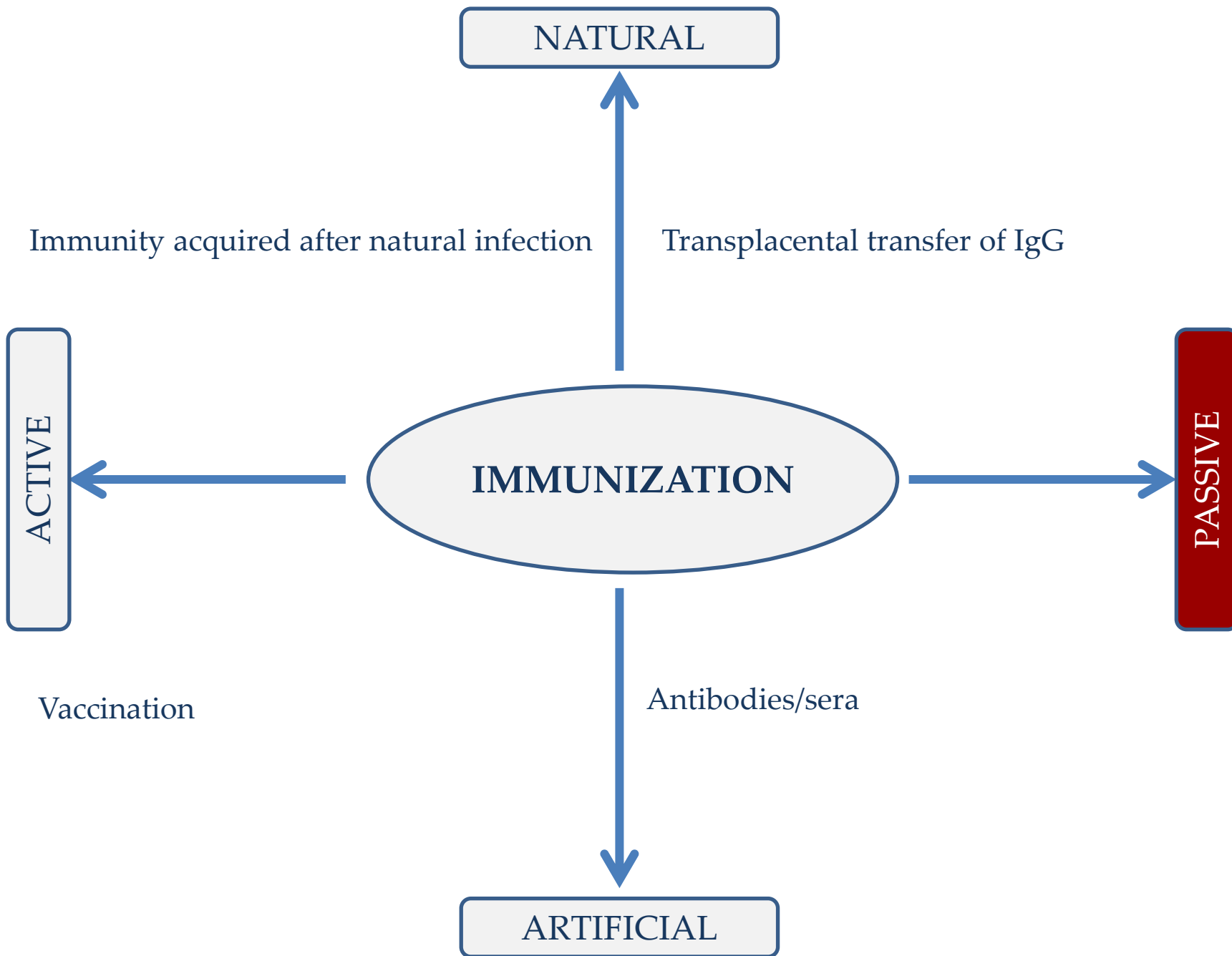


# **Immunization and vaccination**

**Immunity** means protection from disease and, more specifically, infectious disease.

**Immunization** is the process whereby a person is made resistant to a disease.



# Passive immunization...

... Transfer of mostly humoral immunity, antibodies from actively immunized individual to naïve individual...

it was developed in the early twentieth century as a therapy for infective diseases

We distinguish two entities of passive immunity:

- *Naturally acquired passive immunity*
  - *Arteficially acquired passive immunity*
- 
- Passive immunity can occur naturally, when maternal antibodies are transferred across the placenta to the fetus, or it can be induced artificially, when a high concentration of human (or equine) antibodies specific for a pathogen or toxin is given to an unimmunized individual.
  - It is used when there is a high risk of infection or insufficient time for the host to develop an active immune response, or when it is desired to alleviate the symptoms of the disease.

Passive immunization involves the administration of antibodies, mainly specific for a particular antigen.

Since the treated person receives pre-formed antibodies, this type of treatment has a rapid (almost immediate) effect, but this immunity is temporary (due to the half-life of the immunoglobulins) and lasts for several weeks, up to a maximum of several months.

# Passive immunization

## *Application:*

- preventing the development of some viral diseases (after being pricked with a needle contaminated with blood containing the hepatitis B virus or after being bitten by a animal with rabies)
- protection of persons with immunodeficiencies (premature infants or persons with deficiency of humoral immunity)
- therapy of diseases, that are mediated by toxins (diphtheria and botulism) or in the case of exposure of humans to animal venom (e.g., snakebite)

# Passive immunization

The most commonly used are immunoglobulins obtained by pooling the plasma of a large number of people, and in some cases animal serum is used

There are two groups of human immunoglobulin preparations:

- **Immunoglobulins obtained from the plasma of seropositive people** with a high titer of antibodies specific for a particular infectious antigen (an ordinary person who has recently had a particular infection or vaccinated persons) – high titers of specific gamma immunoglobulins. These preparations are used in the prophylaxis of viral infections: hepatitis B, rabies, respiratory syncytial virus infection, chickenpox and some bacterial diseases: tetanus.
- **Intravenous immunoglobulins** or human serum globulins (formerly called gammaglobulins) are obtained from randomly selected healthy blood donors and consist primarily of IgG of various specificities that are included in the normal immune repertoire of adults. These are used in the prophylaxis of infectious diseases in people with humoral immunity deficiency, as well as in those diseases for which specific immunoglobulins do not exist or are not always available, e.g. measles, hepatitis A, rubella (in the first trimester of pregnancy).

