Introduction to Serology and Vaccines

Serology: is the scientific study of blood serum in practice, the term usually refers to the diagnostic identification of antibodies in the serum. This antibodies are typically formed in response to:

1- Infection (microorganisms like bacteria, virus, fungi and parasite)
2- Foreign proteins (mismatched blood transfusion)
3- Owen proteins (autoimmune disease)

Serological tests:

Serological tests are diagnostic methods carried out on a sample of blood serum, the clear liquid that separates from the blood when it is allowed to clot. The purpose of such a test is to identify antibodies and antigens in a patient's sample. Serological tests used to diagnose infections and autoimmune diseases and blood groups. Serological tests may also be used in forensic serology to investigate crime scene evidence.

Several methods can be used to detect antibodies and antigens, including ELISA, agglutination, precipitation, complement-fixation, and fluorescent antibodies.

The study of serum is serology

Serum is the fluid and solute component of blood which does not play a role in clotting. It may be defined as blood plasma without fibrinogens. Serum contain proteins, electrolytes, antibodies, antigens, hormones, and any exogenous substances (drugs or microorganisms). Serum does not contain leukocytes, erythrocytes, platelets, or clotting factors. Serum is used in numerous diagnostic tests as well as blood typing. Measuring the concentration of various molecules can be useful for many applications, such as determining the therapeutic index of a drug candidate in a clinical trial.

The serum of convalescent patients successfully recovering (or already recovered) from an infectious disease can be used as a biopharmaceutical in the treatment of other people with that disease, because the antibodies generated by the successful recovery are potent fighters of the pathogen. Such convalescent serum antiserum is a form of immunotherapy.

Serum therapy, also known as serotherapy, describes the treatment of infectious disease using the serum of animals that have been immunized against the specific organisms or their product

Antiserum:

Antiserum is a blood serum that contains specific antibodies against an infective organism or poisonous substance. Antiserums are produced in animals (horse, sheep, ox, rabbit) and humans in response to infection, intoxication, or vaccination and may be used in another individual to confer immunity to a specific disease or to treat bites or stings of venomous animals. Antiserums from animals are most often used, but in persons allergic to animals, human antiserums have proved valuable.

In 1891 **Emil Behring** saved the life of a young girl with diphtheria by injecting antiserum for the first time in history. Serum horses proved to be saviors of diphtheria-infected people. Based on his observation that people who survived infection with the diphtheria bacterium never became infected again, he discovered that the body continually produces an antitoxin, which prevents survivors of infections from being infected again with the same agent. Subsequently, treatment of tetanus, rabies, and snake venom developed, and proactive protective vaccination against diphtheria and other microbial diseases began.

In 1901, Behring won the first Nobel Prize in Medicine for his work in the study of diphtheria.

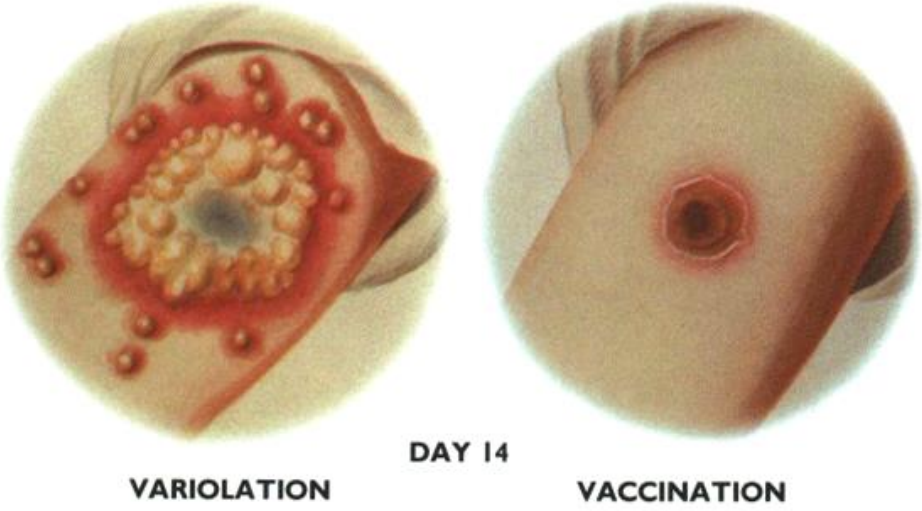
Vaccine: A vaccine is an antigenic material that stimulate adaptive immunity to a disease. Vaccines can prevent the effects of infection by many pathogens. Vaccine's are generally considered to be the most effective method of preventing infectious diseases. The material administered can either be live but weakened forms of either bacteria or viruses, killed or inactivated forms of these pathogens, or purified material such as proteins.

Variolation (inoculation) was the method first used to immunize an individual against smallpox (*Variola*) with material taken from a patient or a recently variolated individual, in the hope that a mild, but protective, infection would result. The procedure was most commonly carried out by inserting/rubbing powdered smallpox scabs or fluid from pustules into superficial scratches made in the skin.

Smallpox was the first disease people tried to prevent by purposely inoculating themselves with other types of infections. smallpox inoculation was started in India before 200 BC. In 1796 British physician **Edward Jenner** tested the possibility of using the cowpox vaccine as an immunization for smallpox in humans for the first time. The word vaccination was first used by Edward Jenner. **Louis Pasteur** furthered the concept through his pioneering work in microbiology.

Vaccination (Latin: *vacca* mean cow) is named because the first vaccine was derived from a virus affecting cows, the relatively benign cowpox virus, which provides a degree of immunity to smallpox, a contagious and deadly disease. Vaccination and immunization have the same meaning but is different from inoculation which uses un-weakened live pathogens. The word "vaccination" was originally used specifically to describe the injection of the smallpox vaccine.

Variolation	Vaccination
Variolation is a method of immunization where administration of live viruses takes place against a viral infectious agent	Vaccination is a method of immunization where administration of an attenuated virus takes place against a viral infectious agent
Form of immunization: live smallpox virus	Form of immunization: attenuated viruses, DNA vaccine or edible vaccine
Example: Smallpox vaccine	Examples: Hepatitis, Malaria, Rubella, etc.



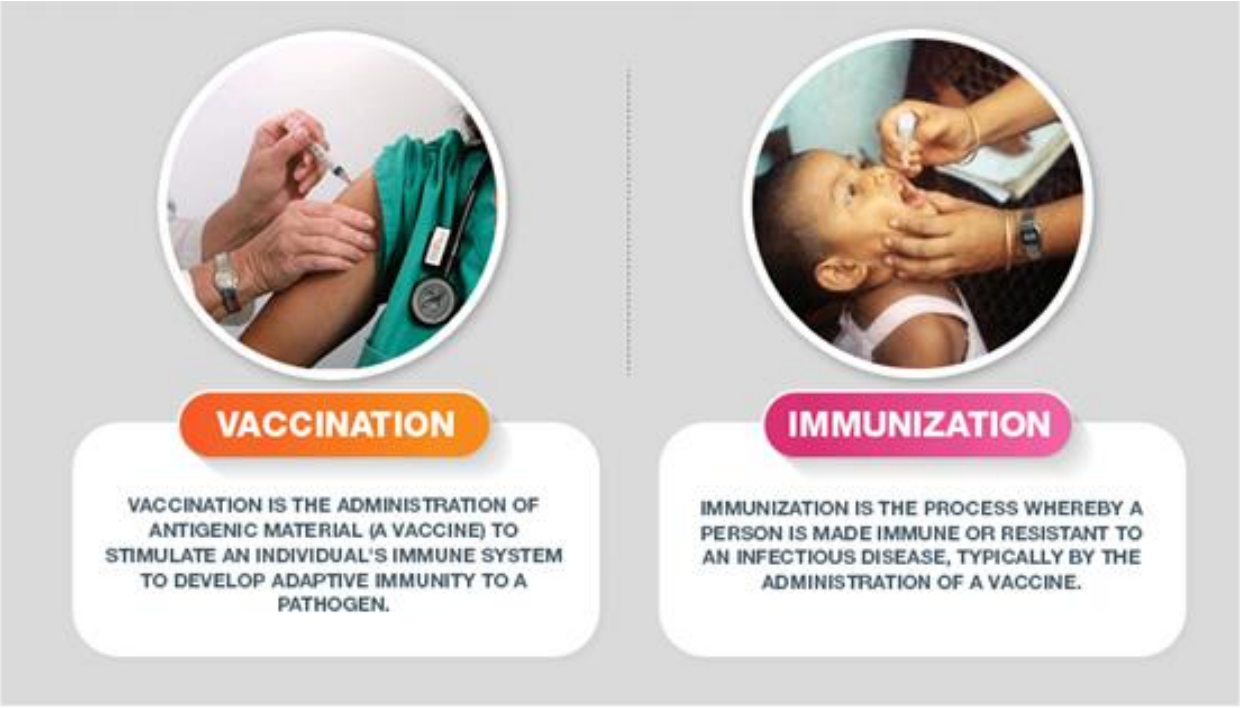
Immunization is the process whereby a person is made immune or resistant to an infectious disease, typically by the administration of a vaccine. Vaccines stimulate the body's own immune system to protect the person against subsequent infection or disease.

Immunization means to make someone immune to something. Vaccination just means to inject a suspension of attenuated or killed microorganisms administered for prevention or treatment of infectious disease.

No vaccine is 100% effective in preventing disease.

