, the weaknesses of DNA vaccination regarding the capability or efficiency are still in scientific investigation leading to less application of this technology in designing vaccines. In this generation, the application of deoxyribonucleic acid (DNA) vaccination is currently less applicable in humans and more common for the usage of veterinary, researchers are making efforts to utilize this approach in finding vaccines against some cancers and severe contagious diseases such as the Middle East respiratory syndrome (MERS) coronavirus [8]. This approach should be also more investigated and implemented in the development of vaccines against the most current corona disease 2019 (COVID-19) which is also a contagious disease that leads to dangerous acute respiratory infections by coronavirus.

5.3 ADVANTAGES OF VACCINE FOR CONTAGIOUS DISEASES COMPARED TO CONVENTIONAL METHOD

With the development of the first vaccine in the past 200 years, humans have been benefited through the decreasing burden of contagious diseases. As an example, smallpox is the first disease that was eradicated and successfully eradicated to whole countries with the help of vaccination in the years of 1980 [15]. The prevention of contagious diseases through the eradication until the elimination of certain diseases by vaccination has proven to be one of the greatest achievements in medical biotechnology. The improvement in biotechnology techniques has further enhanced the rapid growth of vaccine production and led to the introduction of new products that can be spread to the marketplace and accepted by society.

In the past, the conventional or traditional vaccine was developed based on the pathogen of the relative disease in inactivated, killed, or live-attenuated form [15] according to the Pasteur's principle of isolation, inactivation, and injection of particular microorganisms [16]. These pathogens either produce artificially or through the *in-vitro* cell culture propagation [15] where the vaccine is produced in a culture dish with the supportive growth medium. This is a cost-effective method for larger production of vaccines and helps to overcome the vaccine shortages during the pandemic of contagious diseases. *In-vitro* cell culture system also allowed the research and investigation to carry out. For

example, the development of measles, mumps, and rubella (MMR) vaccine in chick embryo cell cultures and allowed the large production of live-attenuated viruses through the growth in non-human cells medium [15]. These pathogens help to boost up human immunity but don't cause infection to human cells. Through the immunization program carried out by many countries at the same time together with vaccination, the number of cases of contagious diseases has gradually decreased. For example, before the vaccine for measles was introduced, the number of cases and deaths related to measles was estimated for about 3044 cases per million per year in the years of 1953. After the vaccine for measles was introduced, the cases were reduced to 0.2 cases per million per year in the years in 2006. It shows a gradual reduction of 99.99% [17].

Figure 6 shows a graph from a report by WHO which demonstrated that the reduction in the reported number of cases for measles has followed by the increasing percentages of immunization coverage of MCV1 [19]. The actual immunization coverage percentages of MCV1 are also higher than the expected number. This situation shows that people are willing to accept the advantages that bring along with the development of vaccine production through biotechnology techniques.

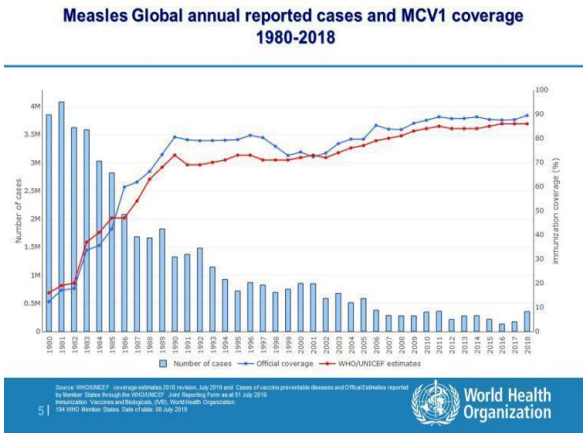

Figure 6: The graph shows the global reported number of cases of measles and immunization coverage percentages of the first dose of live-attenuated viral measles vaccine (MCV1) from the years 1980 to 2018 [19]. The red line graph shows the estimated immunization coverage percentage by WHO and UNICEF while the blue line graph shows the actual coverage.