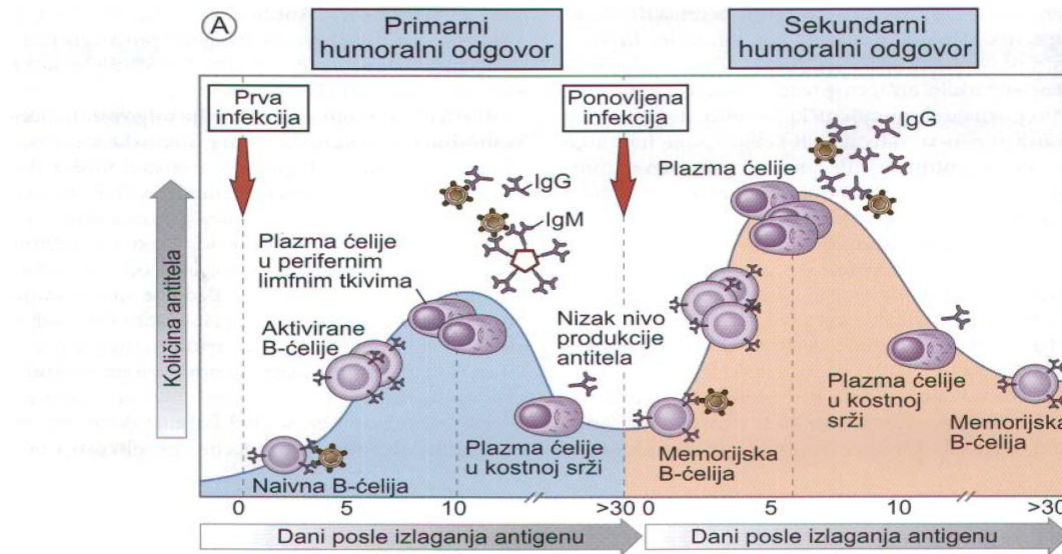
# Vaccination

The fundamental principle of vaccination is to administer a killed or attenuated form of an infectious agent, or a component of a microbe, that does not cause disease but elicits an immune response that provides protection against infection by the live, pathogenic microbe.



# Vaccination

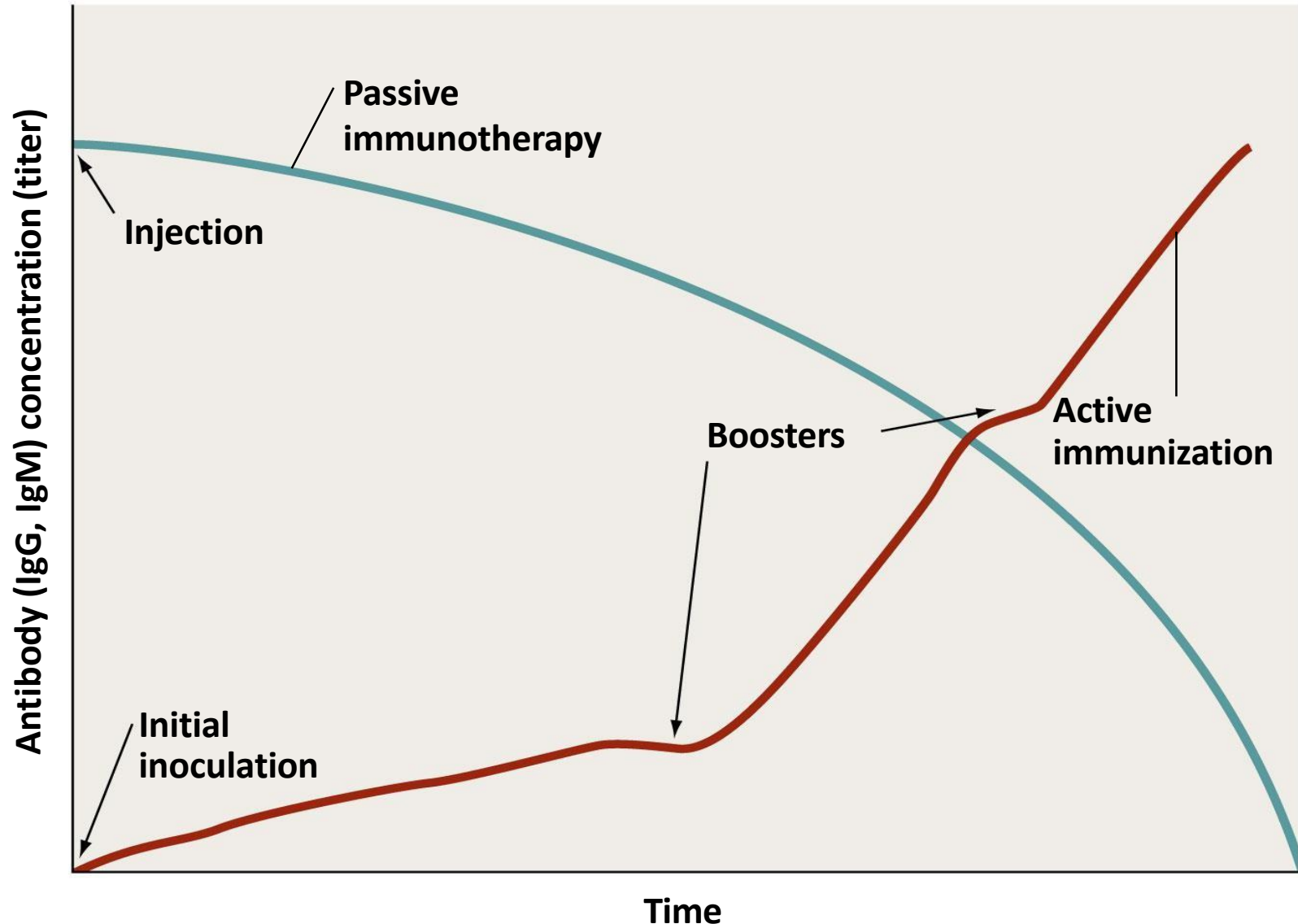
Induces immune memory without causing the disease, the immunized person after the first contact with the microbe develops secondary immune response



