

Main types of vaccines

1. Live attenuated vaccine
2. Inactivated/Killed vaccine
3. Toxoid vaccine

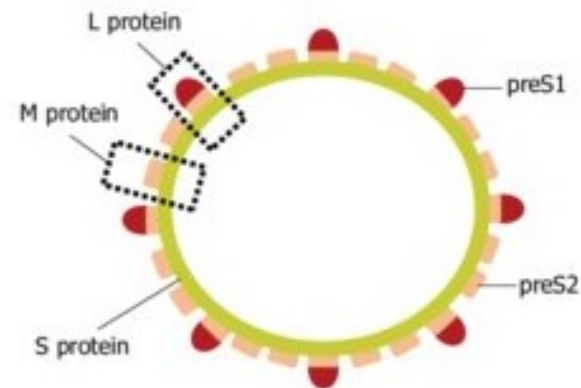
4. Subunit vaccine
5. Pseudo-typed virus vaccine
6. DNA/RNA vaccine

4. Subunit vaccine

Contains purified parts of the pathogen that are antigenic.

Hepatitis B (virus surface antigen: HBsAg)

Structure of HBsAg

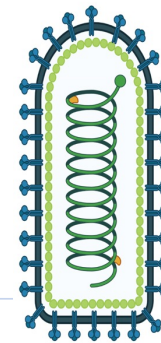


Subunit vaccine

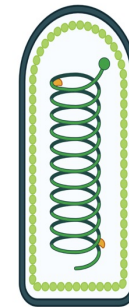
- The hepatitis B vaccine is a recombinant **subunit vaccine**, meaning it contains only a specific component (antigen) of the hepatitis B virus (HBV) rather than the whole virus.
- This protein is produced using **recombinant DNA technology**: the HBsAg gene is inserted into **yeast cells (usually *Saccharomyces cerevisiae*)** or, in some formulations, **mammalian cells**, which then produce the surface antigen.
- The purified HBsAg is then formulated with an **adjuvant** (typically aluminum hydroxide or aluminum phosphate) to enhance the immune response. Aluminum hydroxide forms a gel-like matrix that **adsorbs and slowly releases the antigen** at the injection site. This creates a “**depot effect**”, prolonging antigen exposure to immune cells.

5. Pseudo-typed virus vaccine

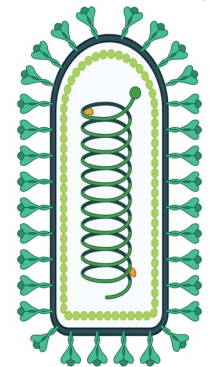
- Used in case of highly pathogenic viruses (e.g. Rabies, Ebola, Lassa fever)
- Based on engineered, replication incompetent (or competent) viruses that present foreign foreign viral envelope proteins (e.g. Vesicular stomatitis virus (VSV))
- VSV can incorporate foreign viral glycoproteins in the absence of the VSV G glycoprotein (VSV Δ G)
- VSV infection (asymptomatic or mild fever)