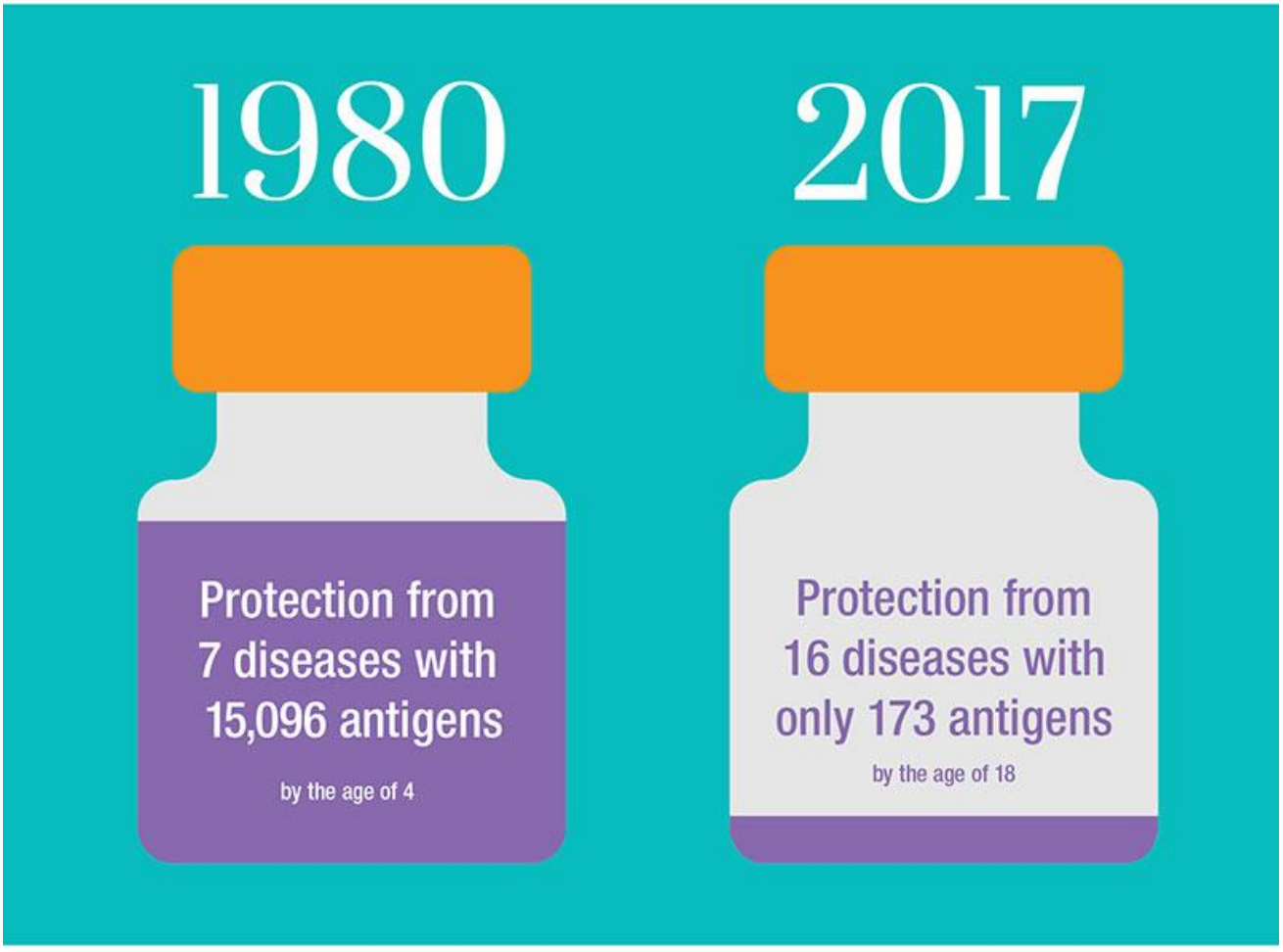
Since no vaccine is 100% effective, vaccination does not automatically mean the person is immunized against the disease.

vaccines have very high effectiveness rates, they are not completely effective for 100% of the people who receive them. For example, a full series of measles vaccine will protect 99 of 100 children from measles and polio vaccine will protect 99 of 100 children from polio. This means when there is a disease outbreak, the very small number of people for whom the vaccine did not work may still be able to catch the disease. Because almost all of our children are immunized and only few are not, it can be the case that during an epidemic the majority of cases occur among children who were immunized. However, the fact remains that those who have not received the vaccine are much more likely to catch the disease."



Vaccines Today Work Better Than Ever

Since 1980: More protection, fewer antigens.



Based on CDC Recommended Vaccine Schedule U.S. for children birth to 18 years.
Source: Plotkin's Vaccines (Seventh Edition)

Herd Immunity

Vaccination can provide excellent protection to a population, even if not every individual in a population is vaccinated, because of phenomenon known as herd immunity. As the population that is vaccinated increase, the chance of an infectious agent becomes smaller.

There are limits to herd immunity, however, if a significant number of unprotected individuals become infected, infection could spread rapidly through the unprotected members of the population.

Herd immunity: is a form of immunity that occurs when the vaccination of significant portion of a population (or herd) provides a measure of protection for individuals who have not developed immunity.

The herd immunity arises when a high percentage of the population is protected through vaccination against a virus or bacteria, making it difficult for a disease to spread because there are so few susceptible people left to infect. This can effectively stop the spread of disease in the community, it is particularly crucial for protecting people who cannot be vaccinated, these include children who are too young to be vaccinated, people immune system problems, and those who are too ill to receive vaccines (such as cancer patients). See figure 1.

Requirments of herd immunity

1- Disease agents restricted to a single host species within which transmission occurs (e.g. Smallpox, no reservoir).
2- Direct transmission (direct contact).
3- Infection must induce solid immunity.

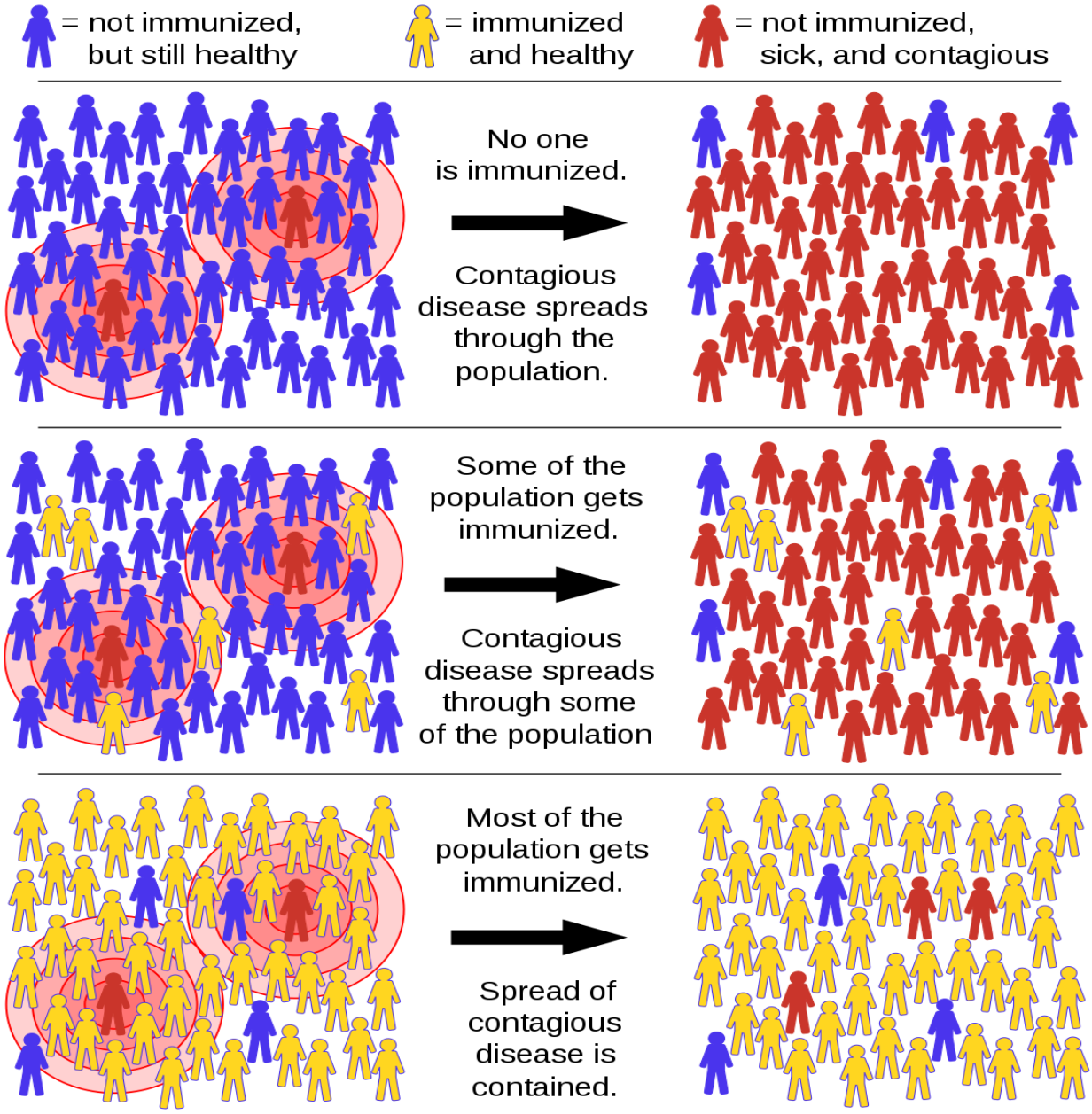


Figure 1. Herd immunity (with and without immunization)

The aim of immunization:

1- The prevention of disease in individuals or groups.

