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Industrial processes for vaccines production

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June 2022

UNIVERSITAT DE BARCELONA

El pesimista se queja del viento, el optimista espera que cambie y el realista ajusta las velas

William George Ward

Querría agradecer a mi tutor durante este trabajo, el Dr. Roger Bringué Tomàs el haberme apoyado y ofrecido ayuda siempre que lo pudiera necesitar durante este trabajo. También querría agradecer tanto a mis amigos como a mi familia el apoyo y la paciencia que han tenido conmigo ya no solo durante este trabajo sino durante todo el grado. Y, por último, pero no por ello menos importante, quiero agradecerme a mí mismo el hecho de no haberme rendido nunca y de haber perseverado hasta conseguir mis objetivos.

Muchísimas Gracias

CONTENT

SUMMARY

The idea of protection and immunization against diseases that vaccines have generated since their conceptualisation has forever changed the way in which humanity has dealt with epidemics, allowing us to go on the offensive and dream of eradicating previously lethal plagues. But this progress has not only come from the hand of science, society as a whole has had to adapt to this new reality and regulatory bodies and laws have emerged to guarantee and provide security to the general population.

With society increasingly able to access information and therefore able to speak up when doubts or uncertainties arise, it is the responsibility of science and engineering to be fully transparent and to work flat out to obtain results that satisfy everyone.

In the field of vaccines, this has translated into the development of new types of vaccines different from those traditionally used, such as messenger RNA vaccines like those approved for the fight against COVID-19.

But it has not stopped there. The entire production process, from the selection of the cell culture to be used to the addition of stabilisers just prior to distribution, has been innovated as needed.

In upstream processing, higher and higher yields of cells are achieved in which to grow the pathogen or antigen needed for the vaccine, and improvements have also been made in the design of stirrers and the growth medium itself.

While in downstream processing, with increasingly severe restrictions on quality control with respect to contaminants or residues, higher and higher yields are being achieved using technologies such as chromatography, diafiltration or gradient centrifugation.

All of this has made it possible to dream that dealing with increasingly complex diseases caused by infectious agents is no longer a utopia. There are still many open fronts and the publication of articles investigating or opening up new possibilities is enormous, which is why the review of the industrial process for obtaining vaccines carried out in this work does its bit to help disseminate the knowledge accumulated so far.

Keywords: Immunization, quality control, upstream processing, downstream processing.

RESUMEN

La idea de protección e inmunización frente a las enfermedades que han generado las vacunas desde su conceptualización ha cambiado para siempre la manera en la que humanidad se ha enfrentado a las epidemias, han permitido pasar a la ofensiva y soñar con erradicar plagas antes letales. Pero este avance no ha venido solo de la mano de la ciencia, la sociedad en conjunto ha tenido que adaptarse a esta nueva realidad y han surgido órganos reguladores y leyes que garantizan y otorgan seguridad a la población general.

Con una sociedad con cada vez más capacidad para acceder a la información y, por ende, habilidad para alzar la voz cuando surgen dudas o inseguridades es responsabilidad de la ciencia y de la ingeniería ser totalmente transparentes y trabajar a destajo para obtener unos resultados que satisfagan a todos.

En el ámbito de las vacunas esto se ha traducido en el desarrollo de nuevos tipos de vacunas diferentes de las tradicionalmente utilizadas, como por ejemplo las vacunas de ARN mensajero como las que se han aprobado para la lucha contra el COVID-19.

Pero no se ha detenido aquí, el proceso entero de producción, que va desde la selección del cultivo celular a utilizar hasta la adición de estabilizantes justo antes de su distribución, ha ido innovándose a medida que ha ido haciendo falta.

En el apartado de upstream processing se consiguen cada vez producciones más altas de células en las que cultivar el patógeno o antígeno necesario para la vacuna, también se ha mejorado en el diseño de agitadores y en el propio medio de crecimiento.

Mientras que en el apartado de downstream processing, con cada vez restricciones más severas en lo que a control de calidad se refiere respecto a contaminantes o residuos, se logran rendimientos más elevados empleando tecnologías como la cromatografía, la diafiltración o la centrifugación de gradiente.

Todo en su conjunto ha permitido que soñar con que enfrentar enfermedades cada vez más complejas producidas por agentes infecciosos deje de ser una utopía. Aún hay muchos frentes abiertos y la publicación de artículos investigando o abriendo nuevas posibilidades es ingente, es por eso que el repaso sobre el proceso industrial para la obtención de vacunas llevado a cabo en este trabajo pone su granito de arena en ayudar a difundir el conocimiento acumulado hasta ahora.

Palabras clave: Inmunización, control de calidad, upstream processing, downstream processing.

SUSTAINABLE DEVELOPMENT GOALS

In the framework of the Sustainable Development Goals or SDGs, it is easy to see where this work fits in, because as mentioned in the summary, the world of vaccines and thus the pharmaceutical industry has been instrumental in taking the concept of public health to a whole new level.

Thus, understanding that this work is part of the goal of good health and well-being, several aspects of it can be highlighted.

The first of these is the enormous effort being made by science to find new solutions and vaccines for increasingly different diseases, and not only that, existing vaccines are being redesigned to achieve greater effectiveness.

It would also be important to highlight the fact that much emphasis is being placed on improving the downstream purification and elimination of impurities in order to further limit the possible harmful effect that some of the components used during manufacturing could have on people.

And finally, it is also worth mentioning that vaccines are increasingly being designed to contain less and less of the original pathogen, as, although the risk of infection from vaccination is practically non-existent, it can still pose a very serious health problem if it were to happen to a person with a weak immune system.

1. INTRODUCTION

Humanity has been trying to defeat diseases and improve its quality of life from time immemorial. Although during centuries progress was limited due to the fact that scientists and doctors did not know the nature of what caused illnesses, the invention of the microscope and the publication of the germ theory of disease paved the way to an innovation, vaccines, that would end up helping to eradicate smallpox, one of the deadliest pandemics that humankind has ever suffered.

Nowadays it is undeniable the effect that such a discovery has had in our conception of health because it has offered the possibility to anticipate to the disease and minimize its effect on the patient or even avoid contagion.

The big pharmaceutical laboratories are the ones responsible of keeping up with the pace of progress and investigation and then mass producing the vaccines to make them available to the population, although universities and some national and international public agencies such as the Consejo Superior de Investigaciones Científicas (CSIC) from Spain also do play an important role in the research part of the process.

Vaccines' impact does not limit itself to the health aspect, it is also important to acknowledge its relevance to the economy of the pharmaceutical industry, which is one of the most lucrative businesses in the world.

To put it into perspective let's take a look on French company Sanofi's, the world's main influenza vaccine producer, report on their results from 2020. Even though it was a year greatly affected by the COVID-19 world pandemic the company announced an increase in their total sales revenue, reaching €36,041 million, mainly due to an 8,8% increase in their vaccine sales, that accounted for 16% of the aforementioned total (Sanofi, 2021).

A company that has been much more mediatic since the beginning of the pandemic is the American pharmaceutical Pfizer, this is because it has developed the most widely used COVID-19 vaccine in Europe and almost the entire world in association with the German laboratories

BioNTech. They have also published their results for 2021, and they break down product by product all the income generated, and it is remarkable that Comirnaty, their vaccine designed to protect people from COVID-19, has earned the company $36.700 million, this represents 46% of their total revenue and multiplies by seven the results from the whole vaccine department the previous year (Pfizer, 2022). This vaccine's sales are almost equal to the total revenue of Sanofi during 2020 when changed to Euros and clearly surpass the Gross Domestic Product (GDP) of some European Union countries such as Cyprus.

