MICROBIAL BIOTECHNOLOGY

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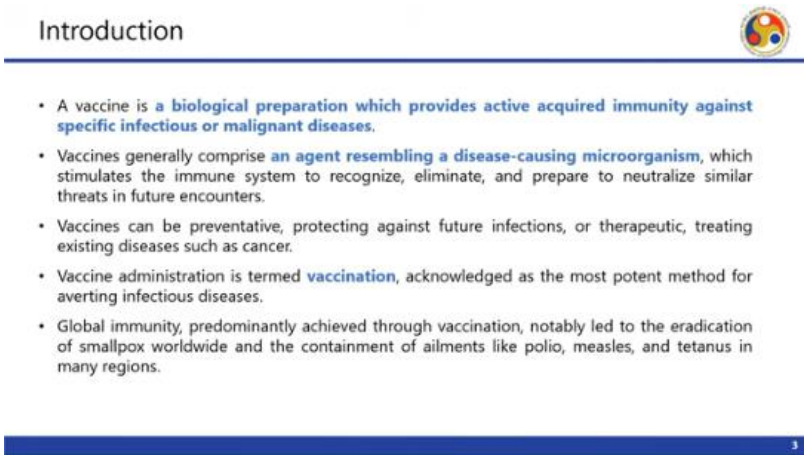
Lecture-35
Lec 35: Production of vaccines

Hello friends, welcome to my course on microbial biotechnology. We are in Module 10, discussing microbes in medical biotechnology. Today, we will discuss the production of vaccines. This lecture is divided into four broad sections. In the first section, we will discuss the basics of vaccine technology.

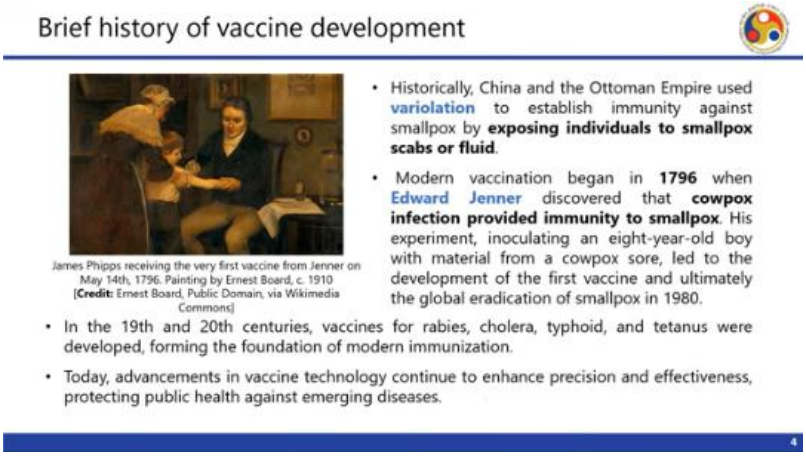
In Section 2, we will discuss vaccine research. In Section 3, we will discuss how vaccines are manufactured. And in Section 4, we will discuss some advances in vaccine biotechnology. So, a vaccine is a biological preparation that provides active acquired immunity against specific infections or malignant diseases. It generally comprises an agent resembling a disease-causing microorganism,

which stimulates the immune system to recognize, eliminate, and prepare to neutralize similar threats in future encounters. Vaccines can be preventive, protecting against future infections, or therapeutic, treating existing diseases such as cancer. Vaccine administration is termed vaccination, acknowledged as the most potent method for averting infectious diseases. Global immunity, predominantly achieved through vaccination, notably led to the eradication of smallpox worldwide and the containment of diseases like polio, measles, and tetanus in many regions.



Let us now look into the history of vaccine development. Historically, China and the Ottoman Empire used variolation to establish immunity against smallpox by exposing individuals to smallpox scabs or fluid. Modern vaccination began in 1796 when Edward Jenner discovered that cowpox infection provided immunity to smallpox. His experiment, inoculating an eight-year-old boy, James Phipps, as you can see in this photograph illustration, with material from a cowpox sore, led to the development of the first vaccine and ultimately the global eradication of smallpox in 1980.

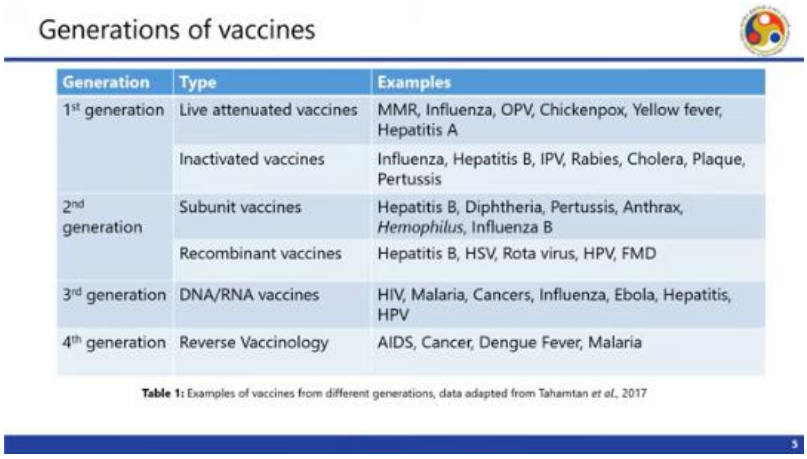
In the 19th and 20th centuries, vaccines for rabies, cholera, typhoid, and tetanus were developed, forming the foundation of modern immunization. Today, advancements in vaccine technology continue to enhance precision and effectiveness, protecting public health against emerging diseases. So if you look into the development of vaccines over time, we can divide them into several generations. The first generation basically refers to live attenuated vaccines and also inactivated vaccines. Some examples of the first are the MMR, influenza, OPV, smallpox, yellow fever, and hepatitis A,



and examples of inactivated vaccines are influenza, hepatitis B, IPV, rabies, cholera, plague, and pertussis. The second-generation vaccines are called subunit vaccines. Some examples are hepatitis B, diphtheria, pertussis, anthrax, Haemophilus influenzae type B. And also some of the recombinant vaccines like hepatitis B, HSV, rotavirus, HPV, and FMD. The third-generation vaccines are basically the DNA and RNA vaccines against malaria, HIV, cancers, influenza, Ebola, hepatitis, and HPV.

Now there is a fourth generation of vaccines coming up. These are also sometimes called reverse vaccinology, targeting AIDS, cancer, dengue fever, and malaria. Vaccines stimulate the immune system to recognize and remember specific pathogens, such as viruses or bacteria. They contain antigens, which are parts of the pathogen that trigger an

immune response but are modified to be harmless. Either by being weakened, killed, or genetically engineered, upon administration, the immune system identifies these antigens as foreign, prompting the production of antibodies—specialized proteins that neutralize the pathogen.



Generations of vaccines

Generation	Type	Examples
1st generation	Live attenuated vaccines	MMR, Influenza, OPV, Chickenpox, Yellow fever, Hepatitis A
	Inactivated vaccines	Influenza, Hepatitis B, IPV, Rabies, Cholera, Plaque, Pertussis
2nd generation	Subunit vaccines	Hepatitis B, Diphtheria, Pertussis, Anthrax, *Hemophilus*, Influenza B
	Recombinant vaccines	Hepatitis B, HSV, Rota virus, HPV, FMD
3rd generation	DNA/RNA vaccines	HIV, Malaria, Cancers, Influenza, Ebola, Hepatitis, HPV
4th generation	Reverse Vaccinology	AIDS, Cancer, Dengue Fever, Malaria

Table 1: Examples of vaccines from different generations, data adapted from Tahamtan *et al.*, 2017

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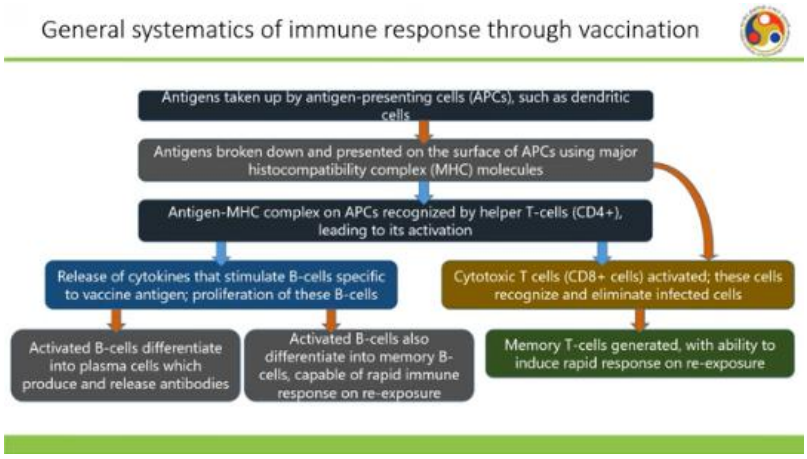
Additionally, immune cells like T cells and B cells are activated, creating a memory of the pathogen's antigens. The primary goal of vaccination is to prepare the immune system to respond quickly and effectively to future exposures, enabling a rapid defense that prevents infection or reduces disease severity. These are the general systematics of immune response through vaccination. We will also discuss this in detail with a little more illustration. The first step is antigens being taken up by antigen-presenting cells,

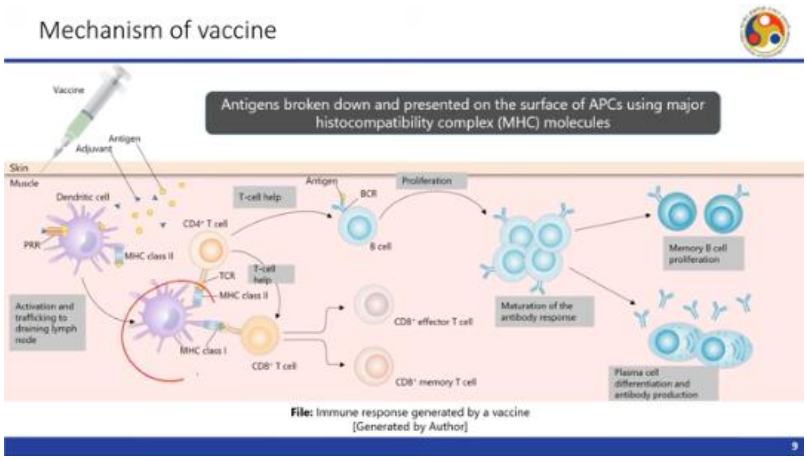
such as dendritic cells. They are broken down and presented on the surface of APCs using major histocompatibility complex molecules. Then, this antigen-MHC complex on APCs is recognized by helper T cells, leading to their activation. The release of cytokines stimulates B cells specific to the vaccine antigen; the proliferation of these B cells leads to activated B cells differentiating into plasma cells, which produce and release antibodies. Additionally, activated B cells differentiate into memory B cells, capable of a rapid immune response upon re-exposure in the future.

