# Vaccines and Their Properties

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# History

**1100s: In** Turkey, Africa, India, China, and Europe.

The variolation technique was developed, involving the inoculation of children and adults with dried scab material recovered from smallpox patients.

The terms *inoculation* and *variolation* were often used interchangeably.

**1721:** Variolation was introduced to Great Britain by English aristocrat Lady Mary Wortley Montague.

# History

**1798:** Edward Jenner published his work on the development of a vaccination that would protect against smallpox.

The word ***Vaccine*** is derived from the Latin ***vacca***, meaning ***cow***.

A virus that mainly affects cows (Cowpox) was used by ***Edward Jenner*** to protect the young boy (***James Phipps***) from smallpox by scratching liquid from cowpox sores into the boy's skin.

- *Louis Pasteur first used term vaccine*

# History

- **1879:** Louis Pasteur created the first live attenuated bacterial vaccine (chicken cholera)
- **1882**: Robert Koch identified the tubercle bacillus as the cause of tuberculosis, subsequently called Koch's bacillus.
- **1884:** The first live attenuated viral vaccine (rabies) was developed by Louis Pasteur, using dessicated brain tissue inactivated with formaldehyde.
- **1885**: Louis Pasteur first used rabies vaccine in humans.

  *Joseph Meister* (21 February 1876 – 24 June 1940)

# Terminology

- A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing, and is often made from weakened or killed forms of the microbe, its toxins, one of its surface proteins or genetic material.
- **Immunogen**
  - ❖ **Immunogenicity**
- **Antigen(Ag)**
  - ❖ **Antigenicity**

- ***Vaccinology***

It is the science to study vaccine and it's properties for prevention and control of diseases.

