

Vaccines

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Foreword

By Prof. Peter Piot

Vaccination against infectious diseases has changed the course of human history. Every year, vaccines save the lives of millions of adults and children. They prevent harmful microorganisms from causing disease and permanent injuries. Not only do they prevent childhood diseases such as measles, whooping cough (pertussis), diphtheria (which often caused croup), meningitis, and mumps, they can also save the lives of adults and the elderly by averting tetanus, pneumococcal disease, and flu.

Vaccines can even prevent cancers, such as cervical cancer (caused by the human papillomavirus) and liver cancer (caused by the hepatitis B virus). Thanks to vaccinations, we have eradicated smallpox, and polio will soon go the same way. Even when we travel or vacation, vaccines against yellow fever, typhoid, hepatitis A, tick-borne encephalitis, and other diseases ensure that we get home healthy.

Vaccinations have given much more to society than they have cost. It's not just in terms of improved health either, as they are also an excellent investment in purely economic terms. Thanks to vaccinations, children can continue to go to school, their parents continue to work, and we save on healthcare. The American Centers for Disease Control and Prevention (CDC) have called vaccinations the public health success story of the 20th century.

According to the World Health Organization (WHO), vaccines for more than 25 infectious diseases are widely available worldwide today. Moreover, scientific progress is opening up exciting prospects for new vaccines: Ebola vaccines have recently been approved, a promising malaria vaccine is being tested in Africa, etc. And, hopefully, as you read this, several successful COVID-19 vaccines will be coming onto the market.

We have all followed the developments surrounding the COVID-19 vaccines very closely. The corona pandemic has dramatically increased interest in vaccine development. Understandably so in a world that had to go into lockdown time and time again to control outbreaks of the SARS-CoV-2 virus and prevent our health system from collapsing. COVID-19 vaccines were therefore developed at an unprecedented rate. Yet, despite the numerous successes of vaccinations, despite the extraordinary return on investment, despite the recent scientific and technological tour de force of researchers and physicians, the world's population has great needs that are still not being adequately met. Moreover, vaccine skepticism and even a refusal to be vaccinated is a growing problem.

This leads to large variations in vaccine coverage between countries and regions. Many population groups are deprived of life-saving vaccinations. Even in supposedly rich Western countries, vaccination programs for adults and the elderly are underperforming. Consider, for example, the annual flu vaccination or the pneumococcal vaccination that reach too few people in these vulnerable target groups.

Also, the development path of new vaccines - starting with the research phase and ending with sustainable application in the wider population - is usually long, difficult, and lined with hurdles. Governments and industry often provide insufficient financial resources for development, and philanthropic organizations have to help. People often encounter unforeseen official barriers during approval procedures and the move to large-scale application. Or there are increasing difficulties in finding acceptance with the general public.

That is therefore something we will have to keep working on. Not only by consolidating and even expanding scientific research but also by prioritizing health prevention from a policy perspective and having vaccinations play a key role in this. We need to distribute successful vaccines more fairly around the world. We will have to set up social and target group-oriented vaccination programs that appeal to and motivate people. Only then can we restore public confidence in vaccines. This requires leadership from both health professionals and policymakers.

Vaccination may have been the best investment in health in the past. And it needs to continue being one in the future. This can only be achieved by making vaccinations a permanent priority in research, industry, public health, and society at large. This VIB Facts Series dossier aims to help with this. Not only by highlighting the achievements of vaccinations, but also by clearly showing the challenges that lie ahead.

Wishing you a pleasant reading experience!

Peter Piot

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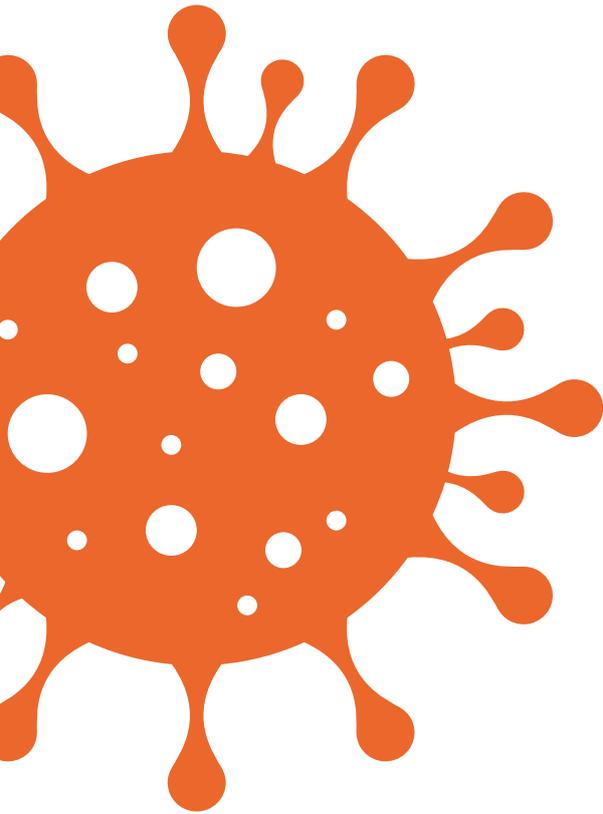
I. A matter of life and death

Infectious diseases

Bacteria and viruses can make us very sick. Just think of childhood diseases such as measles, whooping cough (pertussis), diphtheria (croup), meningitis and mumps, or seasonal flu. Even when we travel, we run the risk of unpleasant infectious diseases. These include yellow fever, typhoid, and hepatitis A. And we recently saw outbreaks of Ebola, Zika fever, and SARS. Today, there is the global COVID-19 pandemic caused by the SARS-CoV-2 virus.

Usually - but certainly not always - young children and elderly people are the most susceptible to infectious diseases. They are most at risk of serious complications or even death. Despite this, even if they are perfectly healthy, people of any age can still be badly affected by an infectious disease.

There are, however, also many harmless viruses and bacteria. These microorganisms don't make us sick. On the contrary, they contribute to our health, are indispensable in agriculture and the food industry, and clean up our waste. For example, our gut is packed with bacteria that help us to digest food. And we use bacteria to make cheese and yogurt, and to purify sewage.



THE DIFFERENCES BETWEEN VIRUSES AND BACTERIA

Viruses - are particles too small to see with a traditional light microscope, which consist of hereditary material (DNA or RNA) packaged in a protein shell. Sometimes that shell is itself surrounded by an envelope of fatty molecules. Viruses cannot reproduce by themselves and do not have their own metabolism, which is why some scientists say that viruses are not actually living things.

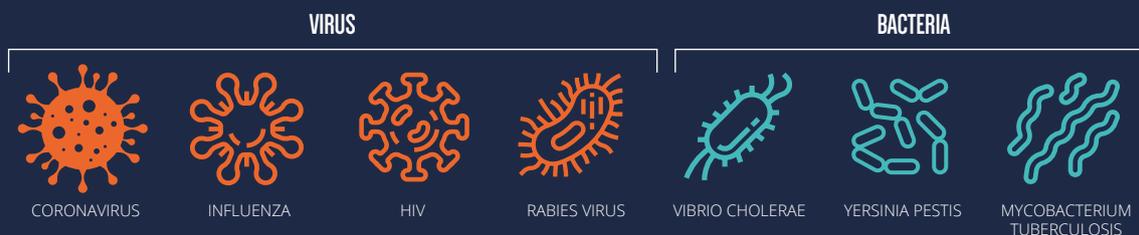
Most viruses are 20 to 300 nanometers across. A nanometer is one-billionth of a meter. They are therefore not visible, even with a light microscope. You need an electron microscope to see them. To multiply, viruses introduce their genetic material into the cells of their host. They then hijack the cell's internal molecular machinery to produce new virus particles (see box 'HIV' in Section 7).

Viral diseases include influenza (the flu), colds, smallpox, polio, hepatitis A and B, rabies, measles, mumps, rabies, chickenpox, AIDS, SARS, Ebola, and COVID-19.

Bacteria - are single-cell microorganisms whose genetic material floats free in the cellular fluid. In other words, they do not have a cell nucleus, unlike, for example, plant, animal, or human cells. The genetic material (DNA) of bacteria consists of a single ring-shaped chromosome. In addition, bacteria usually have even smaller ring-shaped DNA molecules (plasmids) that they exchange with each other. This constantly creates new strains of bacteria. Bacteria multiply by themselves through cell division.

Most bacteria are between 1 and 5 micrometers (one-millionth of a meter) in size. They can be seen with a light microscope. They can have very different shapes: round, comma-shaped, or rod-shaped, and some even look like a corkscrew.

Some bacteria produce harmful substances (toxins) that cause disease symptoms. Examples include cholera, bubonic plague, and tetanus. Some other examples of bacterial diseases are typhoid, pneumococcal disease, tuberculosis, diphtheria (croup), whooping cough (pertussis), Lyme disease, and syphilis.



They're everywhere - Microorganisms such as viruses and bacteria are found all over the world. That's why people can become infected by contaminated food or drink, by breathing in viruses or bacteria that float in the air, or by contact with an infected person.

Resistance and vaccinations

If people become infected by a harmful bacterium or virus, their immune system comes into action. This is a complex system of tissues, cells, and molecules that can render the intruder harmless (see also Section 4).

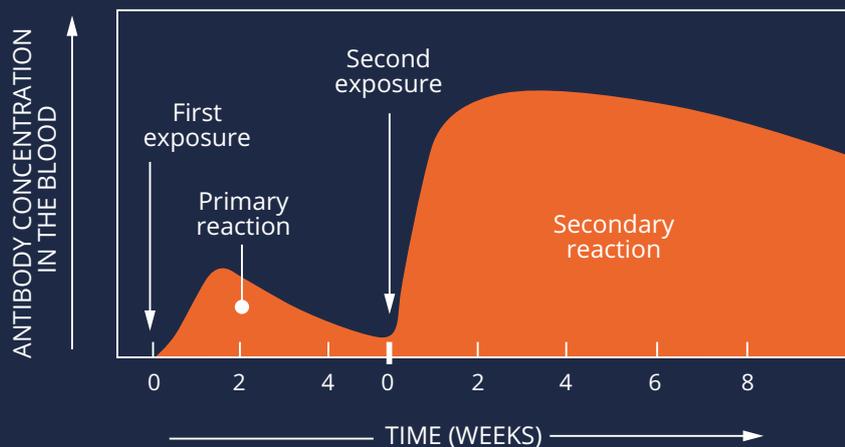
First of all, the immune system makes antibodies against the intruder. It also activates a form of 'defensive memory'.

This memory ensures that a second exposure to the same virus or bacteria will make the immune system immediately sound an alarm and react much more forcefully and effectively to the intruder. We therefore talk about a 'primary' immune response, which takes several days to get going properly, and a 'secondary' immune response that is much more intense (see illustration 'Primary and secondary immune responses').

That's why, for example, children only get measles, mumps, or chickenpox once. As soon as they are exposed to these viruses again, their defenses react immediately. The problem is that they can be quite sick or even develop serious complications during the initial infection.

Vaccines reduce the risk of serious illness and complications, and they make dangerous infectious diseases less common.¹ How do vaccines do that? Vaccines consist of weakened pathogenic viruses or bacteria or small fragments of these. They stimulate the immune system in a similar way to the pathogenic microorganisms themselves but do not make people sick. The body's immune response to the vaccine can cause some discomfort, but this is usually short-lived.

PRIMARY AND SECONDARY IMMUNE RESPONSES



In the primary immune response, the concentration of antibodies peaks after about ten days and then declines. In the secondary immune reaction, we see a very rapid increase in the concentration of antibodies, which is much higher than in the primary response and lasts longer.

The body makes antibodies and activates other parts of the immune system against the components of the vaccine. When people then come into contact with the real viruses or bacteria against which they have been vaccinated, their immune system quickly recognizes these and renders them harmless. As a result, people either no longer get sick or the disease is milder and free of complications. Repeated vaccinations are required for some diseases to build up optimal immunity.

According to the World Health Organization (WHO), vaccines against 26 infectious diseases are available today (see also the box 'Infectious diseases that are prevented (prophylactically) by vaccines' on page 12)². Between 300 and 400 vaccines are under development. More than 200 candidate vaccines are being tested against SARS-CoV-2 alone, the coronavirus that causes COVID-19².

However, the path that a vaccine takes from the laboratory's design stage to use in everyday life is long and complex. Firstly, because some microorganisms make it very difficult for us to develop a suitable vaccine against them, despite all the knowledge we have built up over decades about how the human body, the immune system, and pathogenic viruses and bacteria interact with each other. The search for effective vaccines against, for example, AIDS or malaria has been going on for decades without leading to a widely-used vaccine. We also expect vaccines to be safe as well as effective. We keep putting that safety bar higher. And not without reason, because vaccines have been used that did not meet this requirement well enough. Think, for example, of

the smallpox vaccine we used in the last century that left a scar on the skin and sometimes led to serious complications. A vaccine with that kind of safety profile would not be authorized today (see also the box "From the Ancient Greeks, Chinese and British to Modern Vaccines" on page 8).

The danger of rushing things conflicts with the great pressure placed on researchers and industry to look for vaccines against, for example, COVID-19. Compared to other vaccines, this has been developed very quickly. Nevertheless, we still want the necessary checks to be built in for a high-quality, safe and effective vaccine. And rightly so. That is why VIB has made this Facts Series Dossier available. On the one hand, we want to emphasize that vaccines are among the very best investments in healthcare. They have been in the past and still are. On the other hand, we also explain why vaccines are not put onto the market overnight. Why sound scientific and clinical research should remain the basis of any research program into new vaccines. This scientific basis for a vaccine requires not only knowledge, insight and expertise, but also time. Even though every vaccine researcher knows that society is often impatiently awaiting the future rollout of life-saving vaccines such as those against malaria, Ebola, and, recently, COVID-19.

FROM THE ANCIENT GREEKS, CHINESE AND BRITISH TO MODERN VACCINES^{3 4}

Without any insight into the molecular and cellular basis of the human immune system - or even the existence of viruses and bacteria - the Ancient Athenians were already making reliable observations about infectious diseases and immunity. During the plague epidemic in 430 BC, they found that people who were lucky enough to survive an infection then became immune to it. On a second exposure, the disease was at least much milder and the risk of dying from it seemed to have disappeared. These 'survivors' were therefore used to care for the sick.

The Chinese went a step further in the 15th century. To prevent their children from getting smallpox, they experimented with administering extracts from the dried skin crusts of infected people. The children then usually had a mild and less dangerous form of the disease and were (largely) protected against a subsequent infection. The technique is called 'variolation', after the variola virus that causes the disease.

Variolation was introduced to Europe by British diplomats in the early 18th century. They had learned about the Chinese procedure during their diplomatic missions in the East. Despite this, the Europeans did not rush to use it themselves. A major reason was undoubtedly the fact that about 3% of people who had the procedure died of smallpox (compared to nearly 10 to 30% with an ordinary infection).

However, it had meanwhile got the attention of some English doctors. Some found that people in the countryside often did not respond to variolation at all. Eventually, a causal relationship was established with a milder variant of the human variola virus - the cowpox virus. Many farmers regularly suffered cowpox infections they had picked up while milking cattle, and this gave them significant immunity to the human variant of smallpox.

Doctor Edward Jenner set to work with this idea. He isolated an extract from cowpox sores on the hands of a local milkmaid and used this to infect his gardener's 8-year-old son. Two months later he infected the boy with the human variant of the smallpox virus (see the image opposite). The boy remained healthy. In doing so, Jenner demonstrated that infection with a related pathogen from an animal can cause immunity in humans. This is all the more remarkable if you know that even at that time nobody knew that smallpox was caused by a virus and that people had no idea how human immunity worked.

Jenner called his technique 'vaccination', from the Latin word for cow, vacca. Some vaccines that we still use today, such as those against polio, measles, and rubella (German measles), were also developed when we only had a rudimentary understanding of the mechanisms that lead to immunity.

Unfortunately, not all attempts to create vaccines have been successful. For example, young American children vaccinated in 1967 against RSV disease (respiratory syncytial virus, which can cause serious respiratory infections in young children) became much sicker when infected with the real RS virus. Some even died from it.⁵ Many years later, research revealed that this vaccine activated the wrong parts of the immune system. As a result, the immune response to infection with the real RS virus spiraled out of control. Vaccinated children consequently became much sicker from the infection than unvaccinated ones.⁶

The above story shows that vaccine development is not a matter of free experimentation, but must be strictly controlled and monitored. The 15th-century Chinese variola experiments and Jenner's groundbreaking research would both be impossible today. Strict governmental supervision and internal mechanisms and procedures in research institutions, hospitals, and the pharmaceutical industry no longer allow such reckless experiments in humans. And this is a very good thing, although it does come at a price as it means that developing a vaccine now requires time, extensive expertise, and a lot of money. There will be more about this in Section 6 of this dossier.



The difference between a vaccine and a medicine

A vaccine keeps people from getting sick. People are given a vaccine in advance, when they are still healthy. That is, before they become infected with a disease-causing bacterium or virus. Vaccines 'train' the immune system to prepare it for a possible infection. In other words, vaccines belong to the preventive branch of healthcare. They are just about the only medical intervention recommended for every human on the planet¹. Because vaccines are given to healthy people, we expect them to:

- Meet the strictest safety standards
- Cause minimal side effects, which are also constantly monitored
- Effectively protect against infections
- Be affordable

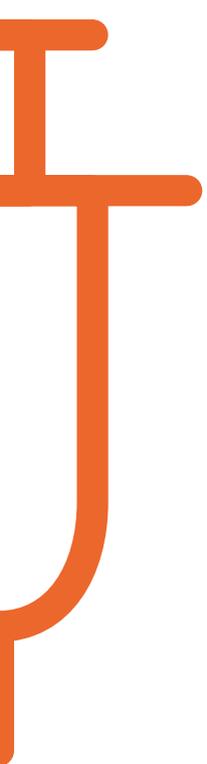
These conditions need to be met for vaccines to have the confidence of the public and to be available to anyone who needs them, including those in poorer countries¹.

Medicines against bacteria and viruses are given to people who have already been infected and may be (and usually are) already sick. Some medicines try to stop these bacteria or viruses from multiplying by acting on them directly (e.g. antibiotics or antivirals). Other medicines alleviate symptoms (e.g. painkillers and fever-reducing medicines) or help the body and the immune system withstand the infection (e.g. vitamins). Medicines assist the healing process and therefore belong to the curative branch of healthcare.



Who are we vaccinating at the moment?

- **Children and young people** - The government enables all children and young people in Flanders to be vaccinated free of charge against 12 infectious diseases under the basic vaccination program⁷. Children receive their first series of vaccines under the basic vaccination program from the age of eight weeks. This program runs until the age of 14. The diseases against which children and adolescents are vaccinated are polio, diphtheria (croup), tetanus, whooping cough (pertussis), *Haemophilus influenzae* type B, hepatitis B, pneumococcal disease, measles, mumps, rubella (German measles), meningococcal disease, and cancer caused by the human papillomavirus. Vaccination against rotavirus at a very young age is optional, but not free.
- **Pregnant women** - Despite significant progress in reducing mortality rates in children under the age of five (including vaccinations), newborn children are still at risk from some bacterial diseases. Because babies do not have a fully-developed immune system, this risk can be reduced by vaccinating (or re-vaccinating) pregnant women against whooping cough and influenza. This relies on the passive transfer of antibodies from the mother to the unborn child so that they are protected in the first months of life⁸.
- **Cocoon vaccination** - To stop infants from getting infected from their surroundings, vaccination against whooping cough is recommended for expectant parents, parents of young children, grandparents, close family contacts, and professionals in pediatric services and kindergartens. Vaccinating people who come near infants is also called 'cocoon vaccination'.

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- **Adults** - Certain vaccinations, such as tetanus, measles, mumps, rubella, diphtheria, and whooping cough, are recommended for adults as well, as immunity gradually decreases and, for some diseases, the risk of complications increases with age.
 - **The over 65s** - Some additional vaccines are recommended if you are over 65. Some diseases occur more often later in life or can have more serious consequences from that age onwards. Examples include pneumococcal infections and shingles (zoster).
 - **Travelers** - Depending on the destination and the circumstances of the trip, travelers are advised to get vaccinated before they go. Some travel vaccinations are even compulsory. The most commonly used travel vaccines are aimed at hepatitis A, measles, yellow fever, and tick-borne encephalitis. For travel vaccinations, we refer you to the Belgian Institute of Tropical Medicine (www.wanda.be).