Antigens are sometimes broken down and present on the surface of APC using mesohistocompatibility complex MSC molecules and they directly activates the cytotoxic T cells which recognize and eliminate the infected cells or they may go through the antigen MSC complex on APC, recognize the helper T cells leading to its activation and then end up in the same point in the immune system pathways. And these actually generate the memory T cells with ability to induce rapid response on re-exposure. Let us have a little bit of explanation of these systematics of immune response in a little bit more elaborate

way, more illustrative way, how a vaccine works when a person is vaccinated. So, the person is vaccinated here, as you can see, this is the skin and underlying the skin is the muscle. So, these are antigens and these are some additives which are called as adjuvants we will learn about them later.



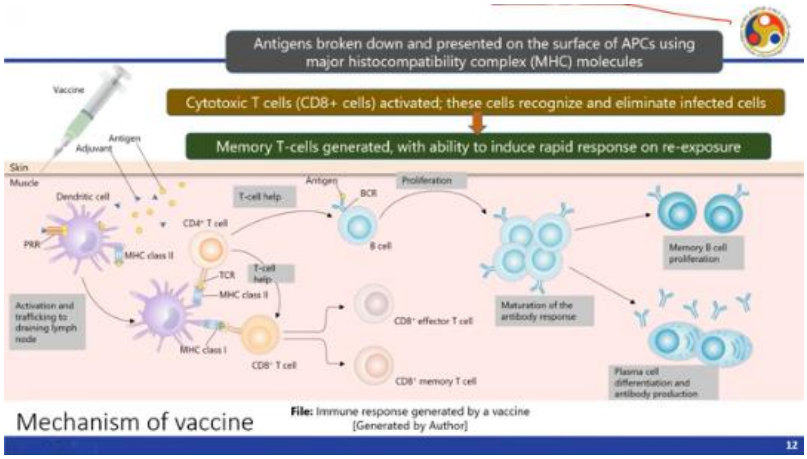
So, these antigens as you can see are taken up by antigen presenting cells such as these dendritic cell. And then this leads to activation and trafficking to drain the draining lymph nodes. So these are broken down and presented on the surface of antigen presenting cells using major histocompatibility complex molecules. So, as you can see over here, this class 1, class 2 major histocompatibility complex molecules are involved in this step. So, these antigen MSC complex on the APCs are recognized by helper T cells.



This helper T cells, the CD4 plus marker leading to its activation. And then there is a release of cytogenes that stimulates the B cells specific to vaccinate the antigen proliferation of these B cell will then follow. These activated B cells differentiate into plasma cells here as you can see which produces and releases the antibodies. That is the number one outcome

of this entire effect or these activated B cells will differentiate into memory B cells capable of rapid immune responses on re-exposure.

Separately, the cytotoxic T cells here have CD8 plus markers; these are activated, recognize, and eliminate the infected cells. So, this step, as we discussed earlier, may also happen directly without going through the cytotoxic T cell pathway. These memory T cells are generated with the ability to induce a rapid response upon re-exposure. So, now we know how a vaccine works through different pathways, involving different cells like B cells, T cells, and memory cells. So, what are the various types of vaccines that are used?



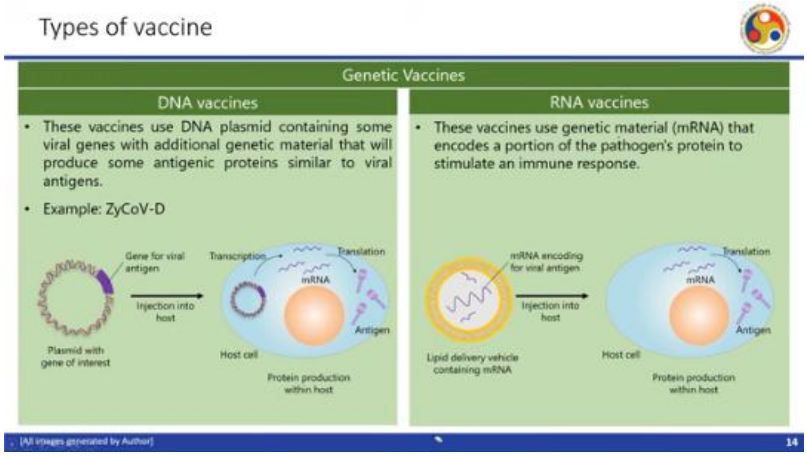
We may use a whole pathogen that causes the disease as a vaccine, and these may be further divided into live attenuated vaccines. These vaccines use a weakened form of the actual pathogen. The potent or strong one would actually cause disease. We use a weakened form of that pathogenic entity. These cannot cause disease but can still stimulate a strong immune response.

Here, this is a disease-causing virus with some spike protein that acts as an antigen. This is weakened with different treatments sometimes, and we use it directly. These are some examples of live attenuated vaccines, like measles, mumps, rubella, yellow fever, and oral polio vaccines. Then we have inactivated vaccines. These vaccines use pathogens that have been killed or inactivated, so they cannot replicate in the body. We may use heat or certain chemicals to destroy their potency, and then these are used as vaccines. Some examples include polio, hepatitis A, rabies, and influenza. Then, we may have genetic vaccines. For example, we may have DNA vaccines.

These vaccines use DNA plasmids containing some viral genes with additional genetic material that will produce antigenic proteins similar to viral antigens. So, this is a gene for

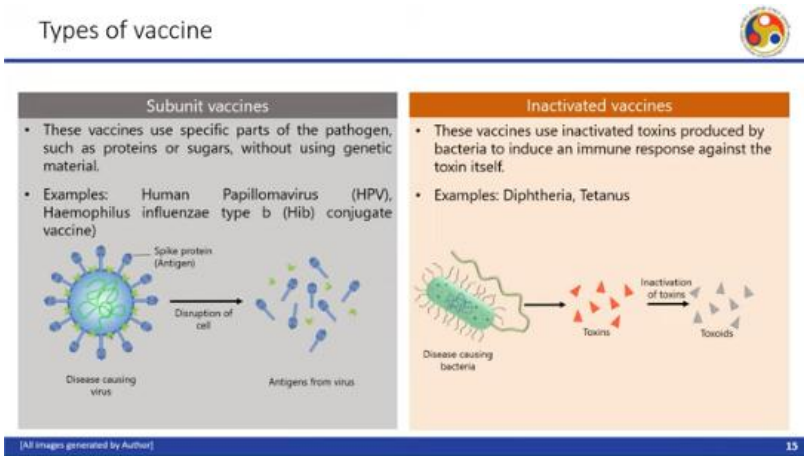
a viral antigen, and this is a plasmid which carries this particular gene. This is injected into the host. Due to transcription and translation, the antigens are produced within the host. Some examples include Zykov-D. Then we have RNA vaccines.

These vaccines use genetic material, mRNA, that encodes a portion of the pathogen's protein to stimulate an immune response. So, this is the mRNA encoding for the viral antigen here, and for delivery, we use a lipid vesicle. This is injected into the host, and then this mRNA is translated directly into antigens.



The difference between DNA vaccines and RNA vaccines is easily visible from these two illustrations. Some examples of RNA vaccines are the Pfizer-BioNTech COVID-19 vaccine and the Moderna COVID-19 vaccine. We may also have subunit vaccines. These vaccines use specific parts of the pathogen, such as proteins or sugars, without using genetic material. Examples include the human papillomavirus vaccine and the Haemophilus influenzae type B conjugate vaccine.

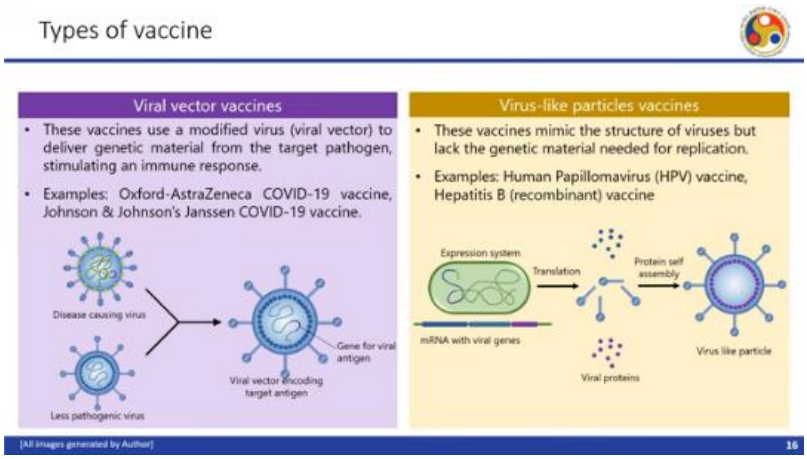
Or you may have inactivated vaccines. These are inactivated toxins produced by bacteria to induce an immune response against the toxin itself. So, in the earlier case, we were using the entire organism, killed it, and used it. But here we are only using the particular toxin, which is inactivated or denatured. Some examples include the diphtheria or tetanus vaccines.



So, now we have the viral vector vaccines. These use a modified virus, a viral vector, to deliver genetic material from the target pathogen, stimulating an immune response. So, we have a disease-causing virus over here. This is a less pathogenic virus.

