UNIT III

MEDICAL BIOTECHNOLOGY

VACCINES

A vaccine is an antigenic preparation of microorganisms such as bacteria, viruses or their products administered for prevention of infectious diseases. A vaccine for diseases is prepared from the microbes that causes disease.

The vaccine is administered in advance so as to give the body time to set immunity, before the invasion of pathogens occurs. Vaccines improves the immunity to a particular disease. An injected vaccine induces the host to generate antibodies against the disease-causing organism; therefore, during the further exposures, the infectious agent is inactivated, its proliferation is prevented and disease state is not established. Effective vaccines stimulated the immune system by promoting the development of antibodies which quickly and effectively attack disease-causing microorganisms when it enters the body, preventing disease development. A vaccine contains live-attenuated or killed microorganisms or parts from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity. A vaccine stimulates a sufficient number of memory T and B lymphocytes to yield effectors T cells and antibody-producing B cells from memory cells of neutralizing antibodies.

Vaccination is injection of antigens into the body to produce immunity and to protect against diseases. It is a process of active immunization. The first injection of the vaccine is called as primary dose. The subsequent injection of the same vaccine is called booster dose. Vaccines are given orally or subcutaneous injection or intracellular injection

Edward Jenner is called father of vaccination. The first vaccine used by Jenner is (cowpox) inoculation of humans confers immunity to smallpox but does not cause smallpox.

Discovery of vaccines

Jenner took the blisters from milk maids who were suffering cowpox 9-year-old healthy. Throught life the boy did not develop small-pox throught his life time and become immune.

Mechanism of vaccination

The immune system is a network of cells, tissues and organs that work together to help fight off infection from harmful bacteria or viruses. When a disease-causing agent, such as virus or bacteria, invades your body, your immune system recognises it as harmful and will trigger a response to destroy it. One of the ways our immune system fights against infection is by creating large proteins known as antibodies. Each antibody is specific to the bacteria or virus that it has detected and will trigger a specific immune response. These specific antibodies will remain in the immune system after the infection has gone. If the same disease-causing bacteria is attacked again, our immune system has a ‘memory’ of the disease and is ready to quickly destroy it before we get sick and any symptoms can develop.

Vaccination is the safest and most common way to gain immunity against a bacteria or virus that your body has yet to encounter. Vaccines contain a very small amount of bacteria or virus that causes the disease The bacteria or virus will be killed, greatly weakened, or broken down into small parts before use in the vaccine so that they can trigger an immune response without making body to develop disease. Our immune system recognizes the harmless form of bacteria or virus from the vaccine and will produce antibodies to fight it off. The immune system then keeps a memory of the disease, so if a vaccinated person encounters the disease years later, their immune system is ready to fight it off and prevent an infection from developing. Thus, the main aim of the vaccines is to stimulate the production of antibodies without the person actually having the to develop disease. The pathogen entering the cells produces cell mediated immune response and pathogen present in the intracellular space produces humoral immune response.

Importance of vaccines: -

- The body develops immunity
- Infectious disease is prevented.
- Certain disease are treated
- Small pox, polio are eradicated from humans by vaccines.

The vaccines are classified as following types: -

I) Live attenuated Vaccines/Attenuated vaccines.

II) Inactivated whole vaccines/Killed vaccines

I)Live attenuated Vaccines/Attenuated vaccines.

The vaccines created by reducing the virulence and harmful effects of pathogens are called attenuated vaccines. It is an alive vaccine. Attenuation makes the pathogen harmless and less virulent. The pathogens are attenuated in a such way their antigenicity is retained thereby stimulating the production of antibodies. **Attenuation vaccines is easier for the host immune system to destroy the pathogen and thus create the immunological memory cells. The memory cells will protect the patient from the infection of similar pathogen.**

Attenuation can often be achieved by growing a pathogenic bacterium or virus for prolonged periods.

Methods followed to inactivate or attenuate the pathogen: -

1) **Ultraviolent inactivation: -**

UV rays are used to inactivate the virus particles, since virus particles are small and UV-rays can reach the genetic material, inducing the dimerization of nucleic acids. Once the DNA dimerized the virus particles cannot replicate their genetic material.

2) **Solvent detergent inactivation: -**

When the lipid coated viruses are treated with detergents, the detergents disrupt the interaction between the molecules in the lipid coat stopping the replication of virus because most of the virus cannot live without their lipid coating so they cannot survive when exposed to detergents. Some viruses may survive but cannot replicate thus becomes in-effective. The detergent typically used is Triton-X 100.

3) **Use of chemical agents: -**

Formaldehyde is used to inactivate the bacteria and virus.