Although Pasteur's principle has become the main choice in vaccine production for many years, it also brings some issues. Some pathogens have the difficulties to cultivate *in-vitro* or develop into live-attenuated form. These kinds of pathogens might induce the unfavourable or destructive immune response of the human body. As an example, HCV, papillomavirus types 16 and 18 [16]. Pasteur's principles are also not suitable for microorganisms that are antigenically hypervariable such as influenza and HIV. The antigenically hypervariable microorganism has a genetic mechanism to evolve a stable genome and create antigenic changes [18] in the host to protect themselves and survive against human immunity. As a consequence, the host cells will become a microbial reservoir for the particular antigenic hypervariable microorganisms and induce the subsequent transmission [18]. This situation will also cause the microorganism to be resistant to human immunization. For microorganisms that will kill the infected host cells such as cytotoxic T cells, Pasteur's principle does not provide clear guidelines in the way to induce the particular pathogens and it will lead to viral replication [16]. The conditions to culture the live bacteria or microorganism into live-attenuated form was much more limited, restricted by unprecedented mutations caused by the antigenic hypervariable microorganism. Selection will also occur among the cultured pathogens if the growth medium is not optimum for them. Some pathogens will change their growth habits and cause mutations, hence some diseases may emerge or persist even though various vaccines have been created against them. For example, Figure 7 demonstrated incidence numbers of various contagious diseases from the years 1991 to 2003 [16]. Through the trend of the graph, advanced techniques and new methods through medical biotechnology are needed to overcome the emerging and persisting diseases.

Besides that, the level of immunogenicity of live vaccines also can be lowered by their degree of attenuation. The attenuated vaccine is still considered alive, it can recover back its virulence and cause diseases. For example, the Sabin Oral Polio Vaccine (OPV). In the beginning, OPV was proven to be highly effective as it reduced the transmissibility of neurovirulence and wild-type poliovirus [20]. However, the vaccine strains in a vaccine recipient undergo selection pressure and cause the attenuated nucleotide substitutions to occur reversion. The transmissibility of vaccine-

derived poliovirus (VDPV) allowed the strain to evolve and cause the outbreak of wild type poliovirus [20].