So, here we go for a viral vector encoding the target antigen. So, this will finally produce the viral antigen. Then some of the examples are the Oxford-AstraZeneca COVID-19 vaccine and Johnson & Johnson's Janssen COVID-19 vaccine. Then we also use virus-like particle vaccines. These vaccines mimic the structure of viruses but lack the genetic material needed for replication.

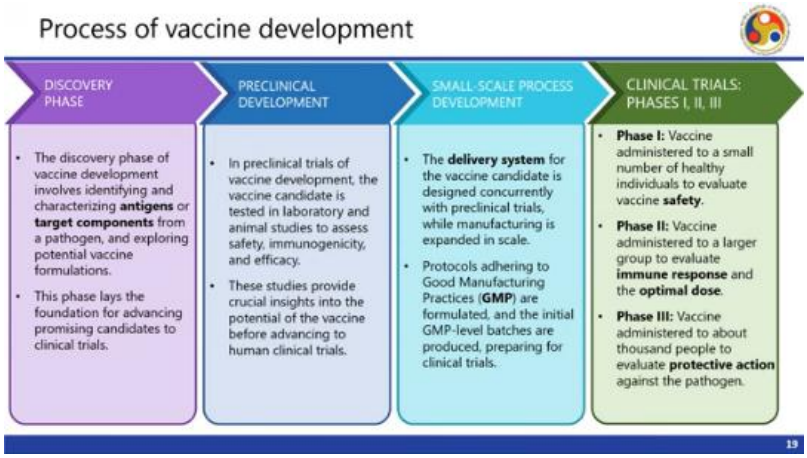
So, some of the examples include the human papillomavirus vaccine and the hepatitis B vaccine. Let us now briefly discuss the research on vaccines. Here, we will discuss the process of vaccine development. This includes the research and development phase, preclinical trials, clinical trials, approval, and finally, licensing. The process of vaccine research or development includes the discovery phase.



The discovery phase of vaccine development involves identifying and characterizing antigens or target components from a pathogen and exploring potential vaccine formulations. This phase lays the foundation for advancing promising candidates to clinical trials. The next phase is preclinical development. In this phase, the vaccine candidate is tested in laboratory and animal studies to assess safety, immunogenicity, and efficacy. These studies provide crucial insights into the vaccine's potential before advancing to human clinical trials.

Next comes small-scale process development, where the delivery system for the vaccine candidate is designed concurrently with preclinical trials while manufacturing is scaled up. Protocols adhering to good manufacturing practices are formulated, and initial GMP-level batches are produced to prepare for clinical trials. Then, the clinical trial phases begin. In Phase 1 clinical trials, the vaccine is administered to a small number of healthy individuals to evaluate its safety.

In phase 2, the vaccine is administered to a larger group to evaluate immune response and the optimal dose. In phase 3, the vaccine is administered to about 1000 people to evaluate protective action against the pathogen. Then comes the evaluation and decision stage. The preclinical and clinical trials are documented, and the vaccine must be approved by concerned authorities such as the WHO, the Food and Drug Administration in the US, and the Central Drug Standard Control Organization in India. If approved, a license to market is issued for the vaccine.



Then comes the manufacturing stage. The manufacturing process is designed and optimized as per GMP standards. Vaccine production is done on a large scale to ensure that sufficient doses are available. Then there is a safety monitoring clinical trial phase 4, also known as post-marketing, where vaccines are continuously monitored to assess any side

effects reported by patients or health professionals. So, during the initial phase of vaccine development, researchers

investigate their ideas for a potential vaccine. This phase typically spans 10 to 15 years of laboratory research, commonly conducted in private industry with frequent collaboration with university researchers. The primary goals of this phase can be summed up as below. First is the identification of targets. Researchers identify pathogens or antigens associated with significant diseases.

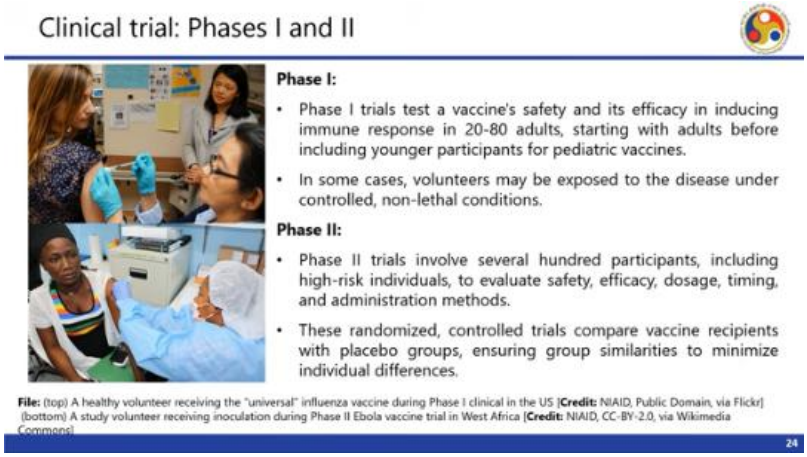
Then comes the antigen discovery. Where researchers isolate, characterize and study antigens from the target pathogen to identify suitable candidates for vaccine development. Then comes the formulation and design. Scientists design the vaccine considering factors such as the type of vaccine, adjuvants and delivery methods. So, these photographs you can see Dr. E. Hoopes and associates working on rubella vaccine in 1965 on the top picture.

And then there is the development of COVID-19 vaccine in the National Institute of Allergy and Infectious Diseases recently. So, before a vaccine undergoes human testing, it undergoes preclinical tests using lab-grown cells and animal models to evaluate the vaccine's safety and efficacy in eliciting a protective response. These tests provide insights into potential human reactions and determine a safe starting dose for human trials. If the vaccine fails to induce the desired immune response at this stage, it typically does not progress further. This phase, often lasting one to two years, is primarily conducted by private companies.

In parallel to preclinical studies, suitable delivery systems for the vaccine testing is also developed. Investigational New Drug, IND, program helps pharmaceutical companies or resources to secure permission for initiating human clinical trials prior to the approval of a

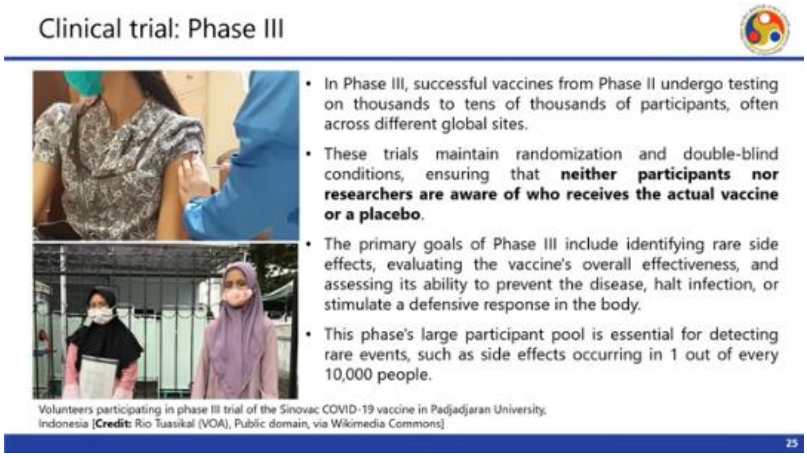
marketing application. The program is controlled by FDA in the US and CDSCO in India. Researchers are required to provide the details on vaccine manufacturing and testing, summarize laboratory findings and outline the proposed human study. Approval from an ethics committee at the trial institution is essential and the authoritative organization has a 30-day window for reviewing and approving the application.

Upon approval, the vaccine proceeds to three phases of human testing, which we have already discussed. The phase one basically tests the vaccine's safety and efficacy in inducing immune responses in around 20 to 80 adults, starting with adults before including younger participants for pediatric vaccines. In some cases, volunteers may be exposed to the disease under controlled, non-lethal conditions. In Phase II trials, several hundred participants are involved, including high-risk individuals, to evaluate safety, efficacy, doses, timing, and administration methods. These randomized controlled trials compare vaccine recipients with placebo groups, answering groups' similarities to minimize individual differences.



In Phase III, successful vaccines from Phase II undergo testing on thousands to tens of thousands of participants, often across different global sites. These trials maintain randomization and double-blind conditions, ensuring that neither participants nor researchers are aware of who receives the actual vaccine or a placebo. The primary goals of phase three include identifying rare side effects, evaluating the vaccine's overall effectiveness, and assessing its ability to prevent the disease, halt, infection or stimulate a defensive response in the body.

This phase large participant pool is essential for detecting rare events such as side effects occurring in one out of every 10,000 people. Then comes the approval and licensing stage. Vaccine licensor follows the successful completion of the development cycle including clinical trials in phase 1 to 3, demonstration of the safety, immunoactivity, and proven effectiveness in preventing infection for target populations. The World Health Organization expert-competent biological standardization establishes international guidelines for manufacturing and quality control, providing a framework for national regulatory agencies in the licensing process.

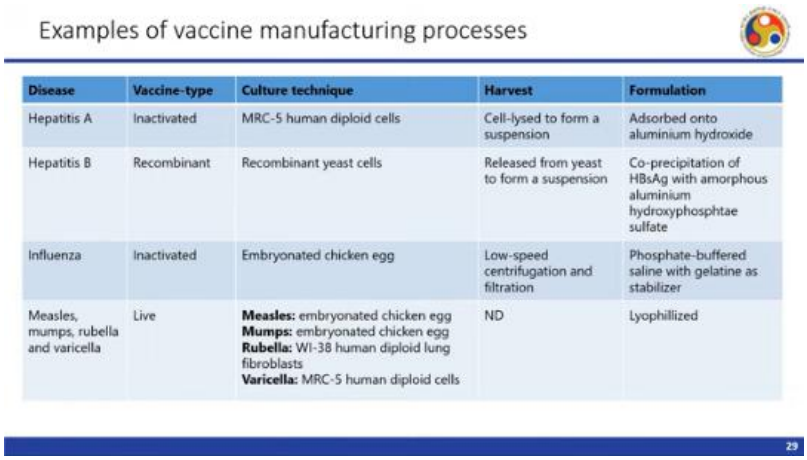


Manufacturers receive licensing only after a comprehensive clinical development cycle, scientific review by regulatory bodies such as the Central Drugs Standard Control Organization of the Government of India or the US Food and Drug Administration, and confirmation of safety and long-term effectiveness. In adopting WHO guidelines for vaccine development, Individual countries take on the responsibility of issuing national licensure and managing vaccine deployment and monitoring within their borders. Let us now discuss how vaccines are manufactured. So here we'll have an overview of the vaccine manufacturing process, then strain selection and growth of the microorganisms,

inactivation and attenuation, vaccine formulation, quality assurance, and phases for monitoring.