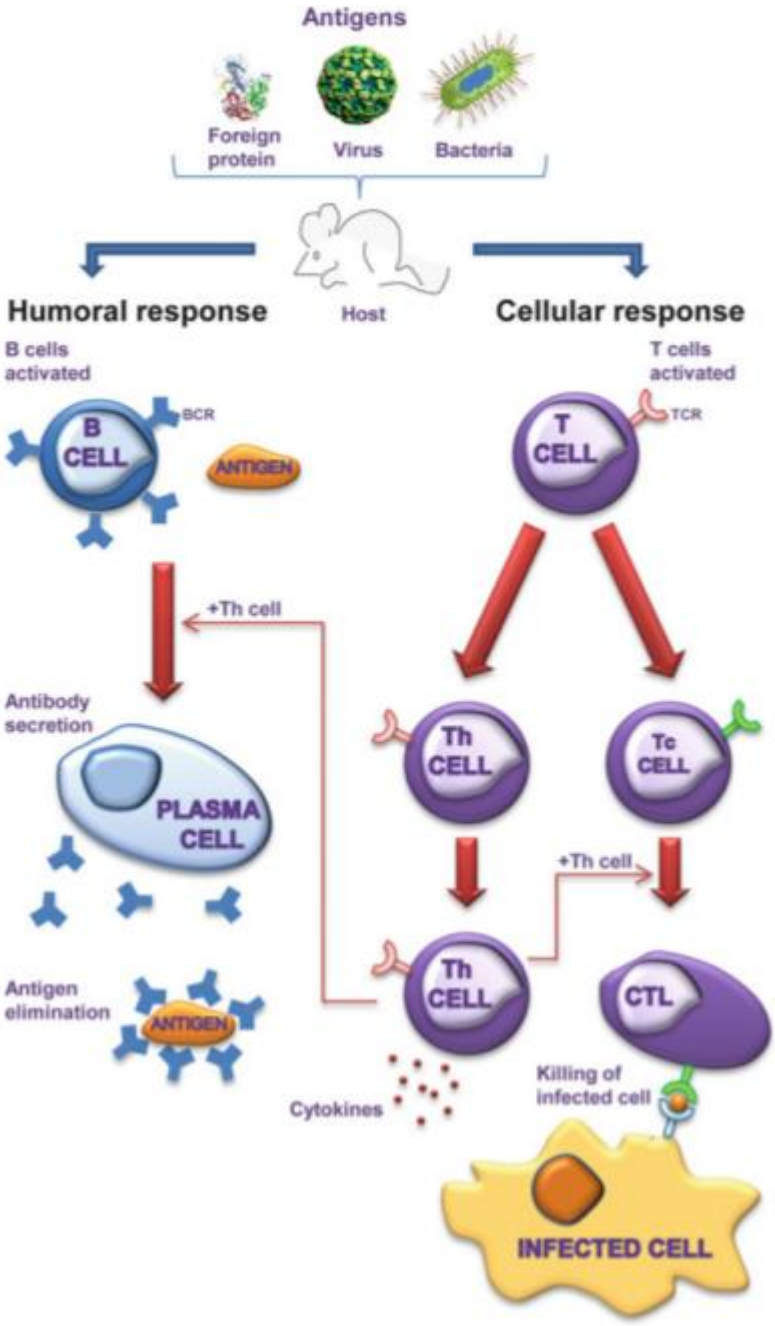
Advantages: -

- Single dose
- Produces both antibody mediated and cell mediated Immunity.
- No need of adjuvants
- Low cost
- Easy of transportation
- Quick action.
- Prolonged exposure to immune system
- Induce cell mediated and humoral immunity.
- Induce cell mediated to immunity.
- Induce humoral immunity

Disadvantages: -

Chances of Reversion of Virulence due to mutation which may lead to the development of infection.

Causes side effects in some cases.



Examples of vaccines prepared by attenuation of virulence

Disease/pathogen	**Method of attenuation**
Rabies	Cultivation in chick embryo
Yellow fever	Cultivation in chick embryo
Small pox	Animal passage
Polio	Mutant strain (Sabin oral polio vaccine)
Anthrax	Cultivation at 40^0-50^0 C
B.C. G	Cultivation of organism on media containing bile.

II)Inactivated whole vaccines/Killed vaccines: -

Inactivated vaccines are made from microorganisms (virus/bacteria) that are killed by heat or formaldehyde. The DNA cannot replicate, the capsid remains intact. It is enough to evoke the immune response. The vaccine is not infectious, since the vaccine does not reproduce, booster doses not required. These killed organism cannot cause disease. It is critically important to maintain the structure of epitopes on surface antigens during inactivation. Inactivated vaccines have a good stability profile compared to live attenuated vaccine. Chemical inactivation with formaldehyde or various alkylating agents has been successful. When the dead pathogen is injected into organism (host), it stimulates the production of antibodies without causing infection.

Immune response: -

Inactivated whole vaccines may not have induce a significant immune response and response are not long lived. Several doses of inactivated whole vaccines are required to produce a sufficient immune response.

Vibrio Cholera

Pure cultures of suitable strains of Vibrio Cholera are grown in the solid media at 37 ^{0} C for few days. The strains are carefully selected for retention of antigenic property. After the incubation period the organism is harvested in the solution, the suspension is centrifuged, the supernatant fluid is discarded and the cells are resuspended in the isotonic saline. The cells are killed by minimal heat treatment for 1 hour 56 ^{0} C or by treatment with chemical bactericide. The approximate number of dead organisms in the suspension is determined by total bacterial count method (TBC method) and the suspension is diluted with the bacteriostatic saline to contain standard number of organisms which is set for standards for labelling and storge.

Pertussis vaccine

Suitable strains of Haemophilus pertussis are grown on the liquid media for 24-72 hours. The organism is harvested in the saline, the suspension is centrifuged, the supernatant fluid is discarded and the cells are resuspended in the isotonic saline containing the bactericide solution. The suspension is stored in cold for several months to reduce its toxicity and then final dilution is made and then standards are set for toxicity, sterility and requirements for storage and labelling.

Advantages: -

- Safer and more stable compared live attenuated vaccines.
- Since they have no live components there is no risk of infection.

Disadvantages: -

- There is a need for adjuvants
- Since responses are not long-lived multiple doses are required.
- Less strong immune response compared to live vaccines.