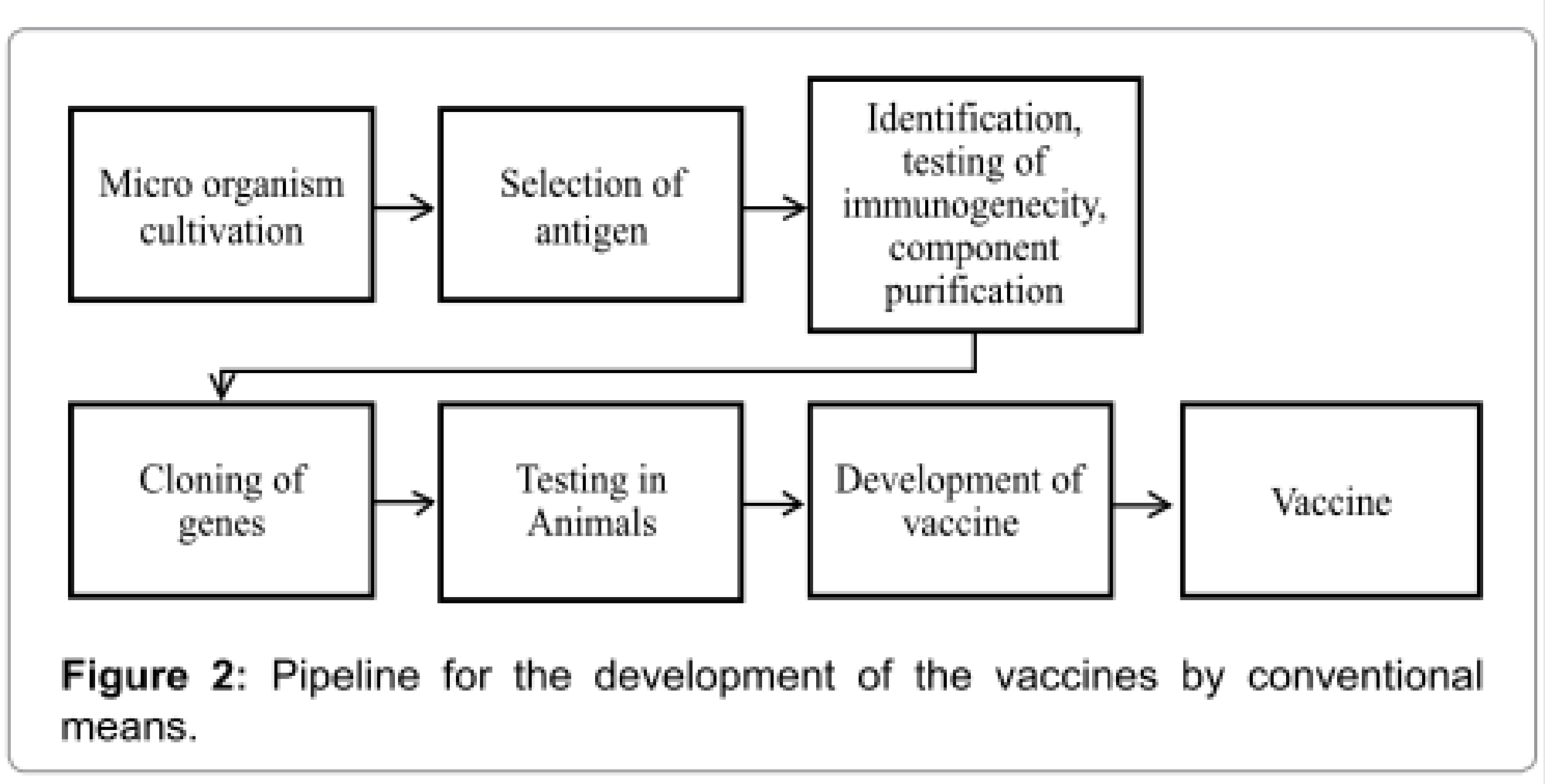



**Figure 2:** Pipeline for the development of the vaccines by conventional means.

# Reverse Vaccinology

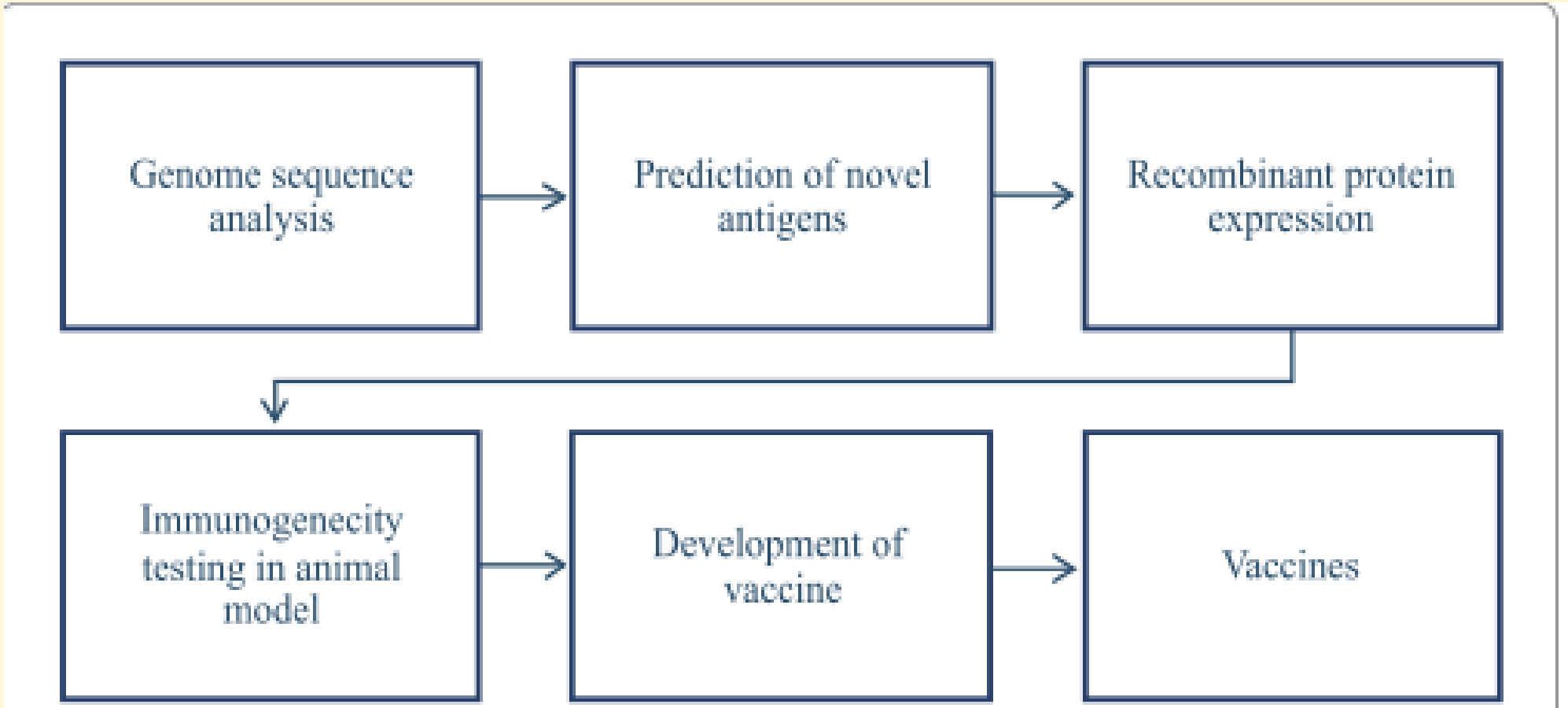



**Figure 3:** Pipeline for the development of the vaccines by reverse vaccinology.

# Properties of Ideal Vaccine

- Immunogenic
- Long lasting immunity
- Safe
- Stable in field conditions
- Combined
- Single dose
- Affordable (and accessible) to all

# Types of vaccines

- ***First generation vaccines*:-** are whole-organism vaccines – either live and weakened, or killed forms.
- ***Second generation vaccines*:-** subunit vaccines, consisting of defined protein antigens or protein components.
- ***Third generation vaccines*:-** DNA vaccine or marker vaccine and synthetic peptide vaccine compose of DNA Sequence code for antigenic protein of pathogen.