Some examples of vaccine manufacturing processes from the vaccine type point of view include inactivated vaccines, recombinant vaccines, or live vaccines, which we have discussed earlier in this lecture. So, corresponding to these diseases—hepatitis A, B, or measles— There are different culture techniques involved. For example, in the case of hepatitis A, which is an inactivated vaccine, we use MRC5 human diploid cells. It is harvested by lysing the cells to form a suspension and is formulated by absorption onto aluminum hydroxide.

Hepatitis B recombinant vaccines are produced by culturing recombinant cells, harvested by releasing them from yeast to form a suspension, and co-precipitated with HBsAg and amorphous aluminum hydroxyphosphate sulfate as a formulation. Influenza vaccine is an inactivated vaccine produced in embryonated chicken eggs, obtained by low-speed centrifugation and filtration, and formulated using phosphate-buffered saline with gelatin as a stabilizer. Measles, mumps, rubella, and varicella are used directly in live forms as vaccines. Measles and mumps are produced in embryonated chicken eggs, rubella in WI-38 human diploid lung fibroblasts, and varicella in MRC5 human diploid cells. Various harvesting techniques are used to obtain them, depending on

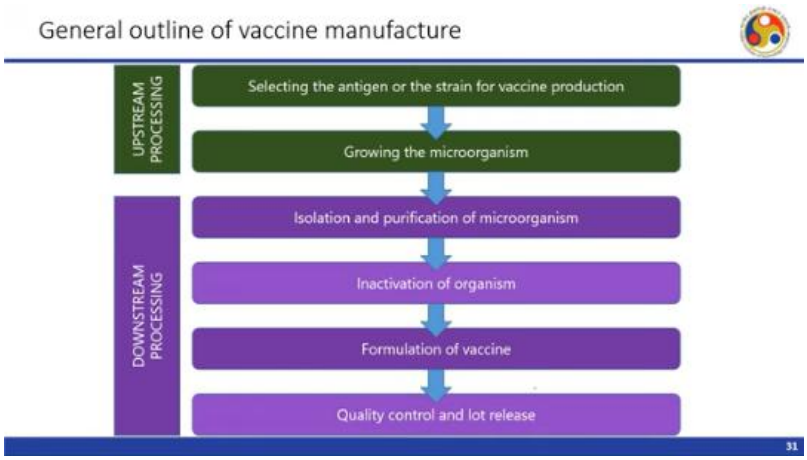


The organism, and these are mostly formulated by lyophilizing them into dry powder. Then, similarly, we have pneumococcal vaccines, which are basically conjugate vaccines. And here, we use Streptococcus pneumoniae serotypes grown on soy peptone broth. And then, Corynebacterium diphtheriae is grown on yeast extract-based media. These are obtained by polysaccharide isolation through centrifugation.

And for formulation, we use an aluminum hydroxide suspension. Polio is an inactivated vaccine. These are cultivated in Vero cells and obtained by clarification and concentration. And these are formulated in medium M199. Then you have rabies, which is again inactivated, produced on human diploid fibroblast.

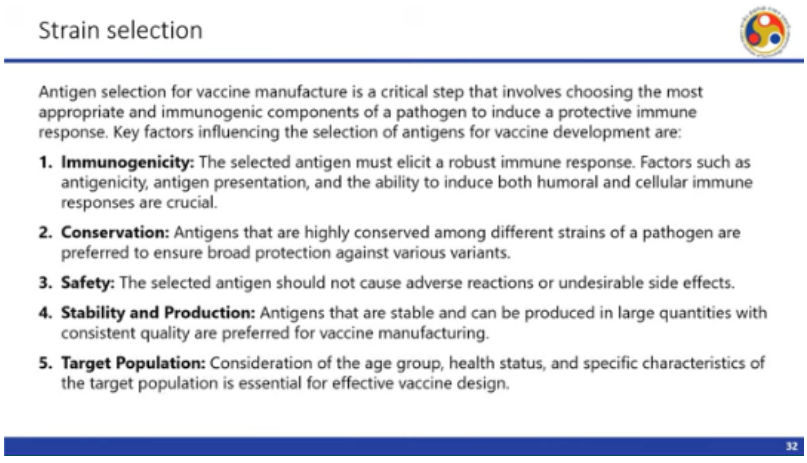
These are obtained by inactivation with beta-propiolactone. For formulation, these are stabilized with buffered polygelin and potassium glutamate in lyophilized form. So, let us look into the general outline of vaccine manufacture, which has an upstream processing phase where we select the antigen or the strain for vaccine production. Then, we grow the microorganism. Then, in the downstream processing step, we isolate and purify the microorganism.

Then we proceed with the inactivation of the organism and finally the formulation of the vaccine. After that, we check the quality, and that particular lot is ready to be released if it passes the QA step. In the strain selection phase, antigen selection for vaccine manufacturing is a critical step that involves using the most appropriate and immunogenic components of a pathogen to induce a protective immune response. Key factors influencing the selection of antigens for vaccine development are, number one, immunogenicity. The selected antigen must elicit a robust immune response.

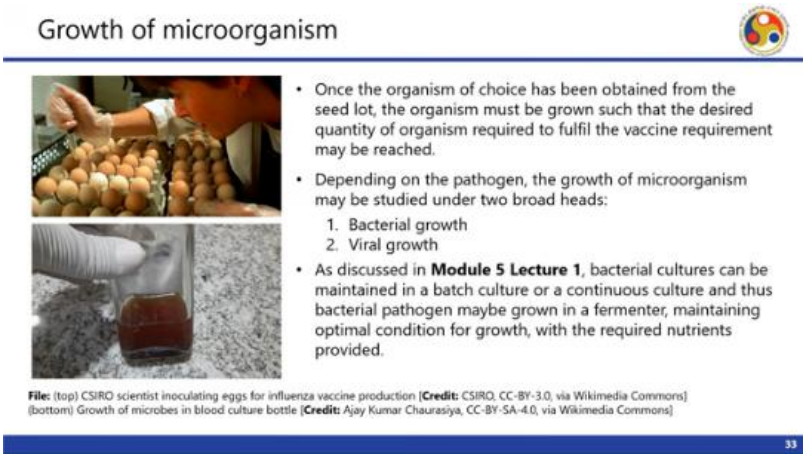


Factors such as antigenicity, antigen presentation, and the ability to induce both humoral and cellular immune responses are crucial. Antigens that are highly conserved among different strains of a pathogen are preferred to ensure broad protection against various variants. The selected antigens should not cause adverse reactions or undesirable side effects. Safety is an important aspect. Antigens that are stable and can be produced in large quantities with consistent quality are preferred for vaccine manufacturing.

Stability is very important for production. Considering the age group or target group, the health status, and the specific characteristics of the target population is essential for effective vaccine design. We should always keep in mind the target population when designing a vaccine. Once the organism of choice has been obtained from the seed lot, the organisms must be grown to reach the desired quantity required to fulfill the vaccine demand. Depending on the pathogen, the growth of microorganisms may be studied under two broad categories.



Number one, the bacterial growth. Secondly, the viral growth. Earlier, we discussed in Module 5 that bacterial cultures can be maintained in a batch culture or a continuous culture, and thus bacterial pathogens may be grown in a fermenter, maintaining optimal conditions for growth with the required nutrients provided. In the case of viral growth, cultivating viruses to produce viral materials necessary for vaccine production involves introducing the virus into a suitable host cell culture, providing an environment that allows the virus to replicate and multiply. Most vaccine producers use either different cell lines or chicken embryos for virus cultures.

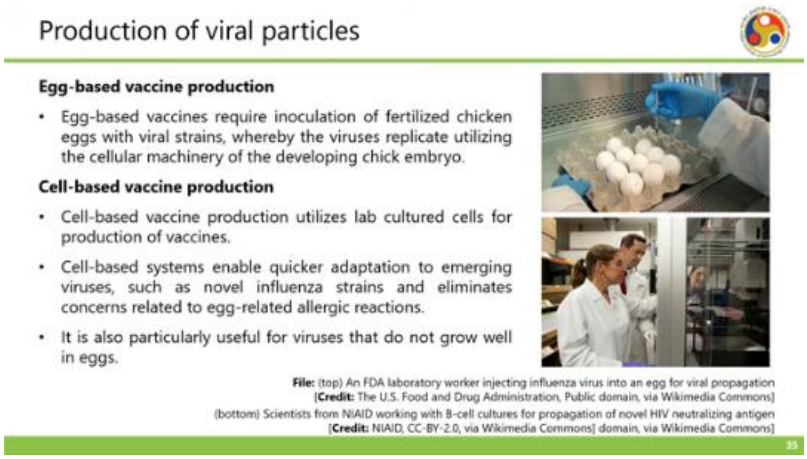


Live animals have sometimes been used for vaccine production. Once inoculated, the host is placed in a controlled environment, typically an incubator, where temperature, humidity, and other conditions are optimized for viral replication. Once a sufficient amount of virus has been produced within the host cells, the culture is harvested. After harvesting, the viral particles are purified and quantified. Now, let us look into the production of viral particles where we are using egg-based culture systems for vaccine production.