Examples: -

- Anthrax vaccine
- Cholera Vaccine
- Pertussis vaccine

- Influenza vaccine

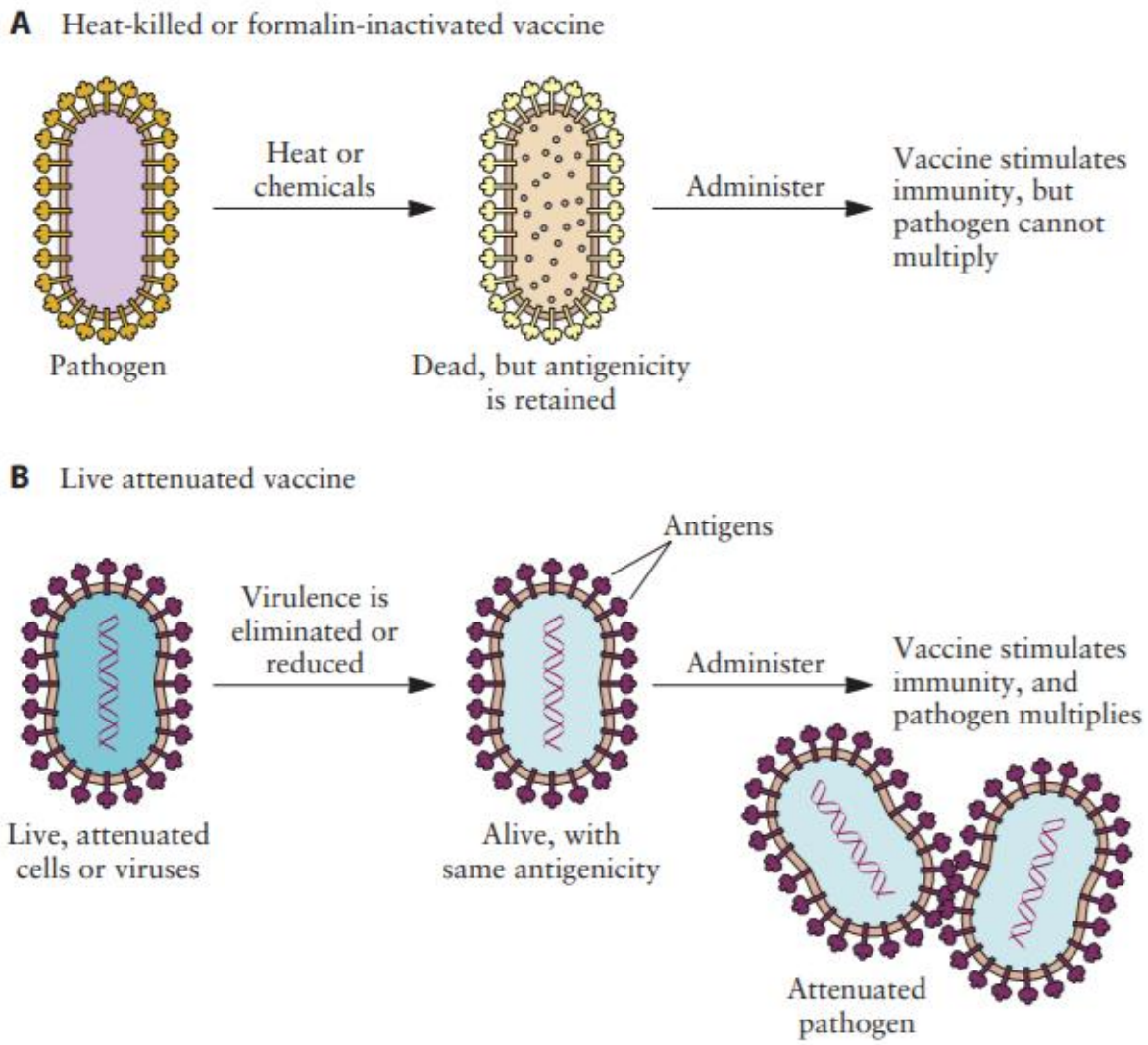


Figure showing the types of vaccines. (A) Heat-killed vaccines (B) Live attenuated vaccines

Difference between Live vaccine and live attenuated vaccine: -

Features	**Live Vaccine**	**Killed Vaccine**
Dose administered	Single dose	Multiple dose
Stability	Less stable	More stable
Immunity induction	Both Antibody mediated and Cell mediated Immunity	Mainly Antibody mediated immunity
Cell mediated Immunity	Good	Poor
Duration of Immunity	Many years	less
Antibody response	IgG	IgG, IgA
Need of Adjuvant	No	Yes
Reversion of Virulence	Possible	Not Possible
Production	Selection of avirulent organism by growing virulent organism in	Directly virulent pathogen is killed or inactivated by chemicals

	adverse culture condition Selection of avirulent organism by prolong passage of avirulent pathogen through different host	(formaldehyde or Alkylating agents), gamma rays or heat
Examples	Sabin's Polio vaccine, Measles vaccine, BCG	Anthrax vaccine, Cholera Vaccine, Pertussis vaccine

Steps involved in the production of vaccines: -

1) Manufacturing of vaccines begins with the small number of specific pathogens. The pathogens should pass through the tests of sterility. The pathogen should be free from impurities and other bacteria, ideal conditions are maintained usually frozen –so that it does not become weaker and do not further divide.
2) Selecting strains: -The strain of pathogen to cause pathogenicity is selected. Restriction fragment length polymorphism (RFLP) is a method for identifying bacterial strains using unique fingerprints which relies on the presence of variations (polymorphisms) in homologous DNA sequences.
3) The strain is cultured in a petridish with the appropriate media which act like stock culture.
4) Bulk production: - This is carried in fermenters with controlled pH, temperature, aeration by providing appropriate media. The cells are harvested till it reaches 10^{8}/cells.
5) Cells are separated from the media by using different techniques like centrifugation, chromatography and filtration.
6) An adjuvant improves the immune response to the vaccine, sometimes by keeping the vaccine at the injection site for a little longer or by stimulating local immune cells. Eg: Aluminium phosphate, aluminium hydroxide or potassium aluminium sulphate. The adjuvant may be a tiny amount of aluminium salts (like aluminium phosphate, aluminium hydroxide or potassium aluminium sulphate). Aluminium has been shown not to cause any long-term health problems, and humans ingest aluminium regularly through eating and drinking.
7) Surfactants keep all the ingredients in the vaccine blended together. They prevent settling and clumping of elements that are in the liquid form of the vaccine.
8) Stabilizers prevent chemical reactions from occurring within the vaccine and keep the vaccine components from sticking to the vaccine vial. Stabilizers can be sugars (lactose, sucrose), amino acids (glycine), gelatine, and proteins (recombinant human albumin, derived from yeast).
9) Lyophilizer: - is a low temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation. This is in contrast to dehydration by most conventional methods that evaporate water using heat. Because of the low temperature used in processing, the quality of the rehydrated product is excellent