1.1. HISTORICAL EVOLUTION OF VACCINE PRODUCTION

Although the idea of immunizing the population by using vaccines is relatively recent there is plenty of information regarding medical procedures that date back to the beginning of the Modern Age, this is centuries before the publication of Edward Jenner's "Inquiry into the Causes and Effects of the Variolæ Vaccinæ" in 1798, that consisted on deliberately infect healthy people with contaminated material to avoid them from catching the disease naturally and even reduce the symptoms.

The first official record of this technique known as variolation comes from a report written by a doctor that worked in the Chinese royal court during the 16th century. He described a procedure used to prevent smallpox in which scabs from infected people were dried and then snorted by the noninfected, who would suffer a mild version of the disease but would not get infected by it if exposed again (Fenner et al., 1988).

There is also evidence of a similar procedure being carried out in India during the 17th century by itinerant practitioners, they extracted purulent fluid from the diseased and then injected it on a healthy patient's arm, this clearly resembles in a certain way how people get vaccinated nowadays. It was this injectable variolation and not the snorting one the version that was finally introduced in Europe around 1721 by Lady Mary Wortley Montagu, the wife of the British ambassador in Constantinople (Boylston, 2012).

This procedure was proven successful as it reduced smallpox lethality from 30% to a value between 1% and 2%, but it also came with some limitations, as it was impossible to carry out without at least a diseased person to extract the pus from, and if this patient suffered from a blood-borne illness such as syphilis there was a risk of contagion.

Doctors and scientists acknowledged these inconveniences, therefore when Edward Jenner published his work about vaccination stating that using cowpox instead of smallpox was safer and almost as effective it soon replaced variolation as the main form of immunization. An example of the early effort made to spread the vaccine comes from the Balmis Expedition (1803-1813), a mission to Latin America traveling with dozens of vaccinated orphans carrying in their bloods the solution to the frequent epidemics that took place in that region.

Now the vaccine could be disseminated arm-to-arm because there was no need of a local outbreak of smallpox to get inoculated with it, a doctor just needed a sample of tissue from a recently vaccinated person to produce more serum. This was the system used during the following decades until 1864, when after a Medical Congress in Lyon an alternative was proposed, to use calves instead of people to produce the vaccine. The idea was simple, by having many animals inoculated with the vaccine the production rates would increase exponentially and the risk of getting infected with a blood-borne disease would be greatly reduced, the implementation of this alternative resulted in the construction of what ended up being called vaccine farms, the first vaccine factories (Esparza et al., 2020).

The spread of these factories and their high production rates soon outcompeted the humanized vaccination, and after some time, in 1898 in the case of the United Kingdom, the original form of arm-to-arm vaccination was outlawed. The following step would be regulating the farms, as it became common to find reports in the newspapers talking about contaminated lots and negative side effects, in 1901 two clusters of tetanus resulting in the death of 20 kids as a result of a contaminated lot of vaccines angered the public opinion a year later, the Congress approved the “Biologics Control Act” to establish quality controls and regulate their sale.

From that point onwards the main efforts were aimed at reducing the dependency on livestock and offering alternative ways to produce the vaccine, thus paving the way to develop other vaccine types rather than attenuated vaccines or inactivated vaccines, the first step was using fertilized eggs as culture medium, this procedure is still used in the vast majority of Influenza vaccines nowadays, and then another breakthrough was made with Salk’s vaccine for polio in 1955, because it was the first commercial vaccine fully designed and produced in cell cultures (Unchern, 2000). This milestone allowed the development of more modern factories using bioreactors and also provided a fully sterilized medium that definitely eliminated the risk of infection from contamination.

Once the production part of the process achieved this point the industry started facing more restrictive quality controls and needed to adapt to new regulations aimed at providing the population with safer vaccines. This resulted in a general increase in the innovation budget for the downstream processes, which are the ones responsible of purifying the serum and getting rid of impurities.

1.2. **CONTROL, REGULATION AND LEGAL ASPECTS OF VACCINES**

Vaccination has not only changed the way society sees diseases; it has also started a debate about civil liberties and public health that is still going on. Soon after the establishment of vaccination as a safer way to immunize people than variolation it spread rapidly across the globe, and to help with this effort some countries started passing laws making illegal variolation, by 1821 Sweden and many little German states had already done it. However, it was not enough, vaccine production rates and the serum's quality were far away from the standards we know today, so to keep up with the pace and achieve a greater global immunity many countries made vaccination mandatory. The Vaccination Act of 1853 from the United Kingdom is a clear example of this, it made compulsory that every new-born had to be vaccinated within three months or the parents would be fined (Wolfe and Sharp, 2002).

Eventually, as the results ended up appearing and death rates dropped clearly among those who had been vaccinated the public opinion started accepting it, but there was also a mindset change in the public administration as it started recognizing conscientious objection as an argument to refuse getting vaccinated, this being added to the Vaccination Act of 1898 also in the United Kingdom. This was the first law in the country to recognize such an exemption and not punish those not willing to vaccinate.

Spain followed a similar path to that of other countries, making vaccination mandatory during epidemics in 1815. However, there was a strong resistance towards vaccination that resulted in a slower progress towards general immunization, this is why in 1903, the government, acknowledging the little progress made in comparison with other European countries, passed an act explaining clearly the responsibilities of each administration when an outbreak was to be managed and specifying the procedures needed. Soon after, in 1921, vaccination was declared mandatory regardless of the epidemiological situation, that meant that there was no need for an ongoing outbreak to be obliged to get vaccinated (Jiménez, 2021).

This was reinforced by a special law from 1944 (Ley de Bases de Sanidad Nacional, 1944, ref 26), it clearly specified that vaccination against smallpox and diphtheria were mandatory and that any other vaccine could be also made mandatory if there was an epidemic. After eradication of smallpox and the beginning of democracy in Spain another law was approved (Ley 22/1980, 1980, ref 27) that modified the one from 1944 and made vaccination recommended again, recognizing the right to refuse being inoculated.

Nowadays vaccines are classified in Spain as special drugs (Real Decreto 1/2015, 2015, ref 28), this means that they need a specific regulation and need to be approved following the procedure explained by another law (Real Decreto 1345/2007, 2007, ref 29), which means that each batch needs to be analysed by the Spanish Agency of Medicines, an Agency from another member state of the European Union or the European Medicine Agency before being approved for usage.

This approval process has also changed during years, until the mid-20th century there was no legislation regulating this aspect and the only available methods to study the quality of the vaccine was to test its potency and ensure that there was no contamination from any other pathogen, the only thing that was legislated were the sanitary conditions of the factories. With the birth of the World Health Organization there was an effort to create an international standard and in 1958 the first smallpox vaccine standards were established, stating the need to endure a certain amount of time at 37°C, bacterial counts, how to perform the potency assay and heat stability parameters (Fenner et al., 1988).

From there onwards these regulations were applied to every other vaccine and many countries approved laws to enforce the resolution. In the European Union the European Medicines Agency (EMA) was created and it developed and evaluation and approval process that every drug, not only vaccines, was to follow if the producers wanted to commercialize it (European Medicines Agency, s.f.). This process has been very mediatic during the COVID-19 pandemic because it has been shortened to achieve a faster vaccine approval and the public opinion doubted if it would be as effective checking the vaccine's safety, but it is important to remember that every decision that the EMA makes it is also checked and ratified by the agencies of each country, thus ensuring that the right decision is taken.

2. OBJECTIVES

The vaccine production and manufacturing process is a key part in the whole public health concept humanity has developed over the decades and centuries. It plays an important role on improving life standards and it has helped eradicate one of the deadliest diseases ever known by humanity.

Thus, an in-depth look on how the whole process takes place and which are its characteristics, paying special attention to the many differences that come with the fact that there are many types of vaccine that do not resemble each other, is the main goal of this work.

The understanding of how different technological advances from different branches of science, from biology to chemistry, have helped to improve the way all the pieces from the process assemble with each other is also an object of study in this work.