# Live, Attenuated vaccines

- Contain a version of the living microbe that has been weakened in the lab so it can’t cause disease.
- They elicit strong cellular and antibody responses
- But some time vaccine could revert to a virulent form and cause disease
- Need to be refrigerated to stay potent
- Difficult to create for bacteria because bacteria have thousands of genes and thus are much harder to control.

# Merits of live vcaccine

- Fewer dose required
- Adjuvants unnecessary
- Less chance of hyper sensititvity
- Induction of interferon
- Relatively cheap
- Smaller dose needed
- Can be given by natural route
- Stimulate both humoral and cell mediated immunity
- Long lasting protection

Ex:

- *Brucella abortus* strain 19 vaccine
- BCG vaccine (Bacillus of Calmette and Gaurin) vaccine developed by growing *Mycobacterium bovis* in bile saturated for 13 years

# Killed/ inactivated vaccines

- Produce by killing the disease-causing microbe with chemicals, heat, or radiation.
- These are more stable and safer than live vaccines
- Usually don’t require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries.
- Here the organism is inactivated using various inactivating agents like formaldehyde, ethylene oxide, ethyleneimine, acetyl ehtyleneimine
- Salk polio vaccine is inactivated with formaldehyde

# Merits of Killed /inactivated vaccine

- Stable on storage, unlikely to produce disease through residual virulence, do not replicate in recipient, unlikely to contain live contaminating organism
- Will not spread to others
- Safe in immuodeficient patients
- Easier to storage
- Lower development cost
- No risk of reversion

# Subunit vaccines

- Instead of the entire microbe, subunit vaccines include only the antigens that can best stimulate the immune system.
- Since vaccines are contain only the essential antigens, these are very specific and sensitive.

  These can prepared by one of two ways:

- They can grow the microbe in the laboratory and then use chemicals to break it apart and gather the important antigens.
- They can manufacture the antigen molecules from the microbe using recombinant DNA technology. Vaccines produced this way are called “recombinant subunit vaccines.”

# Subunit vaccines

- Inactivated exotoxins, capsular polysaccharides, and recombinant microbial antigens
- Bacterial polysaccharide capsules are used as subunit vaccines
- The current vaccine for *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, consists of 23 antigenically different capsular polysaccharides
- The vaccine for *Neisseria meningitidis*, a common cause of bacterial meningitis, also consists of purified capsular polysaccharides.
- Diphtheria and tetanus vaccines, for example, can be made by purifying the bacterial exotoxin and then inactivating the toxin with formaldehyde to form a **toxoid**.

# Toxoid vaccines

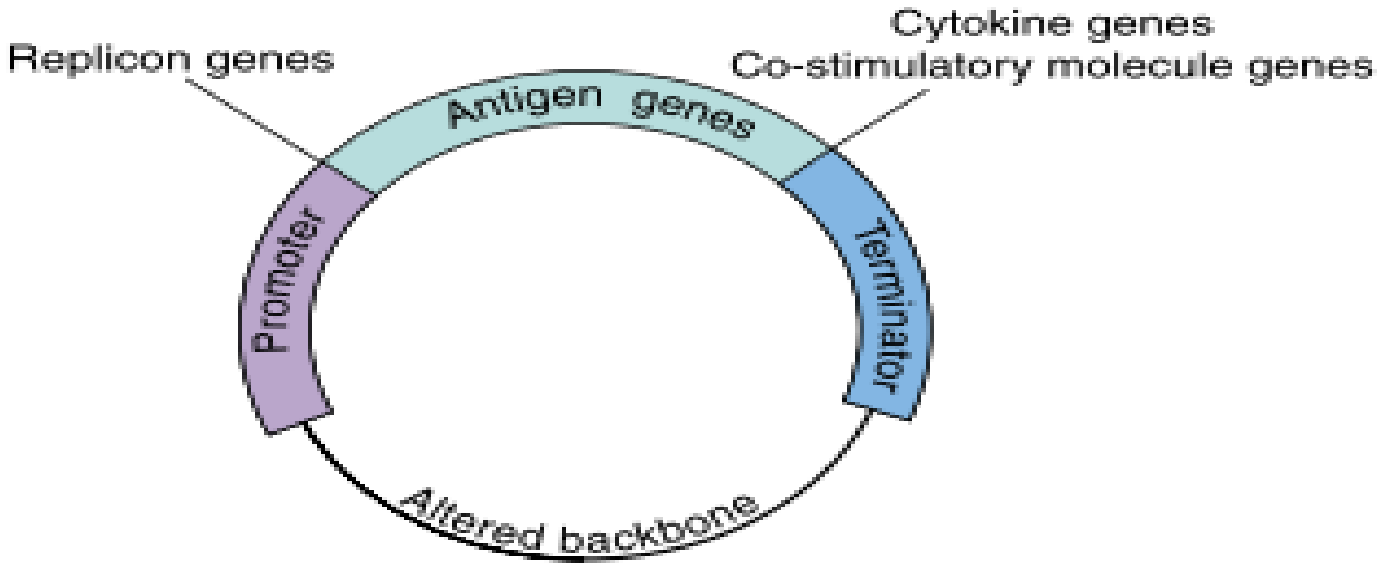
- Most of pathogenic bacteria secrete toxins, or harmful chemicals such harmful agents known as virulence factors, responsible for diseases.
- These can inactivate by treating with formalin, a solution of formaldehyde, heat treatment and sterilized water. Such “detoxified” toxins, called toxoids, are safe for use in vaccines.