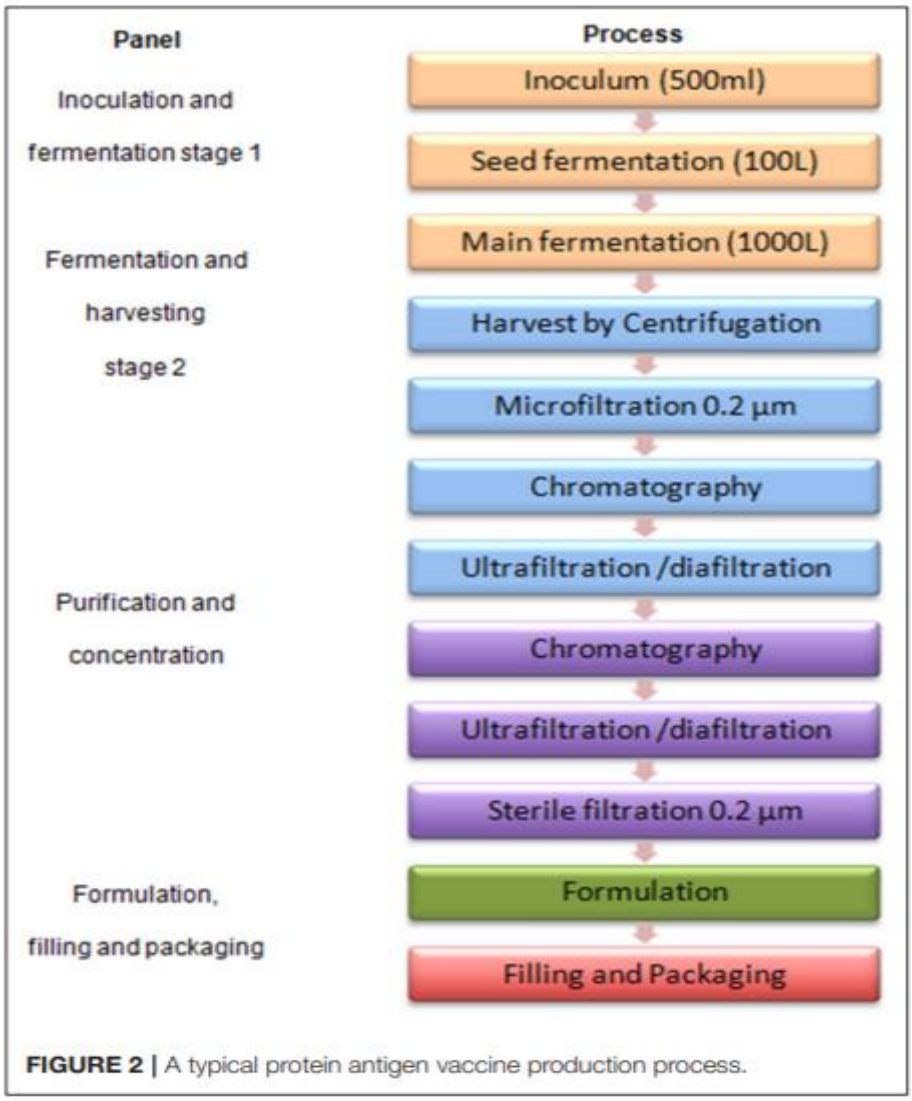


FIGURE 2 | A typical protein antigen vaccine production process.

Steps involved in the production of vaccines

Limitations of Conventional vaccines/traditional vaccines: -

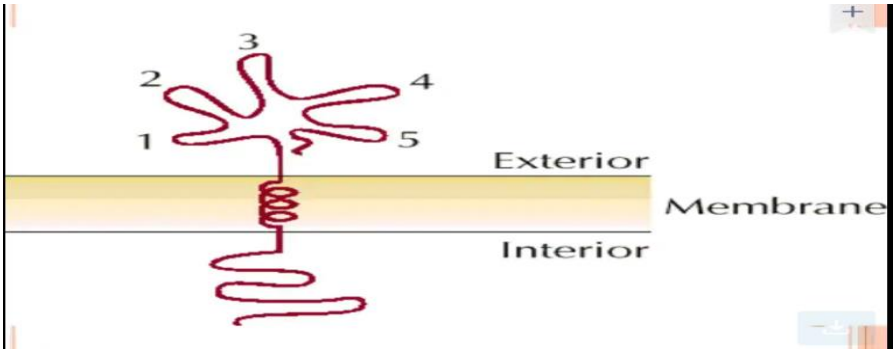
- Not all the infectious agents can be grown on the media, so vaccines are not developed for several diseases.
- Both the yield and rate of production of animal and human viruses in culture are often low thereby making vaccine production costly.
- Extensive safety and precautions are necessary to ensure the laboratory and production, so that person is not exposed to pathogenic agent.
- Attenuated virus may revert so chances of mutations and infections are more which requires continuous testing to ensure the virulence has not occurred.
- Not all the disease are preventable through the use of traditional vaccines. Eg: -AIDS.
- Production of vaccine is time consuming.
- Batches of vaccines may not be killed or may be insufficiently attenuated during the production process, thereby introducing the virulent organisms into the vaccine and spreading the diseases.
- Many vaccines have limited shelf life an often require refrigeration to maintain to potency. This requirement creates a storage problem in countries with rural areas which are not equipped with electrical facility

Peptide vaccines :-

Peptide-based synthetic vaccines are contain peptides which are the mimic the epitopes of the antigen that triggers immune responses. Peptide vaccines are synthesized peptides of 20–30 amino acids, which are highly immunogenic and to trigger the desired immune response. These subunit vaccines are composed of antigens purified from microbes which are usually administered with an adjuvant. The immunologically accessible regions of pathogens are used to develop a peptide vaccine, remaining part is ignored.Purified outer surface viral proteins either capsid or envelope proteins are alone sufficient for electing neutralizing the antibodies in the host organism.

Peptides used in these vaccines are 20–30 amino acid sequences that are synthesized to form an immunogenic peptide molecule representing the specific epitope of an antigen. The peptides which are present on the exterior surface are immunologically accessible, so only those peptides are used as vaccines.

A peptide vaccine in which peptide of the original pathogen is used to immunize the organism. Peptide vaccines are stable and relatively cheap to manufacture. Peptide vaccines are highly immunogenic in their free form provided they contain in the addition to the B- cell epitope-cell epitope recognized by T-cell helper cells. Such T-cell epitope can be provided by carrier protein molecules, foreign antigens or synthetic peptide molecule.



Generalized envelope bound protein with external epitope (1to 5) that elicts the immune response.

Examples of peptide vaccines:-Influenza (injection), Haemophilus influenzae type b (Hib), Pertussis Human papillomavirus (HPV).

The key feature of peptide-based vaccines is as follows:

- Production of peptides is simple, easily reproducible, fast and cost-effective
- Chemical synthesis practically removes all the problems associated with the biological contamination of the antigens.
- Peptide vaccines are typically water-soluble, stable under simple storage conditions (generally does not require "cold chain") can be freeze-dried and their stability can be easily assessed using standard physicochemical characterisation methods.
- The immune responses can be directed against naturally non-immunodominant epitopes. By the use of a multi-epitope approach, single peptide-based vaccine can be designed to target several strains, different stages of life cycle or even different pathogens.