(B)

	Primarni odgovor	Sekundarni odgovor
Period kašnjenja posle imunizacije	Obično 5–10 dana	Obično 1–3 dana
Maksimalan odgovor	Manji	Veći
Izotip antitela	Obično IgM>IgG	Relativan porast IgG, a u određenim situacijama i IgA i IgE (promena klase teških lanaca)
Afinitet antitela	Niži prosečni afinitet, veća varijabilnost	Viši prosečni afinitet (sazrevanje afiniteta)

# Characteristics of immunity induced by active and passive immunization

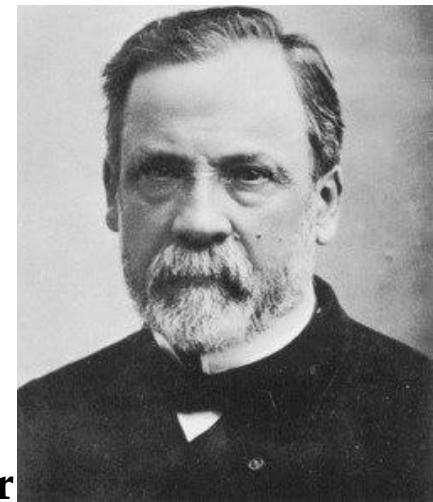


# Ideal Vaccine should be:

- **Effective** – highly immunogenic and induces complete and long-lived immunity (stimulates the production of high-affinity antibodies and memory cells)
- **Safe** – without side effects
- **Stabile**
- **Low-cost**

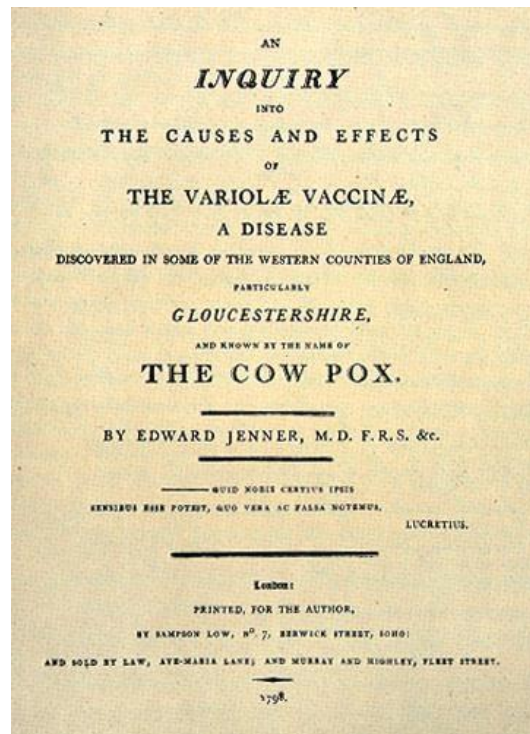
# History of Vaccination

- Thucydide, in the fifth century BC in Athens, first mentioned immunity to an infection that he called “plague”.
- However, the concept of protective immunity existed long before, as suggested by the ancient Chinese custom of making children resistant to smallpox by having them inhale powders made from the skin lesions of patients recovering from the disease (*variolation*).
- By 1700, variolation was employed by many societies in Africa, India and the Ottoman empire, and it was in use in England and France in 1700s.
- The first clear example of induction of immunity was Edward Jenner’s successful vaccination against smallpox (18<sup>th</sup> century).
- The principles of infectious diseases and vaccination were firmly established by the work of Louis Pasteur and Robert Koch (19<sup>th</sup> century), vaccine for rabies.



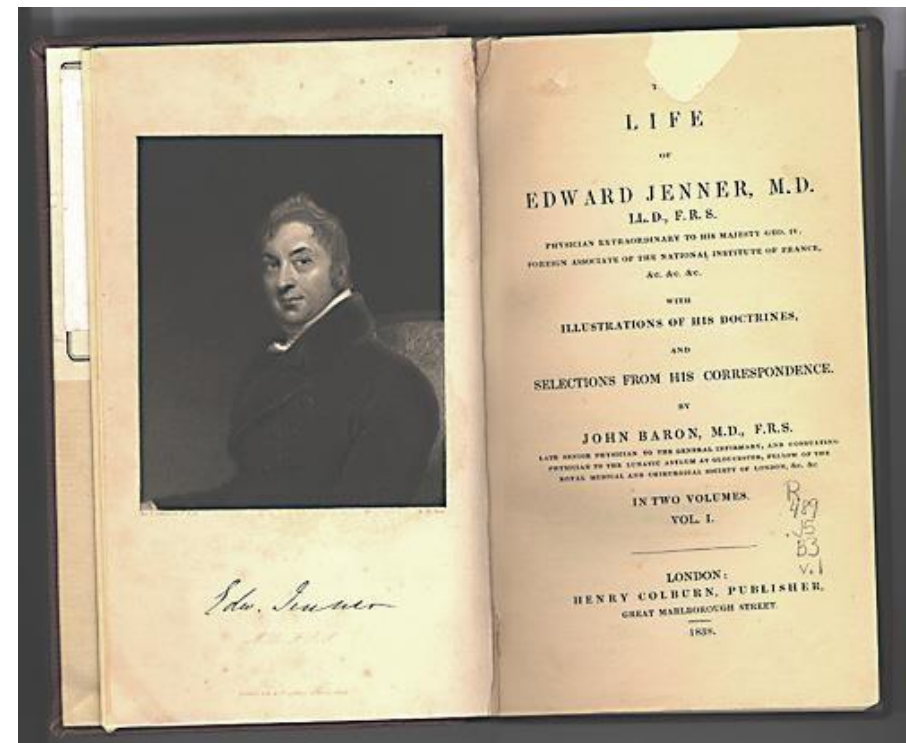
Louis Pasteur

# Edward Jenner



An inquiry into the causes and effects of the Variolæ Vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow-pox

Third edition. London: printed for the author by D. N. Shury, 1801.