Wild-type VSV



Bald VSV Δ G



Spike pseudotyped VSV- Δ G

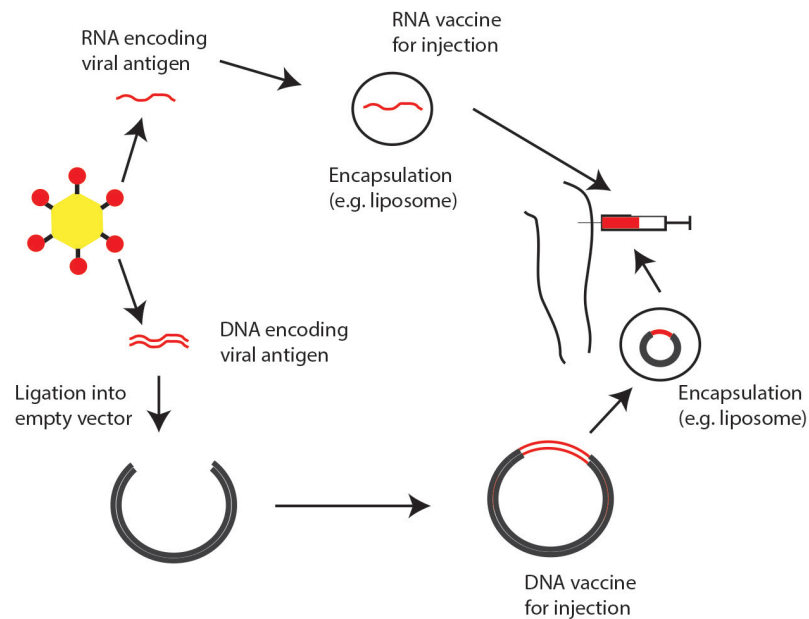
Pseudo-typed virus vaccine

- A **pseudo-typed virus vaccine** uses a **non-replicating viral particle** that has been **engineered to display surface proteins (antigens)** from another virus, the *target pathogen*, but lacks the ability to cause infection or disease.
- In other words, the pseudo-virus **“mimics” the real virus’s outer structure** (so the immune system recognizes it) but **does not contain its pathogenic genome**, making it **safe and non-infectious**.

Pseudo-typed virus vaccine:

- There is at least **one approved vaccine** that uses a pseudo-typed/exchange-viral-vector approach:
- **ERVEBO[®] (rVSVΔG-ZEBOV-GP, live)** is approved by the U.S. Food & Drug Administration (FDA) for prevention of disease caused by **Zaire ebolavirus**.

DNA/RNA vaccines



- Intramuscular injection of genetically engineered DNA (or RNA) encoding pathogen-derived antigen(s). In situ production of the target antigen in transfected myocytes. Release of produced antigen by exosomes. This material is endocytosed by dendritic cells to initiate an immune response.

Advantages of DNA/RNA vaccines

- **Avoids potential risks** of working with live pathogens
- **More rapid vaccine production** as no recombinant antigen has to be produced and purified.
- **Personalized vaccines possible** (Engineering of DNA/RNA vaccines encoding tumor-specific antigens)

Genetic toxicity (DNA vaccine)

- Insertional mutagenesis (vaccine DNA may accidentally insert into essential genes)
- DNA insertion may lead to chromosome instability
- Turn ON oncogenes
- Turn OFF tumor suppressor genes

Other disadvantage

- DNA vaccines are generally poorly immunogenic with weak stimulation of antibody (humoral) responses.

Advantages of an RNA vaccine versus DNA vaccines

- As mRNA is a non-infectious, non-integrating platform, there is no potential risk of infection or insertional mutagenesis.
- Additionally, **mRNA is degraded by normal cellular processes**, and its *in vivo* half-life can be regulated through the use of various modifications and delivery methods.
- **RNA vaccines** deliver **mRNA**, which only needs to reach the **cytoplasm** to be translated into protein not the nucleus, as in case of DNA vaccine
- mRNA vaccines begin producing antigen within **hours**, whereas DNA vaccines can take **days** due to slower transcription and expression.
- Faster antigen presentation generally leads to stronger adaptive immunity

Disadvantages of RNA vaccines

- The RNA evokes strong inflammatory responses caused by the **immune system recognizing the RNA and lipid nanoparticles as danger signals**, leading to **cytokine production**
- RNA is unstable, degrades rapidly and needs specific transportation and storage conditions

Since the COVID-19 pandemic mRNA vaccines have attracted world-wide attention

- **Until recently no DNA/RNA vaccines have been approved for human use.**
- FDA approved only small interfering ribonucleic acid (siRNA) to treat a rare nerve disease (August 2018)

Pfizer and BioNTech Receive U.S. FDA Approval for 2023-2024 COVID-19 Vaccine

- The updated COVID-19 vaccine is targeted *against circulating Omicron-related sublineages, including XBB.1.5, BA.2.86 (Pirola) and EG.5.1 (Eris), which currently account for the largest portion of cases.*

Approved DNA vaccines only for veterinarian use

- (2005) DNA vaccine for horses against West Nile Virus (WNV) infections. Fort Dodge Animal Health, (US)
- (2005) DNA vaccine to protect farm-raised salmon against Haematopoietic Necrosis Virus (HNW) (Novartis)
- (2017) DNA vaccine for chicken (encoding an antigen of the Avian Influenza High Pathogenic H5 subtype. US - AgriLabs®)