- **Occupations** - Certain vaccinations are recommended for specific professional situations. These include people who come into contact with food, children, the elderly or with weakened people, or people who work in the waste industry or in unsanitary conditions. Examples of this include hepatitis A, whooping cough, measles, and tetanus.

And, finally, there is the **annual flu vaccine** that is recommended in the fall for e.g. pregnant women, people with a chronic disease (diabetes, heart, lung, liver or kidney disease, etc.), people with reduced immunity, health workers, and the elderly. For a detailed and up-to-date overview of which vaccines are recommended at what time in life, we refer you to the websites of the Flemish government (www.laatjevaccineren.be and www.zorg-en-gezondheid.be/basisvaccinatieschema).



INFECTIOUS DISEASES AGAINST WHICH WE VACCINATE PREVENTIVELY (PROPHYLACTICALLY)^{9 10}

Included in the vaccination program for children and adolescents

Mumps - is caused by a virus that typically infects the salivary glands. Mumps is transmitted by coughing and sneezing. Before a vaccine was available, almost every child developed mumps. That was usually mild. Nevertheless, mumps can have serious complications such as meningitis. In boys from puberty onwards it can lead to inflammation of the testes (orchitis), which can cause sterility.

Diphtheria (croup) - is an infectious bacterial disease that affects the throat, heart, and nervous system. Diphtheria is usually transmitted through the air, by coughing or sneezing. Ingestion of contaminated (raw) milk or contaminated food can also cause diphtheria. Complications such as breathing difficulties, impaired vision, heart problems, paralysis of the limbs or damage to the nervous system occur in 10 to 20% of diphtheria patients. The complications in 3 to 12% can even be fatal. The risks are greatest for young infants and the elderly.



Hib (Haemophilus influenzae type B) - is caused by a bacterium and can lead to meningitis. Children from 0 to 4 years old are especially at risk. They can suddenly get very sick. Because the disease can worsen so quickly, irreparable damage often occurs before treatment can be started.

Hepatitis B - is an inflammation of the liver caused by infection with the hepatitis B virus. Two variants of the condition are known: acute and chronic hepatitis B. Infected people often do not notice much of an infection. They may feel tired and fluish and/or have muscle pain. The characteristic yellowing of the skin (jaundice) can also occur. In some cases, the liver becomes so inflamed that potentially fatal complications can occur. A hepatitis B infection usually gets better by itself, but the infection can become chronic, especially in children. Then the virus remains in the body and the liver can stay inflamed for years without serious complaints. This can eventually lead to severe damage to the liver (cirrhosis of the liver) or even liver cancer.

HPV cancer - caused by the human papillomavirus. This virus is common and is mainly transmitted through intimate and sexual contact. There are more than 150 types of human papillomavirus. A small number of these types can cause cancer in the long term. Cervical cancer is the most common of these, but cancer in the anus, pubic area (vagina, penis, and labia), and throat can also be caused by HPV.

Whooping cough (pertussis) - is an infectious bacterial disease of the respiratory tract. Coughing, sneezing, and talking cause the bacteria to become airborne and people become infected. Whooping cough is also known as the '100-day cough' because the characteristic 'whooping' cough can last for months. The cough is especially exhausting for infants. They can become so tired and short of breath that they stop breathing. There is then a risk of brain damage. In recent years whooping cough has been on the rise, including among adolescents and adults. The great danger is that a mother, father, or relative could infect a young baby who has not yet been vaccinated against whooping cough. That is why these people are advised to get (re) vaccinated against whooping cough.



Measles - is a serious, highly contagious infectious disease caused by the measles virus. Measles is transmitted through the air, by coughing, sneezing, or talking. The first symptoms are fever, cough, and red eyes. After a few days, a red, somewhat rough skin rash appears on the face, neck, and throat. It then spreads all over the body. The spots gradually fade into a general redness. Complications such as earache or pneumonia occur in 10 to 20% of patients. Sometimes acute encephalitis (inflammation of the brain) occurs. This can lead to permanent damage or death.

Meningococcal disease - is a serious bacterial condition that can cause meningitis, encephalitis, or sepsis. The early signs are drowsiness, confusion, fever, nausea, vomiting, headache, oversensitivity to light and sound, pain in the joints, and a characteristic rash with small red spots that spread quickly over the skin and do not disappear or discolor when pressed. Further development to meningitis or blood poisoning can occur rapidly.

Pneumococcal disease - is caused by bacteria. There are many different types of pneumococcal bacteria. Many people carry pneumococci without getting sick from it. You can infect people by sneezing, coughing, or kissing, even if you are not sick yourself. Pneumococci can cause middle ear infections, sinus infections, and bronchitis, but can also lead to severe pneumonia, meningitis, or septicemia (blood poisoning). People can die from these serious forms of the disease.



Polio (myelitis) - is a viral infection that can cause muscle paralysis and/or meningitis in 0.1% to 1% of infected individuals. There is no treatment for polio except for controlling the symptoms. The virus is transmitted from person to person through contaminated food, feces, water or small droplets in the air. Vaccination against polio is the only compulsory vaccination in Belgium. Since the introduction of compulsory vaccination, polio is no longer found in Belgium and is now also extinct in the rest of Europe. Because the disease still occurs in some other countries, however, it is important to continue to give this vaccine to children.

Rubella (German measles) - is a highly contagious disease caused by the rubella virus. People who get infected usually develop some general symptoms such as fatigue, colds, and mild fever. Only then does a rash appear on the face and neck. Some patients also get a sore throat, cough, and inflamed eyes (conjunctivitis). The virus is particularly dangerous for pregnant women because it can cause miscarriage or severe abnormalities in the child (deafness, blindness, disturbed mental development).

Tetanus (lockjaw) - is caused by a bacterium that enters through open wounds. You can also get tetanus from animal bites, even from a pet. Infected persons often first suffer from restlessness, irritability, and headaches. After that, spasm and tightening of the jaw muscles (lockjaw), difficulty with swallowing, and breathing problems may occur. Damage to the musculoskeletal and nervous systems can cause bone fractures, high blood pressure, and abnormal heart rhythms (arrhythmias). Tetanus is fatal if left untreated. Because the most severe problems are caused by a toxin that the bacterium releases, antibiotics offer little help. A specific antitoxin, however, can save the situation.

Other conditions that existing vaccines are effective against

Rotavirus disease - is an infectious gastrointestinal infection that occurs mainly in babies and young children. The symptoms are severe diarrhea, vomiting, and fever. This brings with it a high risk of dehydration. Hospitalization is sometimes necessary. Vaccination against rotavirus is not part of the vaccination program (with free vaccines) but is recommended for all children under 6 months of age. The vaccine is given by mouth (orally).

Influenza (flu) - is caused by the influenza virus. A dry cough, headache and sore throat, fever, muscle pain, and chills are the main symptoms of the disease. Patients usually get better by themselves after a few days, but flu can have serious consequences for some people: People over 65, pregnant women and people with health problems such as diabetes or a disorder of the lungs, heart, liver, or kidneys should therefore be vaccinated every year. Health workers who come into contact with these people are also advised to get vaccinated.

Hepatitis A (jaundice) - is an infection caused by the hepatitis A virus. It causes inflammation of the liver. Infection mainly occurs in unhygienic, unsanitary conditions. People usually get better without permanent harm. The characteristic symptoms are fatigue, weakness, loss of appetite, headache, nausea, and fever. However, in adults, the condition can sometimes last for 2 to 8 months.

Yellow fever - is caused by the yellow fever virus, which is spread by mosquitoes. The disease only occurs in Africa and South and Central America. Yellow fever is usually subclinical: fewer than a quarter of the patients develop symptoms, but these can vary greatly in nature and severity. Complaints can range from flu-like symptoms over muscle pain, general malaise, headache, nausea, vomiting, and jaundice to fever with severe bleeding (hemorrhagic fever). Up to half of the patients with the most severe symptoms die from the condition.

Shingles (zona) - is caused by the same virus as chickenpox. After going through this viral infection - usually in childhood - the virus remains in the body without causing complaints. During periods of reduced resistance, the virus may reactivate and cause shingles, often characterized by a stripe-shaped rash on the body. The disease is accompanied by an itchy skin rash and (sometimes) severe pain. The disease is most common in people over the age of 60, who can be vaccinated to prevent it.

What else is in the pipeline?

The basic vaccination program is constantly evolving. New vaccines are coming on the market, knowledge about infectious diseases and vaccines is increasing and sometimes a new infectious disease emerges, COVID-19 being a striking example. This is why, over the years, various vaccinations have been added to the basic program or recommended for specific target groups. And this is a process that will continue.



From individual protection to herd immunity

A vaccine primarily protects the individual who is vaccinated. However, there is more: vaccination also indirectly protect the people close to this person and even society at large. This is called 'herd immunity'.

The principle behind herd immunity is that contagious diseases are easily passed from person to person (see the 'Herd immunity' illustration below). In this way, entire communities can quickly become infected. The recent COVID-19 outbreak illustrates this perfectly.

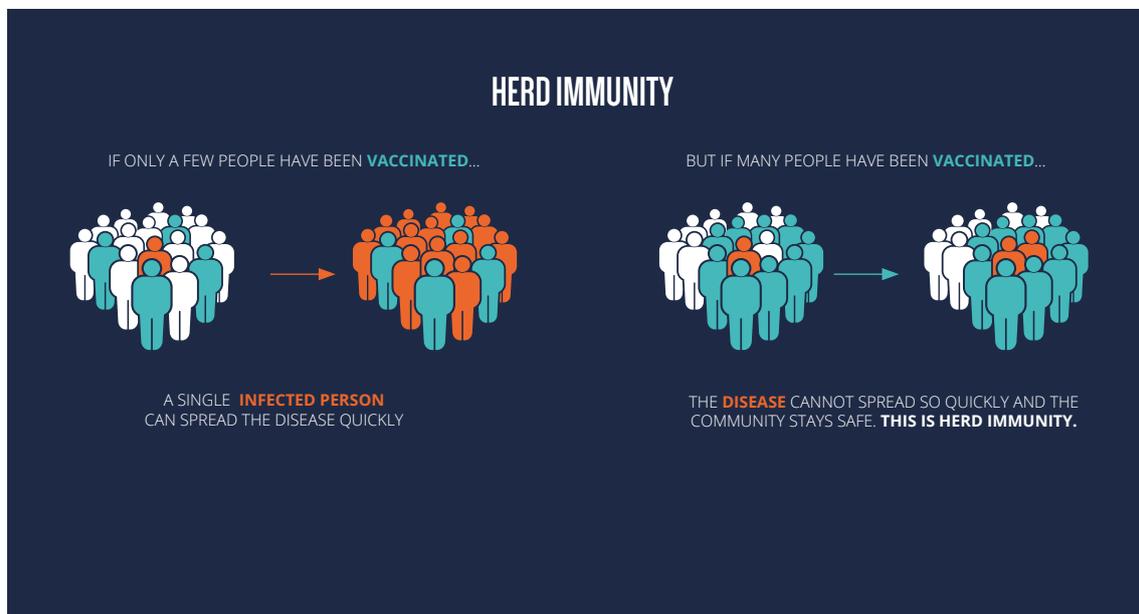
However, if a large part of the population is protected by vaccination, the disease has a much harder time spreading because it finds people all around it who are already immune. The virus or bacterium no longer finds 'fertile soil' in which to grow and will eventually disappear from the population.

Herd immunity is vital for people who cannot be vaccinated. Think, for example, of children who are still too young, people undergoing certain medical treatments (such as those for cancer), or whose immune system is weakened (HIV patients, the elderly, etc.). However, herd immunity only works

if sufficient people are vaccinated. The number of people this should be depends on the disease and how it spreads. For pneumococci, a vaccination rate of only 60% may be enough to create effective herd immunity. The aim should be 80% for rubella, and 95% for measles¹.

This has everything to do with the R_0 value (basic reproduction number) of a specific infection. This is the average number of infections caused by one infected person. For measles, the R_0 in an unvaccinated population ranges from 12 to 18¹¹. By way of comparison, the R_0 for COVID-19 is between 2 and 3 if we do not take appropriate countermeasures. It shows very clearly why measles is one of the most contagious (childhood) diseases we know. It is because of this high level of contagion that herd immunity requires such a high vaccination rate.

In summary, we can say that vaccination is not only a medical intervention to protect yourself, but also an act of solidarity towards society, and especially towards people who are vulnerable because they cannot be vaccinated or because their immunity is too weak.



2. Impact on health and society

Vaccines save lives

Since vaccines have been widely adopted, their benefits have far outweighed their costs. All health experts agree on this¹². Vaccines against infectious diseases have changed the fate of humanity^{1 13}:

- Every year they save the lives of millions of children, adults, and elderly people.
- They prevent sickness, disability, handicaps, and untold human suffering.
- Their enormous benefits to society involve not just public health, but also education, social inclusion, and the economy.
- Vaccination was, and remains, one of the most cost-effective investments in healthcare¹.

And there is no mistaking the achievements of vaccination. The figures speak for themselves. Some examples:

- The most remarkable vaccine success story is the worldwide eradication of smallpox. The

wild-type form of smallpox ceased to exist in 1979. The ongoing program against polio is also getting closer to its ultimate goal of total global eradication. The global polio vaccination program, led by the WHO, has resulted in a 99% fall in the number of infections since 1988. It went from an estimated 350,000 infections at the time to only 175 reported infections in 2019.¹⁴ Africa has since also been declared polio-free. Wild-type polio is now only found in Pakistan and Afghanistan.

- For conditions such as diphtheria, whooping cough, and measles, the impact of vaccines on fatality rates has been impressive. In the United States in the early 1900s, more than 7,500 annual deaths were attributed to measles, 13,000 to diphtheria (1920), and 5,000 to whooping cough (1922). A century later, no diphtheria infections were reported in the US and measles was almost eliminated (see table). In 2012, there were only 18 deaths related to whooping cough (mostly in children under 3 months)^{15 16}.
- We can assume that the figures for West European countries with a high vaccination coverage are similar.
- Recently, the WHO, UNICEF, and the World Bank calculated that 2.5 million deaths are prevented annually by child vaccines². Between 1990 and 2017, vaccination accounted for 55% of the reduction in child mortality (under 5 years of age). These mortality rates fell from 87 deaths per 1,000 births in 1990 to 39 deaths per 1,000 births¹⁷. Between 2011 and 2020, 14 million premature deaths are estimated to have been prevented by the measles vaccine alone.¹⁸ Although there is still work to be done, of the 6.2 million children and adolescents under

TABLE: ANNUAL INFECTIONS IN THE US BEFORE AND AFTER VACCINATION

Condition	Period 1900-1925	1998	Decrease
Diphtheria (croup)	175,885	1	>>99.9%
Tetanus	1,314	34	97.4%
Whooping cough (pertussis)	147,271	5,279	95.7%
<i>Haemophilus influenzae</i> type B disease	20,000	54	99.7%
Measles	503,282	89	>>99.9%
Mumps	152,209	606	99.6%
Rubella (German measles)	47,745	345	99.3%
Polio	16,316	0	100%
Smallpox	48,164	0	100%

Sources: References 15 and 16

the age of 15 who died worldwide in 2018, half could have been saved by, amongst other things, vaccinations¹⁹.

- In a country like Cuba, communicable infections were by far the most important cause of disease and death in children before 1960. In 1962, the government began large-scale vaccination campaigns covering the entire population. The vaccinations were completely free and were integrated into primary healthcare with active participation from local communities. Fifty years later, Cuba leads the world in using vaccination to prevent infectious diseases. The country has managed to completely eradicate numerous diseases in its territory, including polio (since 1962), diphtheria (1979), measles (1993), whooping cough (1994), and rubella (1995). Serious clinical forms of tetanus, meningitis due to meningococci, *Haemophilus influenzae* type b disease, and mumps are also rare. Cuba is therefore doing better than many Western countries. In addition, the country produces most of its vaccines itself^{20 21}.

- Vaccination benefits not only those who receive the vaccine but also their loved ones (see Section 1 on herd immunity)¹. A recent study in Kenya shows this clearly: introducing the pneumococcal vaccine resulted in those who were vaccinated having much better protection against lung diseases caused by pneumococci. However, the incidence of pneumococcal disease was also reduced in infants - who cannot yet be vaccinated - as well as in unvaccinated children and even the population as a whole by a factor of up to a half^{22 23}.

The above examples are just a few of the dozens of case studies demonstrating the added value of vaccines.

Investment in preventive health and prosperity

Vaccines also have a social added value that goes far beyond measures of individual health. They also make an important contribution to the local, national and global economy, political stability, the education of children and young people, reducing the social divide, reducing lost time to maintain family incomes, and much more.²⁴

Recently, researchers calculated that every euro invested in ten child and adolescent vaccines in low- and middle-income countries leads to savings of 10 to 25 euros in healthcare costs²⁵. When the broad economic and social benefits of vaccinations are taken into account, each euro invested generates 44 euros.

Or in other words, twenty years of vaccination against 10 childhood diseases in 73 low- to middle-income countries have yielded savings of \$5 billion in treatment costs and an economic gain of at least \$340 billion²⁶. These savings and gains are achieved because fewer children die or are faced with complications and handicaps. As a result, they are less often absent from school and therefore better educated, their parents are less often absent from work, and once these children grow up, they contribute more to the economy.

In short, vaccines are one of the best investments in healthcare because, for each euro invested, they generate enormous health and economic benefits as well as savings amounting to many times the sums invested. Despite this, it is true that not every vaccine has an equally good cost-benefit score and that for every new vaccine the health benefits must be carefully weighed against the costs and possible disadvantages of the vaccine.

THE ADDED VALUE OF VACCINATIONS SUMMARIZED IN WORDS AND PICTURES

 VACCINES HAVE ERADICATED SMALLPOX WORLDWIDE. POLIO ALSO SEEMS TO BE ON THE WAY OUT.

WORLDWIDE, THE MEASLES VACCINE HAS SAVED **14 MILLION** LIVES OVER 10 YEARS. 

 **HERD IMMUNITY**
AGAINST MEASLES NEEDS A VACCINATION RATE OF AT LEAST 95%.

EVERY EURO INVESTED IN CHILDREN'S VACCINES CAN SAVE UP TO 25 EUROS IN HEALTHCARE AND GENERATE **44 EUROS** IN SOCIAL PROFIT. 

IN FLANDERS, THE VACCINATION RATE IN CHILDREN FOR THE RECOMMENDED AND FREE VACCINES IS BETWEEN **92.9 AND 96.2%**. CHILDREN ARE VACCINATED FREE OF CHARGE AGAINST 12 INFECTIOUS DISEASES. 

 ALMOST 9 OUT OF 10 ADOLESCENT GIRLS IN FLANDERS HAVE BEEN GIVEN THE HPV VACCINE

THE VACCINATION RATE IN FLANDERS FOR INFLUENZA IN PEOPLE OVER 65 WHO ARE NOT STAYING IN A RESIDENTIAL CARE CENTER IS **60.6%**. THAT IS WELL BELOW THE TARGET SET BY THE WHO AND THE FLEMISH GOVERNMENT, WHO ARGUE FOR A VACCINATION RATE OF **75%**. 

SUMMARIZING THE ADDED VALUE OF VACCINES²⁷

DISEASE CONTROL

Eradication	Unless there is an animal 'reservoir' for a pathogen (such as pigs and poultry for the flu virus), vaccination campaigns have the potential to eradicate a pathogen worldwide. The smallpox virus has been eradicated around the world and we are well on the way to do the same for polio with global vaccination programs.
Elimination	Diseases can be eliminated locally by vaccines without global eradication. That, however, requires a high level of vaccination.

CONTROL OF DEATH RATE (MORTALITY), ILLNESS (MORBIDITY), AND COMPLICATIONS

For the individual	Effective vaccines protect people from infections they have been vaccinated against. Even if a vaccine is not 100% effective - and a vaccinated person can still show symptoms of the disease if they get infected - these symptoms are often less severe and there are also far fewer complications.
For the loved ones and the group	If the level of coverage is high enough, vaccines also protect those who have not been vaccinated. This type of immunity at the group or population level is important for those who are too young to be vaccinated, people who cannot be vaccinated due to other medical conditions, or for whom a vaccine is not effective enough.