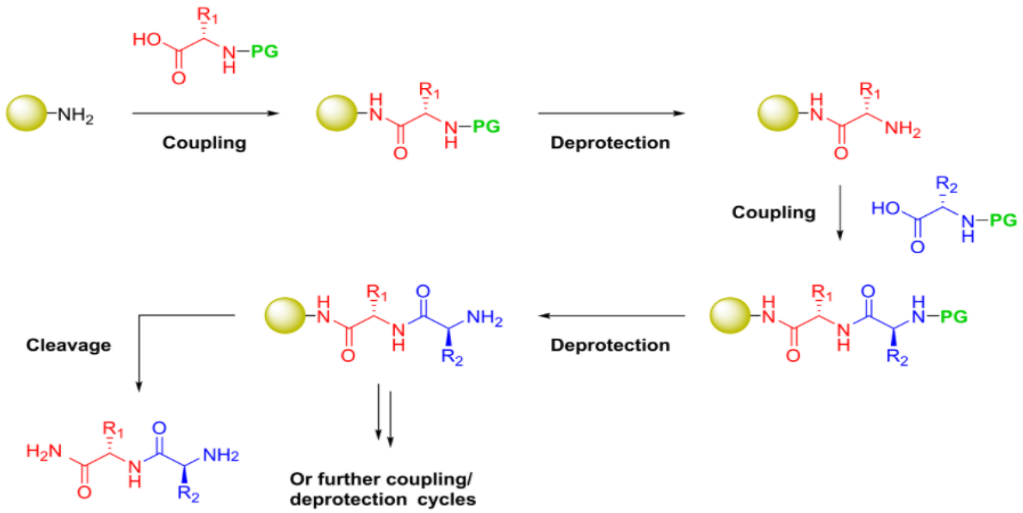
Recombinant vaccine: -

A recombinant vaccine is a vaccine produced through recombinant DNA technology. This involves inserting the DNA encoding an antigen (such as a bacterial surface protein) that stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells and then purifying it from them.

Steps involved in the preparation of vaccines

1)Peptide bond synthesis by solid phase peptide synthesis (SPPS): -

This method of peptide synthesis was first discovered by **Robert Bruce Merrifield** for which he was awarded Nobel prize. In this method the peptide bond is synthesized on the solid phase like polystyrene beads, resin. This is a polymeric reaction in which a good quality of peptide is synthesized with a polymeric reaction which involves coupling and deprotection process.



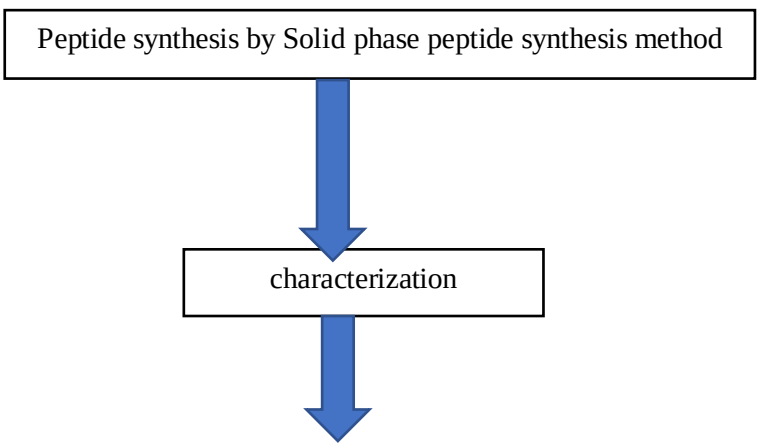
Reaction involved in peptide synthesis using SPPS which involves coupling and deprotection process

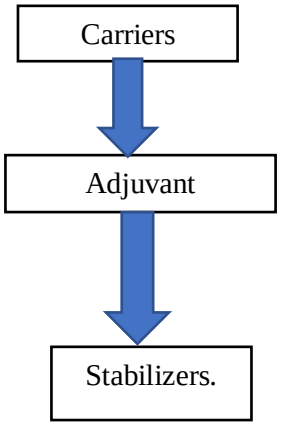
2) Characterization: -The sequence of amino acids synthesized or if any impurities present in the synthesis are characterized by performing NMR (Nuclear magnetic resonance), HPLC (High performance Liquid chromatography).

3**) Carriers**: -Carriers are used depending on composition of the vaccine preparation.

4)Adjuvant: -Some vaccines also contain adjuvants. An adjuvant improves the immune response to the vaccine, sometimes by keeping the vaccine at the injection site for a little longer or by stimulating local immune cells.The adjuvant may be a tiny amount of aluminium salts (like aluminium phosphate, aluminium hydroxide or potassium aluminium sulphate). Aluminium has been shown not to cause any long-term health problems, and humans ingest aluminium regularly through eating and drinking.

5)Stabilizers: -Stabilizers prevent chemical reactions from occurring within the vaccine and keep the vaccine components from sticking to the vaccine vial. Stabilizers can be sugars (lactose, sucrose), amino acids (glycine), gelatine, and proteins (recombinant human albumin, derived from yeast).





Advantages: -

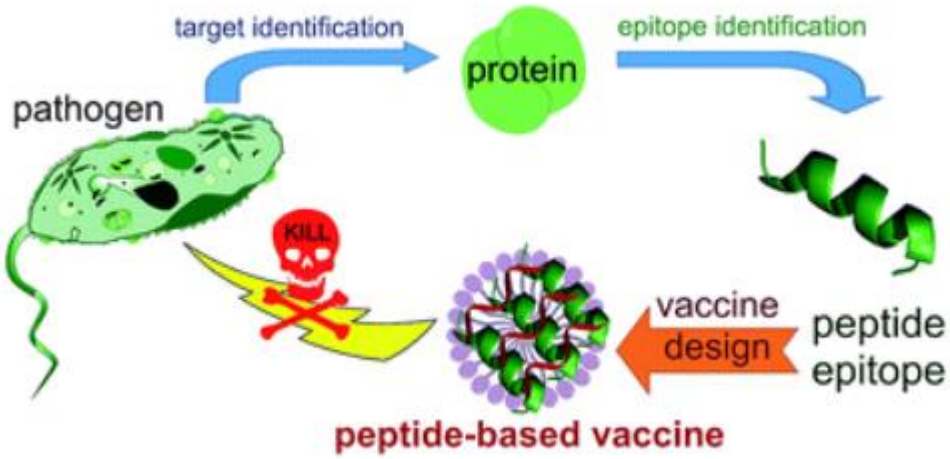
- Production and quality control is simpler.
- Toxicity is lesser
- Safer
- Cost effective
- No infection is present since there is no pathogens are handled.