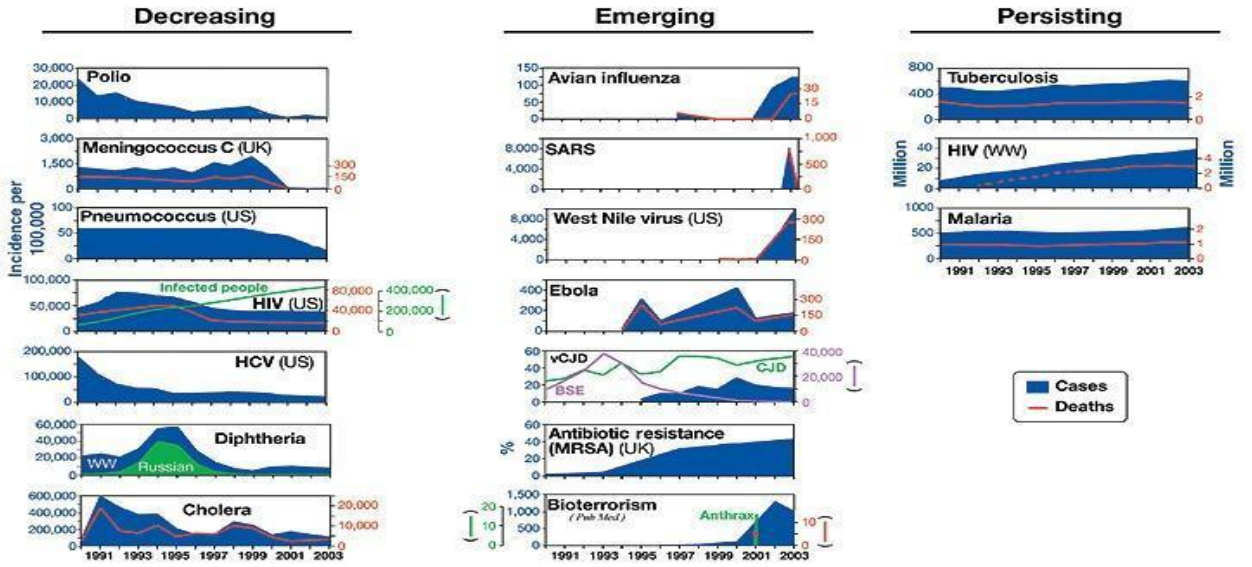

Figure 7: The graph of incidence numbers of various contagious diseases from the years 1991 to 2003 [16]. The left column shows the diseases that are decreasing. The middle column shows the emerging diseases. The right column shows the diseases that are persisting.

Due to these limitations in the conventional method of vaccine production, the development of new vaccine production technology through the research and investigation was carried out to overcome these limitations. With the development of modern biotechnology tools such as genome-based technologies, new techniques and approaches were carried out to overcome the limitation. The new approaches of vaccine production using biotechnologies including recombinant vaccines, reverse vaccinology by reverse genetics techniques, and deoxyribonucleic acid (DNA) vaccination had brought a great movement in vaccine development. The new technology further investigates and formulates the existing vaccine to enhance and maximize their effectiveness, safety, and stability of a vaccine. As seen in Figure 8, the development of medical biotechnology in vaccination such as bacterial culture techniques in the early 1900s allowed the development of vaccines like tetanus and Diphtheria. A combination of medical biotechnology with advanced chemistry and molecular biology led to the production of vaccines against hepatitis B, influenza, ebola, etc. It shows that with advanced medical biotechnology research and investigation, the production of various types of vaccines for contagious diseases also increases greatly with the flow of time.

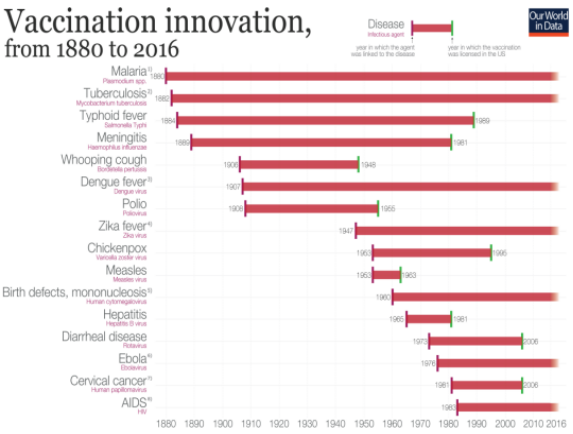

Figure 8: Vaccination innovation from 1880 to 2016 [17]. Each bar shows the time when the agent was first linked to diseases until the time when the particular vaccine is licensed in the United States.

In terms of cost of manufacture, conventional vaccine manufacturing technologies are costly due to the limited range of vaccines. As the product range of vaccines is smaller, each manufacturing process has to be specific to the type of vaccine produced. In low income and under-development countries, the

demand for the vaccine is very high compared to developed countries, thus the specialized apparatus and equipment, raw materials, and skilled labor are needed to be hired and imported from other countries [21]. Therefore, these countries are forced to pay a huge amount of money to meet the high demand for the vaccine. Besides, the vaccine doesn't generate a high amount of revenue due to the insufficient coverage of vaccination costs by the fund and the time when the diseases occur and the availability of vaccines doesn't match.

With the use of new technologies such as recombinant vaccination, reverse vaccinology by reverse genetics techniques, and deoxyribonucleic acid (DNA), the cost of vaccine manufacturing can be reduced. These methods of vaccine manufacturing can produce a large range of vaccines. Thus, the flexibility and efficiency of the particular vaccine manufacturing process will increase and decrease the cost and time consumed. At the same time with the introduction of a new vaccine in low-income countries, the contagious diseases were successfully being eradicated. The actions that had been taken by these countries include providing 100000 doses of vaccines within a week after the introduction of new vaccines and conducting immunization programs to all the infants at a cost approximately $1 per dose [21].