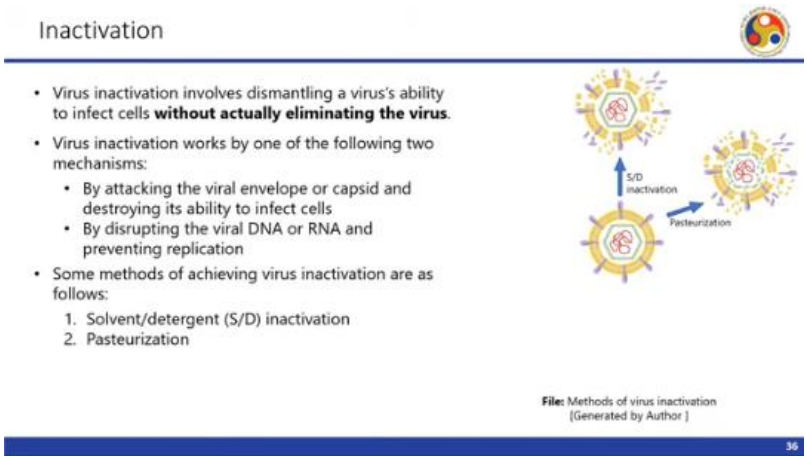
Egg-based vaccines require inoculation of fertilized chicken eggs with viral strains, whereby the viruses replicate, utilizing the cellular machinery of the developing chick embryo. We also have cell-based vaccine production as an alternative. Cell-based vaccine production utilizes lab-cultured cells for the production of vaccines. Cell-based systems enable quicker adaptation to emerging viruses, such as novel influenza strains, and eliminate concerns related to egg-related allergic reactions.

It is also particularly useful for viruses that do not grow well in eggs. Then comes the inactivation step. Virus inactivation involves dismantling a virus's ability to infect cells without actually eliminating the virus. Virus inactivation works by one of the following

two mechanisms. By attacking the viral envelope or capsid and destroying its ability to infect cells.



By disrupting the viral DNA or RNA and preventing replication. Some methods of achieving virus inactivation are as follows. We may use solvent detergent for inactivation. Then we use pasteurization or we use acidic pH for obtaining inactivation particularly low pH treatment or we may also use ultraviolet radiation for the inactivation and we may use some certain chemicals for the inactivation. Attenuation is an important process which is used in the development of vaccines to reduce the virulence or pathogenicity of a microorganism while retaining its ability to stimulate an immune response.



The goal of attenuation is to create a weakened form of the pathogen that is safe for use in vaccines but still elicits a robust and protective immune response. Some methods for achieving attenuation are attenuation by serial passage, attenuation by temperature adaptation, attenuation through genetic modification. Attenuated vaccines elicit a more robust and long-lasting immune response with a rapid onset of immunity when compared

to inactivated vaccines. So, let us look into some of these methods of attenuation for example by serial passage.

This process involves repeatedly passing a pathogen through host or cell cultures under controlled conditions, prompting genetic changes. Over time, mutations reduce the pathogen's virulence while preserving its ability to trigger an immune response. Alternatively, we may opt for attenuation by temperature adaptation, which involves gradually lowering the microorganism's growth temperature to select strains that thrive in cooler conditions. The final attenuated strain replicates well at lower temperatures but has reduced virulence at the host's body temperature.

We may also obtain attenuation by genetic modification, whereby organisms are exposed to mutagens, and mutants with less virulence are selected. Then comes the vaccine formulation stage, where careful design and preparation of the components that make up a vaccine ensure its safety, stability, and effectiveness. So, here we have these antigens; we use stabilizers, adjuvants, preservatives, surfactants, buffers, residual components, and diluents. So, these are the various components in vaccine formulation. So, the key components are the ones we have already shown in this illustration.

The immunogen, although the active ingredient, is present in minimal quantities, with a single dose containing only minute amounts. The adjuvants enhance a vaccine's effects by mimicking pathogen-associated molecular patterns, stimulating a stronger immune response against a specific disease. By activating dendritic cells, lymphocytes, and macrophages, adjuvants amplify the innate immune response, simulating a natural infection. Some commonly used adjuvants are inorganic compounds like potassium alum,

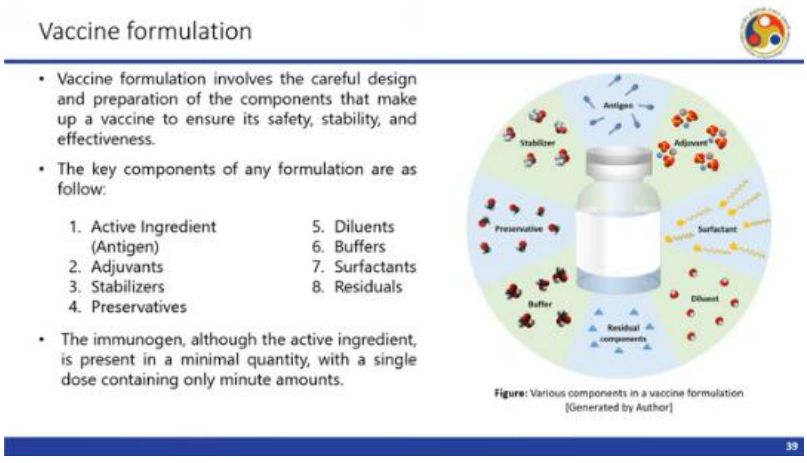
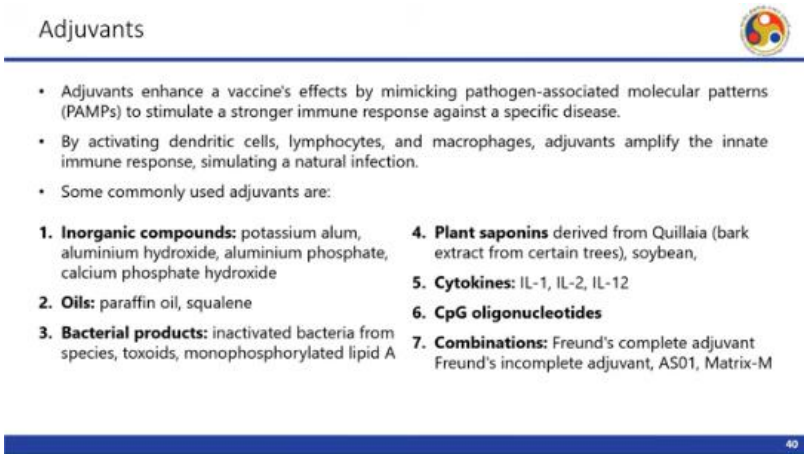


Figure: Various components in a vaccine formulation [Generated by Author]

aluminum hydroxide, aluminum phosphate, calcium phosphate hydroxide. Then we may use oils like paraffin oil and squalene. Then certain bacterial products, particularly inactivated bacteria from species toxoids, monophosphorylated lipid A. Then we have plant saponins derived from quilea, bark extract from certain trees or soybean. Then we have certain cytokines like IL-1, IL-2 and IL-12.

Then we have also CPG oligonucleotides which are used as adjuvants. Then we may have combinations, affluence incomplete adjuvant A01 matrix M and so on. Quality assurance, again quality control is very very important in vaccine production. It ensures vaccine safety, potency, purity and consistency throughout development, manufacturing and distribution. It includes rigorous testing, detailed documentation of results and deviations and post distribution surveillance to monitor safety and effectiveness.

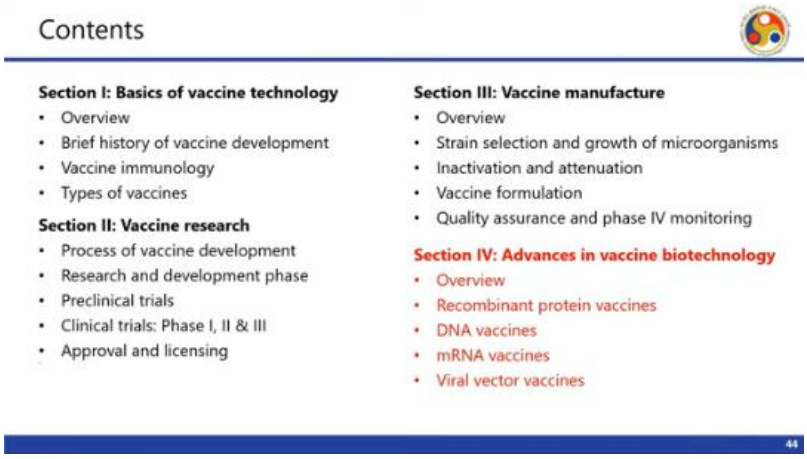


Traceability systems track vaccine batches enabling swift recalls if needed. Specific tests such as virulence tests for live vaccines ensure they do not retain or revert to virulence, reducing risks to animals and the environment. Environmental risk assessments evaluate vaccine shedding, spread and persistence. Stability tests determine the validity of the product's expiry date, ensuring its reliability over time. Then one important phase is the phase four monitoring.

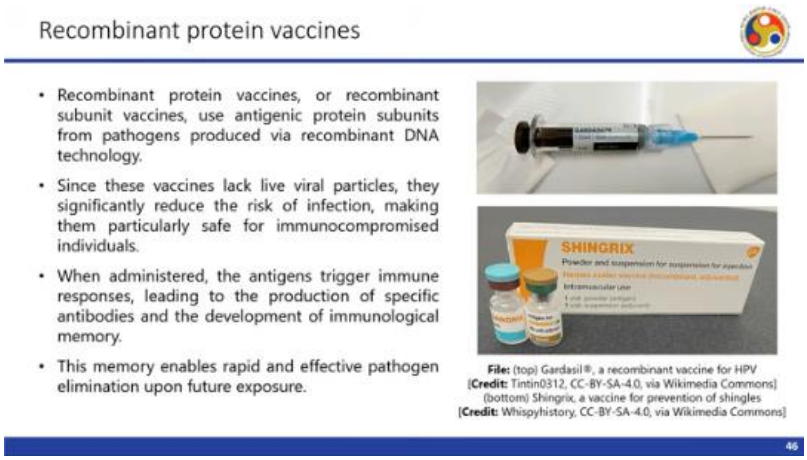
Phase four of clinical trials, also known as the post-marketing surveillance phase, focuses on monitoring the long-term safety and effectiveness of the vaccine in a larger and more diverse population over an extended period. Continuous assessments of the vaccine's safety profile over an extended period are a primary objective, including monitoring for unexpected side effects, adverse events, or interactions with other medications. Phase 4 trials assess how well the vaccine performs under real-world conditions, considering factors such as different patient populations, diverse healthcare settings, and variations in

adherence to treatment regimens. Findings from Phase 4 trials may lead to updates in the product's labeling to include new safety information or changes in recommended usage.