# Conjugate vaccines

- Bacterium possesses an outer coating of sugar molecules called polysaccharides, known as capsule is less immunogenic but more virulent.
- Conjugated to protein (antigens/ toxoid) carriers or adjuvants to enhance immunogenicity and helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium.

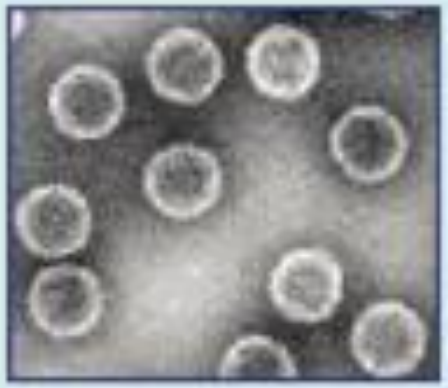
# DNA vaccines

- The DNA vaccines are simple rings of DNA containing a gene encoding an antigen, and a promoter/terminator to make the gene express in eukaryotic cells.
- They are a promising new approach for generating all types of desired immunity: cytolytic T lymphocytes (CTL), T helper cells and antibodies.




 *Journal of Internal Medicine* **253**: 402–410

# Principle of DNA vaccination



Pathogen

Isolation of gene for antigenic protein

Gene of interest
Promoter (ie CMV)
PolyA signal
Bacterial origin of replication
CpG CpG
Antibiotic resistance gene