While keeping in mind and explaining how the differences within each vaccine type affect the overall process, this works aims at explaining with a global vision all the parts and processes involved during vaccine production, taking into consideration how each of them contribute in a way to, in the end, offer to the main public a product able to solve many problems that come from infection-caused diseases.

3. TYPES OF VACCINES

Once general background about how vaccine development and the legal aspects regarding vaccines have changed throughout the years and the impact that such changes have made on the way we as a society see immunization and public health has been presented more in-depth information regarding vaccines and their typology can be explained to understand how affects its production process.

Vaccines can be classified in many different ways, either based on their administration technique (oral, intradermal, intramuscular, nasal, etc.), or the pathogen it helps prevent (bacterial or viral). However, the most useful ways to classify vaccines in order to differentiate their production process is by studying the particle used by the vaccine to teach the human body how to fight it and the technology used in their production.

3.1. **WHOLE PATHOGEN VACCINES**

These are the oldest types of vaccines and the most commonly used nowadays because the mechanism used to generate immunity in the patient has been studied in depth during the past two centuries. The procedure is simple, the whole pathogen that causes the disease that is desired to be prevented is inoculated in the patient and when the immune system recognises the threat that this pathogen represents to the body it mass produces antibodies to fight it off. However, if the pathogen was to be inoculated in a healthy subject without being altered in some way the vaccine would not work, it would just infect the patient without offering any boost to its defences, this is why before producing the serum for the vaccine the virus is rather inactivated or attenuated.

3.1.1. Live, attenuated vaccines

This is the group of vaccines that includes the first vaccine ever developed, the one made by Edward Jenner to prevent smallpox. They produce immunity in the host without harming or representing any health risk because the pathogen used has been attenuated, this means that the pathogen is no longer able to develop inside the host, so it triggers an immune response that will produce antibodies for when a real infection happens, in fact, it is such a close experience to a real infection that they generate a really strong response, so in some cases just one dose confers lifelong immunity and the patient may need only one boost dose after many years.

This strong response also has a downside, since such an immunological threat, although it is not at full strength, requires a healthy immune system, this means that they are not meant for everyone, as it is not safe for people with weakened or damaged immune systems (people with HIV infection or receiving chemotherapy) to receive a live attenuated vaccine because their bodies might not be able to generate antibodies fast enough (University of Oxford, s.f.).

The way these vaccines get attenuated is by passing the pathogen many times through a host that would not naturally get infected, for example embryonated eggs. After many generations being in this different host the pathogen would start to mutate to adapt to these new conditions, losing gradually its original virulence towards humans and eventually not being able to induce a disease, but as it remains the same pathogen the immune system would still recognise it as a threat and would produce antibodies. This procedure shows that these vaccines rely on the natural ability to mutate that the pathogen has, however, as mutation cannot be fully controlled there is a real possibility that a second mutation could happen and get virulent again.

Another consequence on the fact that these vaccines rely on the mutation aspect of the pathogen is that although it represents an easy way to produce viral vaccines it is a lot harder to get the same procedure work with bacteria because they are much more complex when talking about genes.

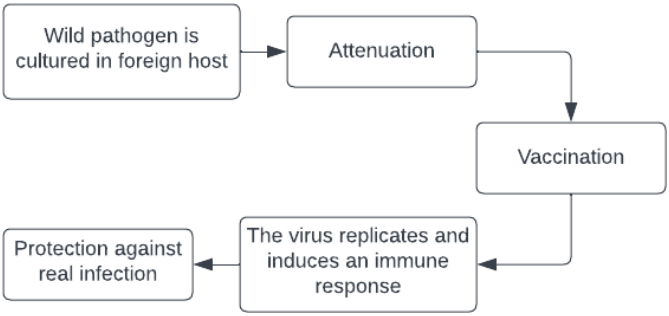


Illustration 1. Steps of a live-attenuated vaccine

3.1.2. Inactivated vaccines

Inactivated vaccines use a killed version of the pathogen instead of an attenuated one to induce an immune response and provide immunity. As the process to obtain the vaccine does not rely on random mutations that may take some time to occur it is a faster way to create a vaccine and in a much simpler way, this is why the flu vaccine developed every year is produced following this method. However, as the pathogen is totally inactivated the vaccine does not trigger such a strong response by the immune system and therefore the immunity does not last as long as the one produced by the live attenuated vaccines, this means that the patient will need many boost doses in order to not lose that protection (Sanders et al., 2014).

Although it may seem that the disadvantages that come from dealing with a killed pathogen outnumber the advantages the truth is that this aspect of the inactivated vaccines is one of their main strengths because if the virus or bacteria is dead it cannot mutate again into a more virulent variant, thus being a much more stable and safer vaccine. Moreover, as the immune response triggered by the vaccine does not have to be as strong as the one that comes after a live attenuated vaccine it makes the inactivated vaccines a more suitable option for those people with their immune system compromised.

They are also easier to transport than the attenuated ones because they not require to be kept cool as the pathogen is completely killed, in the attenuated one there is a range of

temperature that has to be maintained or the vaccine virus/bacteria could start losing efficiency. Bacterial inactivated vaccines are also easier to produce than the attenuated ones as they do not get affected by the mutagenic problematic.

The development and production processes are simple, a wild virus or bacterial strain is cultivated in cells or even in livestock and after being collected the pathogen is killed by chemical (using formaldehyde, β-Propiolactone etc.) or physical (applying heat or radiation) methods and then the vaccine is purified and treated to get rid of the impurities.

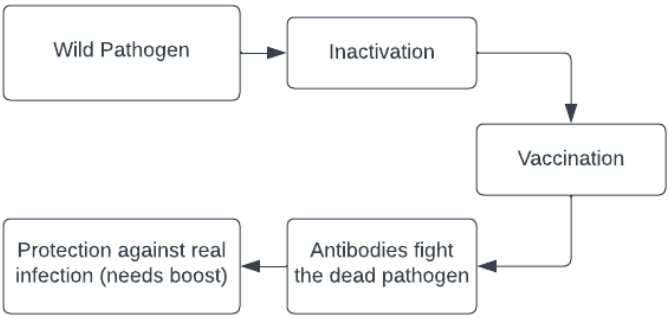


Illustration 2. Steps of an inactivated vaccine

3.2. **SUBUNIT VACCINES**

As science has advanced, so has our understanding of the microorganisms responsible for causing disease and nowadays the main investigation trends in vaccine development are more focused on identifying the specific part of the pathogen that is responsible for the immune response and finding ways to enhance it, this is why newer technologies try to create vaccines that just consist of smaller parts of the virus.

There are many different types of vaccines using this technology because there are many different antigens that can be used to generate such an immune response, this is why although there will be a classification explaining some of them at the end of this section in this document only general characteristics will be talked in detail.

One of the main aspects regarding subunit vaccines is that the complete understanding of the structure of the pathogen is something that needs to be studied in depth, as it is key to identify which parts of it are responsible for triggering antibody production.

If these parts are correctly selected, when the patient receives a shot of the vaccine the immune system will recognise the subunit as the whole virus and start building up defences, thus when the real infection happens it will be protected. However, immunity granted by these vaccines is not as long as the one produced by live attenuated vaccines, this is why substances named adjuvants (they help lengthen the protection) are added (HHS.gov, 2021).

The main consequence of the specific approach that these vaccines have is that they are easier to synthesize and manipulate, making them good candidates to be polyvalent and offer with one shot protection for different diseases and reducing the possible side effects.

The procedure to manufacture these vaccines consists in inserting the genetic code of the specific antigen previously identified as the one responsible for triggering the immune response into yeast cells, these cells are selected because they are easy to grow, then when the antigen has been produced the yeast cell is broken and the antigen purified.