PROTECTION AGAINST RELATED AND UNRELATED CONDITIONS

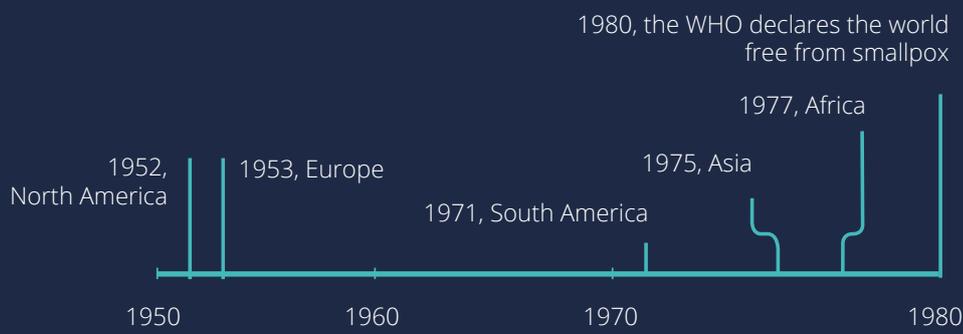
Broad immune activation	In Finland, the US, and other countries, the influenza vaccine has also been shown to partially protect children against ear infections. The measles vaccine may also provide some protection against the complications of dysentery, bacterial pneumonia, and malnutrition.
Cancer	Some vaccines provide long-term protection against cancer. A virus such as hepatitis B leads to chronic hepatitis, which is a major risk factor for liver cancer in some people. Early findings from China and Taiwan suggest that hepatitis B vaccination has reduced the number of patients with liver cancer. The same effect against cervical and other genital cancers is expected from the HPV vaccine.

SOCIAL BENEFITS

Healthcare savings	By preventing disease and mortality, vaccines lead to large savings in healthcare in both the short and long term.
Prevention of antibiotic resistance	Lowering the number of bacterial infections means that fewer antibiotics need to be prescribed. This reduces the spread of antibiotic resistance.
Longer life expectancy	Over the past 100 years, the average life expectancy in industrialized countries has increased by 30 years. This is in large part due to vaccinations.
Safe travel	Each year, millions of travelers are vaccinated against a range of infections they may come into contact with in other countries.
The cornerstone of primary healthcare	In developing countries, the (free) supply of vaccinations is often the only reason why people take their children to a health center or a doctor. These visits allow health workers to better monitor the general health of the children. A double dividend then.
Emancipation of women	The lower infant mortality rate means that women choose to have fewer children. This frees up more time and opportunities for them to participate in economic and social life.
Economic growth	Good public health is a foundation for economic growth. History has shown time and again that diseases, epidemics, and pandemics lead to economic malaise. The recent COVID-19 pandemic is further proof of this. Preventing or fighting epidemics and pandemics with vaccines supports economic growth.
Closing the health and poverty gap	The consequences of infectious diseases are generally felt more strongly by the socially and economically disadvantaged. Preventing these conditions therefore has a greater effect on these vulnerable groups.

Worldwide eradication of smallpox

The years the disease was eradicated for each continent.



North America
1952

South America
1971

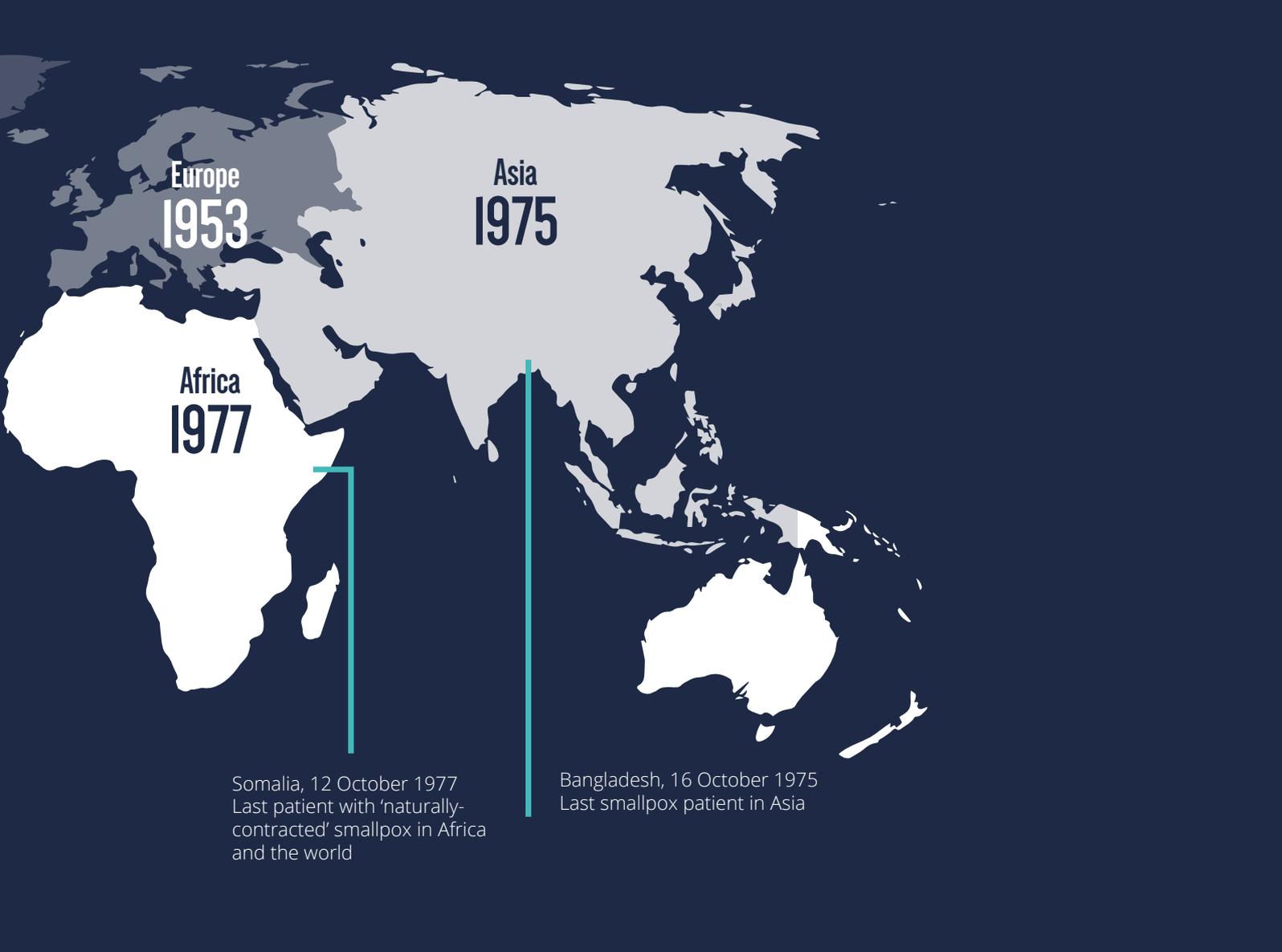
Brazil, 19 april 1971
Last known patient on
the continent

A world free of smallpox ¹⁰⁷

Smallpox was a severe contagious disease caused by the variola virus. People who catch smallpox develop a fever and a characteristic rash. Most people recover, but about one in three die. Many survivors have permanent scars over large areas of their bodies, especially on their faces, and some go blind. Since 1980 we have been living in a smallpox-free world: smallpox is the first disease to have been eradicated by vaccination.

A global eradication program

In 1959 the World Health Organization set out to rid the world of smallpox. By that time North America and Europe were, thanks to vaccinations, already free from smallpox. Unfortunately, this global eradication campaign suffered from a lack of funding and personnel, as well as a shortage of vaccines. The smallpox virus was still widespread in 1966, causing frequent outbreaks in several countries in South America, Africa, and Asia.



Europe
1953

Asia
1975

Africa
1977

Somalia, 12 October 1977
Last patient with 'naturally-
contracted' smallpox in Africa
and the world

Bangladesh, 16 October 1975
Last smallpox patient in Asia

A new, more ambitious, eradication program began in 1967. This time, local laboratories in countries where smallpox was common were able to produce large quantities of high-quality freeze-dried vaccine themselves. A number of other factors played an important part in the success of the intensified efforts, including, just to name a few, the introduction of a surveillance system to detect patients with smallpox much more quickly and large-scale mobile vaccinations in every corner of the world coupled with locally-adapted public information. This time, the program was successful.

By 1971, smallpox had been eradicated in South America. Asia (1975) and, finally, Africa (1977) would follow in the same decade.

A successful program

Nearly two centuries after Jenner published his hopes that vaccination could eradicate smallpox, the 33rd World Health Assembly officially declared the world free of this disease. This was on 8 May 1980. Smallpox eradication is considered one of the greatest achievements in international public health.

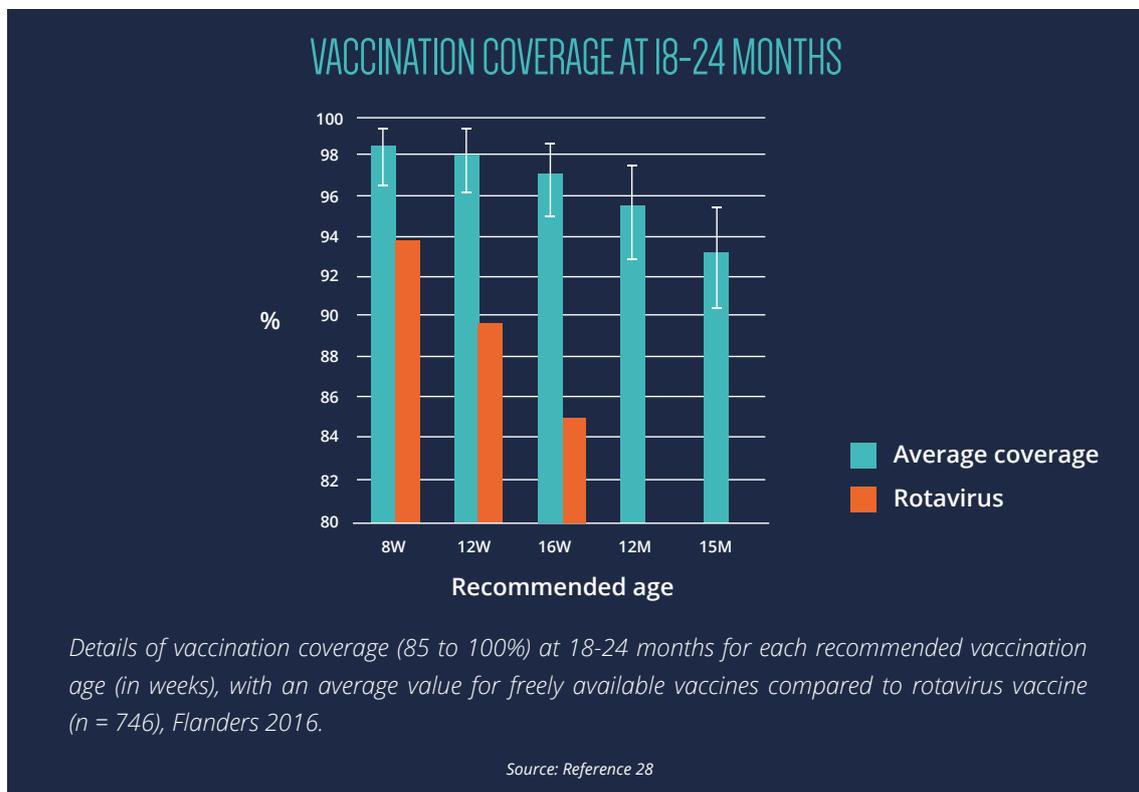
The vaccination rate in Flanders

In 2016, a vaccination coverage study was conducted in Flanders in four different target groups: children up to 24 months of age (born in 2014), their parents, adolescents (born in 2000), and women who had recently given birth.²⁸

The main findings were that the vaccination coverage for the vaccines recommended for young children remains both stable and high in Flanders (92.9-96.2%), except for the rotavirus vaccination (89.7%). The latter is recommended but is not free. We do see that as young children get older, they miss one or more recommended vaccine doses. The vaccination coverage is more than 98% for eight-week-old babies, but the figure for children of 15 months struggles to reach 93% (see the illustration below).

The vaccination rate for adolescents is higher than in previous measurements for all the vaccinations studied, except for hepatitis B (a slight decrease in vaccination rate). The HPV vaccine for girls reached 89.6% of the target group (full three-dose vaccination)²⁸.

For the first time, women who had recently given birth (early 2016) were also asked what their vaccination status during pregnancy was (see the illustration on page 25). The vaccination rate for whooping cough in this group is almost 70%, for influenza, it remains just below 50% even though all these mothers were pregnant in the flu season and were therefore eligible for the seasonal flu vaccination. In addition, 62% of their partners had received a vaccine in the previous 10 years that included whooping cough²⁸.

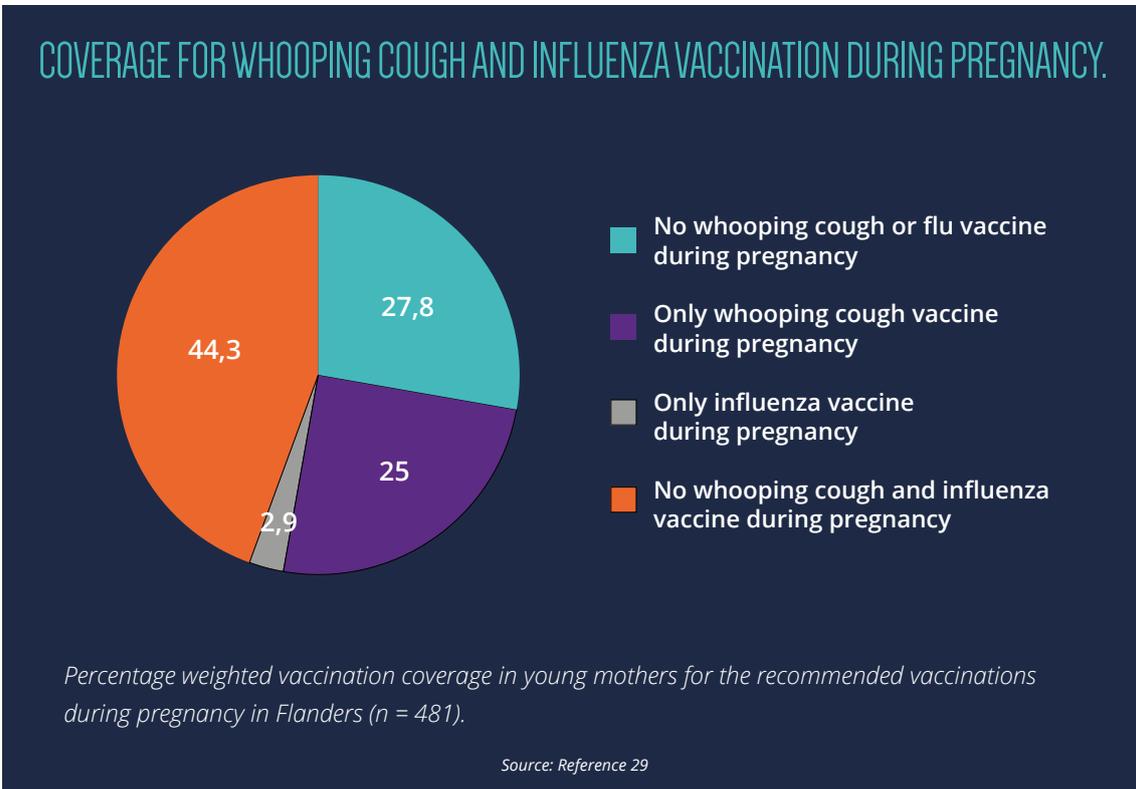


Only a minority of the parents of toddlers recalled being vaccinated with the measles vaccine (45.9% for the fathers and 55.9% for the mothers). The whooping cough vaccination rate of these mothers was 57.6% during their pregnancy (which took place in 2013-2014). The higher vaccination rate among the more recent mothers (69.3%) is consistent with the further implementation of the recommendations in Flanders, including the free provision of the vaccines since mid-2014²⁸.

The situation is more concerning for the vaccination of the over 65s against seasonal flu. Vaccination is considered the most effective precautionary measure for this target group for reducing the frequency and severity of influenza virus infections. In Belgium, this vaccination is therefore also recommended for (among others)

all people aged 65 and over and for all people living in a residential care center. The WHO recommends a target vaccination rate of 75% for this group. This objective was also adopted by the Flemish Government in 2013.

However, based on RIZIV data, we find that in 2016 only 59.5% of people over 65 were vaccinated in Flanders²⁹. Compared to 2009, this was actually a decrease of more than 6%. This downward trend is also evident in other parts of Belgium and the rest of Europe. In Wallonia, the vaccination rate for people over 65 is only 50.1% and in Brussels only 47.8%. Compared to other countries, however, we still score reasonably: we are only outdone by the Netherlands and the UK, which do reach the 75% threshold.



3.

Meeting social challenges

The other side of vaccines

Despite all of the above, vaccines do have their downsides. Sometimes children and adults suffer side effects. These are usually caused by the immune system's response to the vaccine. For example, the site of vaccination can turn red and swell up. Occasionally the entire upper arm or thigh becomes red and swollen. Other more common side effects include fever, crying, headache, listlessness, and vomiting^{30 31}.

Less common side effects include febrile seizures, discoloration of arms or legs, seizures where children hold their breath and turn blue, fainting and reduced reaction time, acute allergic reactions, or, over time, a shortage of blood platelets^{30 31}.

Vaccinations are, at the end of the day, still medical interventions. Just as with any other kind of medical intervention, there is no absolute certainty that no serious side effects will occur. Very rare but still serious side effects - for example, those affecting only one in every million people who are

vaccinated - may go unnoticed even in extensive research. Nevertheless, vaccines are subject to very stringent safety requirements. Even when a vaccine is approved and found to be safe, thorough and critical investigation of side effects continues through intensive monitoring programs^{30 31}.

Vaccines are also rarely 100% effective in everyone who is vaccinated. There are people in whom a vaccine does not always lead to an optimal immune response. These people must then hope to be protected by herd immunity. For example, recent Belgian research shows that the measles vaccine is effective in 96% of the people who were vaccinated. This is 93.3% for mumps and 98.3% for rubella³².

Sometimes infections can also flare up because the dynamics of diseases change over the years. Viruses or bacteria can change (mutate), people can lose their protection prematurely, or the vaccine may be insufficiently adapted to the prevailing or new strains. The latter is, for example, an Achilles heel of the current vaccines against influenza (see also Section 7)¹³.

Furthermore, it cannot be ruled out that vaccines from the past were less powerful and offered shorter-lived protection than was first believed. In this case, whole cohorts of a population sometimes need to be re-vaccinated.

In exceptional cases, it is even possible that vaccines themselves cause the condition they were intended to protect against. A notorious example of this is the watered down oral polio vaccine (see the box 'Antwerp Poliopolis is helping to work towards a new polio vaccine'). In such cases, a proper balance must be made between the risks and benefits of a vaccination program. This is not easy in a world that is increasingly averse to risks, no matter how small.

ANTWERP'S POLIOPOLIS IS HELPING TO WORK TOWARDS A NEW POLIO VACCINE



Polio is now only found in some areas of Pakistan and Afghanistan. The definitive eradication of the disease seems imminent, thanks to the worldwide use of vaccines.

There are two vaccines against polio: a live but attenuated vaccine that is taken orally as a syrup and an inactivated vaccine, which is injected. Because the first vaccine is easy to administer, is inexpensive, and induces broad and long-lasting immunity, it is used in more than 100 countries. In Belgium, too, children were vaccinated for polio 'by spoon'. Today, however, the injectable vaccine is used, as it is in most industrialized countries.

This allows the vaccine to be combined with other vaccines in the vaccination program. But that was not the main reason for the switch. Around the end of the twentieth century, it became increasingly clear that the poliovirus in the oral vaccine can mutate back to the wild type and thus itself cause polio in people who received the vaccine. In addition, there is a risk that these people will pass the disease on to others, especially in populations with a low vaccination coverage³³.

Because it is one of the three polio variants included in the vaccine, virologists talk about 'circulating vaccine-derived type-2 polioviruses (cVDPV2)'.