Disadvantages: -

- Several doses must be given for proper -life.
- Requires adjuvant.
- Duration of immunity is shorter
- Carriers used in vaccine production may cause hypersensitivity reaction.
- Requires primary coarse of infection followed by boosters.

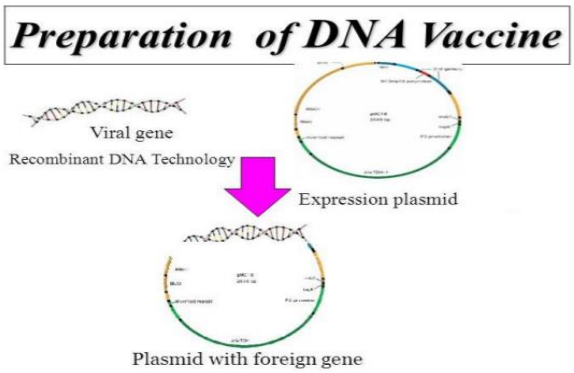
Mechanism of peptide vaccines: -

Peptide-based vaccines are usually made of synthetic B- or T- cells epitopes (class I or class II) that can also be combined. T-cells recognize peptide sequences complexed with major histocompatibility complex (MHC) class I or II molecules on the surface of APCs. $CD8^+$ T-cells (also called cytotoxic T lymphocytes (CTL)), kill infected cells, while $CD4^+$ T helper cells, that recognize MHC II-epitope complex, interact with CTL to reinforce their activity and with B-cells to activate the production of specific antibodies against the pathogen.



DNA vaccines: -

DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. These vaccines contain DNA that codes for specific proteins (antigens) from a pathogen. DNA vaccines have emerged as a safer alternative to standard live and inactivated vaccines for treating human and animal viral diseases.



Schematic representation of DNA vaccine preparation

The direct injection of genetic material into a living host causes a small amount of its cells to produce the introduced gene products. This inappropriate gene expression within the host has important immunological consequences, resulting in the specific immune activation of the host against the gene delivered antigen. In this way, DNA vaccine provides immunity against that pathogen. DNA vaccines are composed of bacterial plasmids. Expression plasmids used in DNA-based vaccination normally contain two units: The antigen expression unit composed of promoter/enhancer sequences, followed by antigen-encoding and polyadenylation sequences and the production unit composed of bacterial sequences necessary for plasmid amplification and selection. The construction of bacterial plasmids with vaccine inserts is accomplished using recombinant DNA technology. Once constructed, the vaccine plasmid is transformed into the bacteria, where bacterial growth produces multiple plasmid copies. The plasmid DNA is then purified from the bacteria, by separating the circular plasmid from the much larger bacterial DNA and other bacterial impurities. This purified DNA acts as the vaccine.

Human disease agents for which recombinant vaccine are currently developed

Pathogen	Disease
Virus	
Yellow fever	Lesions in heart, kidney and liver
Hepatitis A virus	Liver damage
Hepatitis B virus	Liver damage
Human Immunodeficiency virus	AIDS
Herpes simplex virus type 2	Genital ulcers
Bacteria	
Vibrio cholera	Cholera
Mycobacterium tuberculosis	Tuberculosis
Salmonella typhi	Typhoid fever
Clostridium tetani	Tetanus

Advantages of DNA Vaccine

- The immune response focused on the antigen of interest
- Cost-effective
- Less risk for infection
- Antigen presentation by MHC class I and class II molecules
- Long-term persistence of immunogen
- DNA vaccines are very easy to construct and are well-tolerated in humans.
- Large quantities of DNA can be produced in a short time at a reduced cost, and DNA preparations are more stable than other types of vaccines, which are desirable properties for a vaccine.
- They can be made in a short time span – it is easier to make large amounts of a gene
- than make proteins or grow up bacteria or viruses. Speed is important when making
- a vaccine to strains of bacteria or virus that are constantly mutating and changing.
- DNA vaccines are easy to transport and store – DNA is a very stable molecule and does not need to be stored at low temperatures making transportation and storage
- cheaper and easier than conventional vaccines.
- DNA vaccines may be very cheap to make – it is relatively easy to make and purify large amounts of DNA.
- There is no risk to those who are making the vaccine - some conventional vaccines require growing up the infectious bacteria or virus – and this carries a risk (all be it very small) to those who work making vaccine.

Limitations of DNA Vaccine

- Risk of affecting genes controlling cell growth

- Possibility of tolerance to the antigen
- Potential for atypical processing of bacterial and parasite proteins
- Limited to protein immunogens

Applications of DNA Vaccine

- DNA vaccines against cancer – Cancer have been a cause of death for many worldwide. DNA vaccines are reliable forms of immunotherapy and can be effective for people fighting cancer
- DNA vaccines against tuberculosis – DNA-based vaccine can be used to curb Tuberculosis which is a major health problem for people across the world
- DNA vaccines against HIV – Human immunodeficiency virus (HIV) cause acquired immunodeficiency syndrome (AIDS) which is a health crisis and using this type of vaccine, can be treated.

Delivery method of DNA vaccines: -

DNA vaccines have been introduced into animal tissues by a number of different methods. The two most popular approaches are: Injection in saline It is normally conducted intramuscularly (IM) in skeletal muscle or intradermally (ID), with DNA being delivered to the extracellular spaces. This can be assisted by electroporation; by temporarily damaging muscle fibers with myotoxins such as bupivacaine; or using hypertonic solutions of saline or sucrose. Immune responses to this method of delivery can be affected by many factors including; needle type, needle alignment, speed of injection, Volume of injection, muscle type, and age, Sex and physiological condition of the animal being injected. Gene gun delivery the other commonly used method of delivery, ballistically accelerates plasmid DNA (pDNA) that has been adsorbed onto gold or tungsten micro particles into the target cells, using compressed helium as an accelerant. The method of delivery determines the dose of DNA required to raise an effective immune response.

- Nasal spray
- Intramuscular injection
- Intravenous injection
- Intradermal injection
- Gene gun or biolostic delivery

Steps in DNA VACCINS production

The plasmid vaccine carrying the DNA (gene) for antigenic proteins enters the nucleus of the inoculated target cell of the host. This DNA produces RNA

Steps for DNA Vaccine Preparation:

Step 1: The vectors and copied genes have been treated with restriction enzymes, which are agents that cut DNA sequences at known locations.