Cloning into a vaccine plasmid

Production by bacteria

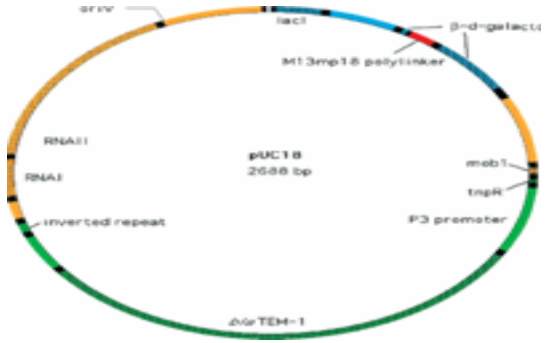
Purification of plasmid

Injection

Immune response

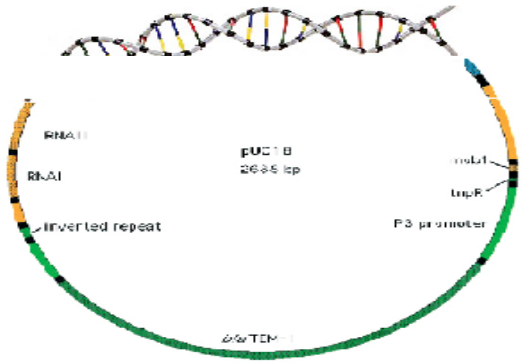
# Preparation of DNA Vaccine

Antigen protein encoding gene



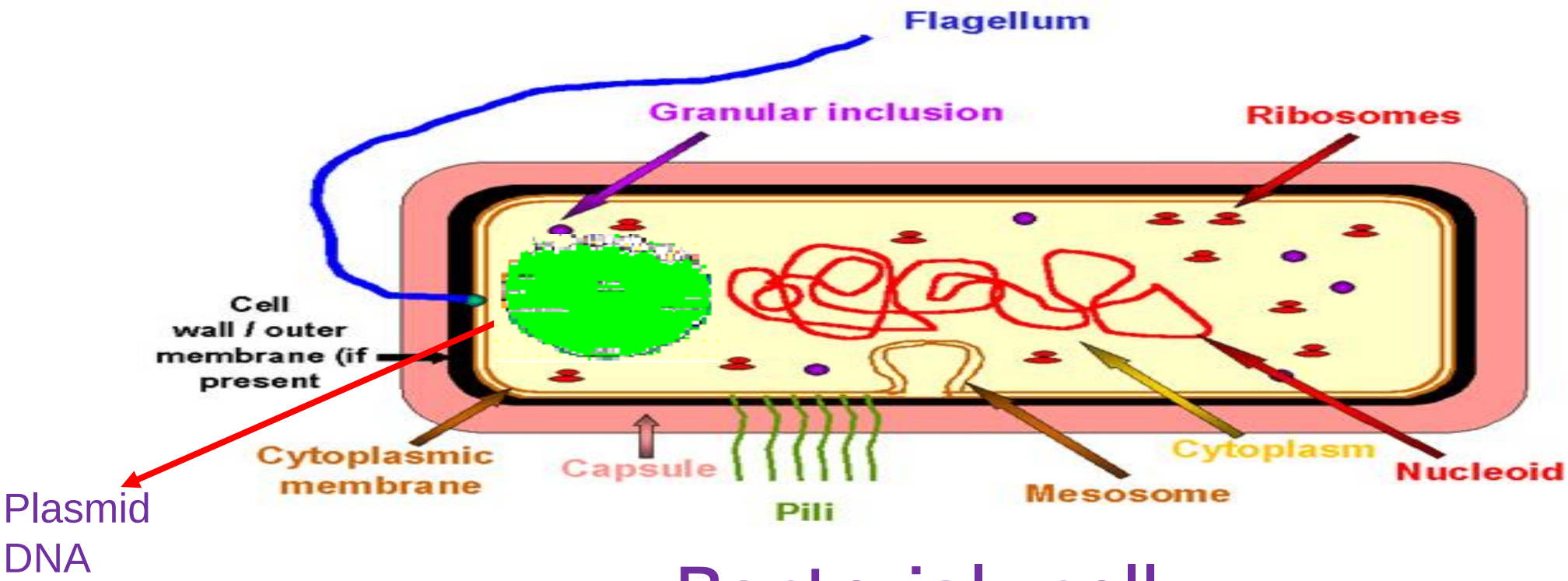

Expression plasmid

Recombinant DNA technology



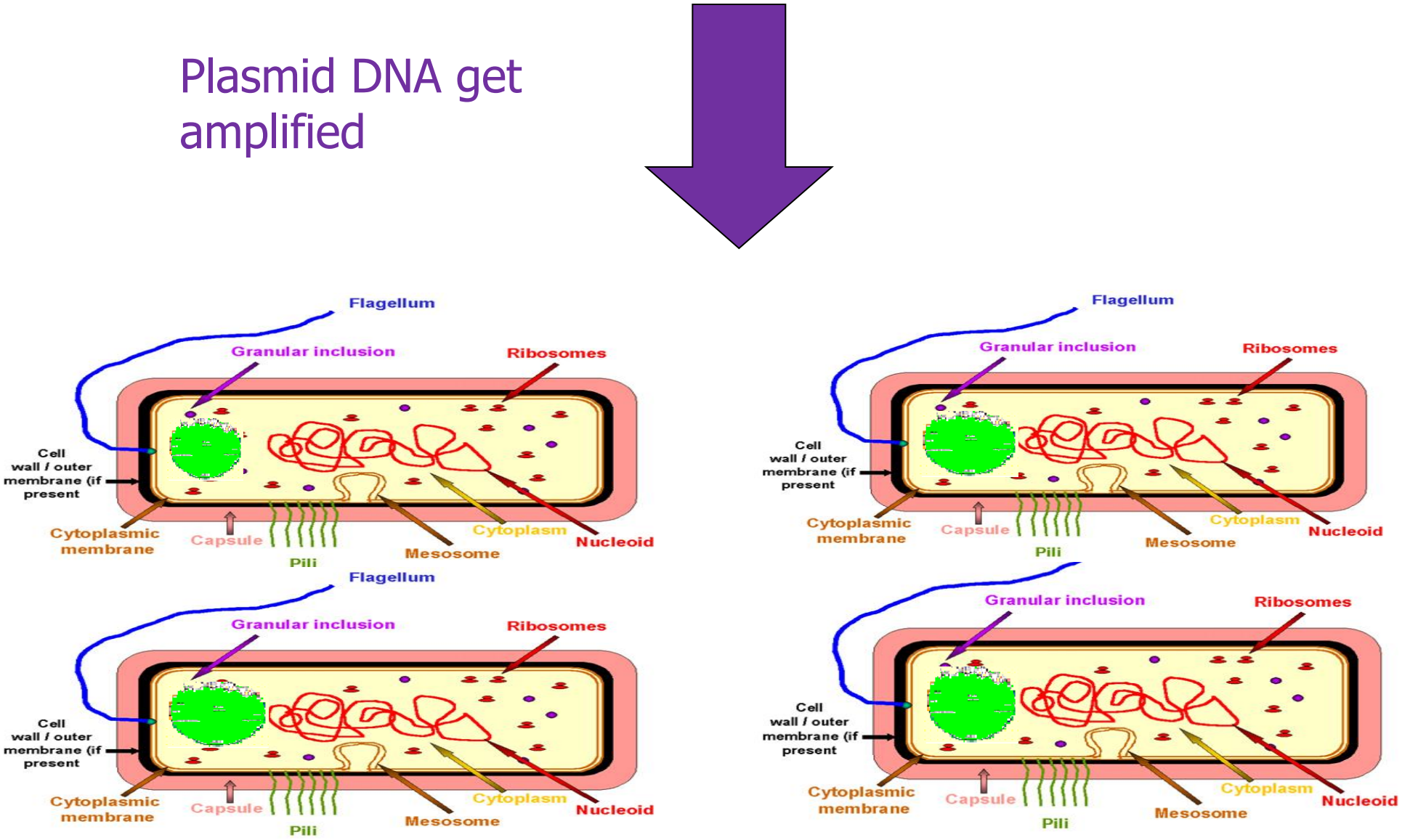

Plasmid with foreign gene

Transform into bacterial cell

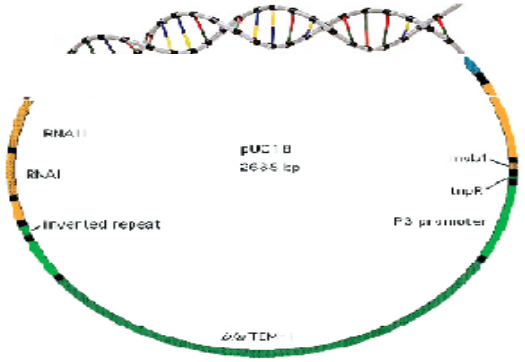



Bacterial cell

Plasmid DNA get amplified



Plasmid DNA Purified




Ready to use

# Delivery methods

| Method of Delivery | | Formulation of DNA | Target Tissue | Amount of DNA |
|---|---|---|---|---|
| **Parenteral** | Injection (hypodermic needle) | Aqueous solution in saline | IM (skeletal), ID, IV, SC and intraperitoneal with variable success) | Large amounts (approximately 100-200 µg) |
| | Gene Gun | DNA-coated gold beads | ED (abdominal skin), vaginal mucosa, surgically exposed muscle and other organs | Small amounts (as little as 16 ng) |
| | Pneumatic (Jet) Injection | Aqueous solution | ED (abdominal skin) | Very high (as much as 300 µg) |
| **Topical application** | | Aqueous solution | Ocular, intravaginal | Small amounts (up to 100 µg) |