This back-mutation of the type 2 variant in the vaccine is rare. In any case, it occurs in fewer than one in a million vaccinations. Nevertheless, in recent years several dozen people have contracted polio directly or indirectly via the vaccine, which is obviously undesirable and can undermine public confidence in it³⁴.

The WHO has taken measures to remove the type 2 virus strain from the oral vaccine. At the same time, an international consortium with financial support from the Bill & Melinda Gates Foundation is working on an improved oral polio vaccine. The University of Antwerp's Poliopolis project is an important partner in this research. In a closed quarantine environment, the new vaccine was trialed for the first time on test subjects³⁵.

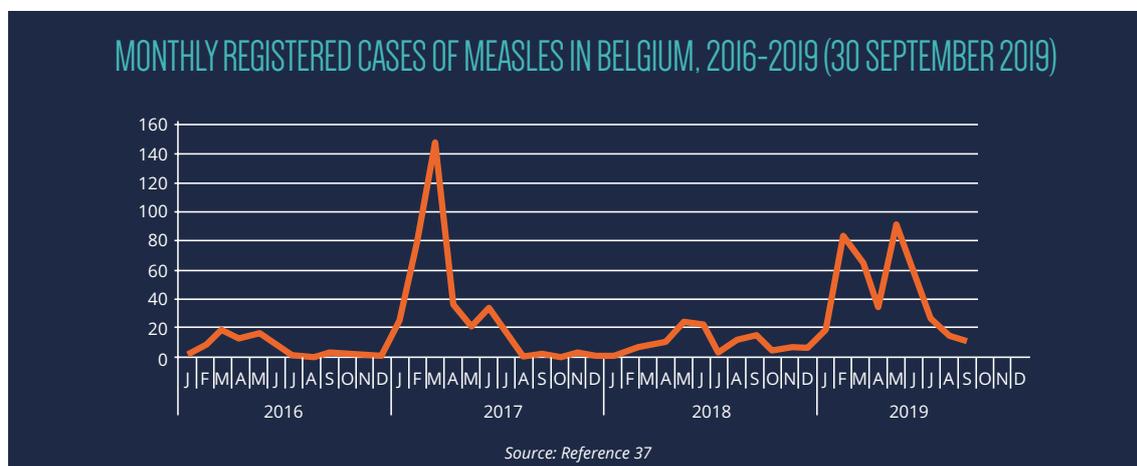
Victim of their own success: vaccination fatigue and mistrust

Vaccines have become the victims of their own success¹. In our country, diseases we vaccinated against in recent decades are much less common than they were about 50 years ago. Hardly anyone knows an acquaintance or family member who has had measles, whooping cough, diphtheria, or rubella. Complications and deaths from these diseases no longer reach the media. The perception is therefore growing that these diseases are just harmless childhood diseases. As a result, the positive effects of vaccinations no longer have a place in our collective memory and the deterrent effect of the diseases against which we vaccinate has faded away. Instead, there is an unreasonable focus on the potential side effects of vaccines, which are getting more and more attention in the media. On top of this, there is a growing group of people who refuse vaccines for religious or philosophical reasons^{12,13}.

The result of all this is that vaccination coverage in several countries is sub-optimal or has even fallen. This endangers herd immunity and leads to local outbreaks of infectious diseases that can spread quickly¹³. An example of this is the repeated flare-ups of measles inside and outside Europe. In 2013

and 2014, there was a major measles epidemic in the Netherlands, mainly among unvaccinated schoolchildren from the 'bible belt'. This region runs from the province of Overijssel via the Veluwe and the rural areas of Utrecht and South Holland to the islands of the Rhine delta in Zeeland¹². There are many people in these areas who, for reasons of faith, do not allow their children to be vaccinated. As a result, the vaccination rate for measles, for example, is below 60%. During the 2013-2014 epidemic, 2,700 measles cases were reported. Of these, more than 180 had to be hospitalized and one 17-year-old died of the disease³⁶.

Other European countries are also experiencing measles outbreaks. The largest of these have been in Ukraine, Romania, and Northern Macedonia, and problems are also reported in Poland, the Czech Republic, Slovakia, Italy, France, Bulgaria, Lithuania, Bosnia-Herzegovina, and the United Kingdom, where there was a particularly large flare-up amongst the Jewish Community of London. All travelers to these areas are advised to be vaccinated against measles if they have not had measles and have not previously been vaccinated against it³⁶.



Belgium has not been spared this either. In 2017 there was a major flare-up in Wallonia, as well as a few smaller outbreaks in Flanders and Brussels (see illustration on page 28)³⁷. A total of 367 infections were detected. Ten of them came from abroad.

In 2018, the number of infections had fallen (n = 117) but clusters continued to occur, often linked to the introduction of the virus from another European country. In 2019, Belgium experienced a new outbreak: 405 measles infections were

recorded in the first 9 months, which was more than 3 times the total for 2018.

At the beginning of 2019, these cases mainly came from travel-related infections, after which the measles virus circulated among the Belgian population, especially in non-vaccinated or incompletely vaccinated subgroups. Fortunately, there has been a significant decrease in the number of new cases since the start of the 2019 summer holiday³⁷.

Restoring trust

The WHO, the European Commission, and various European (scientific) institutions are deeply committed to restoring public confidence in vaccines. Various studies have shown that the safety of vaccines is a significant concern for the general public. People seem to be more concerned with the perceived risks of vaccines - which they estimate much higher than the objective figures indicate - than with the proven effectiveness of vaccines. The risk-benefit ratio is perceived differently than the scientific evidence shows. New challenges take the form of anti-vaccine campaigns and fake news on social media¹.

Although more accessible public communication on complex topics such as safety and risk-benefit analysis is important, building or re-establishing trust must go much further than just communication or providing transparent and easily understandable information about the diseases we vaccinate against. We need a better understanding of why people get tired of vaccination, why mistrust is growing and the questions the public is asking themselves. We must formulate clear and concrete

answers to this. If science does not yet have complete answers to these questions, we must invest in research into them^{1 38}.

An important key to restoring confidence in vaccines undoubtedly lies with the COVID-19 pandemic. If scientists, doctors, and pharmaceutical companies succeed in restoring normality in people's lives worldwide thanks to a sufficiently protective vaccine against SARS-CoV-2, this will give a great boost to public confidence in vaccination and in medical science in general. However, we must be aware of various hidden dangers: it must be possible to manufacture and distribute these vaccines in sufficient quantities to everyone who could benefit from them. And to do all of this at an affordable price. You can read more about the worldwide efforts needed for this in Section 7 of this dossier.

4. How a vaccine provides protection

Antibodies provide defense ...

The immune system in humans consists of a network of cells, tissues and organs that work together to fight infection by harmful bacteria or viruses.

The production and use of antibodies is one of the ways in which the immune system fights infections. Antibodies are proteins that bind to a virus or bacteria and mark it for destruction by other parts of the immune system. Each antibody is specific for a particular bacteria or virus and will therefore trigger a targeted immune response (see the 'Like a key in a lock' box on page 31).

These antibodies will continue to circulate in the body even after the viruses or bacteria of the first infection have disappeared. This means that if that person comes into contact with the same bacteria or virus again, the immune system is ready to respond quickly.

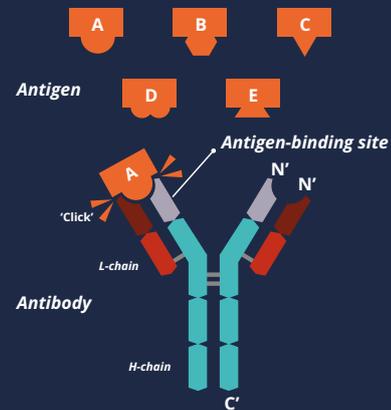
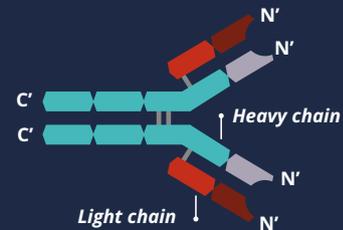
The above is the standard account for how vaccines and our immune system work. Most websites and information leaflets rarely go beyond this presentation. However, the human immune system is a good deal more complicated than described here. We can already deduce this from the description of the primary and secondary immune response in Section 1 (see page 6). It then becomes clear that after an initial infection or vaccination, the concentration of antibodies gradually decreases and may even fall below the detection limit. This phenomenon has already been seen after a few weeks in many patients with a mild form of COVID-19. However, this does not mean that they have not built up long-term immunity. And this is because the story of inducing immunity is much more complicated than the mere production of antibodies.

LIKE A KEY IN A LOCK

Antibodies, called 'immunoglobulins', are proteins produced by B-type white blood cells. Antibodies bind to foreign substances, including bacteria and viruses.

An antibody consists of two identical heavy chains (H-chain; blue in the drawing) and two identical light chains (L-chain; red-brown). Each of these chains has an unchanging 'constant' part (C'-part) and a 'variable' (N'-part). It is with this variable part that the antibody binds to the foreign substance.

One antibody will bind to bacteria A, another to bacteria B, and a third to a virus. In reality, the antibody recognizes a biomolecule on the outside of that bacteria or virus. This can be a fragment of protein or a combination of a piece of protein and a sugar molecule. The foreign molecule to which an antibody binds is also called an 'antigen'. So the antibody and antigen fit together like a key in a lock. Each antibody will therefore recognize and bind to only one antigen.



... but there is much more to it than just antibodies

In reality, it involves an interplay of very diverse cells that together make up the immune system. A system that, on the one hand, must be able to activate quickly to protect us against intruders, but at the same time include checks and balances to prevent the defense from going into overdrive or, just as bad, attacking our own proteins and cells.

We are going to throw some light on how vaccinations activate the human body against potential pathogens. As an example, we will take the influenza vaccine (see also Section 7). Although not every vaccine works in exactly the same way, the molecular and cellular processes are very similar. And these are exactly the broad lines we outline below. From this we learn that it is the memory B-cells that are mainly responsible for the immunity lasting years - sometimes even for life - after an initial infection or a vaccination^{3 39}.

HOW THE INFLUENZA VACCINE ELICITS AN IMMUNE RESPONSE

The antigens neuraminidase and hemagglutinin

The influenza virus has two proteins on its outside that act as antigens - that is, they are recognized by the human immune system. These proteins are neuraminidase (NA) and haemagglutinin (HA).

An influenza vaccine contains inactivated or killed influenza virus particles that are injected into the human body. These particles contain the NA and HA proteins. The NA and HA proteins are recognized as foreign and activate a complex network of immune cells including dendritic cells, T-cells, and B-cells. All these cells are white blood cells, called lymphocytes, and form part of the immune system..

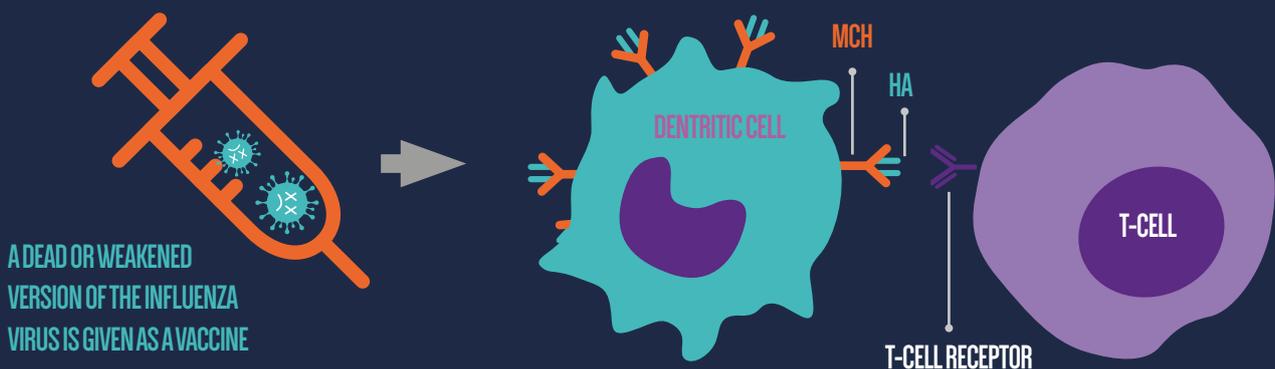
The reaction of dendritic cells and T-cells

An important first step is taken by the **dendritic cells**, which swallow the virus particles and cut the proteins into pieces. The dendritic cell takes fragments of the HA protein and puts them on its own cell surface so it can 'present' them in combination with its own receptor protein, the MHC protein (Major Histocompatibility Complex).

A T-cell binds via its T-cell receptor to the combination of the MHC protein and the HA fragment on the outside of the dendritic cell. This binding causes the T-cell to activate and divide.

This results in three types of T-cells:

- **Cytotoxic T-cells** specialize in killing and disposing of body cells infected by viruses.
- **T-inhibitor cells or T-suppressor cells** ensure that the immune response is moderated and does not get out of hand.
- **T-helper cells** support the activation of B-cells (see below) by giving off signaling substances.

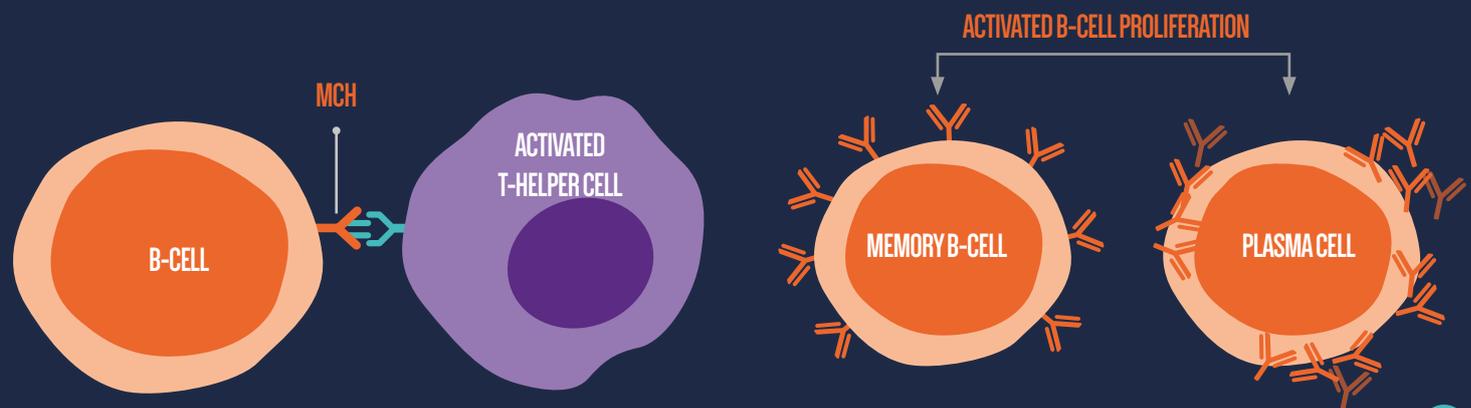


B-Cells

B-cells will also recognize the virus's HA protein. **B-cells** do this via their antigen receptor. Each B-cell has only one type of antigen receptor on its surface that recognizes a specific part of an antigen called a 'motif'. In other words, this is similar to the previously described lock-and-key principle of antibody-antigen binding. So only B-cells with an antigen receptor that matches the HA antigen will bind with it.

The B-cell will take in the HA protein fragment and present it on the surface of its membrane in combination with the MHC complex. That combination of HA protein and MHC complex on the surface of the B-cell is recognized by the T-cells previously activated by the dendritic cells. As a result, the T cell secretes signaling substances (cytokines), which in turn activate and induce B-cells to divide. This results in two types of B-cells:

- **Plasma B-cells** produce antibodies in large quantities. These antibodies bear the same motif as the B-cell antigen receptor that was originally bound to the HA antigen. These antibodies will therefore bind to the HA protein of the influenza virus and render the virus harmless. Most plasma B-cells will disappear from the blood once the infection has been successfully controlled. Consequently, antibodies might not be found in the blood after some time.
- **Memory B-cells** put the same antibodies on their membranes. However, they continue to circulate in the body for a very long time. If we suffer a second infection with the same pathogenic bacterium or virus, the memory B-cells act immediately. They divide very rapidly and some of them transform into plasma B-cells that produce large amounts of antibodies against the HA protein. Although antibodies eventually disappear from our blood, we can still fight a new infection quickly thanks to the memory B-cells.



You need to be aware that we have not covered all the details of the immune system and the immune response in the 'How the influenza vaccine elicits an immune response' box. As mentioned above, this is a complex system in which numerous tissues, cell types, and biomolecules play a role. Explaining it all would take far too long. In addition, many aspects of the human immune system are still unexplored and are the subject of intensive basic research.

Immunity in overdrive

Research shows, for example, that a healthy immune system must not only react quickly and vigorously but also do so in a controlled manner. Otherwise, things will go wrong. For example, we know that some infectious diseases can be fatal due to an overreaction of the immune system^{40 41}. This overreaction is called a 'cytokine storm'.

Cytokines are small proteins that function as signal molecules. They are released by various types of cells, including T-helper cells, that form part of the immune system (see above). Cytokines help coordinate the immune response by attracting various types of immune cells. Sometimes, however, this reaction can go into overdrive. For example, when a pathogen enters the lungs, it provokes a local immune response. Cytokines ensure that all cells necessary for an efficient immune response rush to where the problem is. An inflammatory reaction occurs at the infection site: swelling occurs, the temperature rises (fever), pain stimuli are emitted, the blood vessels dilate (local redness), etc.

In some patients, excessive or uncontrolled amounts of cytokines are released, attracting and activating too many immune cells. Hyperinflammation then develops, which can seriously damage the tissues. It can even kill the patient. Cytokine storms are a common complication of respiratory infections caused by

coronaviruses such as SARS and MERS, including SARS-CoV-2. The high death rate from bird flu in 2005 is also linked to cytokine reactions that get out of control. The phenomenon has also been observed in infections with the cytomegalovirus, Epstein-Barr virus, variola virus (smallpox), group A streptococci, and other microorganisms^{41 42}. Cytokine storms have even occurred in patients taking part in clinical trials for new drugs⁴³.

This is also one of the reasons why a great deal of attention must be paid to safety when developing vaccines. A vaccine cannot be allowed to set off an overreaction of the immune system. We expect a vaccine that stimulates an effective but balanced immune response that provides adequate protection but does not lead to dangerous immunological derailments.

5. The technology behind vaccines

The way vaccines are designed and produced has evolved over the years. The days when children were infected with unpurified cow viruses from the ulcers on a milkmaid's hands, as Edward Jenner did in the 18th century, are far behind us. Nevertheless, even today we still use 'live' vaccines, which are based on weakened variants of the real pathogen. The idea behind such vaccines goes back to Jenner's basic hypotheses.

Many new techniques have been developed to design and manufacture vaccines. Also, all vaccines on the market today, regardless of the underlying technology, must meet the same strict criteria for safety and efficacy. The following is a commonly used system for classifying historical and existing vaccines according to the underlying methodology⁴⁴:

VACCINE TIMELINE¹



Live, attenuated pathogens

The principle of incorporating live, albeit attenuated, variants of a virus or bacterium into a vaccine is still widely used today. Examples of this include vaccines against cholera, tuberculosis, yellow fever, measles, mumps, polio, rubella, and rotavirus disease.

It is important for these vaccines that the virulent or pathogenic nature of the virus or bacterium can be disconnected from the ability to elicit an immune response. This can often be done by selecting mutated strains. Other methods involve culturing the pathogenic strains for many generations and selecting the harmless variants.

Vaccines containing live, weakened strains usually elicit a broad immune response from both macrophages and B-cells that produce antibodies (see Section 4). They also give the best approximation of the normal infection and reproduction cycles of the virulent pathogen. As a result of this combination, vaccines with live pathogens lead to long-term immunity, in some cases even lifelong immunity.

On the other hand, there may be safety concerns with live weakened vaccines such as with the live

polio vaccine. In some patients, viruses in the vaccine mutate back to the wild-type form which is virulent and thus causes polio itself. Obviously, this is a very undesirable side effect. That is why several countries, including Belgium, switched to a 'killed' polio vaccine (see next section).

To gain the advantages of using live vaccines, a consortium of researchers and sponsors is looking for an adapted live polio vaccine that is genetically more stable than the old vaccine (see box in Section 4 'Antwerp Poliopolis is helping to work towards a new polio vaccine' on page 27).

