Vaccine production

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Vaccine

- A **vaccine** is a biological preparation that improves immunity to a particular disease.
- A vaccine is an agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.

Vaccine

- A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe or its toxins.
- Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g. vaccines against cancer are also being investigated; see cancer vaccine).

History

- The term *vaccine* derives from Edward Jenner's 1796 use of the term *cow pox* (Latin *variolæ vaccinæ*, adapted from the Latin *vaccīn-us*, from *vacca* cow), which, when administered to humans, provided them protection against smallpox.
- Sometime during the 1770s Edward Jenner heard a milkmaid boast that she would never have the often-fatal or disfiguring disease smallpox, because she had already had cowpox, which has a very mild effect in humans.
- In 1796, Jenner took pus from the hand of a milkmaid with cowpox, inoculated an 8-year-old boy with it, and six weeks later variolated the boy's arm with smallpox, afterwards observing that the boy did not catch smallpox.
- Since vaccination with cowpox was much safer than smallpox inoculation,[3] the latter, though still widely practiced in England, was banned in 1840.
- Louis Pasteur generalized Jenner's idea by developing what he called a rabies vaccine, and in the nineteenth century vaccines were considered a matter of national prestige, and compulsory vaccination laws were passed.

Types of Vaccines

- Killed
- Attenuated
- Toxoid
- Subunit
  - Non-recombinant subunit vaccine
  - Recombinant subunit vaccine
- Conjugate
- Recombinant virus vaccines or live recombinant vaccines
- DNA Vaccine
- Edible vaccine

Killed vaccines

- Some vaccines contain killed, but previously virulent, micro-organisms that have been destroyed with chemicals or heat.
- Examples are the influenza vaccine, cholera vaccine, bubonic plague vaccine, polio vaccine, hepatitis A vaccine, and rabies vaccine

Attenuated Vaccine

- Many of these are live viruses that have been cultivated under conditions that disable their virulent properties, or which use closely-related but less dangerous organisms to produce a broad immune response; however, some are bacterial in nature.
- They typically provoke more durable immunological responses and are the preferred type for healthy adults.
- Examples include the viral diseases yellow fever, measles, rubella, and mumps and the bacterial disease typhoid. The live Mycobacterium tuberculosis vaccine developed by Calmette and Guérin is not made of a contagious strain, but contains a virulently modified strain called "BCG" used to elicit an immune response to the vaccine. The live attenuated vaccine containing strain Yersinia pestis EV is used for plague immunization.

Toxoid Vaccine

- Toxoid vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism.
- Examples of toxoid-based vaccines include tetanus and diphtheria.
- Toxoid vaccines are known for their efficacy.
- Not all toxoids are for micro-organisms; for example, *Crotalus atrox* toxoid is used to vaccinate dogs against rattle snake bites.

Subunit vaccines (non-recombinant and recombinant)

- Non-recombinant subunit vaccine
  - In case of non-recombinant subunit vaccine antigens must be produced and purified by cultivation of pathogen. Example : the subunit vaccine against Hepatitis B virus previously extracted from the blood serum of chronically infected patients.

- Recombinant subunit vaccine
  - These vaccines are those in which genes for desired antigen are inserted into vector, usually a virus. The antigen is purified and injected as vaccine.
  - Hepatitis B vaccine that is composed of only the surface proteins of the virus now produced by recombination of the viral genes into yeast.
  - Virus-like particle (VLP) vaccine represents a specific class of recombinant subunit vaccine that mimic the structure of authentic virus particles.
    - They are recognized readily by the immune system and present viral antigens in a more authentic conformation than other subunit vaccine.
    - They can be synthesized through the individual expression of viral structural proteins which can be then self assemble into virus-like structure. Combinations of structural capsid proteins from different viruses can be used to create recombinant VLPs.
    - Example: human papillomavirus (HPV) that is composed of the viral major capsid protein, and the hemagglutinin and neuraminidase subunits of the influenza virus.

Conjugate Vaccine

- Certain bacteria have polysaccharide outer coats that are poorly immunogenic.
- By linking these outer coats to proteins (e.g. toxins), the immune system can be led to recognize the polysaccharide as if it were a protein antigen.
- Example: *Haemophilus influenzae* type B vaccine

Recombinant virus vaccines or live recombinant vaccines

- A gene coding for an immunogenic protein from one organism into the genome of other, such as vaccinia virus is introduced.
- The organism expressing that gene is called as recombinant.
- Following injection into the subject, the recombinant will replicate and express sufficient amount of the foreign protein to induce a specific immune response to the protein.
- Can also encode for several antigens from different pathogens, introducing the possibility of a single vaccine for several diseases (Polyvalent vaccine).
- Example vaccine for poultry against fowl pox and new castle disease.

DNA vaccine

- In recent years a new type of vaccine called *DNA vaccination*, created from an infectious agent's DNA, has been developed.
- It works by insertion and expression of viral or bacterial DNA into human or animal cells.
- Some cells of the immune system that recognize the proteins expressed will mount an attack against these proteins and cells expressing them.
- Because these cells live for a very long time, if the pathogen that normally expresses these proteins is encountered at a later time, they will be attacked instantly by the immune system.
- One advantage of DNA vaccines is that they are very easy to produce and store.

Edible Vaccine

- Genes coding for significant antigens are introduced into plants, such that the fruits produced bear foreign antigens.
- This is edible vaccine and is still in experimental stage.
- Transgenic tobacco is successfully engineered for the production of edible vaccines against Hepatitis B antigen using‘s gene of HBV (Hepatitis B Virus). The optimum level of recombinant protein was obtained in leaves and seeds.
- Potato is one of the best sources for vaccine production but the raw potatoes are not palatable and cooking destroys protein antigens. Vaccine for cholera is successfully developed in potato.
- Banana is the ideal plant for oral vaccine production due to its excellent digestibility, palatability and availability throughout the year. Vaccine for hepatitis B is successfully made in banana.