| **Cytofectin-mediated** | | liposomes (cationic), microspheres, recombinant adenovirus vectors, attenuated Shigella vector, aerosolised cationic lipid formulations | IM, IV (to transfect tissues systemically), intraperitoneal, oral immunization to the intestinal mucosa; nasal/lung mucosal membranes | variable |

| Method of Delivery | Advantage | Disadvantage |
|---|---|---|
| **Intramuscular or Intradermal injection** | No special delivery mechanism<br>Permanent or semi-permanent expression<br>pDNA spreads rapidly throughout the body | Inefficient site for uptake due to morphology of muscle tissue<br>Relatively large amounts of DNA used |
| **Gene Gun** | DNA bombarded directly into cells<br>Small amounts DNA | Requires inert particles as carrier |
| **Jet injection** | No particles required<br>DNA can be delivered to cells mm to cm below skin surface | Significant shearing of DNA after high-pressure expulsion<br>10-fold lower expression, and lower immune response<br>Requires large amounts of DNA (up to 300 µg) |
| **Liposome-mediated delivery** | High levels of immune response<br>Can increase transfection of intravenously delivered pDNA<br>Intravenously delivered liposome-DNA complexes can potentially transfect all tissues<br>Intranasally delivered liposome-DNA complexes can result in expression in distal mucosa as well as nasal muscosa and the generation of IgA antibodies | Toxicity<br>Ineffectiveness in serum<br>Risk of disease or immune reactions |