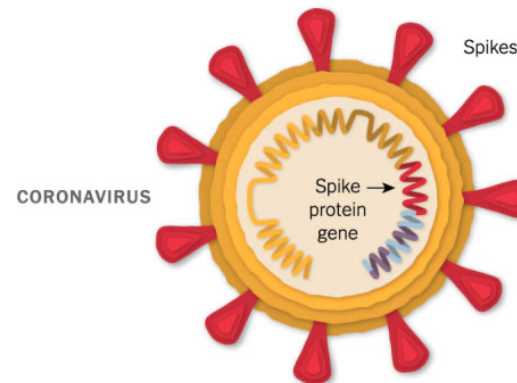
History of mRNA vaccines

- Landmark experiment (1987): Robert Malone graduate student at the Salk Institute for Biological Studies in La Jolla, California
- He mixed strands of messenger RNA with droplets of lipid. Human cells bathed in this mixture absorbed the mRNA, and began producing proteins from it.

Reference: Malone, R. W., Felgner, P. L. & Verma, I. M. *Proc. Natl Acad. Sci. USA* **86**, 6077–6081 (1989)

Inside COVID 19 vaccine

- Based on the SARS-CoV2 gene encoding the spike protein
- The mRNA sequence is adapted to stabilize the spike protein in the shape necessary to fuse with human cells

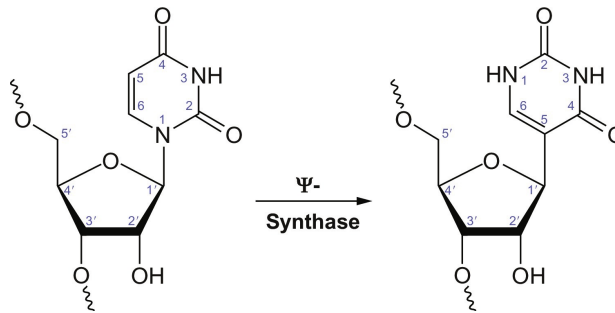


Reference Nature: NEWS FEATURE 14 September 2021

Inside COVID19 vaccine



(encoding the SARS CoV-2 spike protein)



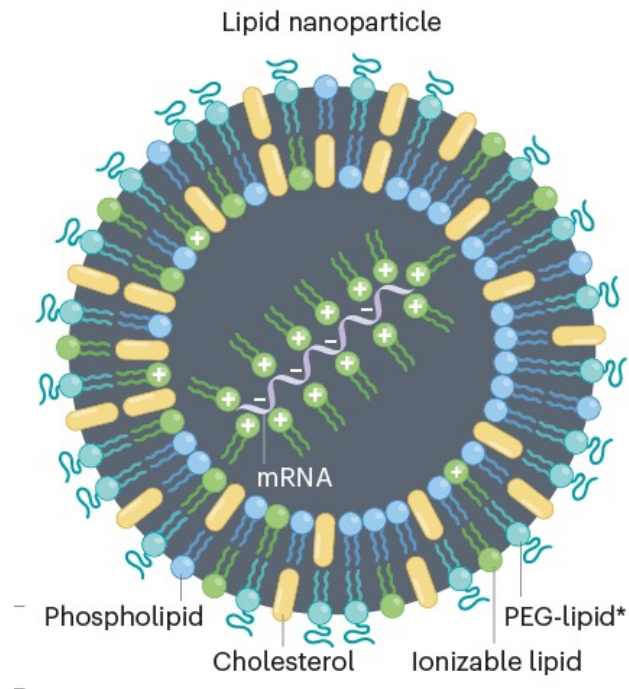
Uridine

Pseudo-uridine

The change is thought to stop the immune system to react against the introduced mRNA

Reference Nature: **NEWS FEATURE** 14 September 2021

Inside COVID-19 vaccine



*Lipid residue attached to polyethylene glycol is thought to reduce toxicity of the lipid nanoparticle in the human body.