Deoxyribonucleic Acid (DNA) vaccination is one of the earliest modern technologies applied in the manufacturing of vaccines in the 1990s. As demonstrated in Figure 9, the DNA vaccine has more benefits compared to live-attenuated vaccine and killed or protein subunit vaccine. It has a higher efficiency in stimulating antibody responses because the DNA vaccine was directly injected into host cells and stimulates antibodies to attack the contagious diseases. The immune response of cellular or humoral is also stimulated without the need of live vectors [22]. Therefore, it is often acting as a preventive vaccine. Besides that, the insertion of multiple variants of antigen into a single plasmid is relatively easier in DNA vaccination. Therefore, it provides the opportunity to further design and experiment during the production of the vaccine. Multi-antigen vaccines can be produced from the evolved strains of the pathogen through genes and antigens. For example, possible production of a type of universal influenza vaccine which can be used for all time compared to the seasonal influenza vaccine. The logistic advantages of DNA vaccines include the relatively lower cost due to the ease of development and production [22]. These advantages are suited for developing countries and bring along the benefits such as the reduction in cost for transportation and storage.

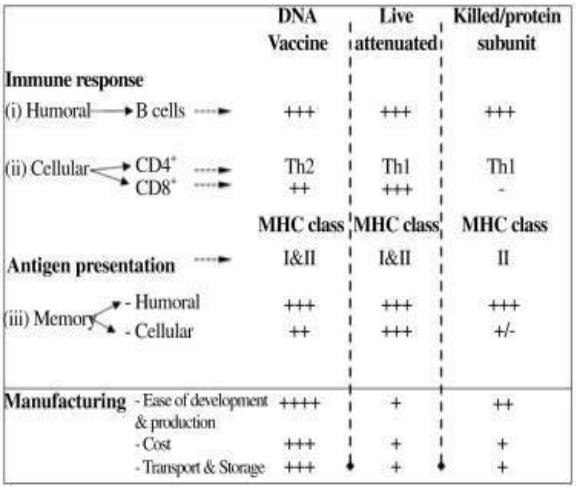

Figure 9: The advantages of DNA vaccine compare to the live-attenuated vaccine and killed or protein subunit vaccine [22]. The positive sign (+) shows the level of advantages, the more the number of a positive sign, the greater the advantages of a particular vaccine.

Besides that, the introduction of recombinant vaccination. The recombinant vaccine was produced by the introduction of genes coding for antigens into the vector genome. The advantages of the recombinant vaccine are they are produced without the use of actual disease agents. Recombinant vaccine viruses will not recombine with other wild viruses and occur in the recovery of virulence. One of the vaccines produced using the recombinant vaccination techniques is the Hepatitis B Virus (HBV) vaccine. This is a highly efficient vaccine due to the immunogenic characteristics of the hepatitis B surface antigen (HBsAg) in yeast cells. With recombinant vaccination, the immune system will recognize the proteins inside the vaccine as a foreigner and mount the immune response. Therefore, it provides future protection against the particular pathogen [15]. On top of that, with the introduction of recombinant techniques in HBV vaccine production, many manufacturers are involved in HBV vaccine production. At the same time, due to the competitions, the prices per dose of the HBV vaccine decreased [15]. This made the HBV vaccine the most affordable vaccine in most developing countries.

For reverse vaccinology by reverse genetic techniques, it is also one of the modern methods of vaccine production that brings a lot of advantages as compared to the conventional method of vaccine production. The basic concepts of reverse vaccinology are the

sequencing of the genome and screening by biotechnologies techniques to explore genes. The selection of genomic sequences of pathogens occurred to eliminate the antigens that are potentially dangerous and provide immunity protection [15]. This method is also used as a prediction test for antigens or pathogens that may potentially cause diseases. With the help of reverse vaccinology, the process of cultivation of microorganisms is deducted. Besides that, it also helps the people to identify the virulence factors of antigens and novel vaccine candidates. This approach allowed the development of a vaccine for those pathogens which isn't suitable for Pasteur's principle. For example, HCV and human papillomavirus 16 and 18 pathogens.