2- Protection of individuals against symptoms, ex: Diphtheria, Tetanus are for example anti-disease rather than antimicrobial vaccines.

Characters of vaccine (properties of ideal vaccine)

Vaccines must fulfill several, criteria to be effective in protecting large numbers of individuals:

1- It is **highly immunogenic**, so that a single vaccine dose provide a complete immunization regimen.

2- The recommended vaccine regimen is highly efficacious in **preventing disease** in individual vaccine recipients.

3- It has **long duration of immunity** so that frequent booster doses are not needed.

4- It **limits spread of infection**, because it prevents vaccine recipients from spreading infection to other people.

5- It is **heat stable**, so that refrigeration is not required during shipping and storage.

6- **Injection is not required** for administration, e.g. nasal spray of vaccine can be used.

7- It can safely be administrated simultaneously with other vaccine either as a part of specific combination vaccine (measles-mumps-rubella) or a separate individual vaccine.

8- **Adverse effect in vaccine recipients are few**, **non-sever, and temporary**, (the microbe used to prepare the vaccine does not cause disease in recipient who have weakened immune system from HIV infection, severe malnutrition, malignancies, or congenital immunodeficiency).

9- The **microbe** used to prepare the vaccine **never reverts to wild type** or otherwise mutates to cause dieses, new mutant forms might arise that could evade the immune system and produce disease, new vaccinated individuals.

10- It is technically **simple to manufacture**, so that it can be produced in less sophisticated settings.

11- It is **inexpensive** to manufacture, distribute and administer, so that it is affordable by the maximum number of people.

Routs of Administration

1- Subcutaneous or intramuscular rout (most vaccines).
2- Oral routs (Sabin, oral BCG).
3- Intradermal (BCG).
4- Scarification (Smallpox).
5- Intranasal (live attenuated influenza vaccine).

Characteristics of disease suitable for control by vaccine and immunization

1- Disease is well known by public and occurs commonly, so that many people are aware of its existence and importance.
2- Disease is recognizable by health workers, (cause rash) so that the consequences of the disease can be linked to a specific type of microbe and disease outbreaks can be recognized.
3- Disease short term or long term effects on individuals can sometimes be severe or permanent, so that the public (parents, health workers) support preventing its feature occurrence.
4- Disease is difficult to control at a population level without the use of immunization programs.
5- Disease incubation period (time between the exposure to the microbe and development of disease symptoms) is not too short, so that vaccine still provide at least partial protection if given often exposure (measles vaccine given soon after animal bite exposure).
6- Microbe has no nonhuman reservoir from which it can be reintroduced into the human population after adequate control has been achieved.
7- Genetic mutation that results in biochemical changes to the microbe outer coat occur very slowly so that the vaccine ability to prevent infection and disease is well maintained over time.
8- Infection with the microbe does not result in mild (subclinical) disease or in a prolonged "carrier state", so that there are no infected people who could easily spread the disease to susceptible contacts because they themselves do not feet ill or appear ill.

Scheme of immunization:

Primary vaccination:

- One dose vaccines (BCG, Variola, Measles, Mumps, Rubella, Yellow fever).
- Multiple dose vaccines (Polio, DPT, Hepatitis B).

Booster vaccine:

- To maintain immunity level after its declines after some time has elapsed.

TYPES OF IMMUNITY

Immunity divided into 2 types: Innate immunity and Acquired immunity figure 2.

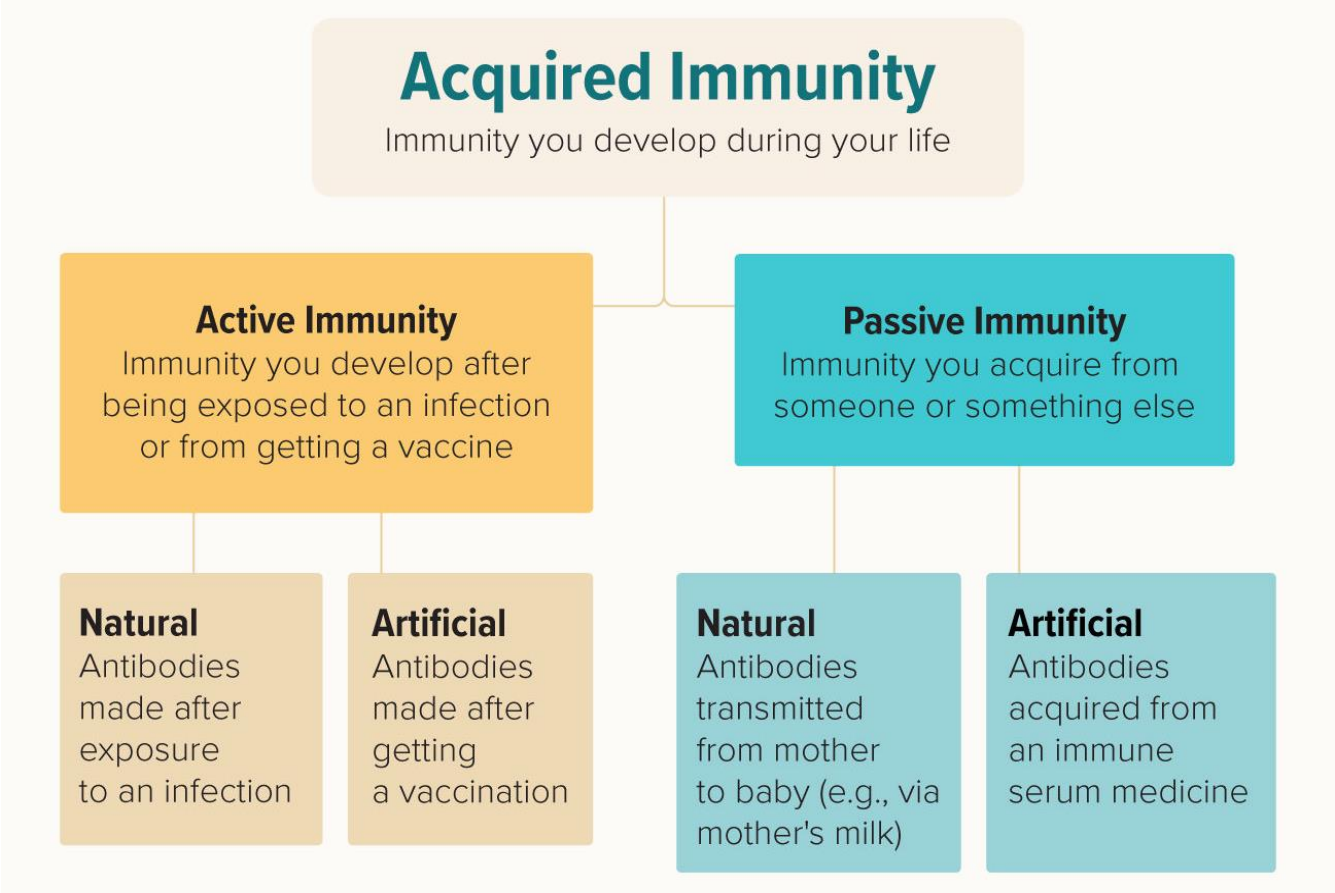


Figure 2. Types of Acquired immunity

Active Immunity

Active immunity refers to the process of exposing the body to an antigen to generate an adaptive immune response. The response takes days/weeks to develop but may be long lasting even lifelong. Wild infection for example with hepatitis A virus and subsequent recovery gives rise to a natural active immune response usually leading to lifelong protection. In a similar manner, administration of two doses of hepatitis A vaccine generates an acquired active immunity leading to long lasting protection.

Passive Immunity

Passive immunity refers to the process of providing IgG antibodies to protect against infection; it gives immediate, but short -lived protection- several weeks to 3 or 4 months at most. Passive immunity is usually classified as natural or acquired. The transfer of maternal tetanus antibody (mainly IgG) across the placenta provides natural acquired immunity for the newborn baby for several weeks/months until such antibodies is degraded and lost. In contrast, acquired (artificial) passive immunity refers to the process of obtaining serum from immune individuals, pooling this, concentrating the immunoglobulin fraction and then injecting it to protect a susceptible person. **Passive immunization is used when:**

1- There is high risk of infection and insufficient time for the body to develop its own immune response.
2- To reduce the symptoms of ongoing or immunosuppressive disease.
3- Can be provided when people cannot synthesize antibodies.
4- Used in the case of immunodeficiency disease (such as hypogammglobulinemia).

Immunization is often required shortly following birth to prevent disease in newborn such as tuberculosis, hepatitis B and pertussis, however, maternal antibodies can inhibit the induction of protective vaccine responses throughout the first year of the life, this effect is usually overcome by secondary responses to booster immunization.

There is potential risk for hypersensitivity reactions, especially from gamma globulin of non-human origin. Passive immunity provide immediate protection, but does not develop memory, therefore the patient is at risk of being infected by the same pathogen later unless they acquire active immunity or vaccination.