Reference Nature: **NEWS FEATURE** 14 September 2021

Long-term risks?

- Approved mRNA COVID-19 vaccines show increased risks of myocarditis (inflammation of the heart muscle) and pericarditis (inflammation of the lining outside the heart), particularly within the first week following vaccination. For COMIRNATY, the observed risk is highest in males 12 through 17 years of age. (BIONTECH press release, 11.09.2023)
- A recent publication documents the intracellular reverse transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 into DNA in a human liver cell line.

Aldén, M. et al., *Curr. Issues Mol. Biol.* **2022**, 44, 1115-1126.

The power of vaccines

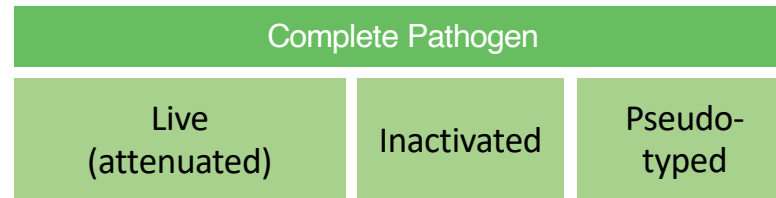
Cases in the US

| Disease | Max. number of cases | Number of cases in 2000 | Percent change |
|--------------------------------------|----------------------|-------------------------|----------------|
| Diphtheria | 206,939 (1921) | 2 | -99.99 |
| Measles | 894,134 (1941) | 63 | -99.99 |
| Mumps | 152,209 (1968) | 315 | -99.80 |
| Pertussis | 265,269 (1934) | 6,755 | -97.73 |
| Polio (paralytic) | 21,269 (1952) | 0 | -100.0 |
| Rubella | 57,686 (1969) | 152 | -99.84 |
| Tetanus | 1,560 (1923) | 26 | -98.44 |
| <i>Haemophilus influenzae</i> type B | ~20,000 (1984) | 1,212 | -93.14 |
| Hepatitis B | 26,611 (1985) | 6,646 | -75.03 |

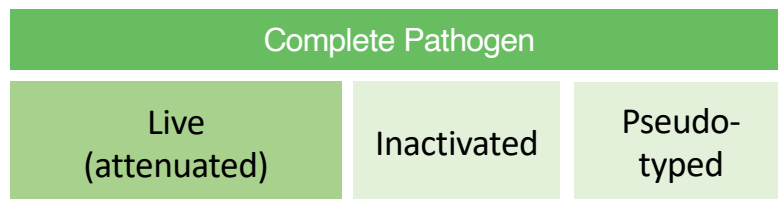
Types of vaccines

| Complete Pathogen | | | DNA / RNA / Protein | | | |
|-------------------|-------------|--------------|-------------------------|------|-------------------|---------------------|
| Live (attenuated) | Inactivated | Pseudo-typed | DNA (Adenovirus Vector) | mRNA | Protein (Subunit) | Toxin (inactivated) |

Types of vaccines



Types of vaccines

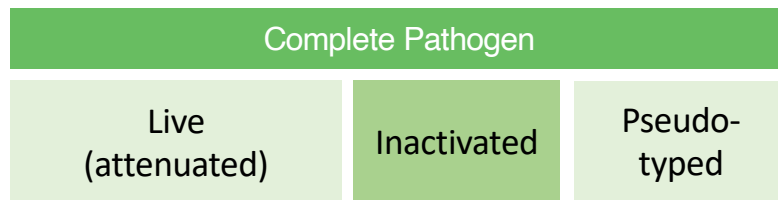


“attenuated”