# Vaccination in 20<sup>th</sup> Century

- In 1924, Gaston Ramon developed the method to produce **toxoids** of the pathogenic toxins of diphtheria and tetanus, producing safer vaccine antigens that retained their immunogenic potential.
- The Nobel Prize in Physiology or Medicine 1951 was awarded to Max Theiler "for his discoveries concerning yellow fever and how to combat it." Theiler, through passage of **yellow fever** virus in mice, developed an attenuated live yellow fever virus variant, which became a highly effective vaccine.
- Laboratory growth of poliovirus in 1940s permitted the development of both the inactivated **polio vaccine** (**IPV; Salk, licensed in 1955**) and the live attenuated oral polio vaccine (**OPV; Sabin, monovalent licensed in 1961, trivalent in 1963**). As a result of these vaccines, still in use today, poliovirus type 2 was eradicated in 1999, and no wild-type poliovirus type 3 has been detected since 2012. Only poliovirus type 1 is still endemic in 2016 in just two countries, Pakistan and Afghanistan.
- Recognition and subsequent exploitation in vaccines of key antigenic substructures rather than whole microbes was another technical advance. This led to safer vaccination with **components (subunits) of pathogens**, as opposed to entire microbes (e.g., the bacterial polysaccharide capsules from *S. pneumoniae* or *Neisseria meningitidis*, or the viral surface antigens [hemagglutinins, or HA] in influenza split-virus vaccines). When delivered as vaccines, these isolated microbial components produced protective antibodies and cellular immune responses in vaccinated hosts but did not cause the disease induced by the complete wild-type organisms.

# Vaccination in 20<sup>th</sup> Century

- Discovering antigenic shift and drift in influenza virus and almost complete changes in HA and NA proteins revealed the necessity to regularly update the vaccine composition to match the changing circulating strains, in 1973, the World Health Organization (WHO) began providing annual recommendations for the composition of the seasonal influenza vaccine based on the current circulating subtypes and strains.
- In 1978, the first trivalent vaccine was licensed (two influenza A strains and one influenza B strain).
- Beginning in 2013, the WHO began including a second type B strain and recommended four vaccine strains annually (A/H3N2, A/H1N1, -B/Victoria, and B/Yamagata), which enabled production and licensure of the new quadrivalent influenza vaccines.
- Several other live, attenuated viral vaccines, such as measles, mumps, and rubella vaccine (MMR), were developed in the second half of the twentieth century and became staples of childhood vaccination programs globally.

The name vaccination was applied to the intervention performed by Jenner, since *Vacca* is the Latin word for cow (he induced immunity to cowpox)



# Herd immunity

Vaccines are not given solely to protect individuals against diseases. Another purpose of vaccination is to protect communities by reducing transmission of disease-causing microbes from vaccinated persons to unvaccinated persons.

The term for this protection is herd immunity or community immunity.

The importance of prophylactic immunization against infectious diseases is best illustrated by the fact that worldwide programs of vaccination have led to the complete or nearly complete eradication of many of these diseases in many countries.

## Effectiveness of Vaccines for Some Common Infectious Diseases

Disease	Maximum Number of Cases (Year)	Number of Cases in 2018	Percentage Change
Diphtheria	206,939 (1921)	1	−99.99
Measles	894,134 (1941)	375	−99.95
Mumps	152,209 (1968)	2,515	−95.82
Pertussis	265,269 (1934)	15,609	−94.11
Polio (paralytic)	21,269 (1952)	0	−100.0
Rubella	57,686 (1969)	4	−99.99
Tetanus	1,560 (1923)	23	−98.52
<i>Haemophilus influenzae</i> type B	~ 20,000 (1984)	38	−99.83
Hepatitis B	26,611 (1985)	3,322	−87.51

This table illustrates the striking decrease in the incidence of selected infectious diseases in the United States for which effective vaccines have been developed.

Data from Orenstein WA, Hinman AR, Bart KJ, Hadler SC. Immunization. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practices of Infectious Diseases*. 4th ed. New York, NY: Churchill Livingstone; 1995; and *Nationally Notifiable Infectious Diseases and Conditions, United States: 2018 Annual Tables*.

# Aims of Vaccination

- To protect individuals with the highest risks (**strategy of selective immunization**)  
or
- To eradicate, eliminate or control the disease (**strategy of massive immunization**)

**Eradication:** pathogen is eliminated worldwide (cow pox)

**Elimination:** disease is eliminated in the most countries, but not all (polio)

**Control:** disease is no longer huge public health problem (tetanus neonatorum)

# **The success of vaccination in eradicating infectious disease depends on several properties of the microbes:**

Vaccines are most effective if the infectious agent

- does not establish latency,
- does not undergo antigenic variation, and
- does not interfere with the host immune response.

It is difficult to effectively vaccinate against microbes such as HIV, which establishes latent infection, is highly variable, and inhibits host immunity.

Vaccines are also most effective against infections that are limited to human hosts and do not have animal reservoirs.

# **Most vaccines in use today work by inducing humoral immunity**

- Antibodies are the only immune mechanism that prevents infections, by neutralizing and clearing microbes before they gain their foothold in the host.
- The best vaccines are those that stimulate the development of long-lived plasma cells that produce high-affinity antibodies and memory B cells.

# **The major challenges in developing effective vaccines against several important infections**

- The immunologic correlates of protection are often poorly defined.
- Fundamental questions about how to maximally stimulate durable memory, effective Tfh cells, and long-lived plasma cells remain unresolved.
- Clinical experience has taught us that the longevity of vaccine-induced protection varies greatly, being lifelong with hepatitis B antigen vaccines and quite short with many others. The reasons for this critical difference are unknown.