Step 2: The enzymes have cut open the round vectors and trimmed the ends of the copied genes.

Step 3: Add bacteria to the vectors to allow the altered vectors to replicate.

Step 4: The ends of the vectors have again come together, but now with a gene spliced into the loop. Many copies of the vector/gene loop for genetic vaccine are needed.

Step 5: These copies can be produced with the help of bacteria. Vectors are capable of self-replicating when within a bacterial host, as long as that host is in an environment conducive to growing.

Step 6: Combine the vectors and bacteria, the vectors will be shocked into the bacteria

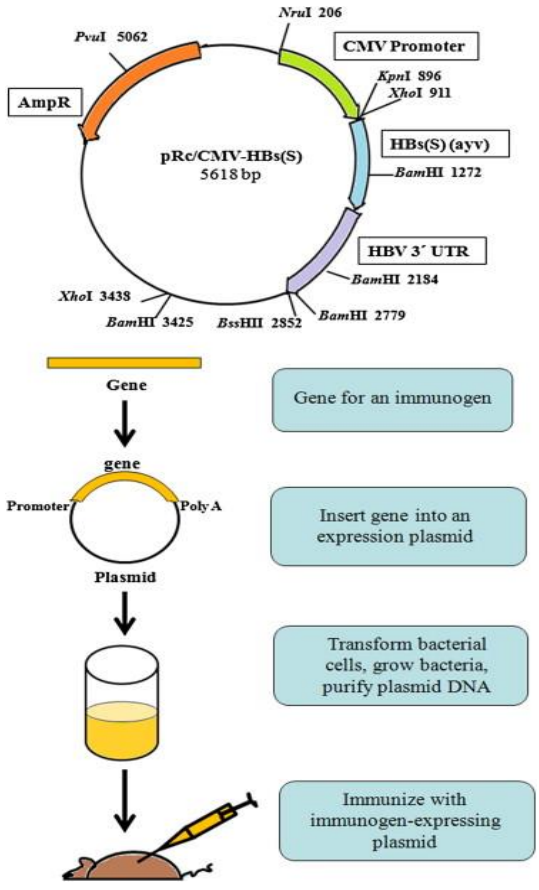
Step 7: Use the purifier to separate the altered vectors from the bacteria.

Step 8: The final vaccine should include only the vectors, and then separate those from the bacteria after enough copies have been produced.

Step 9: This can be done with a detergent, which ruptures the cell walls of the bacteria and frees the DNA within.

Step 10: The relatively large bacterial DNA can then be separated from the smaller DNA loop that makes up the vector.

Step 11: Fill the syringe with the altered vectors Upon inoculation, billions of copies of the altered vector will enter the body. Of these, only 1 percent will work their way into the nuclei of body cells.

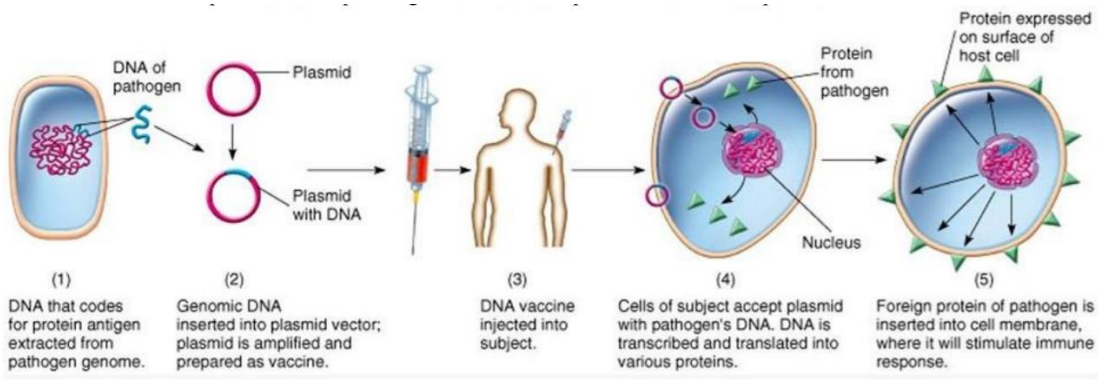


Steps involved in the production of DNA vaccine.

Mechanisms of Action

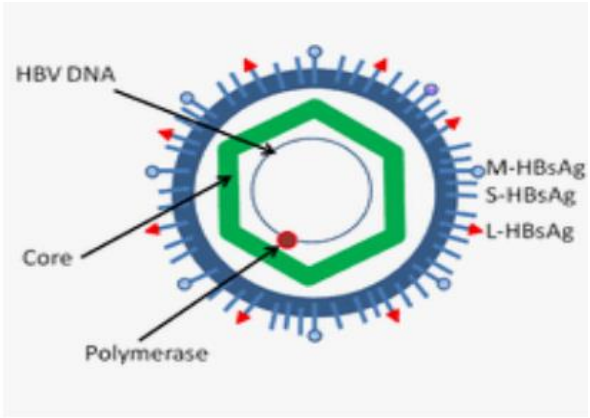
As the antigen molecule bind to B-lymphocytes become memory cells that can protect the host against future infections. It produces humoral and cellular based immunity. A plasmid vector that expresses the protein of interest

(e.g. viral protein) under the control of an appropriate promoter is injected into the skin or muscle of the the host. After uptake of the plasmid, protein is produced endogenously (Antigenic Protein is presented by cell in which it is produced) and intracellular processed into small antigenic peptides by the host proteases. The peptides then enter in to lumen of the endoplasmic reticulum (E.R.) by transporters in the E.R., peptides bind to MHC class I molecules. Subsequent CD8+ cytotoxic T cells (CTL) are stimulated and evoke cell-mediated immunity. CTLs inhibit viruses through both cytolysis of infected cells and non cytolysis mechanisms such as cytokine production. The foreign protein can also be presented by MHC class II pathway by APCs These CD4+ cells are able to recognize the peptides formed from exogenous proteins degraded to peptide fragments and loaded onto MHC class II molecules. Depending on the type of CD4+ cell that binds to the complex, B cells are stimulated and antibody production is stimulated



Production of vaccines using genetically engineered microorganisms (HBV).