Subunit vaccines can be classified by which antigen is the one that produces the immunity after being inoculated (HHS.gov, 2021):

- Recombinant protein and polysaccharide vaccines: In these vaccines the antigens are the proteins or sugars from the pathogen's surface, the body will recognise them as foreign and produce antibodies. In some cases, it is a better option to combine both proteins and polysaccharides in what is called a conjugated vaccine because the immune reaction towards the sugar is bigger when it is attached to the protein.
- Toxoid vaccines: The antigen in these vaccines is a protein released by the pathogen when it infects the host, the vaccine consists in a toxoid inactivated in a way that is no longer poisonous.
- Virus like particles: In these vaccines a molecule that is really similar to the original virus is inoculated, but it is unable to infect because it does not have any viral genetic information, but as it resembles the virus the antibodies that the body will generate will be useful when natural infection occurs.

- Outer Membrane Vesicles: These vaccines contain as antigen the equivalent of viral polysaccharides for bacteria, they work by stimulating a response by just inoculating the non-infectious parts.

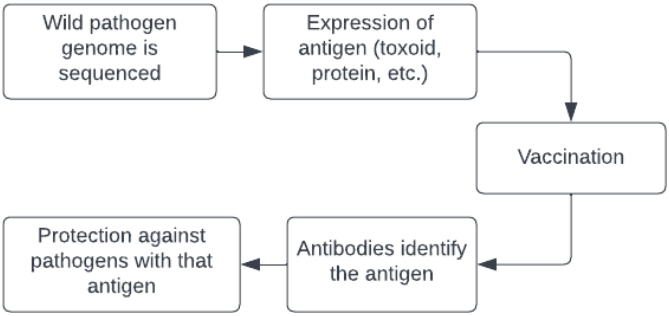


Illustration 3. Steps of a subunit vaccine

3.3. **NUCLEIC ACID VACCINES**

This is a much newer technology in comparison with the previous ones mentioned, in fact, in the United Kingdom there are only authorized nucleic acid vaccines and both of them are developed to protect from COVID-19 infection. They work completely differently from the whole pathogen and subunit vaccines as they do not use any part of the infectious agent to trigger any immune response in the patient, they work by inoculating the instructions on how to produce the antigen itself into the patient's cells and, after some time, these naturally generated antigens will trigger an immune response and then grant protection against infection.

There are two main types of nucleic acid vaccines, the ones that use messenger RNA as the one carrying the antigen genetic instructions and the ones that use DNA, although as of 2022 there is not licensed any DNA vaccine. The first ones are introduced in the body within a lipid membrane that grants the messenger RNA with some protection as it is easy to get damaged and also it helps it go through the cell membrane, meanwhile DNA vaccines are supposed to be able to work properly without this protection, however as it does not need this

lipid membrane it is tougher for the DNA to enter the cell, this is why these vaccines are still being investigated.

One of the main advantages of these vaccines is that as there are no live components within the vaccine there is no risk of real infection, it does work in this aspect in a similar way as the whole antigen inactivated vaccines, and therefore they share a common downside, they both need booster doses as the immune response is not as strong as the one induced by real infection.

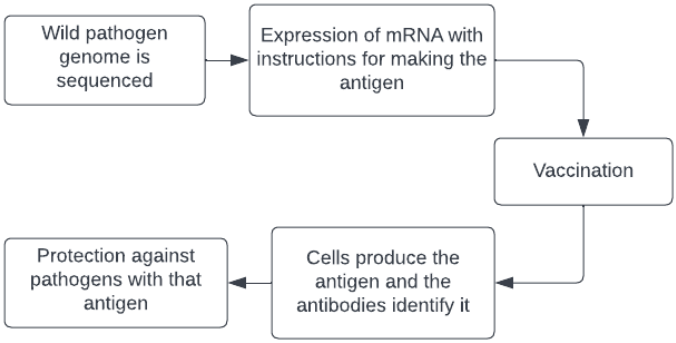


Illustration 4. Steps of a nucleic acid vaccine

Table 1. Vaccine type examples (HHS.gov, 2021)

Type of vaccine		Live attenuated	Inactivated
Viral	Whole antigen	- Smallpox - Yellow Fever	Flu Rabies
	Subunit	-	- Human Papillomavirus (Recombinant protein) - Hepatitis B (Recombinant protein)
	Nucleic acid vaccine	-	Covid-19
Bacterial	Whole antigen	Typhoid fever (oral vaccine)	Cholera
	Subunit	-	- Tetanus (Toxoid) - Meningococcal disease (Conjugated)

4. VACCINE INDUSTRIAL PRODUCTION PROCESS

The industrial production of vaccines is a key part of the global process that goes from detecting a potentially dangerous pathogen and providing general population with immunity. It comes right after deciding which type of vaccine would be more useful depending on the structure of the pathogen as it has been explained before, for example, if the pathogen were a virus, then a real viable option would be developing a whole pathogen live attenuated vaccine as they grant long term immunity to the patient, but if it were a bacteria then it would be more logical to develop a subunit vaccine that focused on the specific antigens that trigger the immune response as the bacterial genes are far more complex that the ones from a virus.

Therefore, as the vaccines are different it is clear that their production processes will be different, the inoculated ones would require a step of chemical or physical inactivation while live attenuated ones would not. However, it is possible to do a simplification of the global process that will be correct for almost all vaccines, and it consists in dividing the whole production in two steps as it is done in every biochemical process: upstream and downstream.

The upstream part is the one responsible for generating the desired pathogen or antigen that the vaccine will carry, thus common operations within this part are selection of the cell that will be used for culture, characterization of the pathogen genetic line that is considered to be the most suitable for vaccine production and cell growth in a bioreactor.

The downstream part is the one responsible for clarification and purification of the antigen as well as recovery of the pathogen and get rid of all impurities present. This is why cellular disruption, membrane ultrafiltration and centrifugation are considered common operations of downstream processing.

4.1. UPSTREAM PROCESSING FOR VACCINE PRODUCTION

4.1.1. Pathogen and antigen characterization and cell culture selection

The first step to successfully produce a vaccine is to characterize and obtain the specific particle (antigen or pathogen) that the vaccine will contain.

In case of a whole pathogen inactivated vaccine this step is not necessary, as the pathogen will be exactly the same found in the wild, the only condition it needs to fulfil is that it is suitable for mass production, but in all the other vaccines this step is crucial. In the live attenuated one a wild string of the virus will be selected and then successively cultivated in cell cultures until some mutations reducing its virulence show up and then considered suitable for vaccine production.

For subunit vaccines the procedure is similar, a cell strain capable of expressing the protein required to act as the antigen is selected and then the DNA sequence responsible for producing the antigen in the pathogen is added to this cell strain and then analysed to determine if it is within the quality parameters according to regulations.

Once the antigen or pathogen has been correctly selected the next step is to determine which culture is the most suitable for the bulk production and then the vaccine compound is adapted to replicate at an optimum level.

When the products are viruses (the most common pathogen used in whole pathogen vaccines) they require that the cells are actively growing and dividing to sustain their growth and reproduction and, moreover, they require a cell strain that is susceptible to them, this mainly happens in mammalian cell strains with a clear interferon expression deficiency. Interferon is a protein that cells produce when they get infected and it acts as an alarm activating an immune response to fend off the infection, thus an interferon deficiency is considered as a factor that contributes to viral propagation.

There are mainly two cell strains used in viral vaccines, one called Vero that comes from epithelial cells from the kidney of an African green monkey and was the first continuous cell line to be approved by the World Health Organization for vaccine manufacturing and the other one called Madin-Darby Canine Kidney (MDCK).

Vero is the most widespread used strain for almost every viral based vaccine, even the Sinopharm vaccine for COVID-19, and MDCK is mostly used for influenza live attenuated vaccines in competition with the egg-based production (Verma, et al., 2020).

However, the most common procedure that is still used for producing flu vaccines is the egg-based one because it is clearly cheaper, the process is almost the same as a cell-based vaccine one, the virus gets inoculated into a fertilized egg and then, the egg, acting as a bioreactor, is incubated to be harvested one week later. The main disadvantage is that the cells from the egg are avian and not mammalian, thus they are not as good as replicating the virus and the vaccine efficiency is lower.