FDA Licensed immunoglobulin

Application of artificial passive immunity

Disease	Product	Source	Use
Botulism	Specific equine IgG	Horse	Treatment of wound and food borne forms of botulism, infant botulism is treated with human botulism immune globulin. The administration of horse antitoxin remains the only specific pharmacologic treatment available for botulism.
CMV	Hyper immune IVIG	Human	Prophylaxis, used most often in kidney transplant patients.
Hepatitis A, Measles	Pooled human Ig	Human serum	Prevention of hepatitis A and measles infection, treatment of congenital or acquired immunodeficiency (it is still indicated following exposure and prior to travel to areas of endemic infection
Hepatitis B	Hepatitis B Ig	Human	Post exposure prophylaxis, prevention in high risk infants (administration with hepatitis B vaccine)
ITP, Kawiski disease, IgG deficiency	Pooling human IgG	Human serum	Treatment of ITP and Kawiski disease, prevention and treatment of opportunistic infection with IgG deficiency
Rabies	Rabies Ig	Human	Post-exposure prophylaxis (administration with rabies virus)
Tetanus	Tetanus Ig	Human	Treatment of tetanus infection
Vaccinia	Vaccinia Ig	Human	Treatment of progressive vaccinia infection including eczema and ocular forms (usually resulting from smallpox vaccination in immunocopromised individual)
Varicella chickenpox	Varicella zoster Ig	Human	Post exposure prophylaxis in high risk individuals

Antitoxin: known as heterologous hyper-immune serum is often given prophylactically to individuals.

Advantages and disadvantages of passive immunization

Vaccines typically need time (weeks or months) to produce protective immunity in an individual and may require several doses over a certain period of time to achieve optimum protection. Passive immunization, however, has an **advantage** in that it is **quick acting**, producing an immune response within hours or days, faster than vaccine.

Passive immunization can override a deficient immune system, which is especially helpful in someone who **does not respond to immunization**.

Antibodies, however, have certain **disadvantages**. First, antibodies can be **difficult and costly to produce**. Although new technique can help produce antibodies in the laboratory, in most cases antibodies to infectious disease must be harvested from blood of hundreds or thousands of human donors, or, they must obtained from the blood of immune animals (as with antibodies that neutralize snake venoms). In the case of antibodies harvested from animals, serious allergic reactions can develop in the recipient.

Another disadvantage is that many antibody treatments must be given via intravenous injection, which is a **more time-consuming** and **potentially complicated** procedure while the injection of a vaccine is less time consuming and less risk of complication.

Finally, the immunity conferred by passive immunization is short lived: it does not lead to the formation of long-lasting memory immune cells.

Types of Vaccines

There are two basic types of vaccines: live attenuated and inactivated. The characteristics of live and inactivated vaccines are different, and these characteristics determine how the vaccine is used.

1- Live attenuated vaccines

Live attenuated vaccines are produced by modifying a disease-producing (wild) virus or bacterium in a laboratory. The resulting vaccine organism retains the ability to replicate (grow) and produce immunity, but usually does not cause illness. Attenuated vaccines can be composed of either whole viruses or bacteria, or fractions. Fractional vaccines are either protein-based or polysaccharide-based. Protein-based vaccine include toxoids (inactivated bacterial toxin) and subunit products. Most polysaccharide-based vaccine are composed of pure cell wall polysaccharide from bacteria. Conjugate polysaccharide vaccines contain polysaccharide that chemically linked to a protein, this linkage make the polysaccharide a more potent vaccine.

To produce an immune response, live attenuated vaccines must replicate in the vaccinated person. Small dose of virus or bacteria is administered, which replicates in the body and creates enough of the organism to stimulate an immune response. Damages the live organism in the vial (heat or light) or interferes with replication of the organism in the body (circulating antibody) can cause the vaccine to be ineffective. Although live attenuated vaccines replicate, they usually do not cause disease. When a live attenuated vaccine does cause disease it is usually much milder than natural disease.

The immune response to a live attenuated vaccine is virtually identical to that produced by a natural infection. Live attenuated vaccine produce immunity in most recipients with one dose, except those administered orally. However, a small percentage of recipients do

not respond to the first dose of an injection live vaccine (MMR or Varicella) and a second dose is recommended to provide a very high level of immunity in the population. Live attenuated vaccines may cause severe or fatal reactions as a result of uncontrolled growth of the vaccine virus. This occurs in persons with immunodeficiency.

Two principle methods are used for attenuation:

1- Serial passage in cell cultured in vitro.

2- Adaptation to low temperatures.

(With development of DNA technology it is now possible to induce the required genetic change)

Table 1. Live attenuated vaccines

Bacteria/Virus	Vaccine	Method	Rout
Vibrio cholera	CVD103 hgr	Generally modified	Oral
Salmonella	Ty21a	Generally modified	Oral
Mycobacterium	BCG	Prolog subculture	ID
Polio	Sabin	Passage in monkey kidney cells	Oral
Yellow fever	17D	Passage in chick embryo cell	SC
Influenza		Temperature sensitive mutant	IN
Measles	MMR	Passage in fibroblast cells	SC
Rubella	Wistar	Wister institute (RA 27/3 strain of atten. Virus)	SC
Chickenpox	Oka/merck	Human diploid cell cultures	SC
Smallpox	Vaccinia	Naturally a virulent	ID

Advantage

1- Infectious microbe can stimulate generation of memory cellular as well as humeral immune response.

2- Its can multiply in the host, fewer quantities must be injected to induce protection.

3- Multiple booster dose may not be required.

4- Some live vaccines can be given orally to induce mucosal immunity and IgA synthesis.
5- They can lead to elimination of wild type virus from the community.

Disadvantage

1- May very rarely revert to its virulent form and cause disease.
2- Live vaccines cannot be given safely to immunosuppressed individual.
3- Since they are live and because their activity depends on their viability, proper storage is critical.

2- Killed vaccines

Killed or inactivated organisms are used where attenuation has not been achieved, the reversion to wild type occurs too easily.

These vaccines include organisms that are dead because of the treatment with physical or chemical agents. In the case of toxins, they will have been inactivated (toxoid). They should be incapable of infection, replication, or function but still able to provoke immunity.

Table 2. Killed or inactivated vaccines

Bacteria/virus	Vaccine	Method	Rout
Vibrio cholera	CVD103 hgr	Phenol	SC or ID
Salmonella typhi	TAB	Heat, phenol, acetone	SC
Yersinia pestis	Haffkine	Formalin	SC
Bordetella pertussis	Sabin	Merthiolate	IM
Poliomyelitis	Salk	Formalin	IM
Rabies virus	Semple	Phenol	SC
Influenza virus	MMR	Formalin	IM
Hepatitis A	HM175	Formalin	IM

Advantage

1- Safe to use and can be given to immunodeficient and pregnant woman.
2- Cheaper than live attenuated vaccine.
3- Storage not are critical as live vaccine.

Disadvantage

1- Since the microorganism cannot multiply, a large number are required to stimulate immunity.
2- Periodic booster must be given to maintain immunity.
3- Only humoral immunity can be induced.
4- Most killed vaccines have to be injected.
5- Inactivated such as formaldehyde may alter immunogenicity.

3- Sub-cellular fraction

1- Polysaccharide capsule of pneumococci (Haemophilus and Meningitis).
2- Surface coat of hepatitis B virus (can be purified from the plasma carriers).
3- Pili of *E. coli* and *N. gonorrhoeae* removal of all infectious material is obvious a vital element is safety control and such vaccines.
4- Peptide vaccine consist of those peptide from the microbial antigen that stimulate protective immunity.

4- Toxoid

Bacterial toxins inactivated (usually by formaldehyde) so that they are no longer toxic but still induce protective antibodies.

For example, the tetanus toxoid is derived from the tetanospasmin produced by *Clostridium tetani*.

5- Microorganisms as a vector for cloned genes

The idea is the use of expression vector (M.O.) complete with inserted gene as a vaccine. Following, injection into the patient it would proliferate sufficiently to release an immunizing amount of foreign protein without inducing disease itself, Examples:

- Vaccinia virus in 1982 contain gene for hepatitis B surface Ag (HBsAg), influenza and herpes simplex. Their disadvantage is complication
- Bacteria like:

  A) Attenuated Salmonella typhi may act as a general vectors for vaccines against all enteric diseases.

  B) BCG is the latest vector to be proposed its advantage is:

  1- Large genome

  2- The most widely used of all vaccines

  3- It induce mediated immunity, both to itself and to other antigens.

  4- It could be ideal vector for Ag from all intracellular organisms which include: Tuberculosis, Leprosy, Brucella, Leishmania, Toxoplasma and Listeria

6- Vaccine conjugate

Vaccine can produce humoral immunity through B-cell proliferation leading to antibody production, which may or may not involve helper T-cell, for example pneumococcal polysaccharide, and *H. influenza* type b have specific protective Ab without involvement of T-helper, these T-cell independent response are characterized by low Ab titers, particularly in children 3 to 18 months consequently. However by covalently conjugating the *Haemophilus* polysaccharide to protein Ag, such as diphtheria toxin protein, *Haemophilus* vaccine produced a robust T-cell dependent Ab response even in 3 months old infants.