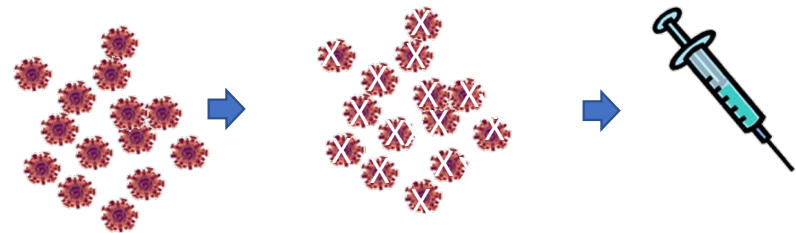


- Smallpox
- Polio
- Tuberculosis (BCG)
- Measles
- Mumps
- Rubella

Types of vaccines

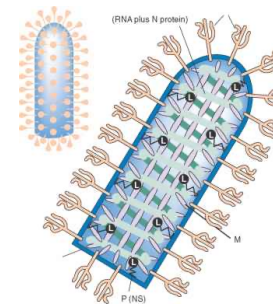
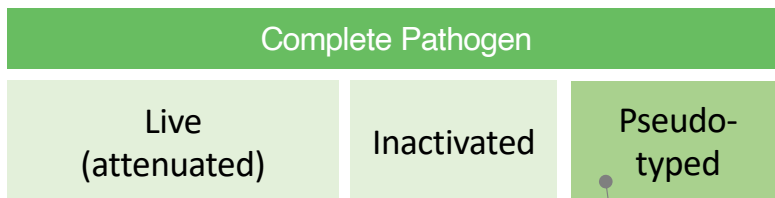


- Injected polio vaccine (Salk vaccine)
- Most influenza vaccines
- Hepatitis A vaccine
- Rabies vaccine
- CoronaVac, Covaxin, Sinopharm, etc



Inactivated
(Formaldehyde)

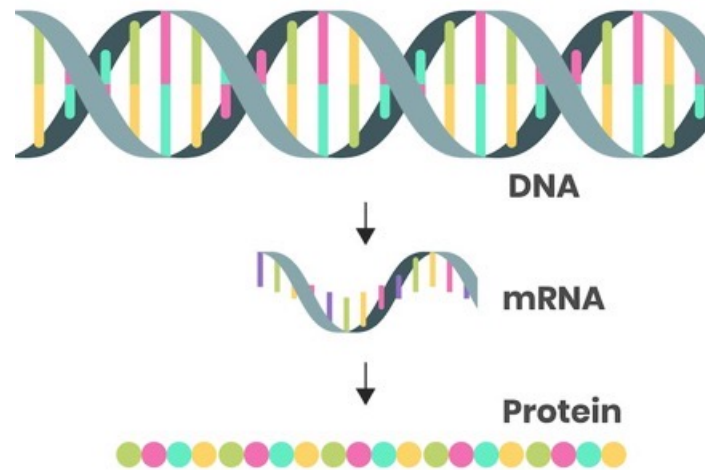
Vaccine types



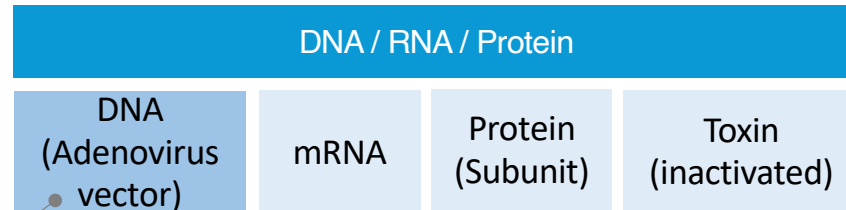
Ebola Zaire Vaccine (rVSVΔG-ZEBOV-GP, live)

DNA/RNA/Protein vaccines

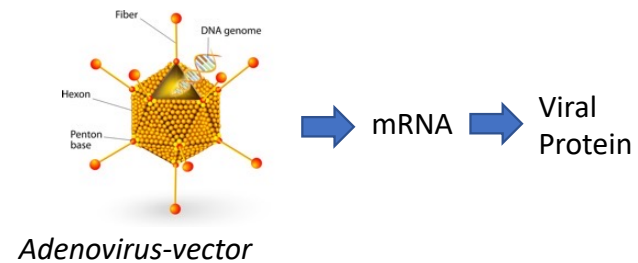
| DNA / RNA / Protein | | | |
|-------------------------------|------|----------------------|------------------------|
| DNA (Adenovirus vector) | mRNA | Protein (Subunit) | Toxin (inactivated) |