When the product is an antigen then options multiply as there are many different proteins that can be used as antigens in a vaccine. Depending on the complexity of the protein needed lower eukaryote cells like yeasts, animal cells or even human cells can be used.

Yeasts provide with easy to manage and highly productive cell cultures, but they do have a downside on how they secrete the protein because they are highly glycosylated and this may affect the protein effectiveness, therefore cellular disruption is used as a way to obtain the protein without getting it altered.

There are also many animal cell strains used for protein synthesis such as the Chinese Hamster Ovary (CHO), that is the most common mammalian cell line used for protein production, or the Mouse Myeloma (NSO) cells. They come both from rodents because they have the ability to continuously divide while they are in culture.

Also lately, as the proteins required by the vaccines have become more complex there is growing investigation in how to use human cell strains as HEK293, that comes from cells from the kidney of an aborted embryo of 1973, because these cells are the closest science has to a real alive human person.

There is also an extra action that can be done to optimize later production, because, as in every industrial process the main goal is to produce the maximum amount of product using the smaller number of raw materials (in this case, cells). This is especially crucial in protein production for subunit vaccines because although all the cells are given the DNA sequence to produce the antigen not all of them will integrate it and thus, produce it.

The solution to overcome this issue is to generate a selective pressure by adding to the DNA instructions another gene that will allow only the transfected cells (these are the cells that have integrated the DNA sequence to code the protein) to survive in the growth medium that will be used later in the bioreactor.

4.1.2. Bioreactor selection and bulk production

The fact that protein-producing cells already have been genetically coded to mass produce the antigen needed for the vaccine whereas the virus-producing cells need to be infected while in the reactor already shows up a critical difference that will alter the way both of them are handled. This step that consists on adding virus within the cell culture is called infection phase and it is clearly favoured when the cells are attached to a surface rather than when they are in suspension. As protein-producing cells do not need this infection phase they can be managed in suspension.

The difference between suspension cultures or adherent cultures lays in what is the parameter used for scalability, while in suspension cultures growth is limited by the amount or concentration of cells in the medium, that can be grown by simply making the vessel bigger, in adherent cultures growth is limited by the surface available for cells to grow in, this has led to the development of porous particles called microcarriers that are suspended in the medium and thus enlarge the amount of available surface.

Another consequence of these difference between suspension and adherent cultures is the way the medium is kept homogeneous during production, because as in every biochemical process it is really important to maintain a good level of nutrients and oxygen to keep cell density as high as possible to maximize production. For suspension cultures it is easy to say that a stirred reactor will work adequately, as it keeps moving the growth medium and does not allow the formation of dead zones within the reactor. This is why for suspension cultures the most common operation mode is batch harvesting in stirred tanks.

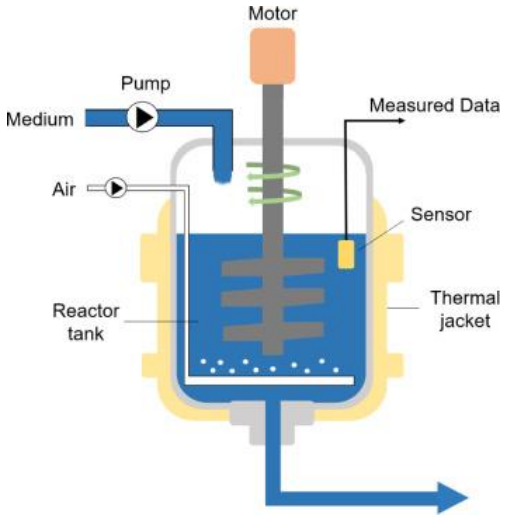


Illustration 5. Stirred-tank batch reactor (extracted from Kim et al., ref 30)

However, for adherent cultures it is not that easy to choose the correct option, moreover, it is important to say that every different virus (the main product obtained by adherent cultures in vaccine production) has different optimal conditions regarding temperature, level of nutrients and virus-cell surface area. So, although it is important to keep a good level of mixing and homogeneity, if agitation is too high this may lead to too strong shear forces that would detach cells from microcarriers, thus reducing production.

The solution to overcome this issue is to work in continuous, then the agitation needed to maintain a high concentration of nutrients is substantially lower, but this comes with another problem, working in continuous would mean that to maintain production there must be a constant inflow of cells and virus, and the way to avoid this is by using perfusion reactors. This perfusion reactors are constantly fed with fresh growth medium while part of the old medium gets removed, and to keep the cells inside the reactor by adding filters that separate the microcarriers that have the cells attached to them from the culture liquid while letting the virus be harvested continuously.

More recently an alternative to perfusion reactors and batch-reactors has been presented, the use of packed-bed reactors that maximize the surface available for cell growth while eliminating the need for agitation. This concept work on the premise that by constantly receiving

fresh growth medium higher cell densities can be achieved as the packed bed used do offer high porosity.

The main disadvantage that comes with these reactors is the fact that oxygen distribution is less optimal than the one obtained by sparging in the stirred tanks.

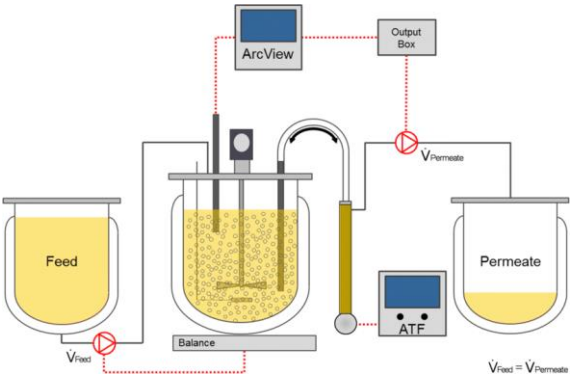


Illustration 6. Perfusion reactor (extracted from Nikolay et al., 2018)

Once each process has been assigned their most adequate bioreactor the procedure followed to bulk-produce the desired product can be explained.

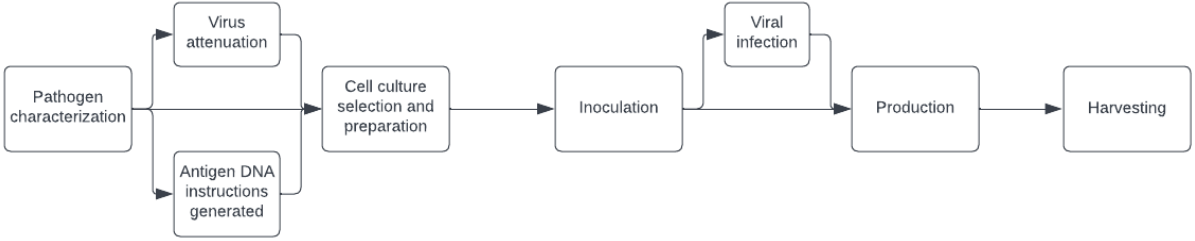
First of all comes the inoculation or inoculum, it consists in generating the maximum number of cells possible in the bioreactor. In order to do this the cells chosen to be used are prepared in smaller vessels and successively passaged to scale them up until they are ready to be added to the reactor. In case of suspended cells this passaging consists in replenishing those nutrients that have been consumed to a point that favours cell growth and reproduction but does not allow the production of the desired protein and in case of adherent cells apart from adding new nutrients the cells have to be detached from the surface and then centrifuged in order to get rid of impurities to be finally attached to the microcarriers in case that a perfusion reactor is going to be used.

The growth medium may vary depending on which cell line is being used or which protein or pathogen is going to be produced, but there are some common parameters that have to be critically controlled in order to ensure a correct operation. These key parameters that have to be within a target range are pH, temperature, dissolved oxygen, glucose level and osmolality.