Production of Vaccine

- **Antigen generation**
- First, the antigen itself is generated. Viruses are grown either on primary cells such as chicken eggs (*e.g.*, for influenza), or on continuous cell lines such as cultured human cells (*e.g.*, for hepatitis A).
- Bacteria are grown in bioreactors (*e.g.*, *Haemophilus influenzae* type b).
- Alternatively, a recombinant protein derived from the viruses or bacteria can be generated in yeast, bacteria, or cell cultures.
- **Antigen generation for inactivated (Killed) vaccine:**
- Inactivated vaccines are produced by killing the disease-causing microbes with chemicals or heat.
- Viruses can be lipid-coated(enveloped) or non-enveloped.
- Virus inactivation works by one of the following mechanisms - By disrupting the viral DNA or RNA and preventing replication.• Solvent/detergent (S/D) inactivation• Pasteurization• Acidic pH inactivation(Low pH Treatment)• Ultraviolet (UV) inactivation
- Solvent/detergent (S/D) inactivation: Most enveloped viruses cannot live without their lipid coating, so they die when exposed to these detergents.• Other viruses may still live, but they are unable to reproduce, rendering them non-infective.• The detergent typically used is Triton-X 100.
- Pasteurization -• Effective for both non-lipid and lipid-coated viruses.• Because pasteurization involves increasing the temperature of solution to a value that will sufficiently denature the virus.
- Acidic pH inactivation (Low pH Treatment) –• Most effective with lipid-coated viruses• Incubation typically occurs at a pH of 4 and lasts any where between 6 hours and 21 days.
- UV inactivation -• UV rays can be used to inactivate viruses since virus particles are small and the UV rays can reach the genetic material• Once the DNA dimerised, the virus particles cannot replicate their genetic material.

…Production of Vaccine

- Antigen generation for attenuated vaccine:
  - To make a live attenuated vaccine, the disease-causing organism is grown under special laboratory conditions that cause it to lose its virulence or disease-causing properties.
  - The attenuation can be obtained by heat or by passage of the virus in foreign host such as embryonated eggs or tissue culture cells. For example To produce the **Sabin polio vaccine**, attenuation was only achieved with high inocula and rapid passage in **primary monkey kidney cells**.
- Inclusion bodies — Bacterial cells often are used to produce proteins that can function as vaccines. Bacteria produce proteins intracellularly and store the produced proteins in internal structures called inclusion bodies. Following bacterial cell collection and lysis, the inclusion bodies are collected and disrupted. This often involves a series of steps involving protein denaturation followed by protein renaturation or folding.
- Membrane extraction —vaccine products can be portions of bacterial or mammalian cell membrane structures.The vaccine product is formulated from the extracted and purified membrane structure. Capsule extraction
- —Some bacteria grow and secrete a complex carbohydrate material forming an external capsule. This capsular material can be isolated and purified to formulate a vaccine.

... Production of Vaccine

- Isolated from the cells used to generate it.
  - A virus may need to be inactivated, possibly with no further purification required.
  - The most common method of vaccine production is based on an initial fermentation process followed by purification.• CENTRIFUGATION• FILTRATION• CHROMATOGRAPHY
  - Recombinant proteins need many operations involving ultrafiltration and column chromatography.
- Formulation
  - the vaccine is formulated by adding adjuvant, stabilizers, and preservatives as needed.

Formulation

- Other than microorganism or its part a vaccine contain the following substance:-
- **Suspending fluids**
  - The liquid which contains the chemicals used during production which kill or weaken the organism for use in vaccines.
  - Sterile water, saline or fluids containing protein,
  - Egg proteins are found in influenza and yellow fever vaccines, which are prepared using chicken eggs
  - Yeast Proteins, Hepatitis B vaccines are made by transfecting cells of Saccharomyces cerevisiae (baker's yeast) with the gene that encodes hepatitis B surface antigen, and residual quantities of yeast proteins are contained in the final product.
- **Stabilizers** - Albumin, Phenols, Glycine - Monosodium glutamate (MSG) and 2-phenoxy-ethanol which are used as stabilizers in a few vaccines to help the vaccine remain unchanged when the vaccine is exposed to heat, light, acidity, or humidity.
- **Antibiotics** , which are added to some vaccines to prevent the growth of bacteria during production and storage of the vaccine. eg. neomycin, streptomycin, polymyxin B, chlortetracyline, and amphotericin B.
- **Thimerosal** is a mercury-containing preservative that is added to vials of vaccine that contain more than one dose to prevent contamination and growth of potentially harmful bacteria. Eg. diphtheria-tetanus-acellular pertussis (DTaP), hepatitis B, and Haemophilus influenza type B (Hib).
- **Inactivating Agents:** It is used to inactivate virus, toxoins. eg. formaldehyde, β-propiolactone, Glutaraldehyde
- **Adjuvants or enhancers** – aluminum gels or salts (Alum) Alum is used in several licensed vaccines including:• diphtheria-pertussis-tetanus• diphtheria-tetanus(DT)• DT combined with Hepatitis B (HBV)• Haemophilus influenza B• Inactivated polio virus• Hepatitis A (HAV)• Streptoccucus pneumonia vaccine• Meningococccal vaccine• Human papilloma virus (HPV)

Questions

- Write an assay on Vaccine production (Go for MS word file for detail of vaccine producion)
- What are the various types of vaccines of vaccine? Explain their method of production.
- What are the types of vaccines explain with their examples.
- Write a short note on types of vaccines.
- Write short notes on vaccine formulation.
- Write in brief various steps of vaccine production.