Giving live vaccines to people with reduced immunity may also entail a risk of increased reproduction of and/or invasive infection by the micro-organism in the vaccine even though it has been weakened. This can lead to complications. Examples of vaccines that these people need to pay attention to are the oral polio vaccine, the measles vaccine and the yellow fever vaccine.



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Inactivated or killed pathogens

A second commonly used method of producing vaccines is by inactivating or killing the virus or bacteria. All the pathogen's antigens for eliciting a defensive response remain available, but the virus or bacterium has been rendered harmless. Examples of this include vaccines against whooping cough, hepatitis A, rabies, and influenza.

However, the components in these vaccines can no longer multiply in the body of the vaccinated person. As a result, they usually lead to a more narrow and less intense immune response than live but weakened vaccines. To stimulate the immune system sufficiently, additives (called adjuvants – see below), such as aluminum salts, are usually added to these vaccines. But even then, amongst other shortcomings, hardly any cytotoxic T-cells are produced that target the vaccine antigens (see Section 4).

This methodology is often chosen for viral vaccines because virus particles are easy to separate and purify from the cells in which they are grown. In addition, the coat and other external viral proteins remain intact during inactivation. As a result, they still evoke a very specific immune response with less risk of side effects than from live vaccines.

The production of inactivated vaccines is usually similar to that of live vaccines. However, once purified, the virus or bacterium is inactivated/killed by treatment with chemicals such as formaldehyde or 1,3-propiolactone.

VACCINE TIMELINE¹



Subunit vaccines

If the specific antigen that elicits an optimal protective immune response after vaccination is known, the researchers often opt for vaccines that contain only that one antigen and not the whole pathogen. These are called subunit vaccines. They fall into different categories:

Toxoid vaccines

The bacteria *Clostridium tetani* and *Corynebacterium diphtheriae* - the causative agents of tetanus and diphtheria respectively - produce and secrete toxins (toxins). It is these toxins that cause the symptoms and not the bacterium itself. It has long been known that specific antibodies are capable of neutralizing these toxins. The symptoms of tetanus or diphtheria then stop. Based on this knowledge, vaccines were developed containing 'detoxified' variants of these toxins. These variants no longer have pathogenic properties but are capable of inducing a protective immune response. This type of vaccine is called a toxoid vaccine.

Polysaccharides and conjugated vaccines

Antibodies against complex sugar molecules on the outside of certain bacteria are also known to have an antibacterial effect. In molecular language, these sugar molecules are called 'polysaccharides'. Vaccines against meningococci (*Neisseria meningitides*) and pneumococci (*Streptococcus pneumoniae*) are based on polysaccharides extracted from these bacteria.

Polysaccharide vaccines are usually effective in adults but less effective in children under the age of two. The reason is that the immune systems of young children are still insufficiently developed and the fact that polysaccharides do not elicit a response from T-cells. These limitations can be eliminated by coupling the polysaccharides to proteins, which does produce a T-cell response. This is known as a conjugated vaccine. With the vaccine against, for example, *Haemophilus influenzae B*, purified polysaccharides are chemically bound to a carrier protein, which leads to a robust immune response even in very young children⁴⁵.



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Recombinant protein vaccines

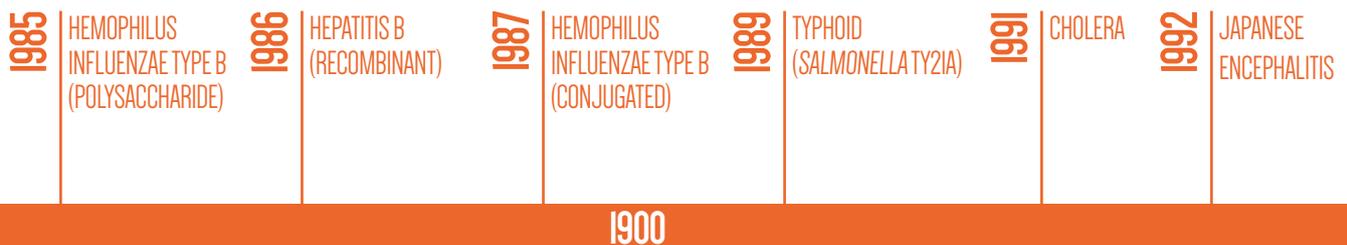
Recombinant protein vaccines are also subunit vaccines. They are manufactured by recombinant DNA techniques. In this process, pieces of genetic material from the virus or bacterium are incorporated into another cell. This can be, for example, another bacterium, but could also be a yeast cell or cells from an insect. Generally, cells are selected that can be grown easily and in large quantities in the laboratory. For this purpose, fragments of genetic material from the pathogens are used that 'code' for an antigen or a group of antigens that are crucial for eliciting an effective immune response against the entire pathogen. The host cells are then cultured, which produce the antigen in bulk. This is purified and forms the basis of the vaccine.

These are therefore pure protein vaccines - there is no DNA in the vaccine itself. Recombinant vaccines include the acellular whooping cough vaccine, the HPV vaccine, and the hepatitis B vaccine. There are many reasons for developing recombinant subunit vaccines, regardless of whether or not they replace existing vaccines. Recombinant protein vaccines are purer and have fewer side effects (e.g. local injection site reactions), they can be modified to elicit a controlled but more potent immune response (e.g., the anthrax vaccine),

are better characterized (the full sequence/ composition is known) and they make it easier to vaccinate against several variants of the same disease maker at the same time (e.g. HPV). The latter are called 'multivalent' vaccines.

Recently, the research community has focused heavily on vaccines produced by recombinant techniques. The following vaccines are currently undergoing laboratory tests: vaccines against the bacterium *Francisella tularensis*, which causes tularemia, also known as 'rabbit fever'. This is a rare but dangerous disease that can be transmitted by animals, flies, ticks, or surface water. Research to link the *Francisella tularensis* O antigen to the ExoA carrier protein of *Pseudomonas* strains via recombinant DNA technology has resulted in a conjugated vaccine that offers protection against the bacteria in laboratory animals. Similar experimental vaccines are under development against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Shigella flexneri*, *Escherichia coli*, and *Burkholderia pseudomallei*⁴⁵.

VACCINE TIMELINE¹

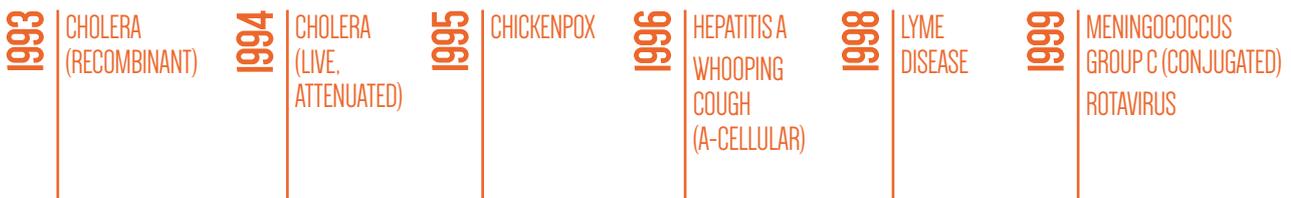


Excipients or adjuvants

In 1925, French vaccine researcher Gaston Leon Ramon found that he could induce a more powerful immune response by adding an adjuvant to a self-developed toxoid vaccine for diphtheria. He used the name 'adjuvant' for substances that boost the vaccine response. Not long after Ramon's discovery, other researchers found that aluminum salts and water-oil emulsions enhance the immune response brought about by inactivated vaccines and subunit, or protein vaccines.⁴⁶

Adjuvants can improve the effectiveness of vaccines in various ways. They can have a 'depot' effect, which means that due to their physical properties they release the antigens in the vaccine slowly so that the stimulation of the immune system lasts longer. They can also cause a local inflammatory reaction at the injection site, attracting more white blood cells⁴⁷.

Numerous substances are currently being studied as potential adjuvants with the aim of giving vaccines better and more targeted immunity or reducing the required dosage so that vaccines can be produced more cost-effectively or adding to the possible ways that vaccines can be administered - for instance, by absorption across the mucous membranes of the mouth, lungs or skin instead of by injection. New adjuvants include cell wall components of bacteria, synthetic polymers, minute fat globules, etc⁴⁸.



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Gene vaccines

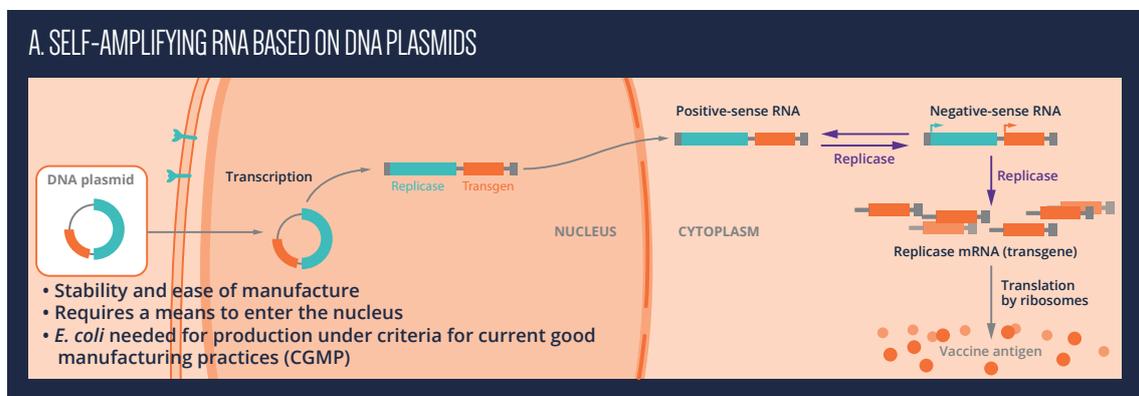
A broad immune response involving both cytotoxic T-cells and memory B-cells is mainly evoked when the antigens are produced in the body cells of the vaccinated person. That is, after all, exactly what happens in 'normal' viral infections: the viruses inject their genetic material into the cells of their host and this then instructs the cell's own molecular machinery to produce new viruses. Live viral vaccines have retained these properties. Gene vaccines, which contain a piece of DNA or RNA from the pathogen, mimic these characteristics^{49 50 51}.

The fragments of genetic material from the virus or bacteria are inserted into a 'genetic vector'. This is a DNA or RNA molecule put together so that it contains the codes instructing our own cells to produce the antigens. Some gene vaccines

are injected in 'naked' form (Figure A), others are packaged in a protein coat (e.g. an empty virus) (Figure B) or introduced into a bacterium.

In 'naked' gene vaccines, the genetic material is delivered in the form of RNA or DNA incorporated into a 'plasmid'. In both cases, absorption efficiency by our body's cells is rather low. This is why experiments are being carried out with, for example, gene vaccines whose mRNA is packaged in tiny fat vesicles that are much more easily absorbed (see drawing on page 68).

Virally packaged gene vaccines inject their genetic material into their target cells (e.g. lung cells) with higher efficiency. The viral packaging thus acts as a kind of courier service that disposes of its genetic package at the host cell's home.



VACCINE TIMELINE¹

2006	HUMAN PAPILLOMAVIRUS (RECOMBINANT)	ROTAVIRUS (LIVE, ATTENUATED) SHINGLES (HERPES ZOSTER)	2008	ROTAVIRUS (MONOVALENT)	2009	MENINGOCOCCUS TYPE A (MONOVALENT)	2010	PNEUMOCOCCUS (CONJUGATED, 13-VALENT)	2014	HUMAN PAPILLOMAVIRUS (9-VALENT) MENINGOCOCCUS TYPE B
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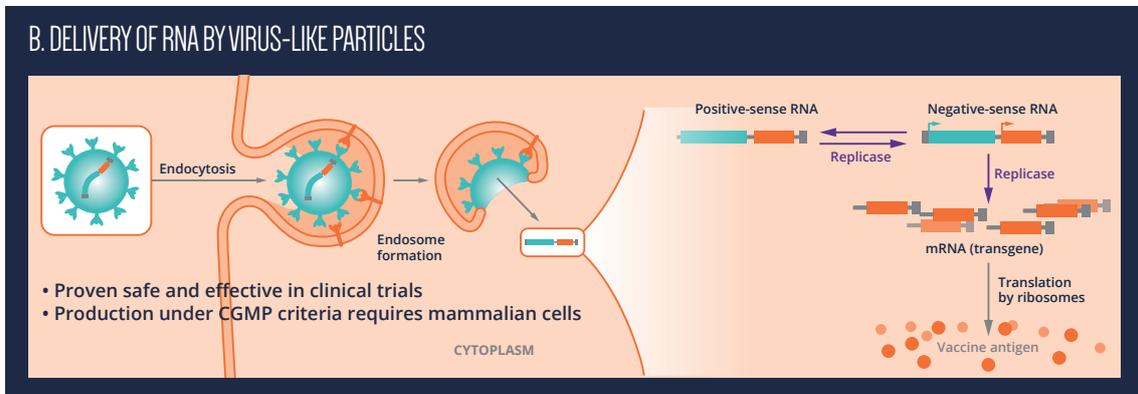
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With gene vaccines packaged in a bacterium, the expectation is that the bacteria will produce the antigens and present them on their own cell surfaces. The immune system will recognize these antigens as foreign and react against them.

Although the basis of the vaccines consists of a substance that is completely new to vaccine development, both naked and viral or bacterial-packaged gene vaccines mimic natural infection as far as possible. At present, the Janssen Pharmaceutica Ebola vaccine (see Chapter 6), is the only gene vaccine to have been approved by the authorities. However, they are the focus of a great deal of research and they are certainly successful in laboratory animals. Gene vaccines are currently under development for HIV, rabies, measles, and the influenza virus. Gene vaccines

are also being extensively used in the recent COVID-19 pandemic (see Chapter 7).

Gene vaccines offer many benefits. DNA and RNA molecules are easy to manufacture and, thanks to today's sophisticated recombinant DNA technology (see, among others, the VIB Facts Series Dossier 'CRISPR-Cas genome editing in medicine'), new vaccines can be developed much more quickly. DNA gene vaccines are also more stable at normal and higher ambient temperatures than traditional vaccines. This eliminates the need for the cold chain. Gene vaccines also allow the specificity and strength of the immune response to be adjusted more flexibly in the desired direction. In short, the platform for gene vaccine development looks very attractive, although obstacles remain to be overcome.



2015	EBOLA (WITHOUT PERMIT, EXPERIMENTAL)	DENGUE FEVER	2016	CHOLERA (ORAL)	2018	TYPHOID (CONJUGATED)	2019	EBOLA (APPROVED)	2020/2021	COVID-19
	MALARIA (IN PILOT SETTINGS IN AFRICA)	MENINGOCOCCUS TYPE B								
2000										

6. From design to impact

The development path of a vaccine

The demand to rapidly develop vaccines against SARS-CoV-2, the cause of COVID-19, comes at a time when there has been a huge acceleration in scientific research. This acceleration has been made possible thanks to a series of technological breakthroughs in genomics, structural biology, systems biology, biomedical data generation and processing, functional analyzes at the individual cellular level, and so on. The merging of these developments has also ushered in a new era for vaccine development. This is also necessary because over the past decade the research community and the vaccine industry have been asked to respond quickly and appropriately not only to the COVID-19 pandemic but also to epidemics such as the swine flu (H1N1 influenza), Ebola, SARS, zika, and MERS.

Nevertheless, despite the explosion of scientific knowledge and potential, vaccine development for many diseases is very difficult and remains an endurance event. For example, despite decades of research, there is still no commonly used vaccine

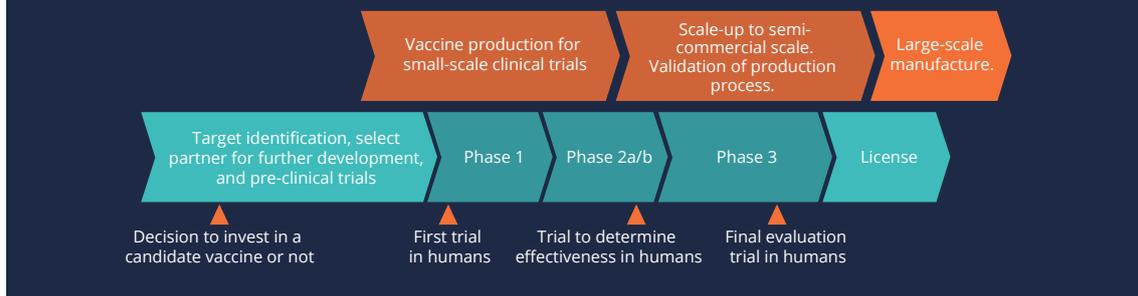
against HIV/AIDS or malaria. Two conditions that cause thousands to hundreds of thousands of deaths every year and on a global scale, in addition to the enormous burden of disease they cause for patients, families, and society.

Although there are points of comparison between the development of a medicine and a vaccine, there are also substantive and procedural differences: with medicines we treat people who are already ill, with vaccines we perform a medical intervention on healthy people to protect them from a risk of becoming infected in the future and whether or not they become (seriously) sick. That is why we believe - at least at a gut-feeling level - that the safety of vaccines must be much higher than that of medicines.

Nevertheless, traditional vaccine development follows a research path similar to that of medicines. We can divide that pathway into several phases: the discovery and preclinical phase, the clinical phase, the approval and registration, and finally the production and market introduction (see illustration "The traditional vaccine development paradigm" on page 45). Each phase is followed by a period of evaluation and consultation to ultimately decide to continue to the next phase or to stop the research.

Vaccine development is therefore a long and costly process, lasting many years and costing hundreds of millions of euros, with no guarantee of success. Under normal conditions, it takes ten to fifteen years for a new vaccine to come on the market.

THE TRADITIONAL VACCINE DEVELOPMENT PARADIGM – 5 TO 10 YEARS ⁵²



Discovery and preclinical phase

Every development of a new vaccine begins with fundamental scientific research into the pathogenic virus or bacterium to gain full insight into its characteristics, functioning, and how it interacts with the human body. Today we have the technology to quickly read out the entire genetic material (the genome) of a virus or bacterium. With the help of computer analyses, this immediately gives us a good insight into the properties of the potential pathogen. This initial insight, together with results of chemical and biological experiments in the laboratory, lets us select antigens with potential for vaccine development (the discovery phase).

Based on all this knowledge, a decision is made as to which underlying technology (see Chapter 5) is most likely to produce a safe and optimally effective vaccine. There is also an investigation into which adjuvants, carrier proteins, and vectors could improve the quality of the vaccine. Subsequently, one or more candidate vaccines are designed that undergo a rigorous selection process based on laboratory and animal model research (the preclinical phase).

The use of laboratory animals is unavoidable (see box on page 48). Without the use of laboratory animals, no new vaccine could be properly evaluated before allowing risky and inadequately tested candidate vaccines to be used on humans. We can all agree that this is totally undesirable.