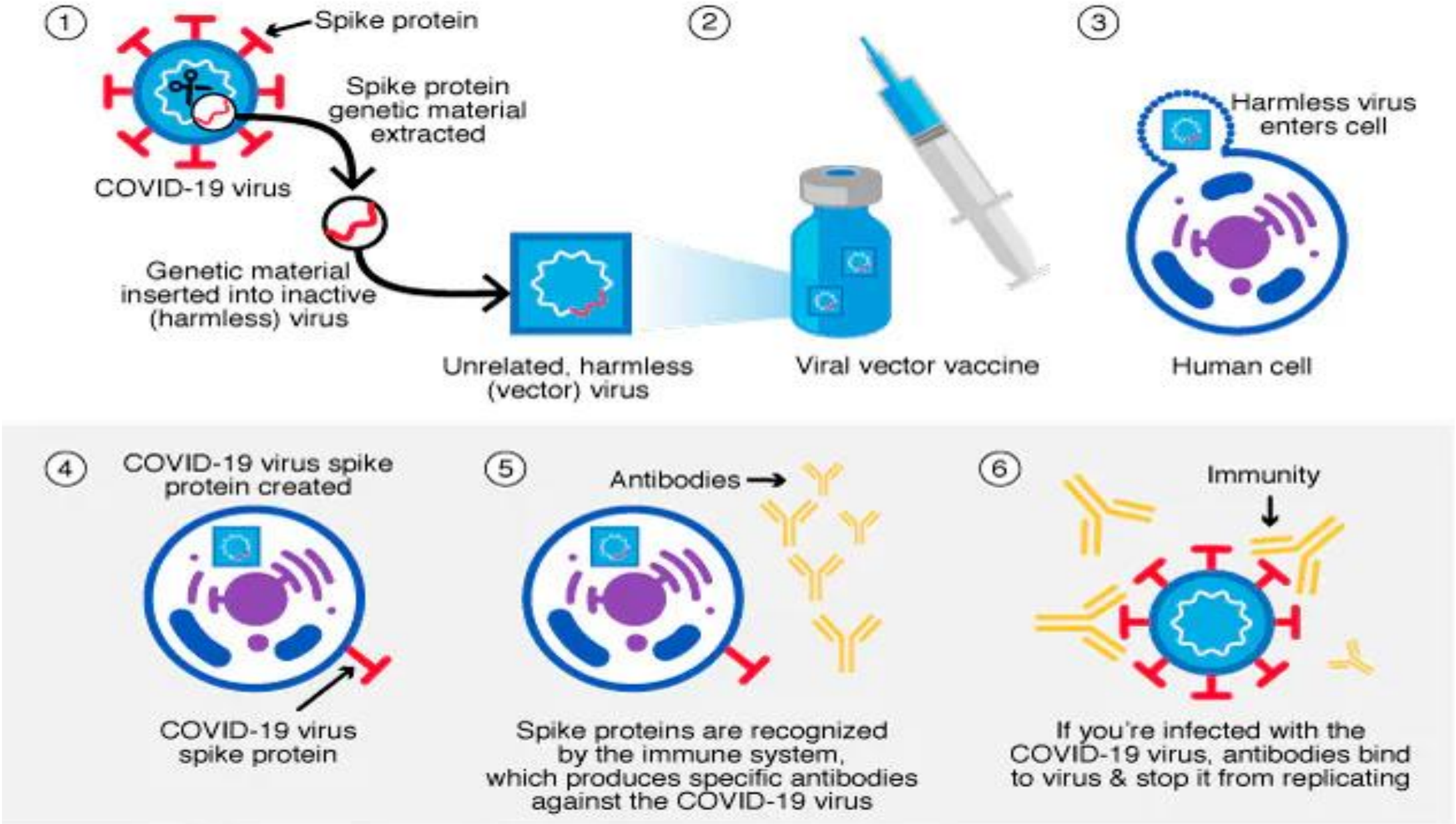
# Advantages and disadvantages of DNA vaccines

| Advantages | Disadvantages |
| --- | --- |
| ❖ **Elicit both Humoral & cell mediated immunity with no risk of infection**<br>❖ **Antigen presentation by both MHC class I and class II molecules**<br>❖ **Immune response focused only on antigen of interest**<br>❖ **Ease of development and production**<br>❖ **Refrigeration not require with stability of vaccine for storage and shipping, Cost-effectiveness**<br>❖ **Obviates need for peptide synthesis, expression and purification of recombinant proteins and the use of toxic adjuvants**<br>❖ **Long-term persistence of immunogen** | • Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides)<br>• Risk of affecting genes controlling cell growth<br>• Possibility of inducing antibody production against DNA<br>• Possibility of tolerance to the antigen (protein) produced<br>• Potential for atypical processing of bacterial and parasite proteins<br>• Extended immuno stimulation leads to chronic inflammation |

# Vector vaccines

- Vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body.
- "Vector" refers to the virus or bacterium used as the carrier vector vaccines (DNA sequence coding for the foreign gene +harmless bacterium, plasmid & virus) closely mimic a natural infection and therefore do a good job of stimulating the immune system.