Different between living and non-living vaccines

	Living	Non-living
Preparation	Attenuation	Inactivation
Administration	Oral, may be single dose	Injection usually multiple doses
Adjuvant	Not required	Usually required
Safety	May revert to virulence	Pain from injection
Heat liability	Required cold chain	Satisfactory
Duration of immunity	Usually years	May be long or short
Immune response	IgG, IgA	Mainly IgG

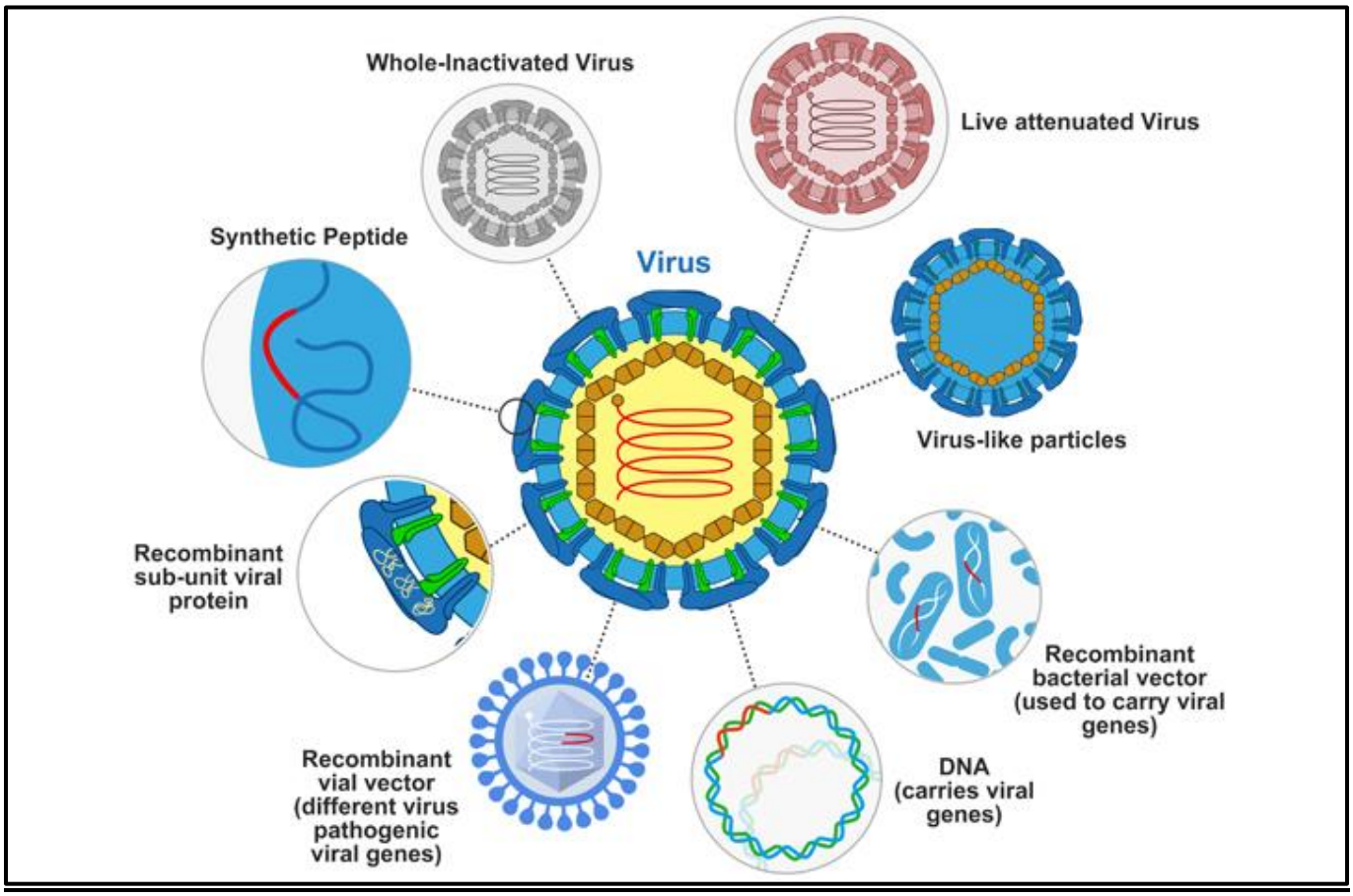


Figure 1. Various approaches for Vaccine Development

Pathological consequences of vaccination:

A) Extrinsic (element in vaccine)

- ❖ Contamination of attenuated viruses with other viruses.
- ❖ Hypersensitivity to egg albumin (vaccine grow in chicken embryo cells).

B) Intrinsic (pathological response induced from vaccine itself)

- ❖ Hypersensitivity ex: type III hypersensitivity to killed measles vaccine.
- ❖ Fever and malaise that follow vaccination with killed typhoid organisms is due to the endotoxin.
- ❖ Autoimmunity as a result of antigenic similarity between host and microbe as in chagas disease (infection with *trypanosome*).
- ❖ Brain damage after vaccination for pertussis.

The immune-compromised host:

Living attenuated vaccine, in the immune-compromised patient is avoided:

1- Vaccinia and BCG in patients with severe T-cell deficiency.
2- In less several T-cell deficiency live measles vaccine in now recommended.
3- In other T-cell deficiency of childhood including treatment with steroid or immunosuppressive drugs (Mumps, Measles, Rubella) is not advised.
4- Non-living vaccine is less dangerous.
5- Vaccines for specific antibody induction e.g. capsular polysaccharide, hepatitis B are recommended in but the most sever B-cell deficiencies.

Vaccine production

The production of vaccine can be divided in the following steps:

1- **Generation of the antigen:** the first step in order to produce a vaccine is generating the antigen that will trigger the immune response. For this purpose the pathogen's proteins or DNA need to be grown and harvested using the following mechanisms:
   - Viruses are grown on primary cells such as cells from chicken embryos or using fertilized eggs (influenza) or cell lines that reproduce repeatedly (hepatitis A).
   - Bacteria are grown in bioreactors which are devices that use a particular growth medium that optimizes the production of the antigen.
   - Recombinant proteins derived from the pathogen can be generated either in yeast, bacteria or cell cultures.

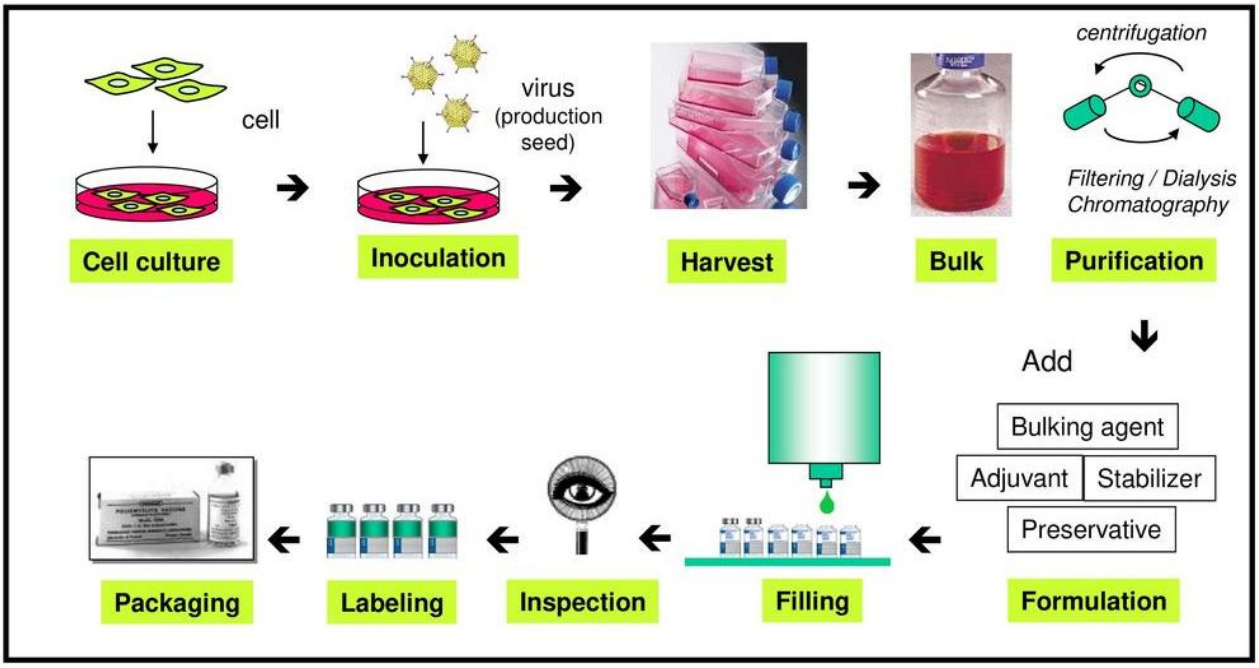
2- **Release and isolation of the antigen:** the aim of this second step is to release as much virus or bacteria as possible. To achieves this, the antigen will be separated from the cells and isolated from the proteins and other parts of the growth medium that are still present.

3- **Purification:** in third step the antigen will need to be purified in order to produce a high purity/quantity product. This will be accomplished using different techniques for protein purification. For this purpose several separation steps will be carried out using the differences in for instance protein size, physic-chemical properties, binding affinity or biological activity.

4- **Addition of other components:** the fourth step may include the addition of an adjuvant, which is a material that enhances the recipient's immune response to a supplied antigen. The vaccine is then formulated by adding stabilizers to prolong the storage life or preservatives to allow multi-dose vials to be used safely as needed. Due to potential incompatibilities and interactions between antigens and other ingredients,

combination vaccines will be more challenging to develop. Finally, all components that constitute the final vaccine are combined and mixed uniformly in a single vial or syringe.