Let us now discuss the advances in vaccine biotechnology. We'll have an overview of the vaccine biotechnology scenario, then discuss a little about recombinant protein vaccines, DNA vaccines, mRNA vaccines, and viral vector vaccines. Research for vaccines that are safer in a broader sense and can also induce a more robust immune response has led to the recognition of genetic vaccines, which utilize DNA or RNA molecules as potential vaccine candidates, although the possibility of using genetic material has been demonstrated time and again by several researchers, including Ulmer and colleagues in 1993. Martinon and colleagues and by Howley and colleagues in 1994. The first RNA and DNA-based vaccines approved for humans were the COVID vaccines recently.

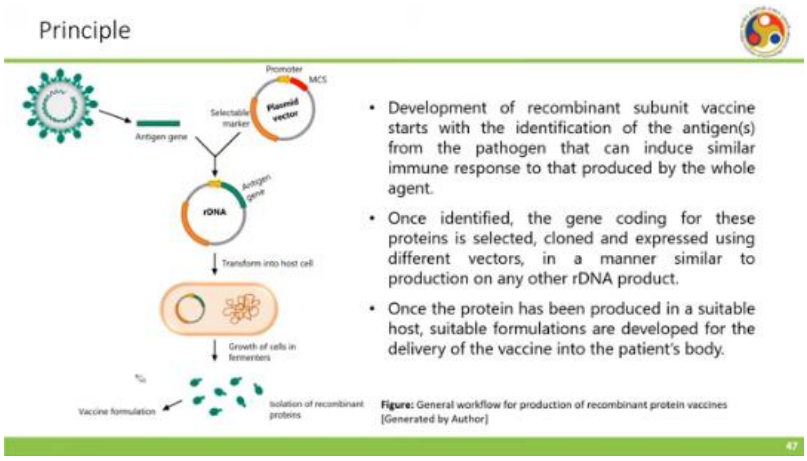


Another type of vaccine now being employed is the viral vector vaccine, first used by Moss and colleagues in 1995. Recombinant protein vaccines, or recombinant subunit vaccines, use antigenic protein subunits from pathogens produced via recombinant DNA technology. Since these vaccines lack live viral particles, they significantly reduce the risk of infection, making them particularly safe for immunocompromised individuals. When administered, the antigens trigger immune responses, leading to the production of specific antibodies and the development of immunological memory. This memory enables rapid and effective pathogen elimination upon further exposure.

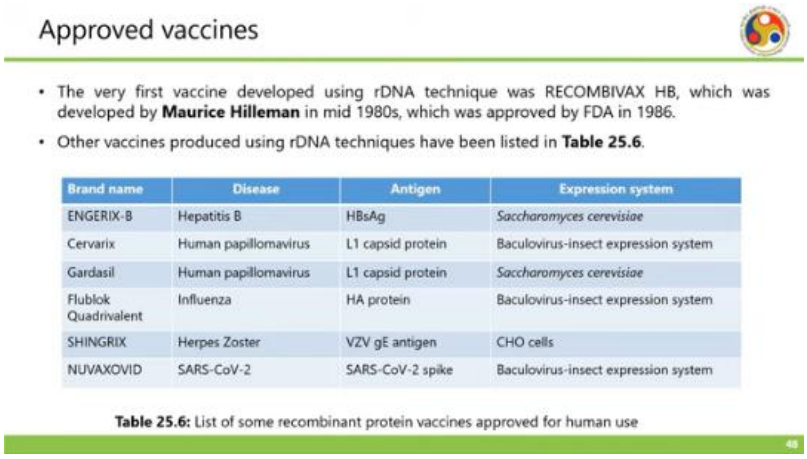


Let us look into the principles involved in this case. The development of a recombinant subunit vaccine starts with the identification of antigens from the pathogen that can induce a similar immune response to that produced by the whole antigen. So, we identify this, and this is the antigen gene. Then we have a vector with a selectable marker, a multiple cloning site, and a promoter to which the antigenic gene will be cloned. Once identified, the gene coding for this protein is selected, cloned, and expressed using different vectors in a manner similar to the production of any other recombinant DNA product.

So here, the antigen gene is cloned next to the promoter in the MCS. Once the protein has been produced, these are transformed into the host cells, and transcription and translation will follow, which will give rise to the protein. Once the protein has been produced in a suitable host, suitable formulations are developed for the delivery of the vaccine into the patient's body. So, we may use fermenters for scaling up, and finally, these are isolated, and vaccines are formulated.



The very first vaccine developed using recombinant DNA techniques was Recombivax HB, which was developed by Maurice Hilleman in the mid-1980s and was approved by the FDA in 1986. Other vaccines produced using recombinant DNA techniques can be seen in the following table, where you have the brand name in the first column, the disease in the second column, the particular antigen (which is the main vaccine component), and the various expression systems used for the production of these vaccines. So, let us just go through one or two examples. For example, the hepatitis B vaccine, sold under the brand name Engerix-B, uses the particular antigen HBsAg, which was expressed in Saccharomyces cerevisiae. Similarly, there is a SARS-CoV-2 vaccine sold under the brand name Nuvaxovid, where the SARS-CoV-2 spike protein gene was cloned into a baculovirus-insect expression system.



Approved vaccines

- The very first vaccine developed using rDNA technique was RECOMBIVAX HB, which was developed by **Maurice Hilleman** in mid 1980s, which was approved by FDA in 1986.
- Other vaccines produced using rDNA techniques have been listed in **Table 25.6**.

Brand name	Disease	Antigen	Expression system
ENGERIX-B	Hepatitis B	HBsAg	*Saccharomyces cerevisiae*
Cervarix	Human papillomavirus	L1 capsid protein	Baculovirus-insect expression system
Gardasil	Human papillomavirus	L1 capsid protein	*Saccharomyces cerevisiae*
Flublok Quadrivalent	Influenza	HA protein	Baculovirus-insect expression system
SHINGRIX	Herpes Zoster	VZV gE antigen	CHO cells
NUVAXOVID	SARS-CoV-2	SARS-CoV-2 spike	Baculovirus-insect expression system

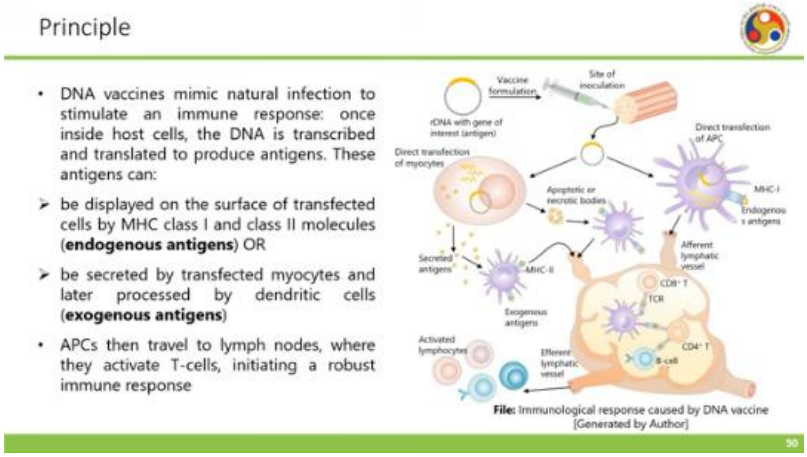
Table 25.6: List of some recombinant protein vaccines approved for human use

So, similarly there are others like Gardasil, Cerverix. So, kindly please go through this table for a full update. So, let's now discuss about DNA vaccines. These vaccines deliver antigen coding DNA sequences into an organism's cells to stimulate an immune response. A genetically engineered plasmid containing the DNA sequence for the desired antigen is injected.

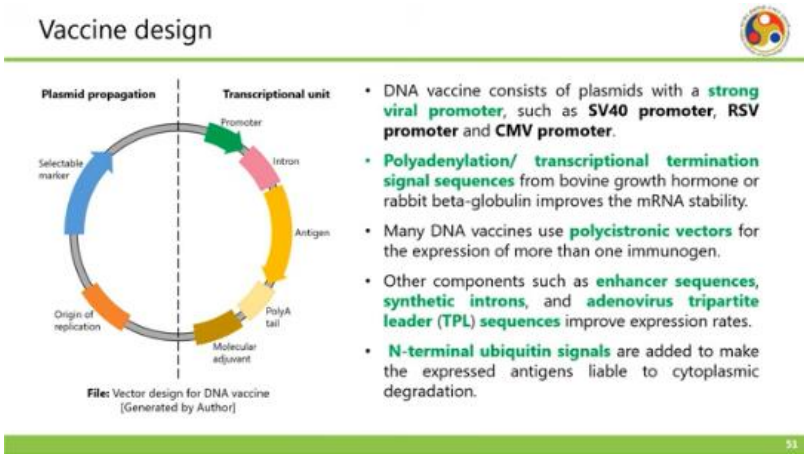
And once inside the cells, the DNA is translated into the antigen triggering immunity. These vaccines can stimulate a broader range of immune response compared to traditional vaccine. In August 2021, Zykov D developed by India's Cadilla Healthcare became the first DNA vaccine to receive emergency approval for human use. Now we have these principles illustrated over here. So, DNA vaccines mimic natural infection to stimulate an immune response.

Once inside the host cells, the DNA is transcribed and translated to produce antigens. These antigens can be displayed on the surface of transfected cells by MSC class 1 and class 2 molecules. Here you can see the site of inoculation once the vaccine is formulated using rDNA having the gene of interest. So, these are getting presented into the antigen presenting cells or it can be secreted by transfected myocytes and later processed by dendritic cells as in the case of exogenous antigens.