5.4 EFFECTS OF BIOTECHNOLOGY APPLIED FOR VACCINATION IN TODAY'S SOCIETY

The arising of biotechnology applied in vaccination had delivered high contributions in both economic and social aspects among various human interventions. Even other influencing factors such as the improvements in management exist, it is undeniable that the application of biotechnology in vaccination contributes to the innovation in producing different types of vaccines against contagious diseases and improving the safety of vaccination in general. Some potential preventive measures for particular diseases that are difficult or impossible to heal are also found with the suitable application of biotechnology in producing vaccines. Vaccination is also an effective way in fighting against the threats of biological weapons in society. However, with the rapid development of biotechnology involved in vaccines preparation process and the uncertain long-term effects caused by the newly invented vaccines, safety issues arise from this condition may lead to a negative phenomenon in the society called "vaccine scares" which may reduce the coverage of immunization of the public and affect the overall public health condition.

A) Improving economic performance with wider vaccination programs

With the wider application of biotechnology in vaccine preparation, various vaccines are invented and more contagious diseases can be prevented in society. It is undeniable that the creation of new vaccines against more contagious diseases definitely helps in extending human life expectancy by providing earlier immunization and lead to a decrease of 50% in the mortality rate while promoting public health quality. The national economic status can be continually prompted with the higher public health quality. There are two methodologies that explain how the improved health quality generates the national economic level including the virtuous cycle and the averted costs through wider vaccination programs.

Population health is fundamental to economic development since healthy individuals make contributions to the economic growth while vaccination can be assumed as the basic foundation of the population health programs. The economic growth of a nation is mainly driven by a healthy individual productivity. Education, investments, tax payments, labor productivity and savings can be driven through a healthy population and eventually forms a virtuous cycle [23]. Figure 10 below shows the cycle formed between population health and economic growth. For instance, a healthy adult is able to give better focus in their professions and contribute to higher productivity or better investments and vice versa [23]. Besides, the health of children should be also prioritized as they can perform better in the education and cognitive ability to serve the country in the future.

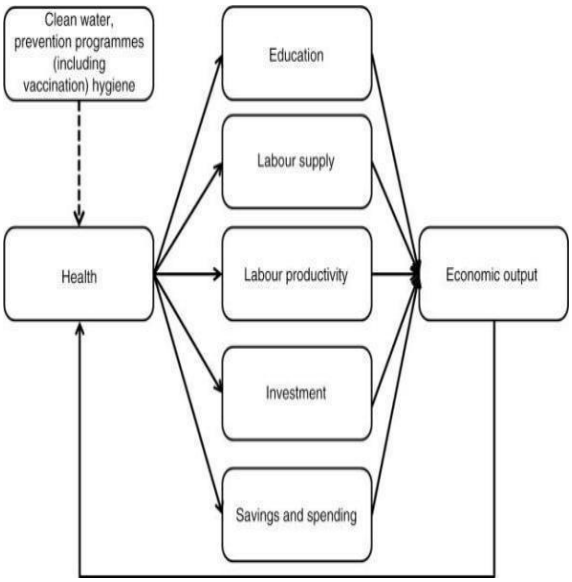

Figure 10: Linkage between health and economic output [23].

Another methodology to measure the economic impact by wider vaccination programs is based on the reduction in cost of illness. According to the evaluated number of cases, deaths and disabilities caused by the contagious diseases, a study from WHO had made an estimation of cost of illness which includes the costs of treatments for those who are infected by the contagious diseases and the productivity losses due to the infections [23]. The costs saved

from the wider vaccination programs can be classified into five main categories which are the treatment cost avoided such as the expense spent on medications and diagnostics, the transportation fees wasted to seek for treatment, the averted downsizing of economic outputs by caregiver, the prevention of productivity loss due to deaths and the avoidance of productivity declination by disabled survivors [24].

The averted costs of illness for different vaccines developed from new biotechnologies are tabulated as in Table 2 below. For example, the hepatitis B vaccine invented from recombinant technology successfully prevented nearly $7000 per death and conjugate vaccine which combines a weak antigen with another strong carrier antigen such as the haemophilus influenza type b (Hib), neisseria meningitidis serogroup A and streptococcus pneumonia vaccines also show great records and even save up to $22000 per death. These costs can be used to develop other sectors in the country such as improving basic facilities such as health equipment or education for the society.