# Vaccine approaches that have been tried

## Vaccine Approaches <sup>a</sup>

Type of Vaccine	Examples
Live attenuated or killed bacteria	Bacillus Calmette-Guérin, cholera
Live attenuated or killed viruses	Polio, influenza, rabies
Subunit (antigen) vaccines	Tetanus toxoid, diphtheria toxoid
Conjugate vaccines	<i>Haemophilus influenzae</i> , pneumococcus
Synthetic vaccines	Hepatitis (recombinant proteins)
Viral vectors	Clinical trials of human SARS-CoV-2 spike protein made by human and chimpanzee adenovirus vectors
DNA vaccines	Clinical trials ongoing for several infections
mRNA vaccines	Approved for COVID-19



# Vaccine Platforms: Classical and Next-Generation

Platform Type	Subtype	Examples
Whole pathogen	Live attenuated	Measles, mumps, rubella, varicella zoster, yellow fever vaccines
	Inactivated	Rabies vaccine
Subunit	Polysaccharide	23-valent <i>Streptococcus pneumoniae</i> vaccine
	Polysaccharide conjugated to protein	13-valent <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Neisseria meningitidis</i> vaccines
	Protein	Influenza vaccine
Next-Generation	Virus-like particle	Human papillomavirus vaccine
	Viral vectored	Dengue, Ebola vaccines
	Nucleic acid based	Zika (in development) and SARS-CoV-2 vaccines
	Nanoparticle based	Influenza (in development)

# Attenuated and Inactivated Bacterial and Viral Vaccines

- composed of intact microbes that are treated in such a way that they are attenuated or killed so they can no longer cause disease while retaining their immunogenicity.
- elicit many of the innate and adaptive immune responses (both humoral and cell-mediated) that the pathogenic microbe would, and they are therefore the ideal way of inducing protective immunity.
- are usually more effective; polio, measles, and yellow fever are three good examples.

# Attenuated and Inactivated Bacterial and Viral Vaccines

The earliest approach for producing such attenuated viruses was repeated passage in cell culture.

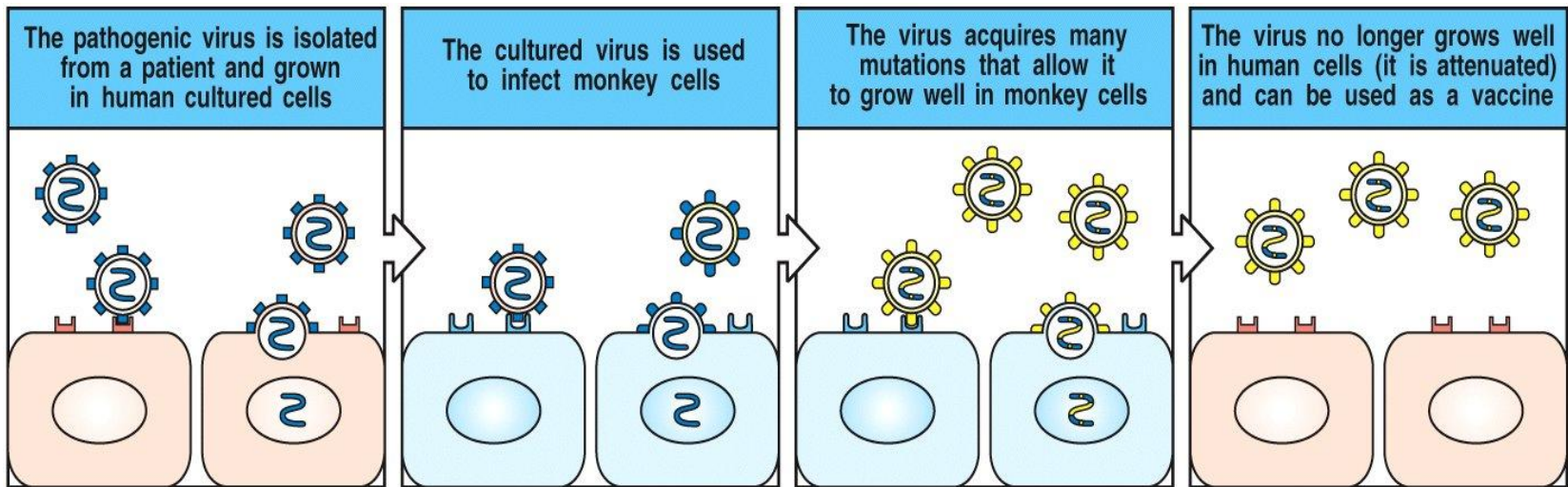


Figure 14-24 Immunobiology, 6/e. (© Garland Science 2005)

More recently, temperature-sensitive and gene deletion mutants have been generated to create attenuated viruses.

Viral vaccines often induce long-lasting specific immunity, so immunization of children is sufficient for lifelong protection.

The major concern with attenuated viral or bacterial vaccines is safety.

The live-attenuated oral polio vaccine has nearly eradicated the disease, but in rare cases the virus in the vaccine is reactivated and itself causes paralytic polio.

# Attenuated and Inactivated Bacterial and Viral Vaccines

A widely used inactivated vaccine of considerable public health importance is the influenza vaccine.

Influenza viruses grown in chicken eggs are used in two types of vaccines:

1. trivalent inactivated (killed) vaccine that is used in the flu shot that is given intramuscularly.
2. influenza vaccine that involves the same three strains, but is made up of live attenuated viruses and is used as a nasal spray.

Two of the major limitations of current influenza vaccines is

1. they do not induce broadly neutralizing antibodies that recognize multiple strains of the virus and
2. antibody-mediated protection is short lived.