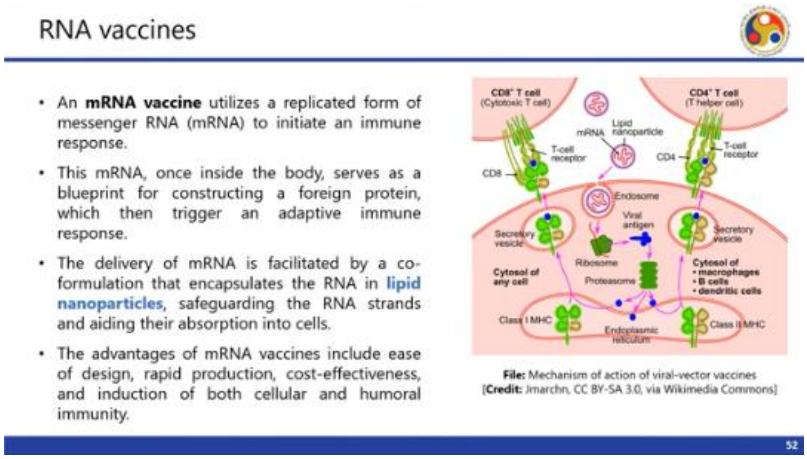
APCs then travel to lymph nodes where they activate T cells, initiating a robust immune response. DNA vaccines consist of a plasmid with a strong viral promoter, such as the SV40 promoter, RSV promoter, or CFB promoter. Polyadenylation transcriptional termination signal sequences, as you can see here from bovine growth hormone or rabbit beta-globin, improve mRNA stability. Many DNA vaccines use polycistronic vectors for the expression of more than one immunogen. Other components include enhancer sequences, synthetic introns, and adenovirus tripartite leader sequences, which improve expression rates.



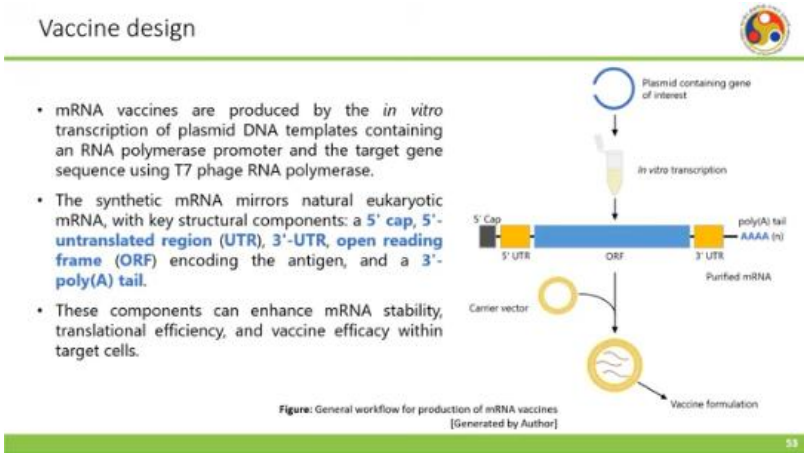
Then there is an N-terminal ubiquitin signal, which is added to make the expressed antigens liable to cytoplasmic degradation. Let us now discuss RNA vaccines. An mRNA vaccine utilizes a replicated form of messenger RNA to initiate an immune response. This mRNA, once inside the body, serves as a blueprint for constructing a foreign protein, which then triggers an adaptive immune response. The delivery of mRNA is facilitated by a co-formulation that encapsulates the RNA in lipid nanoparticles, aiding their absorption into the cells, as you can see here in this picture.



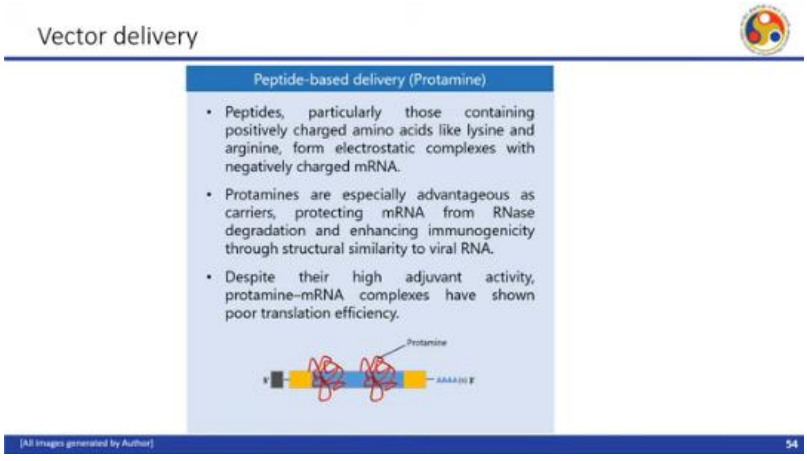
The advantages of mRNA vaccines include ease of design, rapid production, cost-effectiveness, and induction of both cellular and humoral immunity. This viral antigen mRNA is transcribed and translated, and the viral antigen is produced, which then interacts with class 1 and class 2 MHCs, leading to both cellular and humoral immunity. These mRNA vaccines are produced by the in vitro transcription of plasmid DNA templates containing an RNA polymerase promoter and a target gene sequence, using T7 fast RNA polymerase. The synthetic mRNA mirrors naturally occurring eukaryotic mRNA with key structural components. A 5' cap, 5' UTR or untranslated region.



Then you have the 3' UTR here, an open reading frame which encodes the antigen in a 3' poly-A tail. These components can enhance mRNA stability, translation efficiency, and vaccine efficacy within the target cells. So, this is the carrier vector, and this is the vaccine after formulation. Let us now discuss how these vectors are delivered. One of the ways to deliver is using peptides as a delivery agent.

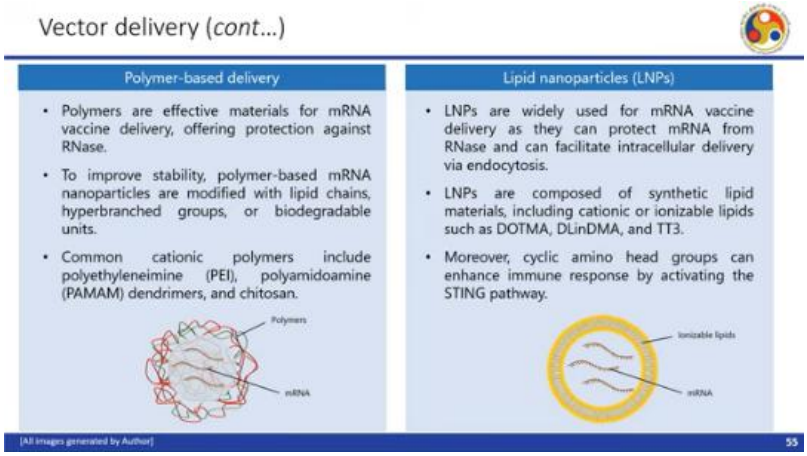


Peptides, particularly those containing positively charged amino acids like lysine and arginine, form electrostatic complexes with negatively charged mRNA. Protamines are especially advantageous as carriers, protecting mRNA from RNA degradation and enhancing immunogenicity through structural similarity to viral RNA. However, despite their high adjuvant activity, protamine-mRNA complexes have shown poor translation efficiency. So, there are other systems available, like the polymer-based delivery systems. Polymers are effective materials for mRNA vaccine delivery, offering protection against RNases.

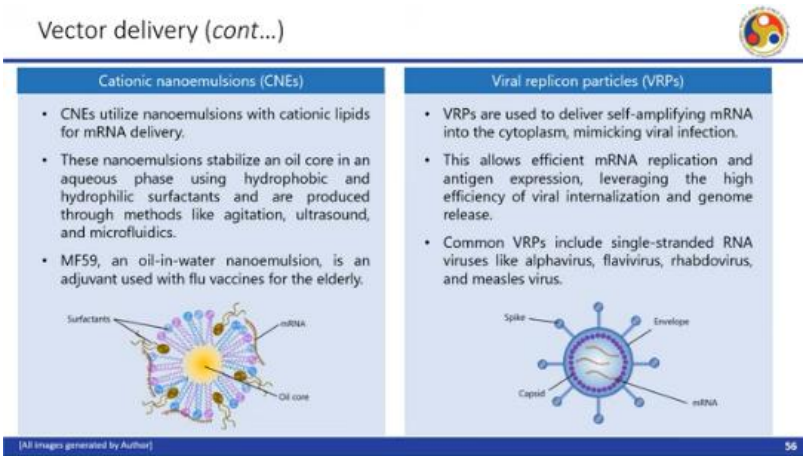


To improve stability, polymer-based mRNA nanoparticles are modified with lipid chains, hydrophilic groups, or biodegradable molecules. Common cationic polymers include polyethyleneimine, polyamidoamine, dendrimers, and chitosans. Then we have the lipid nanoparticles, which are widely used for mRNA vaccine delivery as they can protect mRNA from RNases and can facilitate intracellular delivery via endocytosis. Lipid nanoparticles are composed of synthetic lipid materials, including cationic or ionizable lipids such as DOTMA, DLin-MC3-DMA, and TT3.

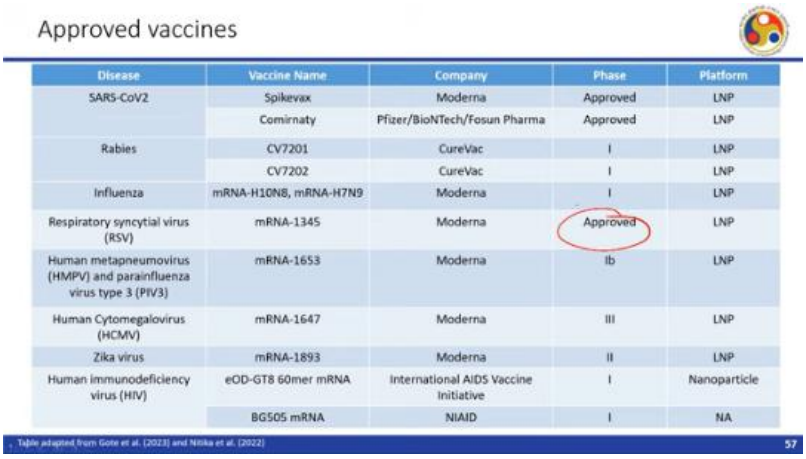
Moreover, cyclic amino head groups can enhance immune response by activating the STING pathway. Then we have the cationic nanoemulsions or CNEs, which utilize nanoemulsions with cationic lipids for mRNA delivery. These nanoemulsions stabilize an oil core. In an aqueous phase using hydrophobic and hydrophilic surfactants, they are produced through methods like agitation, ultrasound, and microfluidics. MF59, an oil-in-water nanoemulsion, is an adjuvant used with flu vaccines for the elderly.