Table 2: Cost of illness averted by different vaccines [24].

	Averted costs of illness (2010 US $)		
Antigen	Per vaccinated individual	Per care-seeking case averted	Per death averted
Hepatitis B	61	12	7000
Haemophilus influenza type b (Hib)	105	48	22000
Neisseria meningitidis serogroup A	25	31	17000
Streptococcus pneumoniae	122	38	19000

B) Potential preventive measures for particular challenging diseases

With more innovations in applying biotechnology in vaccine preparation, the possibility for scientists or researchers to prepare vaccines that are able to prevent certain diseases can be greatly increased. Some diseases or infections are complicated to be cured and even no absolute treatments can be applied on humans to be completely recover from the infections such as the lifelong infections caused by human immunodeficiency virus (HIV) that may develop into acquired immune deficiency syndrome (AIDS) if without proper treatments or another viral disease Saint Louis encephalitis (SLE) that easily spread through an infected mosquito. Most of the treatments given are only based on the symptoms shown but there are still no vaccines or medications that are proven to be effective in fighting against SLE until today [25].

The emergence of HIV-1 infection in 1981 had awakened the awareness of scientists to develop new vaccines against this virus. However, due to the complexity of the virus and difficulties in stimulating neutralizing antibodies towards HIV-1, the researches or trials in preparing the vaccine faced failures. Clinical trials such as the STEP study and Phambili Trial are carried out to investigate the efficiency of recombinant adenovirus 5 vector HIV vaccine but the results are not satisfying. The Ad5-vaccine and another HIV-1 GP120 recombinant protein are not effective to be used as preventive measures against HIV infection [26]. It was suggested that an effective HIV-1 vaccine should be able to regulate the humoral and other cell-mediated immune responses [26].

With the development of biotechnology in vaccine preparation, the researchers put in their efforts in the combination of a DNA-based and viral-based vaccines with the recombinant protein vaccines in order to enhance the immunity induced about 31%. According to the result of recent efficacy test RV144 which incorporates with a multiple-antigen viral-vector prime (ALVAC), the combination successfully stimulates a broader immunity which the DNA-based and viral-based vaccine responsible for T-cell responses while the recombinant protein vaccine stimulates the antibody response [26]. The new approach not only focuses on the power of immunity initiated, but also the overall quality of the humoral response against HIV infection.

Since the DNA vaccine responsible for the T-cell response is not adequate enough to prevent the infection of HIV-1, further trials are followed to improve this problem. With the development in adjuvant study, the further investigations on the application of suitable adjuvant in DNA vaccination against HIV-1 gives another great improvement in the immune response initiated. Molecular adjuvant IL-12 changes the destination of antigen-specific T cells to particular body parts for better immune response and promotes higher immunity level. For instance, mucosal chemokines drive the antigen-specific T-cells to gut mucous membranes to discourage the replication of HIV-1 virus in the human body at the initial stage. The discoveries and developments of new vaccinology due to technological advances help in further clinical trials and induce more effective vaccines to be produced against those challenging diseases.

C) Protection from potential threats of bioweapons

In general, the devices used to spread organisms or pathogens that may cause diseases or infections are known as biological weapons or in short form bioweapons. Those who are infected by the bioweapon may experience an acute effect if the content used are toxins while some others may develop an incubation period which varies with different people from days to weeks depending on the power of organisms in the bioweapons and the immune response of the infected individual. A disastrous epidemic will even happen if the agents used in the biological weapons are highly contagious through the transmission from person to person. The issue of the potential threats brought by biological weapons will result in an irreversible catastrophic effect in society and raise the concerns of public health professionals and political leaders.