This medium must have as nutrients a constant flow of a carbohydrate energy source (glucose), a nitrogen source (amino acids) and lipids (fatty acids), in addition to these three key components it is also important to consider the presence of salts and if needed cholesterol.

After the cells have been inoculated and they have reached the optimum concentration production can begin, in case of the subunit vaccines the conditions are adapted to trigger those genes that have been added to the cells in order to start producing the desired proteins, and in whole pathogen vaccines comes the infection of the culture. In order to infect the culture, the conditions can be changed to favour cell-virus interactions like lowering the pH, reducing agitation to reduce shear forces or change temperature or even add to the nutrient flow some specific proteins that maximize virus production.

Illustration 7. Block Diagram of the upstream process for vaccine production



4.2. **DOWNSTREAM FOR VACCINE PRODUCTION**

4.2.1. Cell disruption

Although it may seem obvious, the first thing needed to purify and perfect a product is the product itself, and in some bioprocesses the product is not ready to treat it just after getting it out of the reactor. In fact, when working with some subunit vaccines, some of the cell cultures used for expressing the proteins, sugars (polysaccharides) or toxins needed to develop the subunit are not able to expel the product and just keep it inside.

This is why just after finishing the batch production (the one mainly used for subunit vaccines) a cell lysis step is required to break up the cells and release the product into the medium. There are many different types of cell disruption, from chemical methods using enzymes or osmotic shock to physical methods that involve heat, agitation or sonication (Yadav and Kale, 2020).

The decision on which method is the most appropriate will change depending on the resistance of the cell, for mammalian cell cultures this resistance is lower than the one that present yeast cultures. However, the most common method is using a homogenizer in presence of a low concentration of detergent, because, as it is a mechanical method there is no risk of altering the intracellular components and thus, destroying the product.

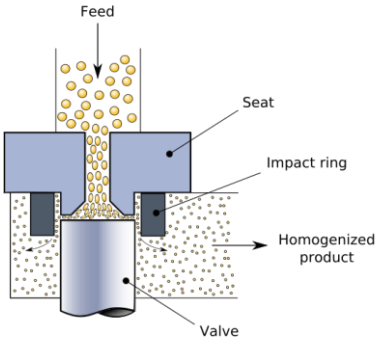


Illustration 8. High Pressure Homogenizer

(Extracted 13/06/22 via Wikipedia Commons, Creative Commons Attribution)

It works simply, it pushes the flow at a high pressure that can reach hundreds or thousands of bars towards a discharge valve, and when the cells within the flow reach that zone, they explode as a result of the drop of pressure. This operation, although it is really necessary also frees many other components into the liquid such as cell debris, other undesired proteins or nucleic acids, this is why after the cell disruption comes a pretreatment and a clarification stage.

4.2.2. Pretreatment

After the intracellular antigens have been harvested from their cells it is reasonable to assume that almost all vaccine production processes are really similar at this point with the desired product, rather a virus, a bacteria or any antigen, suspended in the growth medium with lots of impurities and other compounds around them.

As technology has developed, more intensive and efficient bioreactors have been developed, and with these advances manufacturers have been able to increase the cell density on their processes, thus creating a bottleneck on clarification and purification stages of the downstream process.

For example, a widely used clarification operation that will later be explained more in detail is filtration, and it is greatly compromised when some of these mechanisms occur (Nikolay et, al., 2020):

- Pore blocking: Agglomerates or big particles clog the pores and do not allow the flow to go through the filter.
- Cake layer formation: A layer made by solute particles starts to grow over the filter and ends up blocking the whole flow.

These events tend to happen more easily in high cell density processes as there are more debris capable of generating such blockings. This is why prior to any further clarification a first pretreatment is undergone to diminish the concentration of such undesirable particles.

Flocculation appears as a good solution to such problems because if flocculating agents like polymers are added then many of the cell debris or other impurities can precipitate before going to a filtration stage (Wolf and Reich, 2011). It is also useful in helping to get rid of smaller impurities that could resist a clarification based on centrifugation, as many little particles would not sediment and with flocculation they would.

4.2.3. Clarification

As it has been explained earlier in this paper, vaccines are strongly regulated medicines with defined parameters regarding the levels of contamination and impurities considered acceptable for their approbation. Since the first guidelines were approved for the production of the polio vaccines by the World Health Organization in the 1950s many other ones have followed, thus creating challenges in the development of the purification and clarification stages of the production process, as an example the following table shows the requirements stated by the World Health Organization as the maximum allowed in viral vaccines.

Requirements	Specification
Residual DNA	Less than 10 ng/dose for parenteral inoculation and less than 100 ng/dose for oral vaccine
Reduction of DNA strand size	Less than 200 base pairs
Reduction of host cell protein	Less than 100 ppm or below detectable levels
Purity	More than 95%
Animal serum content	Less than 50 ng/dose

Table 2. Requirements by the World Health Organization in concentration of residues

(Cherradi, 2018)

Such specifications cannot be achieved in a single step, this is why after getting the product harvested and pretreated comes a clarification stage aiming at getting rid of those impurities that lay within the fluid. The parameter used to decide if clarification has been successful is turbidity and it is measured in Nephelometric Turbidity Units (NTU).

Even though at this point of the production process all the vaccines share some similarities like the fact that the wanted particle is already produced and only needs to be purified the impurities are substantially different, thus affecting turbidity and the design of the clarification stage.

For example, in subunit vaccines produced as intracellular product the cell lysis stage has added lots of impurities in the form of cell debris, residual DNA or other proteins that also were

inside the cell, this means that they have a heterogeneous contamination with different sizes and solubility, this means higher turbidity, usually around 400 NTU (Besnard et al., 2016).

Meanwhile, in viral vaccines produced in perfusion reactors there was no need of a cell lysis stage and there was a filter implemented on the out-flow part of the reactor to avoid the loss of cells during continuous operation, this means that the contamination is more homogeneous and the turbidity lower, even under 100 NTU.

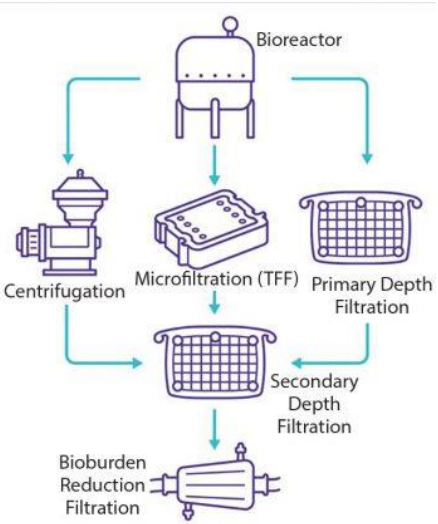


Illustration 9. Clarification process (extracted from Cherradi, 2018)

To simplify this stage, it is usually divided in primary clarification and secondary clarification. The first one aims to remove larger particles while the second one is more focused on removing the smaller ones and the ones that resemble the product itself, thus requiring a more specific separation.

Primary clarification is also influenced by the characteristics and singularities of the contamination getting out of the reactor. As it has been said in harvests needing cell lysis this contamination is rather heterogeneous, with up to 40% solids in the feed in yeasts cultures in comparison with 6-8% in mammalian cells cultures, so it is common to find centrifugation as the first operation.

This centrifugation is usually done in gradient zonal centrifuges that separate the compounds of the flow by their densities because using disk-stack centrifuges poses the risk of

breaking apart the product if it is susceptible to shear forces (Wolf and Reich, 2011). As it has been said before, the addition of polymers to start flocculation can help to improve centrifugation by even enlarging density gradients.

For harvests that show lower turbidity it is more common to find filtration as the first clarification stage because it is easier to scale-up, and many different types can be used, from tangential flow filtration (TFF) to normal-flow filtration (NFF). Both methods are equally useful, but for those particles that may suffer from shear forces TFF poses a bigger risk so NFF using depth filters is a more suitable option (Cherradi, 2018).