5- **Packaging:** once the vaccine is put in recipient vessel (either a vial or a syringe), it is sealed with sterile stoppers. All the processes described above will have to comply with the standards defined for good manufacturing practices that will involve several quality controls and an adequate infrastructure and separation of activities to avoid cross-contamination. Finally, the vaccine is labeled and distributed worldwide.



Vaccine production techniques are evolving. Cultured mammalian cells are expected to become increasingly important, compared to conventional options such as chicken eggs, due to greater productivity and low incidence of problems with contamination. Recombination technology that produces genetically detoxified vaccine is expected to grow in popularity for the production of bacterial vaccines that use toxoids. Combination vaccines are expected to reduce the quantities of antigens they contain, and thereby decrease undesirable interaction, by using pathogen-associated molecular patterns.

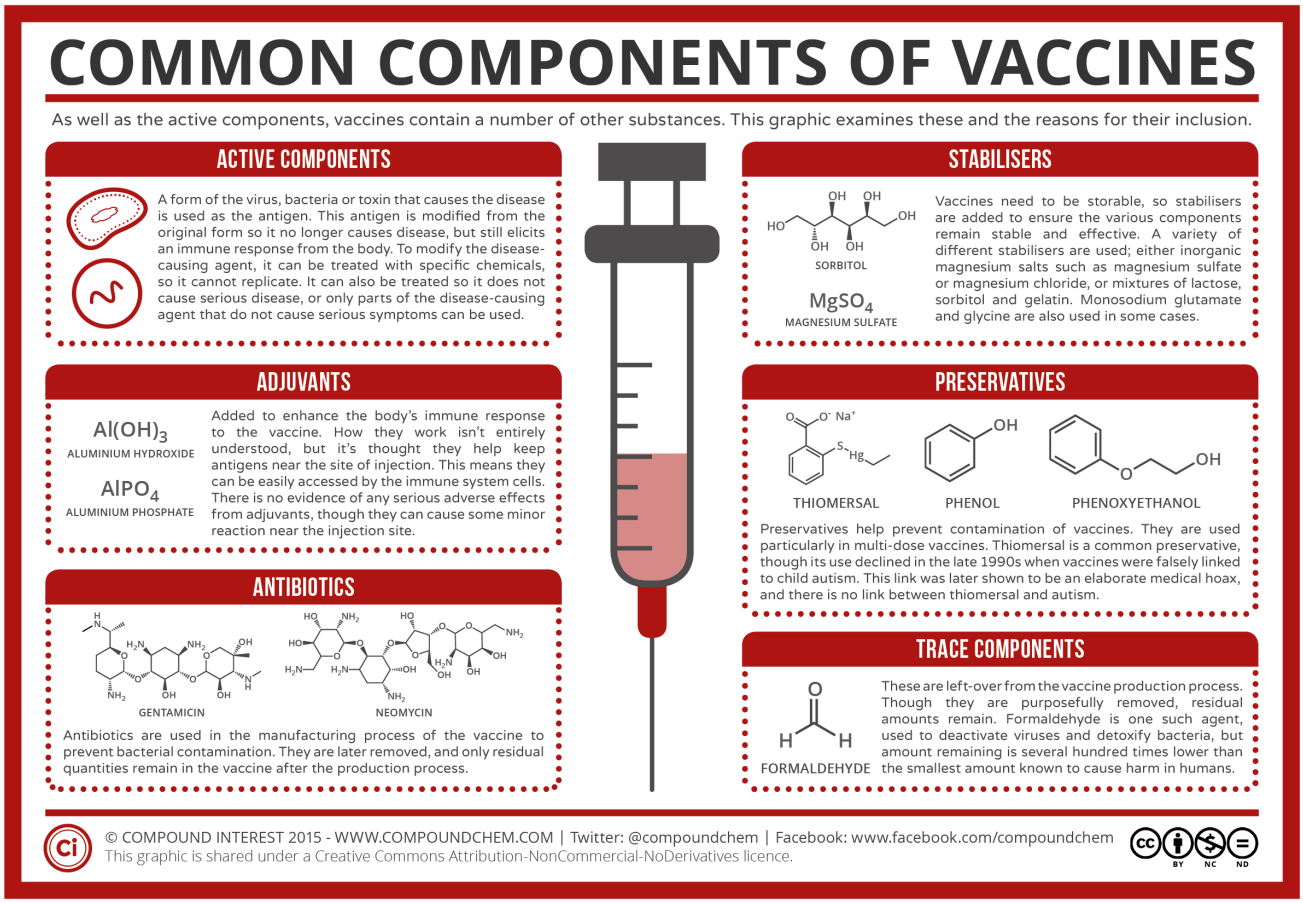
Vaccine composition

Generally, vaccines have several major components.

1) **Antigen (active components):** the active component, or antigen, is the important part, responsible for inferring immunity to the disease or infection the vaccine is designed to guard against. It's composed of a modified form of the virus, bacteria, or toxin that causes the disease; the precise nature can vary between vaccines.
2) **Adjuvant:** chemical compounds added to vaccines to help enhance the body's immune response, these aren't present in all vaccines.
3) **Preservative** (phenol, 2 phenoxyethanol, thimersa: DTap, polio, Hibl): preservatives are used to prevent bacterial and fungal contamination of the vaccine after its manufacture. This is particularly important for so-called "multi-dose" vaccines, where multiple injection doses are drawn for the same rubber-capped vessel.
4) **Additives confer stabilization of live attenuated virus:** stabilizers are added to the vaccine to protect it from adverse conditions which could impact its efficacy, allowing it to be stored for longer periods of time. a range of different possible stabilizers can be used; sugars (sucrose, lactose), amino acids and proteins (gelatin, human serum albumin) can all be utilized for this purpose. They also prevent the vaccine components from adhering to any storage vessel. Many of the compounds used as stabilizers are found naturally in the body anyway, and so do not pose any risk.
5) **Manufacturing residual:**
   - In activating agent (formaldehyde, glutraldehyde): a number of trace components are left behind from the manufacturing process of the vaccine. The concentration of these components in the final vaccine is very low. Compounds such as formaldehyde, one of the agents that can be used to in activate viruses, can be detected, but at levels far below that known to cause harm in humans.

- Antibiotics: in the manufacture of the vaccine, antibiotics will commonly be used to prevent bacterial contamination. Whilst these are removed after manufacture, trace amounts can still remain in the final vaccine. The antibiotics that commonly cause adverse allergic reaction, such as penicillin, are avoided
- Cellular residuals (egg protein, yeast proteins).

6) **Diluents:** vaccines need to be diluted to their required concentration. Most often, this will be accomplished using either sterile water, or a saline solution.



Adjuvant

An adjuvant is an ingredient of a vaccine that helps create a stronger immune response in the patient's body. In other words, adjuvant help vaccines work better. Some vaccines made from weakened or dead germs contain naturally occurring adjuvant and help the body produce a strong protective immune response. However, most vaccines developed today include just small components of germs, such as their proteins, rather than the entire virus or bacteria. Adjuvant help the body to produce an immune response strong enough to protect the person from the disease he or she is being vaccinated against.

Adjuvanted vaccines can cause more local reactions (such as redness, swelling, and pain at the injection site) and more systemic reactions (such as fever, chills and body aches) than non-adjuvanted vaccines.

In some vaccines, the weakened or inactivated virus stimulates a strong immune response so no additional adjuvant is needed for it to be effective to protect against infections.

Factors affecting adjuvant selection:

1) Type of the antigen.
2) Species to be vaccinated: some adjuvant may be safe in one species but not in another.
3) Rout of administration.
4) Side effects.

Classification of adjuvant: (according their mechanism of action)

1) Active immune-stimulants, being substances that increase the immune response to the antigen.
2) Carriers, being immunogenic proteins that provide T-cell help.

3) Vehicle adjuvant; being oil emulsions or liposome that serve as a matrix for antigens as well as stimulating the immune response.

Adjuvant challenge:

1- Toxicity
2- Stability
3- Bioavailability
4- Cost
5- Production difficulty
6- Epitope modification potential during formulation.
7- Pre-existing immunity to carrier protein

Types of adjuvant

1) Aluminum gels or aluminum salts are vaccine ingredients that have been used in vaccines since the 1930s. small amounts of aluminum are added to help the body build stronger immunity against the germ in the vaccine. Aluminum is one of the most common metals found in nature and is present in air, food, and water. The amount of aluminum present in vaccines is low and is regulated by the U.S. Food and Drug Adminstration (FDA).
2) Monophosphoryl lipid A is included in one human papillomavirus (HPV) vaccine. This immune-boosting substance was isolated from the surface of bacteria.

Time of vaccination

Vaccines are designed to prevent diseases affect young children , bearing in mind certain considerations :-

1- The presence of maternally derived antibodies reduce the effectiveness of some vaccines, there for they usually delayed until the third month of life on later.
2- Live attenuated vaccines can cause severe disease in immunodeficiency states .
3- Where the disease is mainly risk to the elderly e.g *pneumococcal pneumonia* vaccination is usually given at a late age.

According to the recommended immunization schedule for persons, children may receive up to **24 vaccinations** to protect them from up to 14 diseases by the time they are 2 years of age. Vaccines are recommended for very young children because their immune systems are not yet fully mature and also because their stomachs produce less acid, making it easier for ingested bacteria and viruses to multiply. These factors leave them the most vulnerable to the devastating effects of these serious diseases.