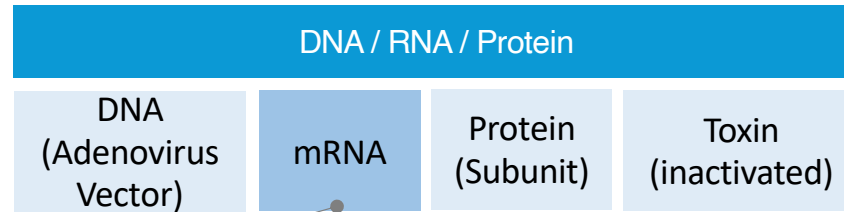
DNA/RNA/Protein vaccines



- Johnson & Johnson (Ad26.COVID-S)
- AstraZeneca (ChAdOX1-nCoV)
- Gamaleya Gam-COVID-Vac/Sputnik V



DNA/RNA/Protein vaccines



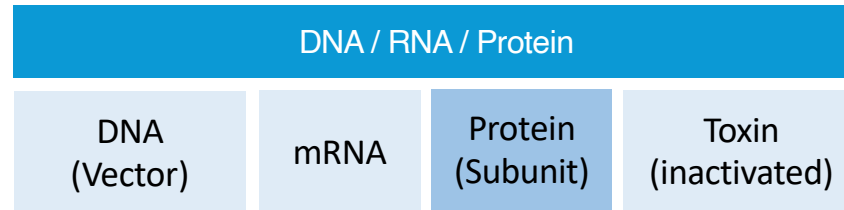
- Pfizer / BioNTech COMIRNATY
- Moderna Spikevax



mRNA

➔ Viral Protein

DNA/RNA/Protein vaccines

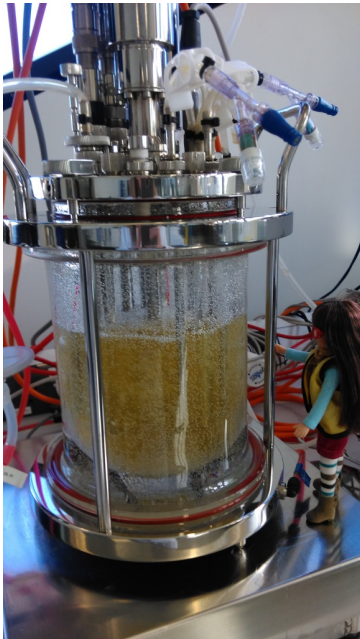


- Novavax NVX-CoV2373
- Gardasil (HPV)
- Hepatitis B - Recombivax HB, Twinrix
- Pertussis (Whooping cough)

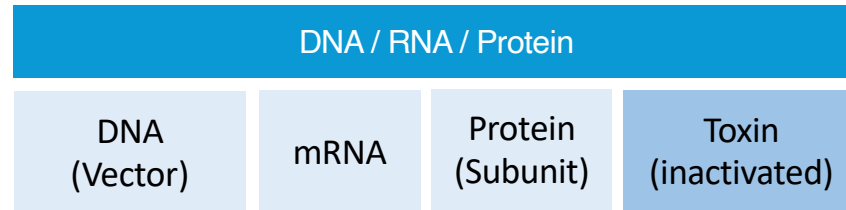
= Viral Protein



DNA/RNA/Protein vaccines



Bacteria



Production:
Bacteria secreting toxins in culture:
Cholera (*Vibrio cholerae*)/Diphtheria (*Corynebacterium diphtheriae*)/
Tetanus (*Clostridium tetani*)



Purification and
chemical inactivation



Vaccination

Vaccine landscape

| | Complete pathogen | | | DNA / RNA / Protein | | | |
|-----------|-------------------|-------------|--------------|-------------------------|------|-------------------|---------------------|
| | Live (attenuated) | Inactivated | Pseudo-typed | DNA (Adenovirus vector) | mRNA | Protein (Subunit) | Toxin (inactivated) |
| Efficacy | ++ | +/- | + | + | ++ | + | ++ |
| Safety | - | + | + | +/-? | +? | + | + |
| Stability | - | + | +/- | + | - | + | + |

Efficacy: Does it work?

Safety: Is it safe?

Stability: Can it be stored?