Filtration using depth filters works based on two mechanisms, size exclusion and adsorption, this means that bigger particles that pore size do not go through the filter and thus are eliminated, and for adsorption this means that some of the impurities get stuck in the filter although they may be smaller than the pore size, this can be enhanced by adding to the filter positively charged material that helps retain negatively charged contaminants (Nikolay et al., 2020).

Once primary clarification has finished the only impurities that remain within the liquid are those that clearly resemble the product in size, form or behaviour, this is why they can only be properly separated after getting rid of those more different first, because they need more specific equipment. The operations that can be used as seconds clarifiers are the same as the ones from the primary stage, this is why usually they are combined to reach the desired level of impurity reduction. Some examples of second clarifiers are ultracentrifugation, depth filters used in NFF or even TFF using smaller pore sizes. (Besnard et al., 2016)

Apart from combining different operations there is also another option, specifically introduce a compound to the flow that alters the composition of the contaminant and helps its differentiation. This is the case for DNA contamination, its size is quite similar to the one of the viruses produced and if the procedure needed to clarify the product was too strong it may be harmful for the virus, thus resulting in a loss of production. To avoid this an enzyme called Benzonase that digests nucleic acids is added, and after some time a filtration takes place to get rid of the enzyme and the reduced DNA (Gousseinov, 2014).

	Type of vaccine	Cell culture	Product location	Cell lysis	Primary clarification	Secondary clarification	References
Meningococcal vaccine	Conjugated polysaccharide	Bacteria	Extracellular	No	0.2 μm hollow fiber cartridges (TFF)	300 kDa cassettes (TFF)	Robinson et al., 2011
Rotavirus vaccine	Live attenuated virus	Vero	Extracellular	No	Centrifugation at 2831 ×g for 30 min and 4424 ×g for 10 min at 4 °C	0.45 μm hollow fiber (TFF)	Zhang et al., 2011
Influenza vaccine	Inactivated virus	MDCK	Extracellular	No	0.65 μm polypropylene depth filter (NFF)	NA	Kalbfuss et al., 2007
pDNA vaccine	Plasmid DNA	Bacteria	Intracellular	Yes	Flocculation	Depth filtration (NFF)	Palmieri et al., 2010
Rotavirus vaccine	Virus like particle	Insect cell	Intracellular	Yes	Centrifugation at 1000 ×g for 10 min at 4 °C	Ultracentrifugation of the supernatant at 100,000 ×g for 1 h at 4 °C	Peixoto et al., 2007

Table 3. Pretreatment and clarification stages for some vaccines

4.2.4. Inactivation

After eliminating those impurities that represent a problem in dealing with the product comes a step that not every vaccine has to get done. This inactivation stage only takes place for those vaccines whose components are a threat to the immune system as they have not been attenuated before, such as the toxins that are used for toxoid vaccines or the wild viruses used for inactivated vaccines.

It is a critical part of the process and if it is not taken seriously, it can constitute a public health problem once distributed because when people get inoculated with non-inactivated vaccines they are essentially being infected with the real disease or with the toxins of the pathogens that also represent a health issue, this being the case of the Cutter incident of 1955, when a batch of poliomyelitis vaccines was not correctly inactivated and 40.000 children contracted the disease and five of them ended up dying.

Inactivation can be achieved by mechanical or chemical methods, but in reality, only two chemical compounds are currently widely used for this purpose in human vaccines, these being formaldehyde and β-Propiolactone (BPL) (Sanders et al., 2014).

For formaldehyde-inactivated vaccines there are some parameters that may vary depending on the pathogen, the first of them is concentration of inactivating agent (from 0,08 to 0,009% w/v), but there is also time of inactivation (some of them may take many weeks) and temperature (from 4 to 37 °C). Too high concentration and temperature may lead to thermal degradation and destruction of epitopes, and too much time would also destroy the pathogen. This is way is key to understand the kinetics of inactivation for each pathogen in order to ensure complete inactivation without losing vaccine efficiency.

For BPL-inactivated vaccines it is also key to fully understand the kinetic of inactivation before further developing the inactivation stage. In comparison with formaldehyde, it takes less time to inactivate any pathogen and the temperatures used during the process can be also lower, this means that thermal degradation is less of a threat. However, formaldehyde is still the most chosen procedure because after many years being used as an inactivation agent it has been widely regulated, thus making developing the inactivation stage easier for manufacturers as they only have to follow those regulations.

4.2.5. Purification

Once most impurities have been eliminated after going through a clarification stage the next step towards final delivery of the product is to purify it and this means enhancing its concentration but also controlling and reducing any other residual impurities that may still be in the fluid.

As the contaminants that are still within the fluid are really difficult to get rid of, the operations mainly used for purification are also much more specific at aiming at them, this is why chromatography is one of the most used purification operations, as it provides with high separation factors while keeping at minimum any possible stress that may alter or damage the product.

But for chromatography to work concentrations need to be relatively high and volumes need to be substantially lowered from those that are handled while using the centrifuges or the depth filters in clarification, this is why prior to any chromatography or some other purification operation a concentration stage is done. The most widely used mechanism to ensure these higher concentrations is ultrafiltration, as by doing it not only impurities can be extracted, but also salts and the solvent used until that moment of the production process (Morenweiser, 2015).

Ultrafiltration is chosen because its membranes reject the product, in this case an antigen or the pathogen, while letting the impurities cross, this means that the part kept after filtrating is not the permeate one but the retentate one.

It also offers, as it has been stated before, the possibility to change the medium or solvent, this is called diafiltration and it is an operation that takes places in most of the virus production found in the literature.

Some aspects to take into consideration while managing ultrafiltration is, again, the presence of shear forces that may tear apart those particles without a strong resistance to it. To avoid this lower work pressure can be selected or filter materials that provide shorter processing times can be installed.

Finally, after being concentrated proper purification can take place in the form of chromatography or even adapted gradient centrifugation, though it is difficult to scale up and really costly.

For the design of chromatography and the whole purification stage the goal is really different to the one in clarification, in the latter the process is designed to get bigger or similar sized impurities out of the flow, while in the former the objective is to get rid of those impurities so small that are impossible to eliminate using those methods.

This is one of the most chromatography used is Size Exclusion Chromatography (SEC) (Morenweiser, 2015) by gel-permeation, it takes place in packed bed columns and achieves its high separation rates by eluting fast the pathogen or antigens that flow through the interparticle volume because they are bigger that the pore size while the impurities get stuck inside the pores and take more time to elute.

The other chromatography widely used is the ion exchange one, mostly working in positive mode, in this case the particle is retained in the resin or the packed bed and the impurities flow through substantially faster, thus purifying and concentrating the product.

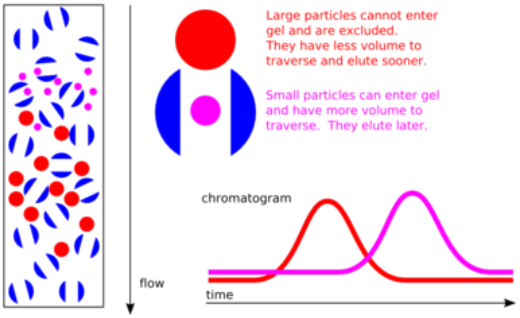


Illustration 10. Size Exclusion Chromatography

(Extracted 13/06/22 via Wikipedia Commons, Creative Commons Attribution)

Both chromatographies can be combined to achieve higher yields and thus ensuring a better product quality.

But this are not the only type of chromatography or the only equipment configuration that could be implemented to ensure a good purified outflow.

Apart from packed bed columns, some membranes and monoliths are also usable as convective chromatography media, the only problem is that the main used method, SEC, is still

limited to the usage of packed bed columns and they still have some issues that need to be overcomed such as clogging in monoliths and poor resolution in membranes (Wolf and Reich, 2011).