Some things should be note when scheduling vaccinations:

- allergic reaction to a previous vaccination or a vaccine ingredient, like eggs or gelatin.
- If a child has a high fever, or a history of fever after receiving a vaccination.

Vaccination schedule in Iraq

- **At birth:** BCG, OPV-0, HBV-1
- **2 months completed:** pentavalent vaccine (DTP-1, Hib1, and HBV-2), OPV1 and Rotavirus1.
- **4 months completed:** quadruple vaccine (DTP-2, and Hib2) OPV2 and Rotavirus2.

- **6 months completed:** pentavalent vaccine (DTP-3, Hib3, and HBV-3), OPV3 and Rotavirus3.
- **9 months completed:** measles
- **15 months completed:** MMR1
- **18 months completed:** Quadruple vaccine (DTP, and Hib) OPV. (booster no.1)
- **4-6years:** DTP, OPV (poster no.2) and MMR2

1- Bacilli calmette-guerin (BCG) vaccine

The live attenuated strain of *mycobacterium bovis* known as bacillus Calmette-Guerin (BCG) uses shared antigens to stimulate the development of cross-immunity to *Mycobacterium tuberculosis*. It lost its virulence in humans by being specially cultured in an medium for years.

Benefit:

1- Prevention of *tuberculosis*.
2- BCG prevents dissemination of the bacterium or the development of other life-threatening complications such as meningitis.
3- BCG is effective at reducing morbidity and mortality in children but is less useful in the prevention of adult respiratory disease.

Route of administration:

- **BCG** is given as a **single** intra-dermal injection at the insertion of the deltoid into the lateral aspect of the left upper arm.
- The insertion of deltoid is most frequently used because the local complication rate is smallest when that site is used.

Successful BCG vaccination:

- A small bleb is raised and a successful vaccination leads to the development of a small local swelling with 2 weeks.
- The lesion progresses to a papule or shallow ulcer of approximately 10 mm diameter and within 12 weeks to form a small, flat scar.

Adverse effects:

1- Local ulceration and regional suppurative adenitis occur in 0.1-1 % of vaccine recipients.
2- If BCG is accidentally given to an immunocompromised patient, it can cause disseminated or life threatening infection

2- Polio vaccines

Poliovirus: Enterovirus (RNA), three serotypes: 1, 2, 3, Human is the reservoir, transmission by fecal-oral or possible oral-oral, communicability 7-10 days before onset, the virus present in stool for 3-6 weeks. viral spread along nerve fibers leads to destruction of motor neurons.

The two vaccines have eradicated polio from most of the countries in the world from an estimated 350,000 cases in 1988 to less than 2000 cases in 2008 and to 359 in 2014.

Salk's polio vaccine "inactivated polio vaccine" IPV

Based on polio grown in a type of monkey tissue culture, which is then inactivated with formalin. Contains 3 serotypes of vaccine virus. The injected Salk vaccine confers IgG-mediated immunity in the blood stream, which prevents polio infection from progress to viremia and protects the motor neurons. It offers no protection to the mucosal lining of the intestine, i.e. people vaccinated with salk's vaccine can still carry the disease and spread it

to unvaccinated individuals. IPV has essentially no adverse effects associated with it other than possible rare hypersensitivity reactions to trace quantities of antibiotics.

Sabin's polio vaccine "oral live-attenuated vaccine" OPV

Sabin's "oral polio vaccine" is a **live-attenuated** vaccine, Contains 3 serotypes of vaccine virus. It replication very efficiently in the gut, the primary site of infection and replication, unable to replicate efficiently within nervous system tissue. The OPV proved to be superior in administration, and also provided **longer lasting immunity than the Salk vaccine.** The trivalent OPV vaccine on very rare occasions has been associated with paralysis (vaccine-associated paralytic poliomyelitis, about 1 case per 750,000 vaccine recipients).

3- DPT vaccine

Diphtheria

- Caused by aerobic gram-positive bacillus; *Corynebacterium diphtheria*
- complication are myocarditis and neuritis, death occurs in 5-10% for respiratory illness

Tetanus

- Caused by anaerobic gram-positive spore-forming bacteria; *Clostridium tetani*
- Complications:- laryngospasm, aspiration pneumonia, and death.

Pertussis

- Highly contagious respiratory infection caused by *Bordetella pertussis*
- Complication :- pneumonia, seizures, encephalopathy.

DPT: mixture of three vaccines, to immunize against diphtheria, pertussis and tetanus **Pertussis, whole heat or formalin killed vaccine with Diphtheria and Tetanus toxoid**

DPT administered in a dose of 0.5 ml intramuscularly five vaccinations before age 7 years (at 2,4,6, and 15-18 month and at 4-6 years)

Adverse effects

- **Minor reaction**:- inflammation, indurations or a painless nodule at the site of injection.
- **Moderate reaction**:- ongoing crying (for three hours or more in the first 12 hours), a high fever (up to 40 ^{O}C).
- **Severe problems**:- happen very rarely include, a serious allergic reaction.

4- MMR vaccine

Measles

caused by *paramyxovirus* (RNA); Complication: diarrhea, otitis media, pneumonia

Mumps

caused by *paramyxovirus* (RNA); Complication: CNS involvement, deafness

Rubella

caused by *togavirus* (RNA); Major concern is **congenital rubella syndrome** as up to 85% of infants affected during first trimester when placenta and fetus infected during viremia; infection may affect all organs, may lead to fetal death or premature delivery, deafness, liver and spleen damage.

MMR vaccine: composed of three live attenuated vaccines (Measles, Mumps & Rubella)

This highly effective vaccine is administered subcutaneously in two doses, the first MMR dose is recommended at age 12 to 15 months and the second at the child's entry into

school (age 4 to 6 years), a dose given before 12 months of age will not be counted. The purpose of the rubella portion of this vaccine is to protect against congenital rubella syndrome by preventing the occurrence of rubella, which, by itself, is a mild disease. Because MMR is a live-attenuated vaccine, non allergy related side effects are noted 5 to 12 days following immunization. Fever and rash are relatively common, experienced by 5-15% of recipients.

Contraindications and precautions:

1- Severe allergic reaction to vaccine component or following prior dose
2- Pregnancy
3- Immunosuppression

5- Hepatitis B vaccine

Hepatitis B infection: caused by Hepadnaviridae fimily (DNA)

Hepatitis B vaccine consists of purified HBsAg particles produced through recombinant DNA technology in yeast. non living antigenic preparation can be derived from the blood of carriers, this is because the surface coat antigen (HBsAg) is over produce by the virus and circulate as free non infectious 22 nm spherical particles, these particles were shown be at least 95% protective. Vaccine usually is given intramuscularly as a three dose series, the second and third doses given 1 and 6 months, respectively, after the first dose (0,1,6). Three doses induce seroconversion in 90-95% of healthy infants, children and adults.

Disadvantage:

1- Derived from human blood
2- Purified with exceptional care because the risks of transmitting live HBV or other.
3- Antibody levels start to fall 1-2 years later so that boosting may be necessary.

6- Rotavirus vaccine

In early childhood, the single most important cause of severe dehydrating diarrhea is rotavirus infection.

- The pentavalent vaccine (attenuated virus) protect against rotavirus gastroenteritis.
- Oral route and three doses; 2,4 and 6 months.

7- *Haemophilus influenza* type b vaccine

Haemophilus influenza is a gram negative coccobacillus, cause severe pneumonia, meningitis and other invasive disease, 15-30% of children who survive (Hib) meningitis may develop permanent neurological disability, including brain damage, hearing loss, 5-10% cases of Hib meningitis are at risk of dying.

- Type of vaccine: conjugate
- Number of doses: three doses (2,4,6 months) and a booster shot at 18 months
- Injection site: outer mid-thigh for infants
- Injection type: intramuscular
- Given as quadruple or pentavalent vaccine.

8- Rabies vaccine

- Killed preparation is used
- Post exposure case 5-6 injection intramuscularly
- Pre exposure 2-3 dose are usually sufficient for protection, with boost every few years.

9- Influenza vaccine

- The most widely used B-propiolactone killed viral vaccine
- Vaccine is offered to high risk groups such as nursing staff and patients with chronic respiratory cardiac disease.
- Revaccination in subsequent is required to maintain antibody level.

10- Chickenpox (*Varicella zoster*) vaccine

- Live attenuated vaccine is highly protective up to 95% protection
- Given to immunocompromised and neonates at risk.

11- Typhoid vaccine

Two vaccines have emerged

- Live attenuated by random chemical mutagenesis, they induce local immunity in the intestine when given orally.
- Polysaccharide vaccine is composed of purified virulence antigen; single dose has given protection into 70% range.