# Attenuated and Inactivated Bacterial and Viral Vaccines

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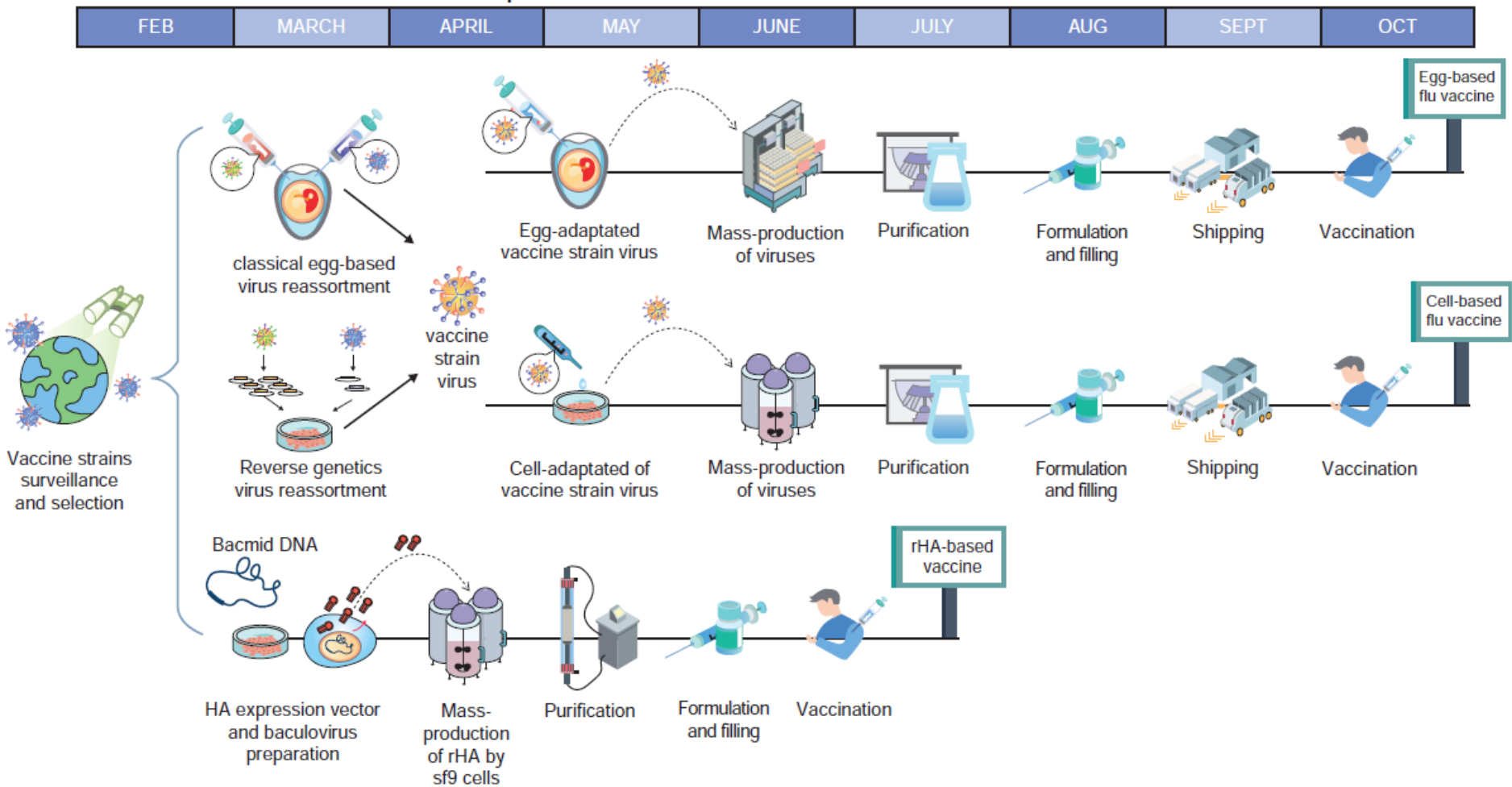
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# Current influenza vaccine productions



# Attenuated Bacterial and Viral Vaccines

## Advantages

- good and long-lasting protection
- one dose and small inoculum
- no need for adjuvant