A large number of candidates are rejected during this preclinical phase. It is estimated that less than half survive this phase.⁵³

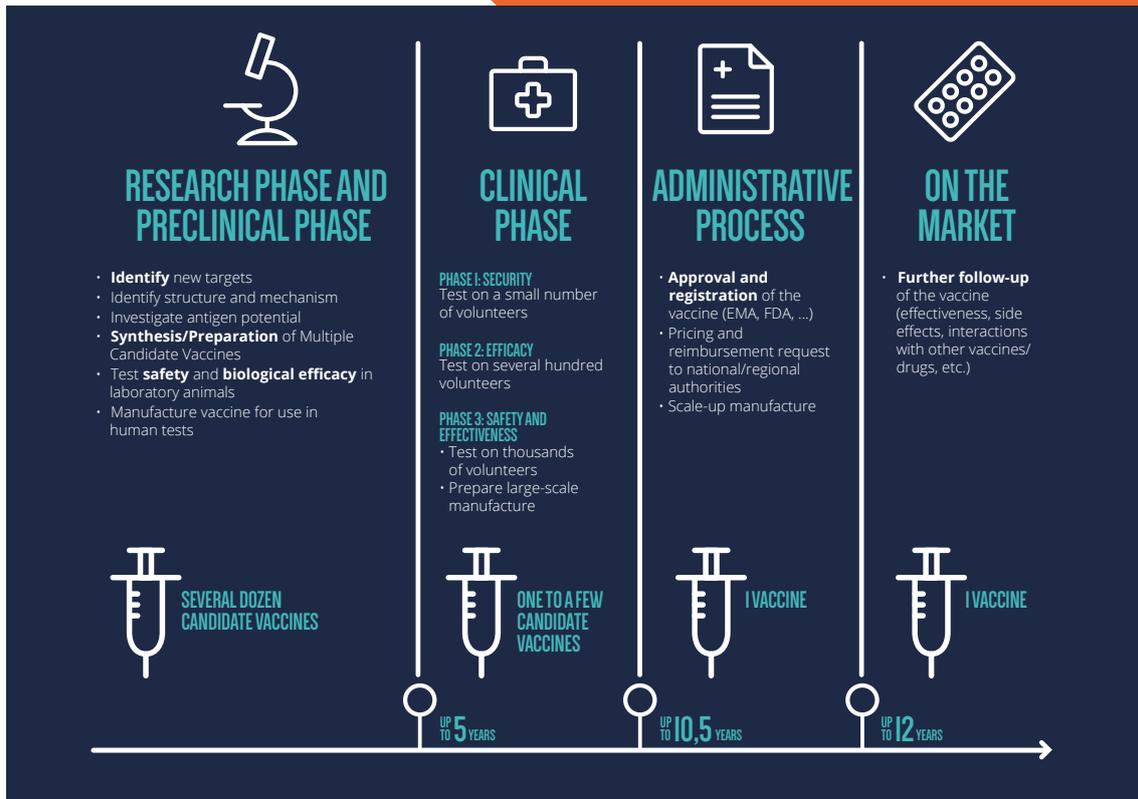
Clinical phase

A candidate vaccine that is judged safe and promising based on the preclinical phase will then move into the clinical phase, where it is tested in humans. There are three different phases:

- In a phase I clinical study, the safety and effect of the vaccine is analyzed in a small number of volunteers. Checks are made to see whether serious side effects occur.
- If everything turns out to be safe, the vaccine will be tested in a larger group of people in phase 2 clinical trials. The researchers first examine which doses and which vaccination schedules can offer optimal protection - for instance, are the correct immune cells stimulated, are neutralizing antibodies produced and how long do they remain measurable and active? Monitoring for side effects continues in this phase.

- If phase 2 yields hopeful results, a phase 3 clinical study will be conducted in which a much larger group will be vaccinated. This phase is designed to answer the key question of whether vaccine recipients are better protected against infection than unvaccinated subjects. The vaccine is tested on thousands to tens of thousands of people. The tests are usually placebo-controlled. That is, one group receives the real vaccine while another group gets a placebo (i.e. fake) vaccine. It is also double-blind, which means that neither the test subjects nor the health professional know whether someone has received a real or placebo vaccine. Only the researchers have this information. Safety is central to the study in this phase too.

Participation in these clinical studies is always on a voluntary basis and people can make informed decisions about this.



Approval, registration and production phase

Only if the results are positive across the board will a candidate vaccine be authorized for general use by the competent public authorities. This approval is granted in the EU by the EMA, the European Medicines Agency, and in the US by the Food and Drug Administration (FDA). For Africa, the WHO grants prequalification status.

After that authorization, the manufacturer in Belgium must submit a dossier to the Superior Health Council, which decides whether the vaccine should be recommended for specific target groups and/or added to the basic vaccination program. A dossier must also be submitted for reimbursement. Without this reimbursement, the cost of the vaccine will be borne by the vaccinated person.

The manufacturer must also ensure that the production of the vaccine is scaled up so that enough doses are available for those who need it. Finally, follow-up research will take place after the vaccine has come onto the market. The aim is to detect new, usually very rare, side effects and to check whether the vaccine offers adequate long-term protection. This is sometimes called phase 4 of the clinical trial.

EXPERIMENTAL ANIMALS IN VACCINE STUDIES

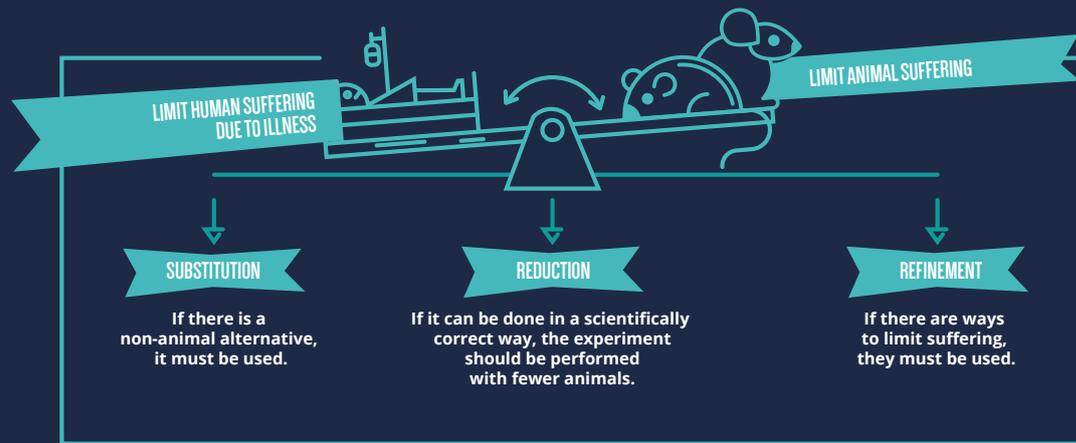
Necessity

Much research into the safety and efficacy of vaccines is done without the use of experimental animals. These are experiments in test tubes, on cell cultures (in vitro research) or with computer models. Nevertheless, research on laboratory animals (in vivo research) is not only important but unavoidable if we are to better understand the mechanisms behind vaccinations and evaluate both the safety and efficacy of new vaccines before starting clinical studies in humans.

Well-considered use

Researchers conduct experiments on animals only after thorough consideration (see the VIB Facts Series Dossier 'Animal experiments'). For every new project, they carefully weigh the use of experimental animals against its importance for human health. Maximizing animal welfare comes at the top of the list of priorities:

- *Researchers may only work with laboratory animals if they have received education and training in animal welfare and the ethical use of laboratory animals in experiments.*
- *Animal experiments can only be started if they have been approved by the 'animal testing ethics committee' of the university concerned. To get this approval, researchers need to put the case for why they need animals for the research, describe in detail the experiments they will perform, say how many animals will be used (and why that many are needed), and demonstrate that the experiments have not already been performed.*
- *Researchers are expected to strictly apply the 3R principle: replacement, reduction, and refinement of animal experiments. In concrete terms, this means that they must strive to replace animal experiments as far as possible with experiments in test tubes, with cell cultures or with computer models. Furthermore, they must limit the number of test animals to an absolute minimum and perform the experiments in such a way that animal suffering is reduced as far as possible and so that animal welfare is maximized. For each experiment, laboratory animals with the lowest possible level of consciousness should also be chosen. Animal suffering is assumed to increase as their level of consciousness increases. Monkeys are higher than mice in that ranking, which in turn are higher than zebrafish.*
- *There are important medical and scientific questions that doctors and researchers can only answer through research on living animals in which complex interactions take place between cells, tissues, and organs. It cannot be emphasized enough that research with laboratory animals is now one of the most heavily regulated research activities. It is often thought that this research is performed on monkeys, cats and dogs, but the most commonly used laboratory animals are, in fact, mice, fruit flies, and zebrafish, which are bred specifically for research. The animals are housed under the best conditions. Their welfare is even individually recorded (e.g. in mice) and monitored.*



Well-considered animal testing has its place in the search for new vaccines

Through the valley of death

If the science is against it

The development of a vaccine is regarded by some as a journey through at least four successive valleys of death: four phases in which even potentially promising vaccines die anyway^{1 54}. The design and preclinical phase is the first valley of death. Candidate vaccines fail because the wrong antigens, additives, or vectors were chosen, because unforeseen safety aspects arise, or the vaccines are not effective enough in laboratory animals. In retrospect, it often turns out that a lack of knowledge about the immune system, the micro-organism, or the interaction between the two is the cause of the problems.

However, much progress has been made in recent years as a result of private and public initiatives helping to overcome this initial hurdle. An example of this is the Coalition for Epidemic Preparedness Innovation (CEPI). This innovative global partnership between public, private, philanthropic,

and civil society organizations was established after the 2014-2015 Ebola epidemic in West Africa⁵⁵. The partners are working together to accelerate the development of vaccines against emerging infectious diseases and to provide fair access to vaccines for people during outbreaks. Belgium, along with several other European countries, is a sponsor of CEPI.

If the financial resources are insufficient for the (expensive) clinical phase

The second obstacle is the clinical trial phase, in which the vaccine is tested on ever-larger groups of people. This is by far the most expensive phase in the development of a vaccine. It accounts for more than two-thirds of the total cost. The last phase in particular requires large investments.

Figures speak volumes: a recent analysis of the cost of new vaccines for 11 priority infectious diseases

indicates that from the design phase to phase 2, the investment ranged from USD 14 million to USD 159 million per vaccine (EUR 13 million to EUR 145 million)⁵³. However, this did not take into account the attrition rate, which is the investment in candidate vaccines that did not reach the finishing line.

Taking into account the depreciation of these investments and considering the cost over the entire development path, including phase 3 clinical trials, the estimated investment increases to 137 million to 1.1 billion dollars for each vaccine that reaches the approval phase (125 million to more than 1 billion euros)⁵³.

The clinical trials are so expensive because:

- The vaccine at this stage must be produced under highly controlled conditions in dedicated production facilities
- Phase 3 trials must be conducted in different countries
- Increasingly larger groups of people are being vaccinated, which requires the commitment of numerous health professionals, doctors, data analysts, and researchers.
- Several independent partners must be involved in the investigation

These major financial efforts often go far beyond the resources of academic research centers or smaller biotech companies. In general, only large pharmaceutical companies, large foundations, or public institutions (governments) have the financial clout to carry out such clinical trials.

However, pharmaceutical companies are not charities. One of their goals is to make a profit. That is why they often face a dilemma: if such a large investment is required for a new vaccine, they will also want a high enough return to recoup that investment if they are successful. This is difficult if the vaccine is aimed at a limited market or is for an infectious disease that occurs mainly in countries with little capital. Unless governments or philanthropic organizations such as the Bill & Melinda Gates Foundation support this, few private companies are willing to invest in such vaccines (see also box 'Vaccines on and off the breakdown lane' on page 51).⁵⁶

VACCINES ON AND OFF THE BREAKDOWN LANE

The research into vaccines for severe acute respiratory syndrome (SARS) and Zika was stopped early because the epidemics came to an end before the vaccine could be tested on large groups. There was no longer a risk of infection in the population - the viruses had gone 'spontaneously' extinct. There was therefore no longer a population to vaccinate in order to test the efficacy. Nor would there have been a population to vaccinate if the vaccine had been approved.

Several governments had earmarked large budgets to vaccinate their populations for these diseases but withdrew these funds. Companies committed to developing these vaccines had made major investments but were left with no income.

It was different for the Ebola vaccine. During the Ebola outbreaks in West Africa in 2013-2016, philanthropic organizations, governments, and pharmaceutical companies joined forces to search for an effective Ebola vaccine. Then too, the outbreak was largely contained before the effectiveness of the vaccines could be tested extensively. So these vaccines also risked being left by the wayside.

However, some companies continued their research efforts so that during more recent outbreaks in Central Africa, this time mainly in Congo and the neighboring countries, two vaccines could be deployed. This occurred at a time when there was little large-scale data on the efficacy of these vaccines and no official approval for widespread use had been given by any government. It goes without saying that the vaccinations were administered on a voluntary basis and only after consultation with and approval by the local authorities, under the supervision of independent experts and in cooperation with medical aid organizations that had previously worked in the field (including Doctors Without Borders).

The first vaccine was a recombinant vaccine consisting of an Ebola virus surface protein packaged in a live attenuated virus. This vaccine was developed by the American pharmaceutical company Merck⁵⁷. The other vaccine was a gene vaccine from the Belgian company Janssen Pharmaceutica, part of the American group Johnson & Johnson (J&J)^{58,59}. The search for the vaccine had taken Janssen Pharmaceutica eleven years and had cost over 700 million dollars (630 million euros). Half of this was paid by the European Commission and the US government. That's why the company donated a million doses of the vaccine to Congo and other African countries for free⁶⁰.

The Ebola vaccines were finally approved by the EMA, the European Medicines Agency, at the end of 2019. Later, the American authorities and various African countries also followed. Hopefully, these vaccines can avert another Ebola outbreak.

If the roll-out is delayed

Once a new vaccine is ready for use, it encounters a third hurdle: the reluctance of national governments to roll it out widely without taking into account the balance between medical and social added value compared to cost. Today it is no longer sufficient that the safety and efficacy of a new vaccine have been extensively tested. Governments want value for money and are asking manufacturers to demonstrate the cost-effectiveness of their new vaccine. This means estimating the gains from improved health and greater life expectancy and setting this against the financial price that society will have to pay for it. If governments think the cost is too high for the expected health benefit, they will not reimburse a new vaccine and/or include it in the advice list for vaccinations. On the other hand, governments will try to keep the cost down by playing off producers of equivalent vaccines against each other by issuing a call for tenders.

Even if the cost-benefit analysis is favorable, a rapid and broad introduction can still be delayed, even in industrialized countries. For example, the meningococcal B vaccine was approved for the United Kingdom in January 2013. In March 2014 it was recommended for general use. However, it wasn't until May 2015 that the vaccine would become part of the standard vaccination program. It then took another twelve months to complete the tender procedure for the roll-out⁶¹.

Another example is the rotavirus vaccine in Flanders. The rotavirus causes infectious diarrhea in infants. Until the introduction of the vaccine, more than 5,000 children were hospitalized each year with severe rotavirus infection. In 2006, two live vaccines were approved by the European authorities. Belgian research from 2010-2011 shows that the vaccines offer 91% protection against hospital-

ization⁶². Although the Belgian vaccination calendar recommends this vaccination for all infants, the vaccines are only partially reimbursed. This contrasts with all other vaccinations in the basic vaccination program. These are provided free of charge. It is therefore not surprising that the vaccination coverage for the rotavirus is much lower than for other childhood vaccinations²⁸.

Whether an approved vaccine ultimately finds its way to the citizen is indeed often a matter of dollars and cents. Countries not wealthy enough to purchase vaccines at current market prices are supported by Gavi, the "Vaccine Alliance". Gavi is a partnership of UNICEF, the WHO, the World Bank, the Bill & Melinda Gates Foundation, and several individual countries. With help from Gavi, more than 760 million children have been vaccinated in the world's poorest countries. These campaigns have so far saved an estimated 13 million lives⁶³.

In addition to the cost of the vaccine itself, many countries have limited capacity to expand their vaccination programs. The inclusion of an additional vaccine requires extra health personnel and infrastructure. These are often not available in countries with poor healthcare. The expansion of vaccination programs is therefore by no means always a priority, despite support from Gavi.

When local problems throw a spanner in the works

The fourth hurdle, often called 'the last mile', is local and has various aspects. Logistical issues such as production, purchasing, transport, and organization can cause problems, especially in areas with poor communications. For example, many vaccines have a limited storage life and need to be transported and stored at low temperatures. Keeping this 'cold chain' intact can be difficult in tropical regions or where electricity is not always available.

There is an additional need to work closely and extensively with local communities to encourage the acceptance of vaccinations and avoid misunderstandings. For example, vaccination campaigns against Ebola in Central Africa were hampered by public opposition to health workers. The doctors, nurses, and caregivers were thought to be causing the disease. Situations like this undermine the success of vaccination campaigns.

Other local problems may arise at a political level. It is not uncommon for vaccination campaigns to be used as a political tool, which seldom benefits the wide acceptance of vaccines. Political instability as a result of - sometimes armed - conflicts and local wars also always harm the vaccination rate. This sometimes leads to catastrophic outbreaks of infectious diseases among the most vulnerable. The warring parties often even make it impossible for aid workers and health workers to do their jobs. In this way, vaccines for protecting adults and children from disease and premature death, are turned into weapons of war.

7. Some focal points in vaccine studies

A universal influenza vaccine

Symptoms, epidemics, and pandemics

We use the expression 'flu-like illness' for an acute respiratory infection with general symptoms such as fever, headache and/or muscle pain, and a feeling of discomfort. More specific symptoms of respiratory disease include a cough, a sore throat, shortness of breath, and/or a head cold. But it's not just the influenza virus that causes flu-like illness: adenovirus, parainfluenza virus, respiratory syncytial virus (RSV), and rhinovirus also cause these symptoms. It is not certain that it really is the flu until the influenza virus has been detected in the patient with a test^{64 65}.

There is said to be an influenza epidemic if at least 15 out of 10,000 inhabitants show this flu-like illness. Normally, just such an epidemic occurs here every year between October and April. Usually, it peaks in the first two months of the year. The 2009-2010 season was an exception to this. In

that season, the 'swine flu' peaked in October and early November.

An influenza pandemic is said to occur when an influenza virus variant enters the human population in a specific place and spreads rapidly from there to the rest of the world. In addition, if the variant has little or no immunological similarity to virus strains already in circulation, most people will have no immunity to the new virus, with the result that some pandemics result in widespread death and disease.

Examples of influenza pandemics include Spanish flu (1918), Asian flu (1957), Hong Kong flu (1968), Russian flu (1977), and Swine flu (2009). Fortunately, the last pandemic was rather mild, in contrast to the 1918 Spanish flu, which killed more people than the First World War.

INFLUENZA IN BELGIUM⁶⁶

- On average, 500,000 people in Belgium are affected by an influenza syndrome each year, which is about 2 to 8% of the population.
- A severe flu epidemic affects about 10% of the population (1.1 million per 11 million inhabitants).
- On average, 1 in 1,000 influenza patients develop complications requiring hospitalization. That works out to an average of 500 per year.
- More than 90% of the deaths strike people aged 65 and above.
- The European Center for Disease Control (ECDC) estimates that a mildly virulent influenza strain leads to 8 additional deaths per 100,000 inhabitants. With virulent strains, this can be as high as 25 to 45.

The influenza virus

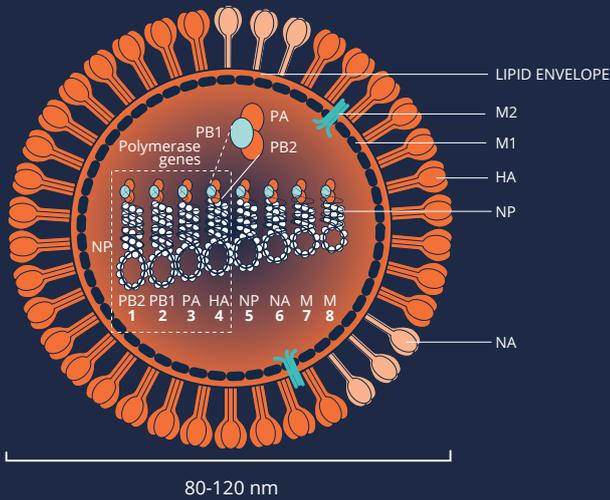
The hereditary material of the influenza virus consists of 8 RNA molecules. These 8 RNA molecules are packed inside a protein shell which is surrounded in turn by an ultra-thin layer of fat, called a lipid coat or lipid membrane⁶⁴ (see the 'The composition of the influenza virus' illustration on page 56). Three proteins are embedded in this lipid coat: hemagglutinin (HA), neuraminidase (NA), and the M2 protein, which forms a channel structure. These proteins are therefore located on the surface of the influenza virus particle.

Three types of influenza virus affect humans: A, B, and C. Influenza A viruses are further subdivided into subtypes according to variations in their haemagglutinin and neuraminidase proteins. There are 16 different subtypes of the HA protein (H1 to H16) and 9 different subtypes of the NA protein (N1 to N9) in birds and a limited number of those subtypes occur in humans. The Spanish, Russian, and Swine flus were all of the H1N1 type. The Asian flu, which appeared in 1957, was of the

H2N2 type and the Hong Kong flu (1968) was of the H3N2 subtype.

We are by no means the only species to be plagued by the influenza virus. Ducks, chickens, pigs, horses, dogs, and cats also have to deal with it. The virus can even jump from animals to humans and from humans to pigs.