For example, recombinant technology has helped in the development of vaccines against Ebola virus. Ebola Virus Disease (EVD) is a highly mortality rate disease that may cause haemorrhage and fever conditions in some certain animals or humans. The disease was first found in the Democratic Republic of the Congo in 1976 through the transmission from wild animals to humans by contacting the contaminated body fluids. The low blood pressure condition due to fluid loss leading to high fatality level of the disease which is approximately 25% - 90% among those who are infected. In the past, a rumour said that the Soviet Union utilize the high fatality rate of Ebola virus to produce bioweapons from year 1986 to 1990 but no clear evidence has been proven on this rumour, yet the virus still exhibits a high potential usage as a bioweapon and dangerous to humans [27]. Scientists and researchers put in all their efforts to find an effective vaccine of the Ebola virus.

During the year 2014, the Ebola glycoprotein (GP) vaccine was developed and announced in the 8th Vaccine and ISV Conference. The immune system was successfully stimulated with the recombinant technology used. The code recombinant protein used in the Ebola GP vaccine is carried by the recombinant DNA according to the genomic sequence of Guinea Ebola strain that results in a wide infection in West Africa in the year 2014 [28]. The immunologic adjuvant added later was then proven to be very high in immunogenicity regardless of the amount of adjuvant used. In addition, another uncommon vaccine technology that produces the vaccine in a tablet form which is relatively more stable to temperature changes was developed by Vaxart Inc. in 2015 and more clinical trials will be carried out in the future to ensure the effectiveness of the vaccine. The availability of effective vaccines against Ebola virus helps to prevent the outbreak resulting from the biological weapons and more vaccines for various highly potential bioweapons are in the progress of researches and preparations such as Anthrax, Marburg virus, Bunyavirus and others.

D) Hidden threats from mishandling of biotechnology

Besides the new discoveries and potential development brought by the biotechnology applied in vaccination, the biotechnologies used still present other risks that may cause unintended negative consequences to humans. The rapid development of biotechnologies can be most likely to be proven harmful to humans through two occasions, including the unplanned effects of proper research or the intended manipulation of biotechnologies to irrupt the confidential DNA information or harm humans.

Although the advance biotechnology applied on vaccination shortens the time of preparation of vaccines, it also fastens the development of bioweapons at the same moment. For instance, the researchers are warned that any mishandling or escape of recombinant DNA derived from the drug-resistant bacteria from the laboratory may result in infectious superbugs that cause wide and catastrophic infections in the society [29]. Bioweapons that contain the viral organisms or

deadly toxins which is similar to the concept of vaccine will also be easily produced with rDNA technology. It is estimated that it only takes about a few weeks to produce poliovirus from scratch with the rDNA technology rather than a few years. This situation leads to a higher possibility and rapid improvements in the development of bioweapons for those bioterrorists or villain scientists. With the more complete genome information of pathogens or harmful organisms, the synthesis of DNA for those species will also offer the bioterrorists to make bioweapons even easier.

Besides, the application of advanced biotechnology in preparing vaccines without passing accurate information of the vaccines to the public lead to ethical and safety issues in the community. Based on a qualitative study investigating this issue, it was found that most of the parents opposed the influenza vaccination program due to the insufficient detailed information obtained regarding the vaccines or the effects of the vaccines [24]. Most of the people who accepted the interviews declared that their knowledge regarding vaccination was not complete and some of them also confused between the seasonal influenza vaccines with the influenza A virus (H1N1) vaccine [24]. The condition results in a temporary halt in vaccination programs and greatly reduces the overall immunization of the community to contagious disease. Thus, the public health officers should be more responsible to solve vaccine safety scares issues by giving clear development technology progress and information of vaccines to the public especially the parents. The authorities should also properly tailor the vaccines information for different audiences according to their requirements and try to make the messages as simple as possible to allow the public to have a better understanding on the benefits of applying biotechnology in preparation of vaccines and the benefits of the vaccination programs.

It is a must and compulsory for us to remember that one of the most cost-effective primary preventive methods to prevent deadly contagious diseases is vaccination. A comprehensive vaccination system with the aid of biotechnologies should be continually developed from time to time to stop the spreading in the earliest stage of contagious diseases, enhancing public health and immunity.

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