## disadvantages

- safety–  
immunodeficiency  
and pregnancy!
- stability

# Inactivated Bacterial and Viral Vaccines

## Advantages

- Complete virulent microbe
- Stability
- Safety

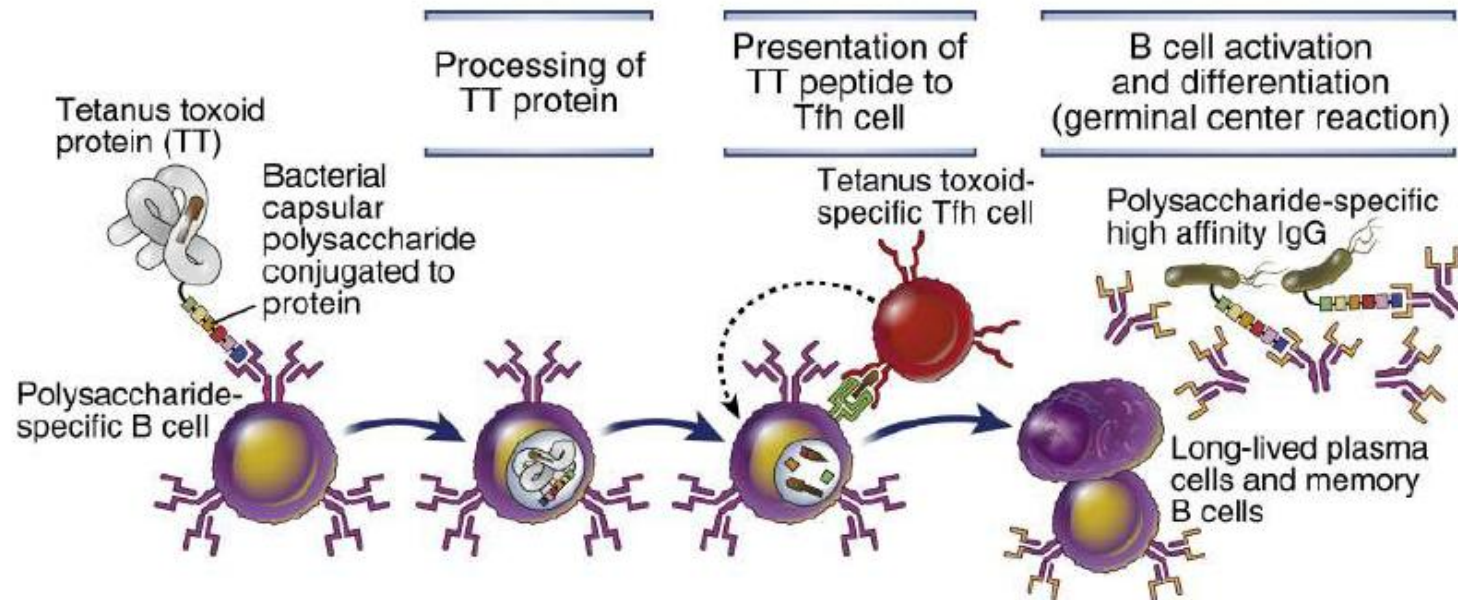
## Disadvantages

- Type and duration of immunity
- Several doses
- Adjuvant is indispensable



# Purified Antigen (Subunit) Vaccine

- These second-generation vaccines were produced to eliminate the safety concerns associated with attenuated microbes.
- **Subunit vaccines** are composed of **antigens purified from microbes or inactivated toxins** and are usually administered with an adjuvant.
- One effective use of purified antigens as vaccines is for the prevention of diseases caused by bacterial toxins. Toxins can be rendered harmless without loss of immunogenicity, and such toxoids induce strong antibody responses.
- **Diphtheria and tetanus** are two infections whose life-threatening consequences have been largely controlled because of immunization of children with toxoid preparations.



# Purified Antigen (Subunit) Vaccine

- Vaccines composed of bacterial polysaccharide antigens are used against pneumococcus and *Haemophilus influenzae*. They are obtained by coupling the polysaccharides to proteins (hapten-carrier conjugates) to form **conjugate vaccines**.
- The currently used *H. influenzae*, pneumococcal, and **meningococcal vaccines** are conjugate vaccines.
- Purified protein vaccines stimulate helper T cells and antibody responses, but they do not generate potent CTLs. The reason for poor CTL development is that exogenous proteins (and peptides) usually enter the class II MHC pathway of antigen presentation (except in the special situation of cross-presentation).
- As a result, protein vaccines are not recognized efficiently by class I MHC–restricted CD8+ T cells.

# Combinations vaccines

Combination of several toxoids and inactivated pathogens applied simultaneously

**MMR – Morbilli, Mumps, Rubella**

**DiTePer – Diphtheria, Tetanus,  
Pertussis**

# Synthetic Antigen Vaccines

- A goal of vaccine research has been to identify the most immunogenic microbial antigens or epitopes, to synthesize these in the laboratory, and to use the synthetic antigens as vaccines.
- It is possible to deduce the protein sequences of microbial antigens from nucleotide sequence data and to prepare large quantities of proteins by recombinant DNA technology.
- Vaccines made of recombinant DNA–derived antigens are now in use for **hepatitis B virus** and **HPV**.
- In the case of the most widely used HPV vaccine, which was developed to prevent cancers caused by the virus, recombinant viral proteins from four strains (HPV 6, 11, 16, and 18) are made in yeast and combined with an adjuvant. HPV 6 and 11 are common causes of warts, and HPV 16 and 18 are the HPV strains most often linked to cervical cancer.

# Live Viral Vaccines Involving Recombinant Viruses

- Another approach for vaccine development is to introduce genes encoding microbial antigens into a noncytopathic virus and to infect individuals with this virus, the virus serves as a source of the antigen in an inoculated individual.
- The great advantage of viral vectors is that they, like other live viruses, induce the full complement of immune responses, including strong CTL responses, against the antigen produced by the foreign gene.
- This technique has been used most commonly with vaccinia virus vectors, and more recently with canarypox viral vectors, which are not pathogenic in humans.

# Live Viral Vaccines Involving Recombinant Viruses

- A potential problem with recombinant viruses is that the viruses may infect host cells, and even though they are not pathogenic, they may produce antigens that stimulate CTL responses that kill the infected host cells. Also, the nonpathogenic virus could recombine with host viruses or gene sequences and become virulent.
- These safety concerns have limited widespread use of viral vectors for vaccine delivery.
- One approach that overcomes many of these issues and concerns is the use of live recombinant hybrid vaccines that are non-replicating. An adenovirus 26 vector (humans generally lack antibodies to this adenovirus) and a chimpanzee adenovirus vector have been used to generate vaccines to a number of viruses, including Ebola virus, Zika virus, and SARS-CoV-2. Non-replicating adenoviruses infect numerous host cells and thus produce a significant amount of the viral antigen.

# DNA Vaccines

- Inoculation of a plasmid containing complementary DNA (cDNA) encoding a protein antigen leads to humoral and cell-mediated immune responses to the antigen. It is likely that APCs, such as DCs, are transfected by the plasmid and the cDNA is transcribed and translated into immunogenic protein that elicits specific responses.
- Bacterial plasmids are rich in unmethylated CpG nucleotides that are recognized by TLR9 in DCs and other cells, thereby eliciting an innate immune response that enhances adaptive immunity. Therefore, plasmid DNA vaccines could be effective even when administered without adjuvants.
- The ability to store DNA without refrigeration for use in the field also makes this technique promising.
- However, DNA vaccines have not been as effective as hoped in clinical trials, mainly because the first generation of these vaccines did not produce adequate amounts of the immunogen.
- Studies with newer vectors for DNA vaccination are currently in progress.