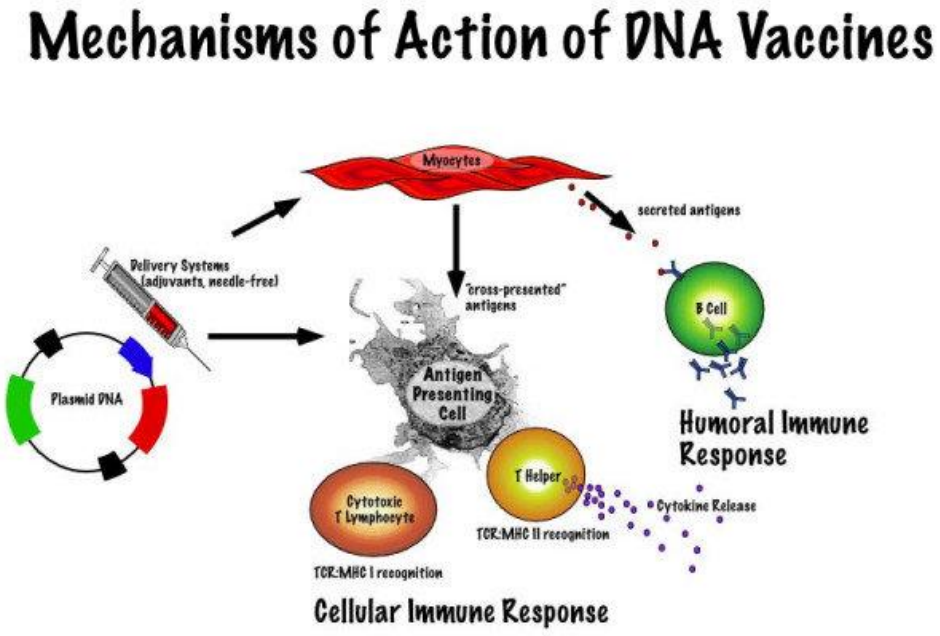
12- Cholera vaccine

- Heat killed – poor protection
- Toxoid using cholera toxin, some success
- Deletion mutant
- Expression cholera genes in an attenuated *Salmonella typhi* vaccine

DNA Vaccination

DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. Nucleic acid vaccines are still experimental, and have been applied to number of viral, bacterial and parasitic models of disease, as well as to several tumor models. DNA vaccines have a number of advantages over conventional vaccines, including the ability to induce a wider range of immune response types.

DNA vaccines are made up of small, circular piece of bacterial DNA called a plasmid that has been genetically engineered to produce one or two specific proteins (antigens) from a pathogen. The vaccine DNA is injected into the cells of the body, where the inner machinery of the host cells reads the DNA and converts it into pathogenic proteins, because these proteins are recognized as foreign, when they are processed by the host cells and displayed on their surface, the immune system is alerted, which then triggers a range of immune responses.

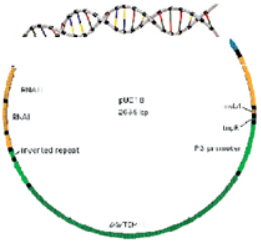


HOW DNA VACCINE IS MADE ?

Viral gene

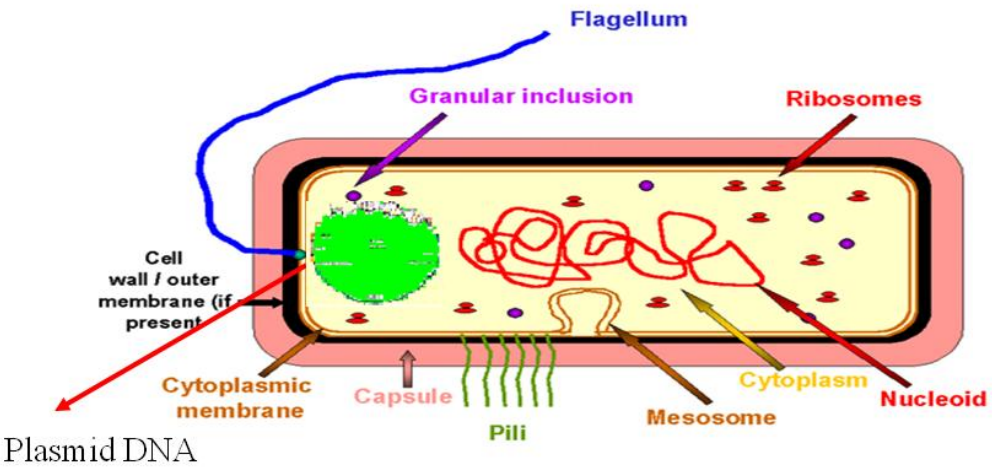
Expression plasmid

Recombinant DNA Technology



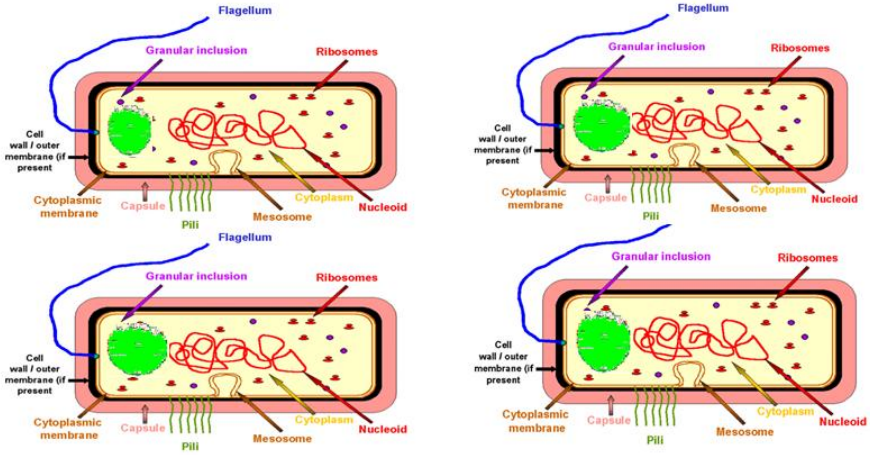
Plasmid with foreign gene

Transform into bacterial cell

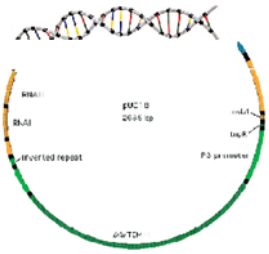


Bacterial cell

Plasmid DNA get Amplified



Plasmid DNA Purified



Ready to use

Potential advantage and disadvantage of nucleic acid based immunization:

Advantage

1. Subunit vaccination with no risk for infection.
2. Antigen presentation by both MHC class I and class II molecules.
3. Able to polarize T-cell help toward type 1 or type 2.
4. Immune response focused only on antigen of interest.
5. Ease of development and production.
6. Cost-effectiveness
7. Stability of vaccine for storage and shipping.
8. Long term persistence of immunogen.
9. Obviate need for peptide synthesis, expression and purification of recombinant proteins and the use of toxic adjuvant.
10. In vivo expression ensure protein more closely resembles normal Eukaryotic structure, with accompanying post-translational modifications.

Disadvantage

1. Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides).
2. Risk of affecting genes controlling cell growth.
3. Possibility of inducing antibody production against DNA.
4. Possibility of tolerance to the antigen (protein) produced.
5. Potential for atypical processing of bacterial and parasite proteins.

Delivery methods:

DNA vaccines have been introduced into animal tissues by a number of different methods. The two most popular approaches are:

1- **Injection of DNA in saline using a standard hypodermic needle**

Conducted intramuscular (IM) in skeletal muscle or intradermally (ID), with DNA being delivered to the extracellular spaces.

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.

2- **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.

Alternative delivery methods have included aerosol instillation of naked DNA on mucosal surfaces, such as the nasal and lung mucosa.

The method of delivery determines the dose of DNA required raising an effective immune response, saline injection require variable amounts of DNA from 10 µg -1 mg, whereas gene gun deliveries require 100 to 1000 times less DNA than intramuscular saline injection to raise an effective immune response.

Alternative approaches for vaccine production:

1- Recombinant viral antigen subunit vaccines

Virus proteins or genetic material from a selected infectious agent is inserted into live microbe that is non-pathogenic, the recombinant microbe will multiply and express the foreign gene, and the vaccine recipient will be immunized against microbial Ag. *E. coli* cell were first to be used for this purpose, vaccinia the virus originally used to vaccinated for smallpox and adenoviruses have proved practical agents for this technique.

These methods are particularly effective in designing vaccines for obligate parasite that are difficult or expensive to culture syphilis spirochete or malaria parasite. Ex: HB virus.

This technology provides mean of isolating the gene that encode various microbial Ag, inserting them into plasmid vectors, and cloning them in appropriate host.

2- Synthetic peptide

Identification of the peptide sequences that trigger a protective immune response (immunogenic site) and to use completely synthetic versions of these as the vaccine substance.

Cowpea mosaic virus was genetically engineered to include: surface antigen from foot and mouth disease virus (pathogenic to human and animal). This virus was used to infect its natural host (black-eyed pea plant), and introduced genes from the foot and mouth disease virus was expressed handsomely in the plant, and the plant needs to be sacrificed a few week after infection. One leaf from the infected plant produced enough surface Ag to serve as a vaccine for 200 dose.

3- Edible vaccine

Edible vaccine are mucosal-targeted vaccines, which cause stimulation of both systemic and mucosal immune response. Edible vaccines are being developed for various diseases, such as measles, cholera and hepatitis B, and many more are in the process of development.

Edible vaccine would not need purification, refrigeration and injection, this made the vaccine cheep. Tomatoes and lettuce have been transformed to produce HBs Ag.

4- Anti-idiotypic vaccine

- This unique amino acid structure in the antibody is known as the idiotype, which can be considered as a mirror of the epitope in the Ag.
- Antibodies can be raised against the idiotype by injecting the antibody into another animal.
- This anti-idiotype antibody mimics part of the three dimensional structure of the Ag. This can be used as a vaccine.
- When the anti0ideotye antibody is injected into a vaccine, antibodies (anti-idiotype antibodies) are formed that recognize a structure similar to part of the virus and might potentially neutralize the virus.