And for the other chromatography types, such as affinity, mixed-mode or hydrophobic interaction, there is still much investigation to do (Coskun, 2016).

For affinity chromatography the main problem comes from the fact that the development, purification and immobilization of the ligands used to retain the impurities or the product is really expensive and not available to scaling it up.

Hydrophobic interaction is also not usable because of the high salt concentrations needed, this can clearly can affect the virus viability, and it would not make sense to add salts after getting rid of them in the concentration stage.

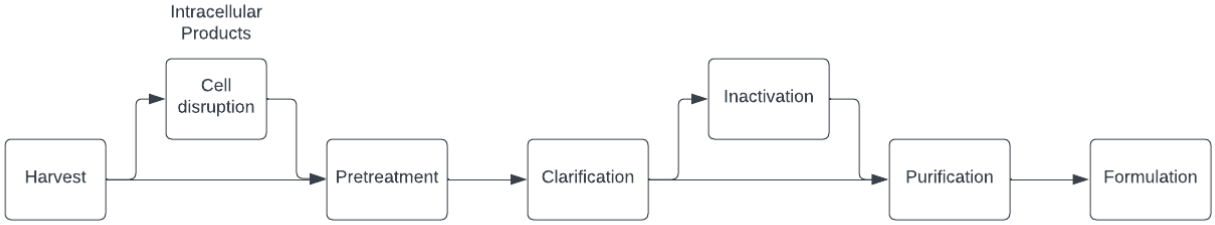
Finally, for mixed-mode chromatography the main problem is the lack of information available right now about the viability of the operation, some studies are starting to show up some results but it is not enough.

So, once chromatography and purification are finally done, it can be considered that the production part of the vaccine has been successfully finished because now the remaining components of the mixture coming out of the process has been carefully designed to fulfil those parameters stablished by the World Health Organization that have been presented earlier.

Vaccine Name	Manufacturer	Virus strain	Cell substrate (all of them use human cell culture)	Concentration	Purification	Inactivation parameters	References
Havrix	GSK	HM175	MRC5	Sterile filtration and ultrafiltration	Gel permeation chromatography	250 µg/ml formaldehyde 15 days at 37 °C	Andre, 1995
Vaqta	Merck	CR326	MRC5	Precipitation in polyethylene glycol	Chromatography	100 µg/ml formalin 20 days at 37 °C	Armstrong et al., 1993
Avaxim	Sanofi Pasteur	GBM	MRC5	Sterile filtration	Chromatography, ultrafiltration and diafiltration	Formaldehyde concentration not specified 14 days at 37 °C	Vidor et al., 1996
Epaxal	Crucell Switzerland	RG-SB	MRC5	Ultrafiltration	Ultracentrifugation	0.25 % formalin (w/v) 10 days at 37 °C	Gluck et al., 1992

Table 4. Purification and inactivation stages involved in some Hepatitis A vaccines

Illustration 11. Block diagram of the downstream process for vaccine production



4.3. **FORMULATION**

As the product has been produced and successfully purified the only step remaining before its commercialization is the formulation of the vaccine itself. This means that some components will be added to the vaccine in order to enhance its properties or to maintain its quality.

When thinking of vaccines as a product is undoubtable that the active component is the antigen or pathogen used to generate an immune response in the patient, but as it has been stated when presenting the different types of vaccines there are some of them whose immune response is not strong enough, thus a special compound called adjuvant is added to improve this situation.

Vaccines more susceptible to need adjuvants are the subunit ones, and the function of those adjuvants is to trigger the formation of those specific immune cells that are responsible of recognising the threat and then generate immunity (CDC, s.f.).

There are two main adjuvants used for commercial human vaccines (De Gregorio et al., 2013), the first ones are aluminium salts or alum and they have been used since a century ago and nowadays is used in almost all protein-based and inactivated vaccines such as the vaccines for rabies, diphtheria and anthrax.

Alum structure adsorb the vaccine antigen and when it is injected it acts as a delivery system, carrying the antigen to the most appropriate place to trigger the immune response because alum presence in the body can induce local inflammation. This means that it attracts and stimulates the creation of antibodies that try to fight the foreign particle and when they reach the aluminium salt, they also find the antigen so the immune reaction is greatly enhanced, thus producing longer immunity.

The other main used adjuvants are the oil-in-water emulsion adjuvants, mainly MF59, a compound that does also work as a delivery system for the antigen traveling with it through the body, then the main difference between alum and oil-in-water emulsion adjuvants is how they help improve the immune response.

Instead of attracting antibodies to attack it induces the creation of the precursors of the antibody cells responsible of defending the body from the infection.

But vaccines do not only have adjuvants, they also need some excipients, these are the substances added to the final product responsible for maintaining its quality (Center for Biologics Evaluation and Research, 2019):

- Buffers: Its goal is to retain the pH and resist changes that may lead to a loss of efficiency. Example: sodium chloride.
- Preservatives: These compounds are the ones responsible of avoiding unwanted contamination. Example: 2-phenoxyethanol.
- Stabilizers: They keep the vaccine effective after manufacturing it by stopping chemical reactions that may lead to a product degradation. Example: Sugars.
- Surfactants: As emulsifiers they are responsible of keeping the particles suspended within the serum. Example: oleic acid.
- Diluents and solvents: They are the liquids used to dissolve or dilute the substances present in the vaccines. Example: water.
- Residues: Although they may not accomplish any function there are traces of some of the compounds used during manufacturing such as inactivator agents, antibiotics or nutrition medium from the cell cultures. Example: formaldehyde.

Vaccine (Trade name)	Adjuvants	Buffers	Preservatives	Stabilizers	Residues
Anthrax (Biothrax)	Aluminum hydroxide	Sodium chloride	Benzethonium chloride	-	Formaldehyde (inactivator agent)
Cholera (Vaxchora)	-	Sodium chloride, sodium bicarbonate and sodium carbonate	-	Sucrose, dried lactose	Ascorbic acid (inactivator agent), hydrolysed casein (after adding lactose)
DTaP (Daptacel)	Aluminum phosphate	-	2-phenoxyethanol	-	Formaldehyde (inactivator agent), glutaraldehyde (inactivator agent)
Hep A (Vaqta)	Amorphous aluminum hydroxyphosphate sulphate	Sodium chlorate, sodium borate	Neomycin	Bovine albumin	Non-viral protein (cell debris), DNA (cell debris), formaldehyde (inactivator agent)
Typhoid (Vivotif Ty21A)	-	-	-	Sucrose, lactose, gelatin	Ascorbic acid (inactivator agent), amino acids (cell debris), magnesium stearate (surfactant)

Table 5. Composition of some U.S. Food and Drug Administration approved vaccines

(Wodi et al., 2021)

5. CONCLUSIONS

In this work the whole vaccine production process has been studied and explained, paying attention to each step needed and implemented during it manufacturing stage. Many conclusions can be obtained after taking into consideration all the information that has been gathered in the process:

- Increasingly restrictive regulations and legislation have put pressure on improving the quality parameters considered acceptable for any vaccine seeking commercialization approval, this has meant that the downstream part of the process requires higher investments to reach those goals in purity and quality.
- As technology has improved and the vaccine market has grown the production output has also grown substantially, this is a challenge that traditional downstream technologies were not capable to deal with and the situation improved with the implementation of pretreatment stages and extra steps to help with this situation.
- As it is a health-related product that is always under the public eye probably the most important part of the whole production process is to ensure that infection or contamination risks are kept at minimum.
- More novel vaccines that are currently in development stages and clinical trial, such as the ones using nucleic acid technologies (like some of the ones approved to protect from COVID-19), will require further improvement on those areas meant to produce them, as they are constantly getting more complex. This may lead to shortage in production if the proper investment is not done soon enough.

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