This was the first successful recombinant vaccine developed. This virus infects the liver and can cause serious damage. This virus has a surface antigen, HBsAg, which is found in blood of infected patients and has been found to produce a significant immune response. Recombivax and engerix -B are the commercially available vaccines.



Schematic reprentation of hepatitis B virus

Hepatitis B virus (HBV)is wide spread in man and produces several chronic liver disorders such as Fulminant chronic hepatitis, cirrhosis and primary liver cancer. HBV DNA is a double stranded circular molecule of about 3Kb size and has a large single stranded gap which must be required with an endogenous polymerase before digestion with restriction enzyme for DNA cloning. After infection in human being, HBV fails to multiply and

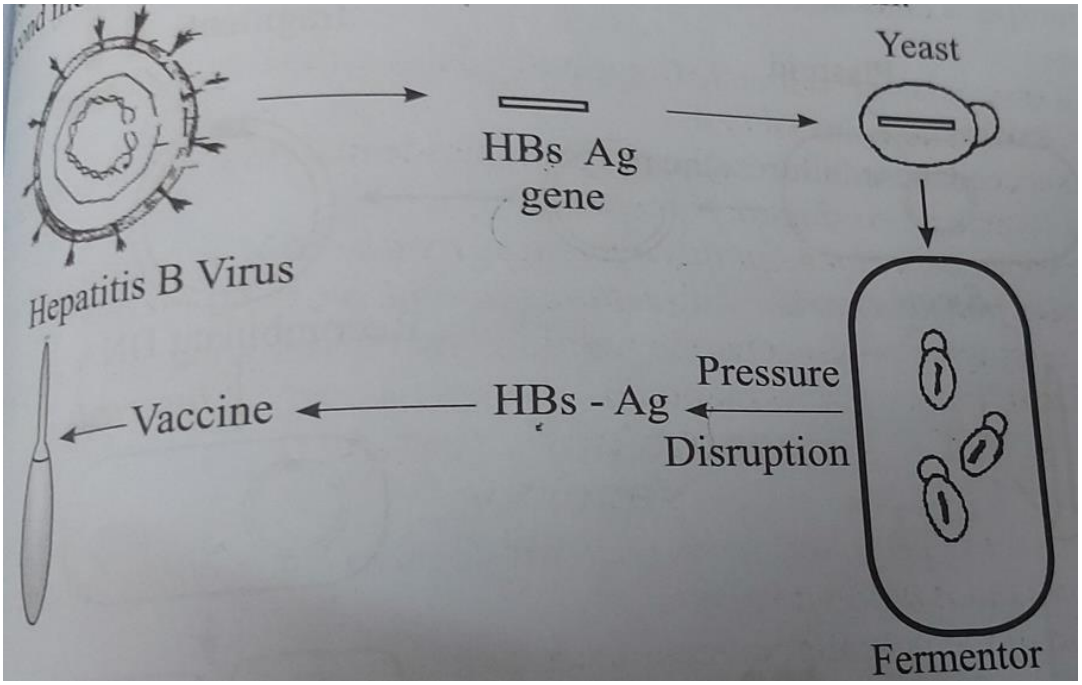
infect a large number of cells and even does not grow in cultured cells. This property has been explained to be due to hindrance of its molecular characterization and development of vaccines. Plasma of human has been detected to have varying number of antigens. Three types of viral proteins are recognized to be antigenic: *(i)* viral surface antigen (HBsAg), *(ii)* viral core antigen (HBcAg), and *(iii)* the e-antigen (HBeAg).

India's first genetically engineered vaccine against HBV developed by a Hyderabad based laboratory was launched on August 18, 1997. India is the fourth country (after the U.S.A., France and Belgium) to develop this highly advanced vaccine. The indigenous yeast-desired HBV vaccine is one third the cost of the imported vaccine.

Recombinant vaccine for Hepatitis B virus. After infection, HBV fails to grow and even in cultured cells it does not grow. This property has been explained to be due to inhibition of its molecular expression and development of vaccines. Recombinant vaccine for HBV was produced by cloning HBsAg gene of the virus in yeast cells. The yeast system has its complex membrane and ability of secreting glycosylate protein. This have made it possible to build an autonomously replicating plasmid containing HBsAg gene near the yeast alcohol dehydrogenase (ADH) promoter. The shuttle vector has leucine biosynthesis marker for selection in yeast and has tetracycline resistance marker for selection in bacteria.

The HBsAg gene contains 6 bp long sequence preceding the AUG that synthesizes N-terminal methionine. This is joined to ADH promoter cloned in the yeast vector PMA-56.
The recombinant plasmid is inserted into yeast cells. The transformed yeast cells arc multiplied in tryptophan-free medium. The transformed cells are selected. The cloned yeast cells are culture for expression of HBsAg gene. The cells are separated are separated by centrifugation, filteration and chromatography. Then the cells are separated by

This inserted gene sequence expresses and produces particles similar to the 22 mm particle of HBV as these particles are produced in serum of HBV patients. The expressed HBsAg particles have similarity in structure and immunogenicity with those isolated from HBV-infected cells of patients. Its high immunogenicity has made it possible to market the recombinant product as vaccine against HBV infection immunoadsorption method.



Production of vaccine using genetically engineered microorganism hepatitis B vaccine.

Features of vaccines

box 11.3
Requirements for an Effective Vaccine

Safe and protective
- The vaccine must not elicit illness or death, and it must protect against illness resulting from exposure to live pathogen. Protection against illness must be sustained for several years (T- and B-cell-mediated immune memory).

Nature of infectious pathogen
- Extracellular: must induce protective neutralizing antibodies
- Intracellular: must induce a protective $CD8^+$ CTL response

Host defense at point of entry of infectious agent
- Mucosal immunity: an important goal of vaccination against many organisms that enter through mucosal surfaces (e.g., oral and nasal)

Preexisting antibodies at the time of exposure to the infection
- Antibodies against extracellular pathogens (diphtheria and tetanus exotoxins) may not protect against infection by these exotoxins and may require vaccination for protection.
- Antibodies to an intracellular pathogen (poliovirus) may not be protective and may require vaccination to activate T cells for protection.

Antibodies and T cells directed against the correct epitopes
- Antibodies against many epitopes may be elicited, but only some of these epitopes may confer protection.
- T-cell epitopes recognized can also affect the nature of the response.

Practical considerations
- Cost-effective (low cost per dose); biologically stable; ease of administration, few (or no) adverse side effects