THE COMPOSITION OF THE INFLUENZA VIRUS

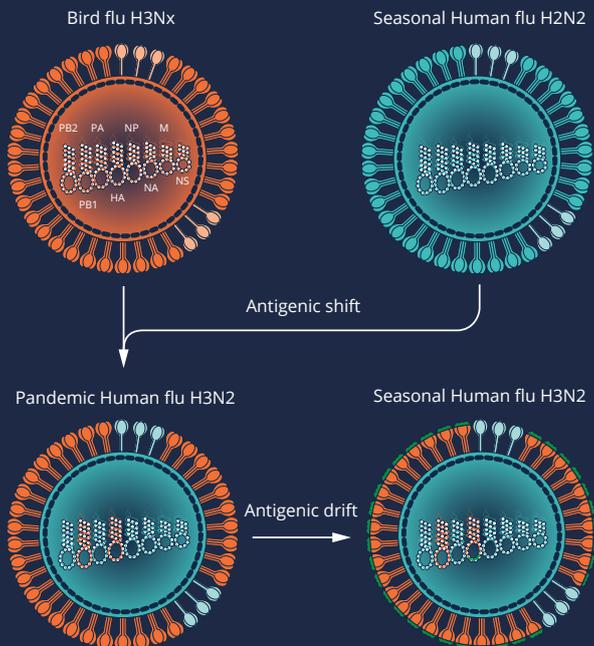


ABOUT DRIFTS AND SHIFTS

Human influenza viruses evolve very quickly because they often mutate (antigenic drift) or exchange entire gene segments between virus strains (antigenic shift):

- Antigenic drift is the process by which the virus's RNA molecules change slightly as a result of spontaneous mutations that lead to limited changes in the hemagglutinin and/or neuraminidase proteins. These mutations are the result of copying errors in the RNA that occur when the virus replicates. Most of these errors are probably neither helpful nor harmful to the virus. However, the errors in the genes coding for the HA or NA can give the virus an important selective advantage.

This is because they allow the virus to evade the immune system. That immune system must then build up a new response each time it encounters a slightly modified virus. That is the main reason why we can get sick from the influenza virus every year even though the virus is still of the same subtype, for example, H1N1. The slight changes in the H1 and N1 proteins mean that our immune systems no longer recognize them effectively.



Source: Reference 64.

- With antigenic shift, new combinations of the 8 RNA molecules arise from the exchange of RNA molecules between two different influenza viruses. This can happen when a human or animal is infected by two influenza virus variants at the same time. When the two sets of hereditary information end up in the same host cell, they spontaneously mix and influenza viruses arise with a new combination of the 8 RNA molecules. Shifts of this type are often the causes of pandemics.

- The example opposite shows how a co-infection of an H3Nx bird flu virus and an H2N2 human virus took place in 1968. This exchange of chromosomes produced an H3N2 virus with parts of both the bird flu virus and the human influenza virus. The almost complete lack of immunity against this virus caused the 1968 pandemic known as the Hong Kong flu - given this name because it was first reported in Hong Kong, although it most likely originated on the Chinese mainland. To date, the H3N2 virus strain returns every year as seasonal flu with its composition slightly changed from the previous year due to antigenic drift.

The influenza vaccine, season after season

The easiest way to prevent flu is to vaccinate. A WHO Committee meets annually to discuss the composition of the vaccine for the following season. Using information from the Global Influenza Surveillance Network - a network of influenza centers spread across 112 countries around the world - the committee identifies and characterizes the strains that caused the previous year's epidemic and tries to predict which strains will be circulating in the current year⁶⁶. Today, that selection is greatly aided by the extension of molecular techniques to characterize circulating virus strains in detail⁶⁷.

The vaccine for the 2020-2021 season includes four different virus strains: two influenza A strains (H3N2 and H1N1) and two influenza B strains (Victoria and Yamagata). The influenza vaccines available in Belgium for this season are all inactivated vaccines⁶⁸. An influenza vaccine is normally only given once per season and the immune response is usually strong enough to last through the flu season. In most cases, the influenza vaccine does not protect for more than six to nine months.

To make an influenza vaccine, the viruses are grown in large quantities. This can be done in either fertilized chicken eggs or cell culture. The 'chicken egg method' dates back to the 1940s but is still used on an industrial scale. However, influenza vaccines are increasingly produced using cell lines. The advantage of this system is that it shortens the development time for a new vaccine and the production system is more reliable. After growth in either system, the viruses are inactivated^{64 67}.

In some countries, live but attenuated influenza vaccines are also used. In 2013, an entirely new

influenza vaccine, obtained through recombinant DNA technology, was approved in the US⁶⁹. The genetic code for the HA proteins of the selected influenza A and B strains are inserted into the genome of baculoviruses that are then used to infect cell lines. These cells, which now produce HA proteins, are then cultured and the harvested HA proteins are purified and processed into the vaccine. This avoids both the need to breed influenza strains and the mass use of fertilized chicken eggs.

A universal vaccine for everyone, and no longer every year

Influenza vaccines can be improved in several ways⁶⁷, but the biggest breakthrough would undoubtedly be a universal vaccine. A vaccine that provides long-term protection against all influenza viruses, regardless of their HA or NA subtypes⁷⁰. In other words, a vaccine that can be used in the same way as the current vaccines for whooping cough, mumps, and measles⁶⁵.

Various research groups, including VIB scientists, are researching just such a universal vaccine. Instead of focusing on the highly immunogenic but constantly changing parts of the viral HA and NA proteins, they are looking for influenza antigens that are much more stable and yet produce a good immune response. There is no shortage of options: the strain of the HA protein, the region with which the HA protein makes contact with the host cell, the extracellular domain of the M2 protein, and certain parts of the M1 and NP proteins⁷¹. Each of these options is being intensely examined in several laboratories. Each option has its proponents and opponents^{67 72}.

Until now, the VIB scientists from Ghent have mainly focused their research on the M2 protein⁷³.

This protein forms an ion channel that pierces the lipid coat of the virus. In other words, the part of it that protrudes from the outside of the coat is accessible to the immune system. However, the M2 proteins are present in much smaller numbers than the NA proteins and, even more so, the HA proteins. It is also obstructed by those other proteins, which restricts the immune system's access to it.

Despite this, antibodies to this outer part of the M2 protein have been shown to slow the multiplication of the virus. By binding to the M2 protein, the antibodies activate other parts of the immune system, including the 'dust cell' macrophages of the lungs, that neutralize the virus particles⁷⁴. Although, admittedly, this immune response remains much less pronounced and robust than against NA- or HA antigens. To increase the ability of M2 to evoke an immune response, alternative strategies are being devised such as fusion with carrier proteins, packaging in nanoparticles, recombinant DNA strategies, linking multiple M2 proteins, etc. (see Chapter 5)^{75 76}.

Laboratory animals vaccinated with the M2 protein - including mice, ferrets, chickens, pigs, and monkeys - were found to build up a defense against not just the virus strain from which the M2 came but also against other strains⁷⁷. Although several M2 vaccines have undergone a first round of clinical studies in humans⁷⁸, none have yet entered a further phase. Consideration is being given to mixing the M2 antigen with other, equally stable, antigens⁶⁷ or even with the antigens that we currently use in the seasonal flu vaccines⁷⁷.

This could have a positive effect on the next pandemic because, if there's one thing we can be certain of, it's that sooner or later we will be confronted with another new influenza pandemic. For that reason alone, the search for long-term protective influenza vaccines will remain a focus of vaccine research.

HIV - a very smart virus

Broken defenses

The human immunodeficiency virus (HIV) targets the CD4+ cells, which are a group of T-helper cells (see Box 4 on page 32). The virus invades the CD4+ cells and hijacks the cell's molecular machinery for its own reproduction (see the illustration on page 60). Ultimately, this hijacking leads to the destruction of the CD4+ cell. The fall in the number of CD4+ cells hinders the functioning of the immune system function and makes the patient more susceptible to secondary infections. This process does not take place overnight but can take several years⁷⁹.

AIDS (acquired immune deficiency syndrome) is the final stage of an HIV infection. The body and the immune system are then affected to such an extent that people can no longer protect themselves against other viruses, bacteria, fungi, and even cancer. Normally harmless infections can then become life-threatening.

Yet today an HIV diagnosis is no longer a death sentence. The current HIV medication gives people the chance to live long and healthy lives without entering the AIDS stage. Antiviral therapy is very effective in inhibiting HIV replication and can even stop it completely. At least for people who have access to these medicines and who faithfully follow their medication schedule⁷⁹.

By making optimal use of the antiviral drugs, the infected person can virtually eliminate the risk of HIV transmission. In this way, medication becomes preventative.

However, due to a lack of widespread HIV testing and limited access to effective medication, much of the world's population is deprived of optimal

screening and treatment (see the box 'HIV and AIDS in Belgium and the world'). This makes it difficult to combat the HIV pandemic. To counter this, better prevention and treatment methods are needed, and empowering information transfer remains a cornerstone of effective prevention policy⁷⁹.

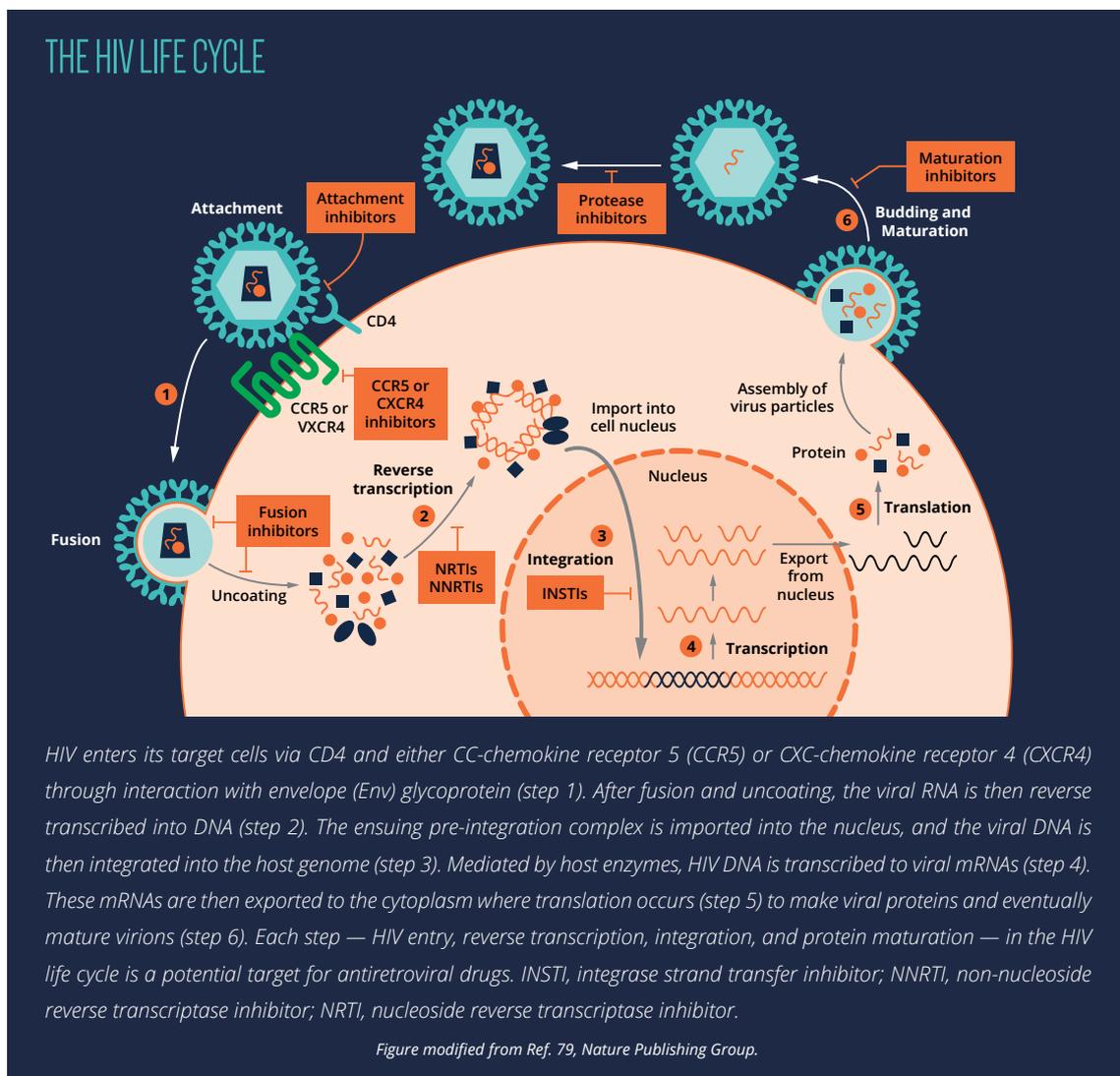
HIV AND AIDS IN BELGIUM AND THE WORLD^{80 81 82}

- *Sciensano recorded 890 diagnosed HIV infections in Belgium in 2017, which is a decrease of 2% in 2016 and 27.5% in 2012.*
- *In 2017, diagnoses in men who have sex with men represented 49% of newly registered HIV infections, and diagnoses in heterosexuals 48%. Transmission via intravenous drug use is exceptional in Belgium and constituted only 1% of HIV diagnoses reported in 2017.*
- *Just under 19,000 people in Belgium were living with HIV in 2017. This corresponds to an HIV prevalence of 1.7 people per 1,000 inhabitants. In 2017, nearly 17,000 people were diagnosed and an estimated 2,000 were not diagnosed. Of all people living with HIV, 10.9% were therefore unaware of their infection.*
- *In 2019, 38 million people in the world were living with HIV and 1.7 million people became infected that year. Almost 700,000 people died of AIDS-related diseases in 2019. Since the start of the epidemic, more than 30 million people have died from this disease.*
- *By the end of 2019, 67% of people with an HIV infection had access to treatment with antivirals. This is an increase of 33% compared to 2009. Nevertheless, 13 million people with HIV still lack antiviral treatment.*

Hidden in the genome

HIV is an RNA virus that converts its RNA genome into a DNA molecule as soon as it enters its host cell. Virologists call this a 'retrovirus' as it copies its RNA into DNA via 'reverse transcription'. This DNA can then be incorporated into the genome of the host cell, where it codes for the production of new virus particles. This incorporation into the host genome makes it particularly difficult to eradicate the virus from infected people⁸³.

The currently-used drugs either obstruct the binding of the virus with the host cell, block the reverse transcription, and/or inhibit the proliferation of the virus. However, once the virus has incorporated its DNA, this cannot be removed with the current generation of medicines. Some form of genome editing would then be required to delete the viral gene sequence, a technology that is the focus of extensive research (see VIB Facts Series dossier 'CRISPR-Cas Genome editing in medicine').



Mutation champion

An important reason why the search for a vaccine for HIV/AIDS is so difficult is the high level of variation of the virus due to mutations^{84,85}. It is estimated that the viral genome incurs one error per replication cycle. This high error rate, combined with a high production of virus particles, leads to a very large number of HIV particle variants, both within the infected person and during transmission to another.

Research has shown that the protein sequence of the most prominent viral antigen - the HIV envelope protein (Env) - changes by 0.6% to 1% each year within the same person^{86, 87}. Envelope proteins can vary by up to 35% between individuals. This great variability is, without doubt, the greatest obstacle to the development of preventive vaccines⁸⁸.

Additional hurdles

Beyond the large sequence variation in the envelope protein, HIV has other tricks to make vaccine development more difficult. The virus can shield the more stable protein sequences of its outer envelope proteins by binding variable sugar groups to them. In addition, it seems that the immune system must be continuously stimulated to organize a sufficiently flexible and dynamic defense⁸⁹.

Moreover, the question arises as to what kind of response by the immune system would protect effectively against HIV. A standard response is clearly far from sufficient because people infected with HIV do produce a robust immune response with antibodies during the first months of their infection. However, this response appears insufficient to block the long-term spread of the virus⁷⁹.

A vaccine may only be able to provide sufficient protection if it can stop the virus from fusing with the host cell in the first place. Viruses would then no longer be able to enter the cell and would also fail to insert their genetic material into the host cell's genome⁷⁹.

In the clinical phase

Despite all these obstacles, researchers have already managed to use vaccination to give macaque monkeys long-term protection against HIV infection⁹⁰, and several clinical vaccination studies have already been conducted in humans. The most notable success was achieved in the RV144 study in Thailand using a recombinant vaccine in which the genetic codes for the HIV-gag, HIV-env, and HIV-pol proteins were inserted into the genome of a canarypox virus. That virus is harmless to humans because it is unable to reproduce itself in human cells.

The study involved 16,000 Thai men and women. The subjects received four injections of this vaccine followed by two injections of HIV-env protein. After 3.5 years, repeated vaccinations were found to result in 31% fewer infections⁹¹. People who produced IgG antibodies, in particular, proved to be better protected (48%) than people who produced IgA antibodies alone. Additional analyses showed that the two types of antibodies compete with each other. However, the IgG antibodies were more effective than IgA antibodies in stimulating a virus-killing response by immune cells. If IgA antibodies were already bound to a virus, this blocked access by the IgG antibodies⁹².

This study has two important takeaways: we are still learning new things every day about the interaction between HIV and the human immune system. First, that this increase in knowledge is indispensable for the development of successful HIV vaccines. Second, that, despite the limited protection generated, vaccination against HIV can be successful. Based on this proof-of-principle, additional clinical studies have been and are being designed with alternative strategies to elicit a more robust response. These strategies include different and later vaccine boosts, the use of neutralizing antibodies, and a focus on a more balanced immune response⁹³.

This means that HIV vaccine research is far from running out of road. HIV is, and remains, one of the 'smartest' viruses we face. A virus that not only manages to bypass a person's natural defenses for many years but also presents us with immense challenges in the search for a safe and effective vaccine.

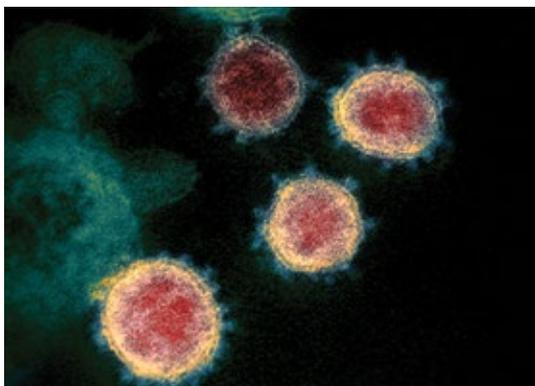
Finding a COVID-19 vaccine at pandemic speed

A market in Wuhan

On December 30, 2019, health authorities in Wuhan, the capital of China's Hubei province, reported a cluster of patients with pneumonia of unknown cause. Almost all the patients had visited the same local food market. The local authorities faced a mystery. The market was quickly closed, but the condition continued to spread. It was transmitted from person to person. But what was the cause? Perhaps a new virus?

A few days later, virologists from Shanghai sent the full genome sequence of the Wuhan virus to GenBank⁹⁴, a public international database for gene and genome information. That information was incredibly important: it launched the development of diagnostic tests, the search for drugs, and a race for a vaccine.

The pathogen appeared to be a coronavirus^{95, 96}. These are single-strand RNA viruses named after the 'crown' of spikes around each virus particle. However, it was also a 'new' (or at least unknown) virus with a genome of 29,903 RNA letters.



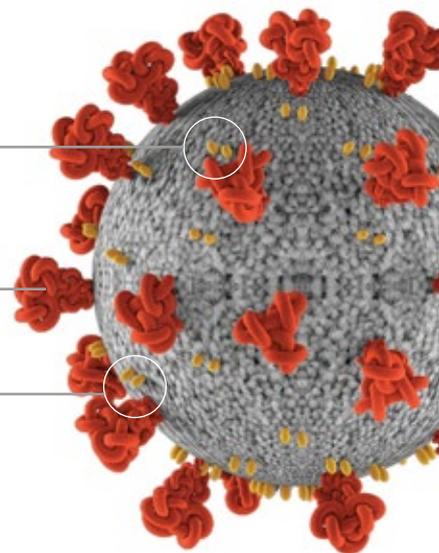
And yet this virus wasn't *entirely* unknown. It turned out to be a close relative of the previously-identified SARS and MERS viruses⁹⁷. That is why it was named SARS-CoV-2. The disease the virus causes has since been named COVID-19, which is short for 'coronavirus disease 2019'. SARS-CoV-2 is believed to have passed from animals to humans. The virus probably originated in a bat because it is known that several coronaviruses infect bats. There may also have been intermediate hosts to facilitate the jump from bat to human. Molecular data points a finger at pangolins⁹⁸.