Then we have the viral replicon particles. These are used to deliver self-amplifying mRNA into the cytoplasm, mimicking viral infection. This allows efficient mRNA replication and antigen expression, leveraging the high efficiency of viral internalization and genome release. Then we have the common VIPs, which include single-stranded RNA viruses like alphavirus, flavivirus, rhabdovirus, and measles virus. So, here in this table, a list of vaccines approved against certain diseases is listed in column 1, for example, SARS-CoV-2.



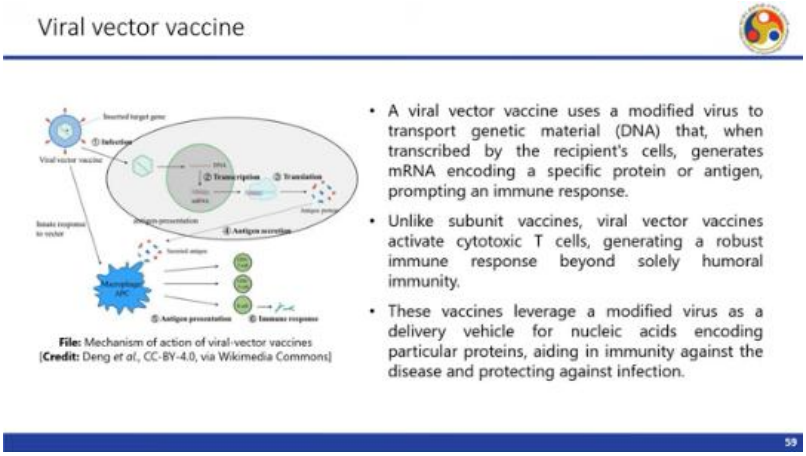
The vaccine names like Spikevax or alternatively Comirnaty are produced by two different companies: Moderna (the first one) and Pfizer (the second one). These have currently received approval, and the formulation they use is lipid nanoparticles. So, we can see that lipid nanoparticles have been used extensively in delivering various vaccines for diseases like rabies, influenza, respiratory syncytial virus, and so on. In various phases of clinical trials, some are in clinical trial 1, some in 3, some in 2, and some are already approved. So, we can see that formulation is an important part of the vaccine development process. So, in 2023, the Nobel Prize in Physiology or Medicine was awarded jointly to Katalin Karikó and Drew Weissman for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19.



Disease	Vaccine Name	Company	Phase	Platform
SARS-CoV2	Spikevax	Moderna	Approved	LNP
	Comirnaty	Pfizer/BioNTech/Fosun Pharma	Approved	LNP
Rabies	CV7201	CureVac	I	LNP
	CV7202	CureVac	I	LNP
Influenza	mRNA-H10N8, mRNA-H7N9	Moderna	I	LNP
Respiratory syncytial virus (RSV)	mRNA-1345	Moderna	Approved	LNP
Human metapneumovirus (HMPV) and parainfluenza virus type 3 (PIV3)	mRNA-1653	Moderna	Ib	LNP
Human Cytomegalovirus (HCMV)	mRNA-1647	Moderna	III	LNP
Zika virus	mRNA-1893	Moderna	II	LNP
Human immunodeficiency virus (HIV)	eOD-GT8 60mer mRNA	International AIDS Vaccine Initiative	I	Nanoparticle
	BG505 mRNA	NIAID	I	NA

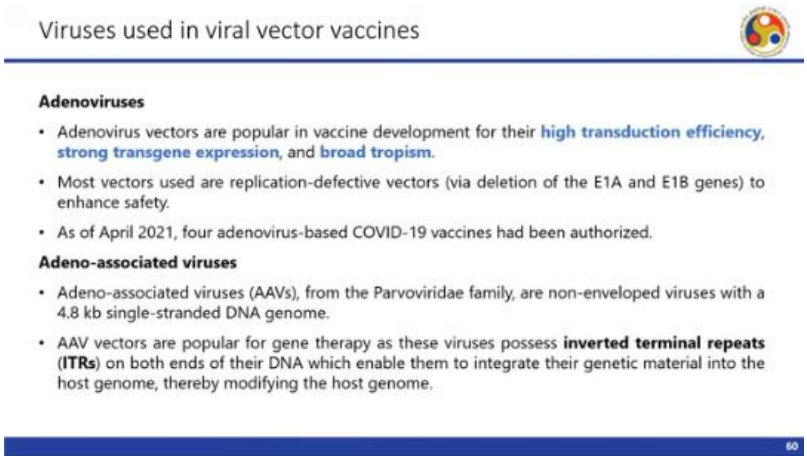
So, now viral vectors are also used as a vaccine. A viral vector vaccine uses a modified virus to transfer genetic material that when transcribed by the recipient cells generates mRNA encoding a specific protein or antigen prompting an immune response. Unlike subunit vaccines, viral vector vaccines activate cytotoxic T cells, generating a robust immune response beyond solely humoral immunity. These vaccines leverage a modified

virus as a delivery vehicle for nucleic acids and calling particular proteins, aiding in immunity against the disease and protecting against infection. Some of the viruses which are used in viral vector vaccines are adenoviruses.

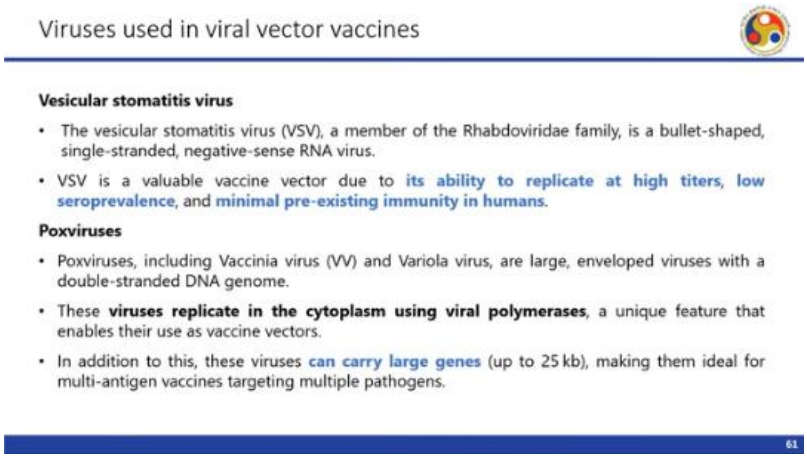


These are popular in vaccine development for their high transduction efficiency, strong transient expression and broad tropism. Most vectors used are replication defective vectors via deletion of the E1A and E1B genes to enhance safety. As of April 2021, four adeno-based COVID-19 vaccines were authorized. Then we have the adeno-associated viruses from the Parvoviridi family. These are non-enveloped viruses with a 4.8 kb single-stranded DNA genome.

These vectors are popular for gene therapy as these vectors possess inverted terminal repeats on both ends of their DNA, which enable them to integrate their genetic material into the host genome, thereby modifying the host genome. Then we have vesicular stomatitis virus which is a member of the rhabdoviridis family. It is bullet sepid single-stranded negative sense RNA virus. It's a valuable vaccine vector due to its ability to replicate at high titers, low seroprevalence and minimal pre-existing immunity in humans. Then another type of virus is the pox viruses which include



Vaccinia virus and variola virus are large enveloped viruses with a double-stranded DNA genome. They replicate in the cytoplasm using viral polymerases, a unique feature that Anabusde uses in vaccine vectors. They can carry large genes up to 25 kb, making them ideal for multi-antigen vaccines targeting multiple pathogens. Here, we can see various vectors listed in column one for carrying different vaccine candidates. Mostly, we see extensive work on SARS-CoV-2 because it was one of the most dangerous diseases in recent times.



In this column, you find the targeted antigen, particularly the spike protein in the case of SARS-CoV-2. For Japanese encephalitis and dengue, you have the viral envelope, PRM, and E. For the Ebola virus, we have the glycoproteins, and so on. Here in this list are the companies that have developed these different vaccines. With this, we finally come to the end of today's lecture.

Approved vaccines

Vector	Vaccine	Target pathogen	Encoded antigen	Developer
Ad5	Ad5-nCoV (Convidecia)	SARS-CoV-2	Spike protein	CanSino Biologics (China)
	Ad5-EBOV	Ebola virus	Glycoprotein	CanSino Biologics
Ad26	Ad26. CoV	SARS-CoV-2	Pre-fusion- stabilised spike protein	Janssen Pharmaceutical Companies
	Sputnik light	SARS-CoV-2	Spike protein	Gamaleya Research Institute of Epidemiology and Microbiology (Russia)
ChAdOx1	ChAdOx1- nCoV-19 (Covishield, Vaxzevria)	SARS-CoV-2	Spike protein with tissue plasminogen leader sequence	University of Oxford/AstraZeneca
VSV	VSV-EBOV (rVSV-ZEBOV, Ervebo)	Ebola virus	Glycoprotein	Merck
YF 17D	ChimeriVax-JE (Imojev)	Japanese encephalitis	Viral envelope (prM and E)	Sanofi Pasteur
	CYD-TDV (Dengvaxia)	Dengue	Viral envelope (prM and E)	Sanofi Pasteur
Ad5/Ad26	Gam-COVID-Vac (Sputnik V)	SARS-CoV-2	Both spike proteins	Gamaleya Research Institute of Epidemiology and Microbiology
VSV/Ad5	GamEvac-Combi	Ebola virus	Both glycoproteins	Gamaleya Research Institute of Epidemiology and Microbiology

Table adapted from McCann et al. (2022)

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Thank you for your kind attention. Thank you.