# mRNA Vaccines

- Another relatively recent mode of vaccination uses messenger RNA (mRNA) encoding microbial antigens.
- The main advantages of mRNA vaccines are the ease with which they can be rapidly developed, the ability to bypass the need for the large-scale manufacture and purification of protein antigens (thereby greatly reducing the cost), and the ability to combine mRNAs encoding many different protein antigens from a pathogen into a single vaccine.
- Although initial attempts to use mRNA were unsuccessful, largely because of stability issues, a number of recent advances have made mRNA vaccination a practical modality. One major advance is modifications of the mRNA itself. These modifications include addition of a synthetic 5' cap and a long poly A-tail to increase stability, altering 5' and 3' untranslated regions of the mRNA to enhance both translation and stability, and codon optimization of the coding portions to enhance translatability.
- Current mRNA vaccines for **COVID-19** retain some ability to activate innate immunity by triggering RNA sensors.
- The mRNA is encapsulated in lipid nanoparticles that facilitate uptake by cells, including dendritic cells, and also function as an adjuvant.

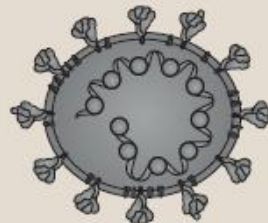


# An Overview of the Different Vaccine Platforms in Development Against COVID-19

## Classical platforms

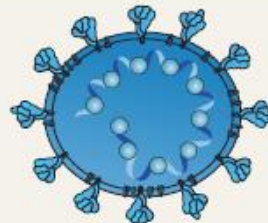
### Whole-inactivated virus

Example: Polio vaccine  
COVID-19:  
PiCo Vacc in phase 1  
clinical trials



### Live-attenuated virus

Example: MMR vaccine  
COVID-19:  
in preclinical stage



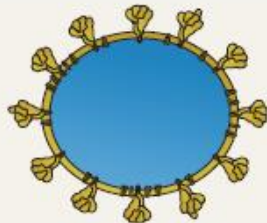
### Protein subunit

Example: Seasonal  
influenza vaccine  
COVID-19:  
NVX-CoV2373 in  
phase 1/2 clinical trials



### Virus-like particle

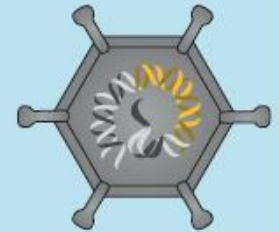
Example: Human  
papillomavirus vaccine  
COVID-19:  
in preclinical stage



## Next-generation platforms

### Viral vector

Example:  
VSV-Ebola vaccine  
COVID-19:  
AZD1222, Ad5-nCoV



### DNA

Example:  
Not currently licensed  
COVID-19:  
INO-4800 in phase 1  
clinical trials



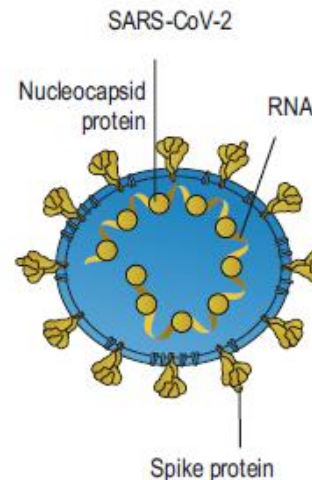
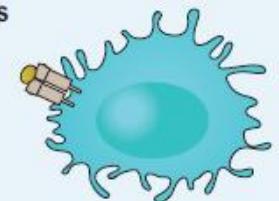
### RNA

Example:  
Not currently licensed  
COVID-19:  
mRNA-1273, BNT162  
in phase 1/2 clinical trials



### Antigen-presenting cells

Example:  
Not currently licensed  
COVID-19:  
LV-SMENP-DC,  
COVID-19/aAPC  
in phase 1/2 clinical trials



# Adjuvants and Immunomodulators

- The initiation of T cell–dependent immune responses against protein antigens requires that the antigens be administered with adjuvants.
- Most adjuvants elicit innate immune responses, with increased expression of costimulators and production of cytokines, such as IL-12, that stimulate T cell growth and differentiation.
- Heat-killed bacteria are powerful adjuvants that are commonly used in experimental animals.
- Only a few adjuvants are approved for patients: 1. aluminum hydroxide gel (which appears to promote mostly B cell responses); 2. a bacterial product, monophosphoryl lipid A, alone or with aluminum salt; and a 3. lipid formulation called squalene that may activate phagocytes.
- Recently, CG-rich oligonucleotides (CpG DNA) have been approved as an adjuvant for hepatitis B vaccines; by activating TLR9, these agents elicit potent innate immune reactions.

	Viruses	Bacteria	Fungi	Parasites
Vaccines for general population	Poliomyelitis	Difteria Tetanus Pertussis		
	Morbili Mumps Rubella	BCG (in some countries)		
	Hepatitis B			
Vaccines for individuals with increased risk for disease	Influenza	BCG		
	Yellow fever	Typhus		
	Hepatitis A	Pneumococcus		
	Rabies	Meningococcus		
	Varicella-zoster virus	Haemophilus		
		Anthrax		
Vaccines that are still not ready for clinical application	Adenovirus	Staphylococcus	Candida	Malaria
	Rhinovirus	Streptococcus	Pneumocystis	Leishmania
	Herpes viruses	Gonococcus		Schistosomiasis
	Respiratory syncicial virus (RSV)	Syphilis		Filaria
	HIV	Лепра		

# Vaccine safety

## -possible side effects-

**The only one medical treatment for healthy people**

### Limitations and problems caused by vaccines

**Inactivated vaccines– microbes are not completely killed**

**Aattenuated microbes can convert to virulent types (example: polioviruses types 2,3)**

**Inclusion of toxic materials (e.g. typhus, whooping cough)**

**Contamination with animal viruses**

**Contamination with egg proteins (hypersensitivity reactions)**

**Cross reactivity (autoimmune diseases)**

### Limitations and problems associated by patients

**Immunodeficiencies (live microbes could cause severe disease)**

**Local inflammatory reaction, frequently to adjuvant**

**Interference between vaccines given at the same time (not always)**