The genome sequence also immediately gave insight into the properties of the virus. As soon as the genome sequence was published, researchers were able to prove that the virus enters the host cell by binding to the ACE2 receptors of lung cells via the main protein on its surface - the 'S' or 'Spike' protein⁹⁸.

E-protein

S-protein or 'spike' protein

M-protein



Getting close to home

The rest of the story has now affected everyone personally. Due to the lack of any immunity among the local population in Wuhan, the virus spread rapidly in the area. Because Wuhan is a global hub city with 11 million inhabitants, airline passengers spread the virus all over China, then Asia, and later Europe and the rest of the world.

Because SARS-CoV-2 spreads through droplet infections from person to person, it is important to meet as few people as possible, keep a safe distance from others, wear mouth-and-nose masks, and disinfect your hands. Starting in March 2020, half the world went into a form of collective quarantine to slow the spread of the virus.

The symptoms of COVID-19 resemble those of influenza: fever, dry cough, fatigue, shortness of breath, sore throat, headache, muscle pain, chills, nausea, blocked nose, and diarrhea. A more distinctive symptom of COVID-19 is the - usually temporary - loss of taste and smell. Those rather mild symptoms can progress to pneumonia, coughing up of blood, thrombosis, blockage of the kidneys, sepsis, heart infarct and stroke, and even death.

Some people who become infected suffer little from the virus, while others - particularly the elderly - run the greatest risk of serious complications, admission to an intensive care unit, or death. Despite this, COVID-19 can strike anyone. Young people have also died from the SARS-CoV-2 virus.

A vaccine at 12 months

It was clear from the outset of the epidemic - and the later pandemic - that a protective vaccine would play a key role in stopping the virus from spreading further. However, as outlined in Chapter 6, the normal procedure for obtaining a vaccine takes ten years and the world did not have that long. Consequently, the pressure on researchers, vaccine developers, and governments to expedite normal procedures became intense.

International collaborations and government measures quickly emerged to drastically shorten the development time for COVID-19 vaccines. In addition, a multi-track approach was used: dozens of academic labs, research institutes, hospitals, biotechnology, and pharmaceutical companies immediately began work and entered the race for a vaccine. Some research organizations and pharmaceutical companies were already experienced with SARS and MERS, while others used alternative platforms based on their own vaccine expertise.

How much could development time be shortened to arrive at a fully-fledged COVID-19 vaccine? The Coalition for Epidemic Preparedness Innovations (CEPI) made a strong commitment to cutting the development time by 90%⁵⁵. It also predicted that, if everything went well, it should be possible to have a vaccine ready in twelve months, so by the spring of 2021. This can only be done if the traditional phases in vaccine development and production, which normally follow each other, are run in parallel instead (see the illustration on page 66). It also insisted that researchers, industry, and the government should join forces and act quickly so that no day, hour, or minute would be wasted on unnecessary administrative formalities⁵².



Indeed, the development of a COVID-19 vaccine really took off. In mid-November 2020, the WHO reported that there were 212 COVID-19 vaccines in development¹⁰⁰. Most vaccines (164) were still in the preclinical phase, but 48 candidate vaccines had already moved on to the clinical phase. Eleven vaccines (see table on page 67) were already in a phase 3 clinical trial by mid-November. This is the phase where the effectiveness of the vaccines is tested in large groups of people (thousands to tens of thousands). These are placebo-controlled studies in which some volunteers receive a genuine vaccine and others a placebo i.e. 'fake' vaccine. By comparing the final infection rate and disease symptoms of the two groups, it is possible to determine how effective the vaccine is.

The eleven most advanced vaccines include four Chinese vaccines (two from the same Chinese state-owned company, Sinopharm), one from Russia, and a number of European or American companies or research institutes. The latter include vaccines from Oxford University Hospitals/AstraZeneca, Moderna/the US government (NIAID), BioNTech/Pfizer and Janssen Pharmaceutica (JNJ).

BioNTech/Pfizer, Moderna, and Gamaleya announced interim results of their phase 3 studies as early as the first half of November 2020. This showed that they would provide 90% to 95% protection. The Oxford/AstraZeneca vaccine provided protection between 60% and 90%, depending on the dose administered.

The various vaccines use different technologies. Chinese vaccines in particular are based on inactivated SARS-CoV-2 viruses. The Russian

and Western vaccines use recombinant DNA techniques and/or gene vaccine technology.

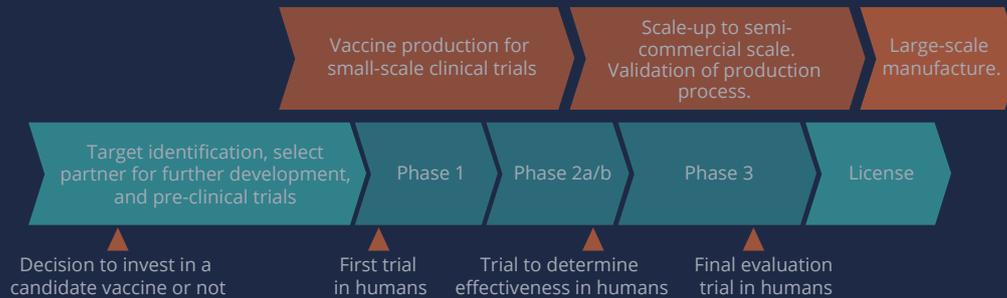
As an example, the composition and development path of the BioNTech/Pfizer vaccine and the Janssen Pharmaceutica vaccine are explained in detail in boxes on pages 68 and 69.

At the time of editing this dossier (November 2020), it is hard to say which of these vaccines you will receive, or have already received. Much will depend on the results of the large-scale phase 3 studies, the ability of the companies to produce enough doses, the European Union's choice of vaccine producers, and how governments will distribute the vaccines amongst the population¹⁰⁴.

But it is clear that everyone involved in vaccine development has done their utmost to make COVID-19 vaccines available as soon as possible.

Because progress in COVID-19 research is very fast, we refer you to a VIB web page that monitors the latest developments: <https://vib.be/en/covid-19-onderzoek-vib>.

THE TRADITIONAL VACCINE DEVELOPMENT PARADIGM – 5 TO 10 YEARS ⁵²



PANDEMIC PARADIGM: OVERLAPPING PHASES, DEVELOPMENT <1 YEAR



The difference between Traditional vaccine development and development using a pandemic Paradigm

The pandemic paradigm requires multiple activities to be conducted at financial risk to developers and manufacturers and without knowing whether the vaccine candidate will be safe and effective, including very early manufacturing scale-up to commercial scale before clinical proof of concept is established. ID denotes identification.

THE PLACE OF BELGIUM IN COVID-19 VACCINE DEVELOPMENT

The Oxford/AstraZeneca vaccine saw the light of day in the university hospitals in Oxford, led by the Belgian Bruno Holthof. Another link to Belgium is that the British-Swedish pharmaceutical giant AstraZeneca uses technology and ingredients from the Belgian company Novasep for the large-scale production of the vaccine¹⁰¹.

The American pharmaceutical company Pfizer is working with the German BioNTech and the Chinese Fosun Pharma on a gene vaccine for COVID-19. The vaccine may be produced on a large scale at the Pfizer site in Puurs, Belgium¹⁰¹. Several of JNJ's Benelux sites have been involved in the development of the Janssen Pharmaceutica/JNJ vaccine. The company made an educational video series 'The Road to a Vaccine' with the scientists involved¹⁰².

Ghent University Hospital was one of the European hospitals that tested the RNA vaccine of the German CureVac on healthy volunteers in a phase 2b study. The hospital was involved in multiple clinical studies from the second COVID-19 wave onwards¹⁰¹.

THE MOST ADVANCED COVID-19 VACCINES (SOURCE WHO - 17 NOVEMBER 2020)¹⁰⁰

Manufacturer	Vaccine platform	Type of vaccine/name	Doses; administration	Protection phase 3 study (on 17 November 2020)
BioNTech/Pfizer	RNA	3 LNP-mRNA BNT162b2	2 / 0 and 28 days	95% (interim results)
Moderna/NIAID	RNA	LNP-encapsulated mRNA mRNA-1273	2 / 0 and 28 days	95% (interim results)
University of Oxford/ AstraZeneca	Gene vaccine	ChAdOx1-S AZD 1222	2 / 0 and 28 days	60% to 90% (interim results)
Janssen Pharmaceutica	Gene vaccine	Adenovirus Type 26 vector	1 2 / 0 and 56 days	Ongoing
Novavax	Protein subunit	SARS-CoV-2 glycoprotein nanoparticle with adjuvant	2 / 0 and 12 days	Ongoing
Sinovac	Inactivated	Inactivated	2 / 0 and 14 days	Ongoing
Sinopharm/Wuhan Institute of Biological Products	Inactivated	Inactivated	2 / 0 and 21 days	Ongoing
Sinopharm/Beijing Institute of Biological Products	Inactivated	Inactivated	2 / 0 and 21 days	Ongoing
Bharat Biotech	Inactivated	Fully inactivated virus particle	2 / 0 and 28 days	Ongoing
CanSino Biological Inc./ Beijing Institute of Biological Products	Gene vaccine	Adenovirus Type 5 Vector	1	Ongoing
Gamaleya Research Institute	Gene vaccine	Adenovirus-based (rAd26-S+rAd5-S) Sputnik V	2 / 0 and 21 days	92.5% (interim results)

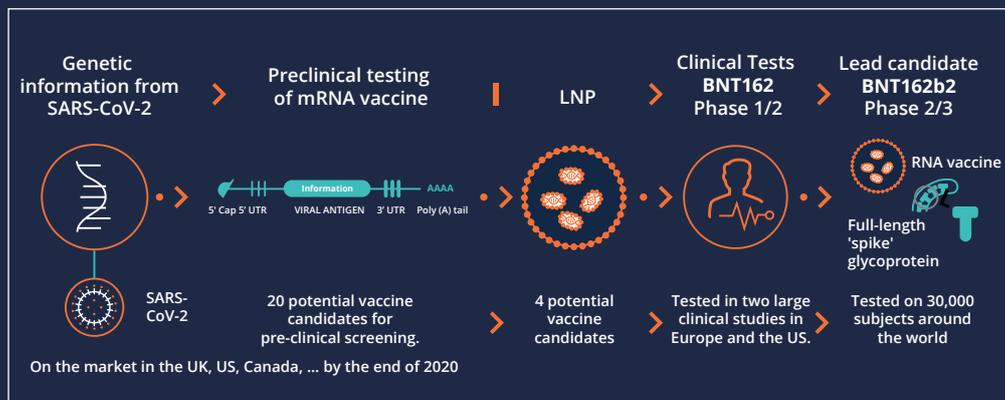
HOW DID THE BIONTECH/PFIZER BNT162B2 VACCINE COME ABOUT?

SARS-CoV-2 coronaviruses consist of round viral particles covered with proteins - the spike proteins - that protrude from their surface (see figure 'SARS-CoV-2: the model in view' on page 63). These spikes give the virus its crown-like appearance. The viruses use these protruding proteins to bind to human cells, which they are then able to enter. Once inside the cell, their genetic material - an RNA molecule - is transcribed and new viruses are formed.

The BioNTech/Pfizer vaccine consists of a short segment of genetic material, messenger RNA, which contains the instructions to create a harmless version of these spike proteins. The messenger RNA is packaged in microscopic fat droplets, called LNPs, which stands for Lipid Nanoparticles. These LNPs protect the RNA against breakdown while allowing it to be more easily absorbed by the cells of our body. In the cell, the messenger RNA is translated into spike proteins, which the immune cells then take action against. Faced with a later infection by the real SARS-CoV-2 virus, these immune cells will recognize and block the spike proteins on the virus.

Unlike, for example, live vaccines, these RNA vaccines do not contain any actual viruses. Moderna's vaccine was developed using the same technology.

The development path of the BNT162b2 vaccine



Based on the genome sequence of the SARS-CoV-2 virus, 20 candidate RNA vaccines were designed. These underwent preclinical studies, after which four candidates were packaged in lipid nanoparticles (LNPs) and tested in phase 1/2 clinical studies. One of these candidates - BNT162b2 - went on to phase 2/3 clinical trials¹⁰³.

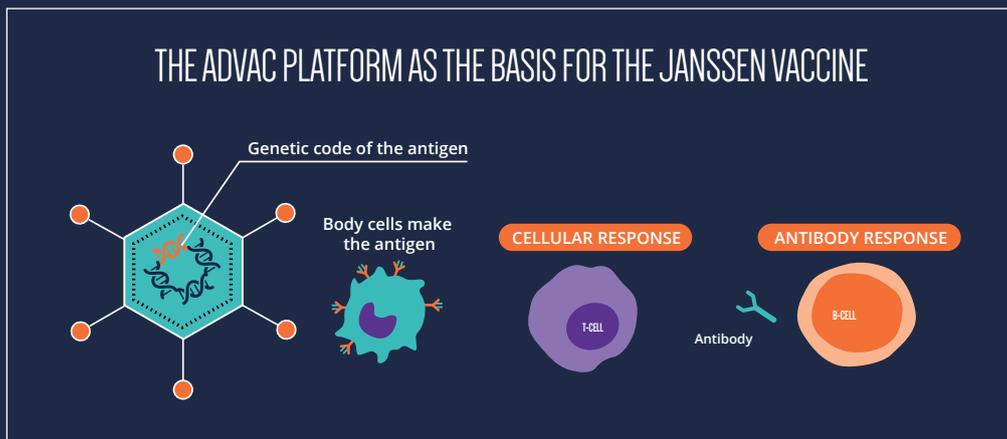
HOW DID THE JANSSEN PHARMACEUTICA/J&J VACCINE COME ABOUT?

For its vaccine against the SARS-CoV-2 virus, Janssen Pharmaceutica used the AdVac technology platform it originally developed for its Ebola vaccine. In other words, Janssen researchers incorporated the genetic code for the SARS-CoV-2 spike protein into a genetically modified adenovirus. In this approach, the adenovirus serves as a vector - a kind of delivery system - for getting a piece of genetic code from the SARS-CoV-2 virus into the cells of our body.

Adenoviruses are already familiar from relatively harmless diseases such as the common cold and the adenovirus used was further weakened by genetic modification.

Once injected into the body, body cells will produce spike proteins that our immune systems can recognize as foreign and develop resistance to. We know from the AdVac platform that it not only triggers a response with antibodies, it also produces memory B-cells, T-helper cells, and cytotoxic T-cells (see Chapter 5).

This broad immune response means that only a single vaccination is needed. The AZD 1222 vaccine from the University of Oxford/AstraZeneca and the Russian Sputnik V vaccine also use adenovirus vectors.



Conclusions

No matter what history book you open, you will quickly conclude that the fate of mankind was determined by struggle, war, insurrections, revolutions, and politics. A tale of blood and treasure, in other words. But is this really the case? Rudy Burgmeijer and Karel Hoppenbrouwers ask themselves this in their 'Handbook on vaccinations, theory and implementation practice', which is the canonical reference work on vaccinations in Dutch-speaking countries¹⁰⁵. They concluded that fateful pandemics have been just as important in the history of humanity.

They argue as follows: "The illustrious Han Dynasty in China came to an end in AD 220, partly because of the plague, brought in by invaders from the north. A major cause of the fall of the Roman Empire in 160 AD was the Antonine plague, in which seven million Romans died from a combination of bubonic plague, smallpox, and measles. In the early 14th century, a quarter to half of the European population died of the plague, leaving no one left to wage war or to maintain economic and social life. And then there is Columbus and his men, who killed eight million people in the Caribbean by giving them smallpox, influenza, tuberculosis, and gonorrhoea. Or Hernando Cortez who introduced smallpox and measles to South America, which eventually killed 95% of the Aztec population. Or, closer to home, there was the Spanish flu. With a death toll of 9 million, the First World War has the reputation of being the deadliest war ever. But the Spanish flu of 1918 far exceeded that, with an estimated 22 to 40 million fatalities worldwide"¹⁰⁵.

Clean water, sanitation, and vaccines against infectious diseases have changed the history of mankind forever. And that brings us to the first sentence of the preface to this dossier, written by Prof. Peter Piot, perhaps the most famous Belgian virologist internationally. Piot also states unequivocally that vaccinations against infectious diseases have made a major contribution to the history of humanity.

However, COVID-19 shows that we need to stay on our guard. The battle is far from won. New viruses, bacteria, or other microorganisms present new dangers. In less than a year, the SARS-CoV-2 virus has infected 53.7 million people and caused 1.3 million deaths¹⁰⁶. And the numbers keep rising. Our greatest hope of tackling this virus, and returning to 'normal life', lies in developing COVID-19 vaccines at unprecedented speed. This is an assignment in which researchers, doctors, pharmaceutical companies, and public authorities - at the time of writing - also seem to be succeeding. Developing and distributing a vaccine within one to eighteen months was considered impossible before COVID-19. This dossier explains why this could be done. A worldwide effort by hundreds - perhaps thousands - of professionals, has achieved the seemingly impossible.

While the spotlight is on the COVID-19 vaccines, we may forget all the other good that vaccinations have brought us. From eradicating smallpox worldwide - and soon polio too - to curbing diseases

such as measles, diphtheria, meningitis, tetanus, yellow fever, and pneumococcal disease. All this leads to less illness and less suffering, longer and more fulfilled lives, lower healthcare costs, and more economic activity thanks to reduced absenteeism at school and work. In short, numerous vaccinologists, epidemiologists, virologists, and health economists come to the conclusion, backed by facts and figures, that vaccination was one of the best healthcare investments ever made.

The history of vaccinology, described *passim* in this dossier in various information boxes, makes it clear not only that vaccines have reduced death and disease over the past hundred years, but also that during the same period this branch of medicine has developed from an empirical 'craft' to a highly innovative science using the latest techniques in genetics, immunology, chemistry, and pharmacology¹⁰⁵.

And yet there are still very important challenges ahead of us¹. To reach everyone who can benefit from vaccinations, every country and every international organization involved in health will have to make vaccines a top priority. Not only must there be more structural funding for vaccine research, but priority vaccines must also be better distributed, especially in countries that lack sufficient resources and infrastructure. This will require leadership from policymakers, goodwill from the vaccine and pharmaceutical industries, and creativity from researchers, doctors, and other healthcare professionals.

Vaccination programs can be more effective if they take into account the local context, specific needs, cultural specificities, and distinctive circumstances of vulnerable populations. This requires microplanning, an adaptive approach, and innovative efforts to integrate vaccination programs in a well-considered way into healthcare, education, and care of the elderly. That an integrated approach to vaccinations can be successful in sectors outside healthcare is proven by our own vaccination programs for children and young people. These are integrated into education through the SGCs (Student Guidance Centres), which provide guidance for elementary and high school students. They achieve high vaccination rates. Another example is provided by residential care centers, which achieve a much higher vaccination rate for the annual seasonal flu than is found amongst elderly people who continue to live at home.

Achieving high levels of vaccination is necessary to achieve herd immunity. This also protects people who, for whatever reason, cannot or do not wish to be vaccinated. In this way, vaccination is an act of solidarity with vulnerable people. We also need to ensure that this principle is better understood by the general public.

We immediately come to the most sensitive area of action, namely raising public confidence in vaccinations. Despite the many successes of vaccinations, there is growing public suspicion of vaccines. This is noteworthy and may have a number of causes. We have only discussed these briefly in this dossier because entire volumes have already been written about it. If we want to increase (or at least maintain) current vaccination rates, both in our own country and worldwide, we will have to pay more attention to the social, historical, and political realities within various target groups. Simply providing information is not enough to counter people's reluctance to get vaccinated in time. This will require a new language and a new model of engagement with the public. This starts with listening to the public more and responding promptly and adequately to concerns, questions, uncertainties, and false claims.

This new relationship with the public also means building local, neighborhood, and citizen-oriented capacity, embracing digital communication, and engaging new partners. This entails taking the risk of acting creatively. For example, the support of religious and traditional leaders has been invaluable in addressing the hesitation surrounding polio vaccination. In some European countries, the use of teenage girls and influencers on social media has had a positive effect on the uptake of the HPV vaccination. It can work, but it requires a different kind of effort than we are used to.

We conclude with the words of Prof. Peter Piot that were quoted at the start of this dossier: "Vaccinations must remain one of the best investments in healthcare. This can only be achieved by making vaccinations a permanent priority in research, industry, public health, and in society at large".

And that is a responsibility we all share.

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