# Vector vaccines



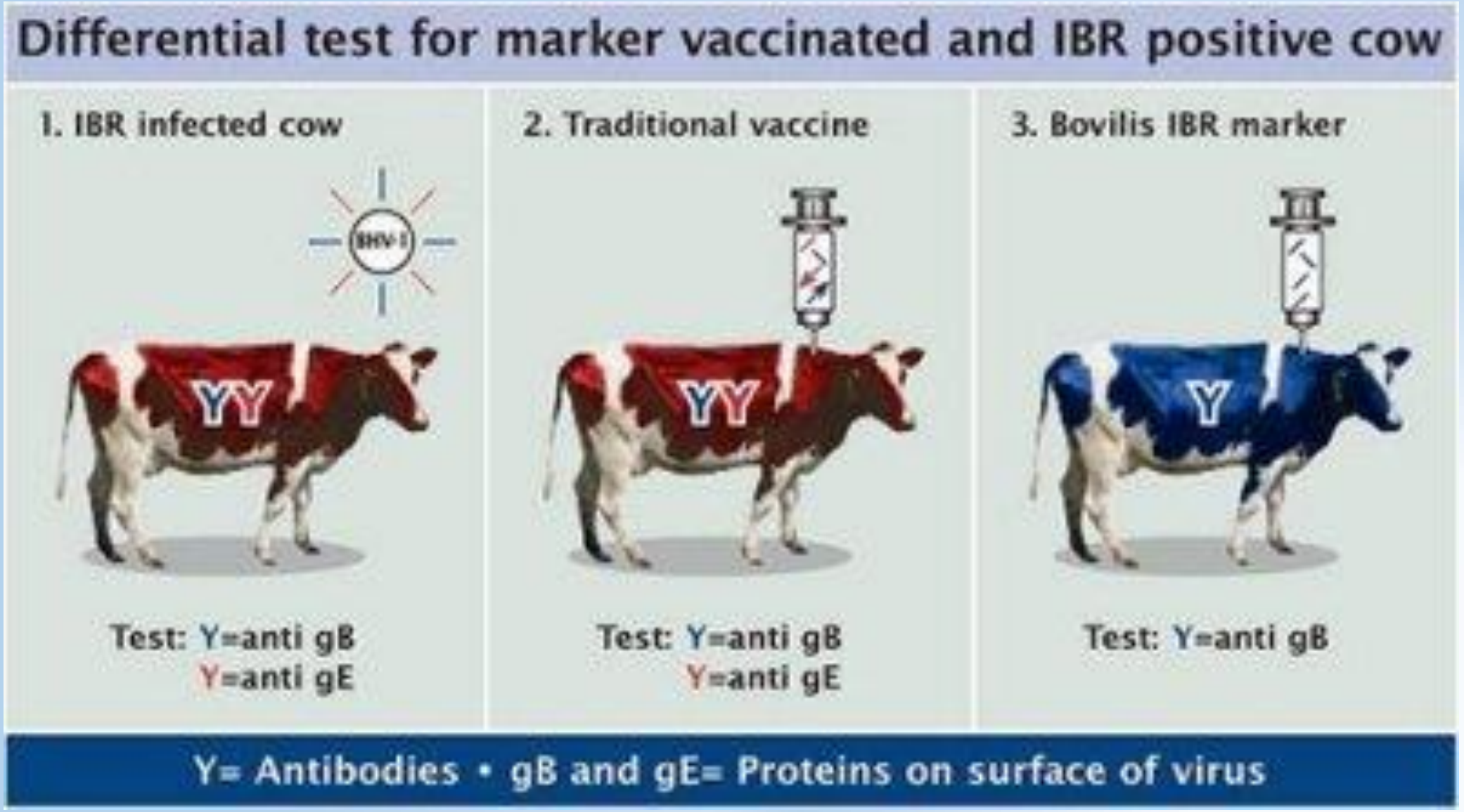
# Marker vaccines

- Also known as "Gene Deleted / DIVA vaccines"
- Marker vaccines, being a form of the virus/bacterium without a particular gene coding for a non protective antigen so, do not produce a key antibody.
- it is a vaccine which allows for the differentiation between infected and vaccinated subjects so it has significant importance in diagnostic aspects.
- The first such vaccines were used to protect pigs against Aujeszky's disease (AD). The same principles were subsequently applied to the development of vaccines against infectious bovine rhinotracheitis (IBR).

# Principle of marker vaccines

- Aujeszky's disease virus vaccines strain lack a specific glycoprotein gene (gG, gE, or gC).
- Infectious bovine rhinotracheitis (IBR) disease virus vaccines strain lack glycoprotein gene gE , gB (Envelope) and *tK* (Thymidine kinase, virulence).
- FMDV serotype O virus (vaccine strain O IND R2/1975) disease virus vaccines strain, lack a specific non-structural proteins 3A and 3B.

# Principle of marker vaccines

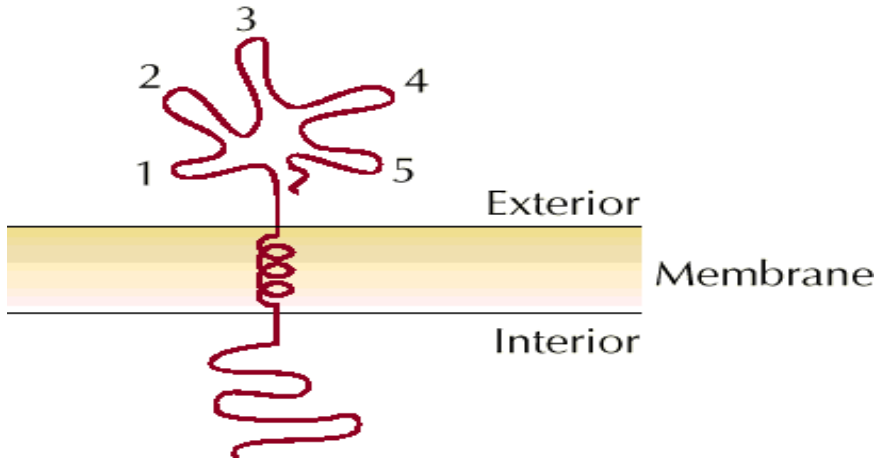


# Synthetic vaccine

Synthetic vaccines mainly consists of

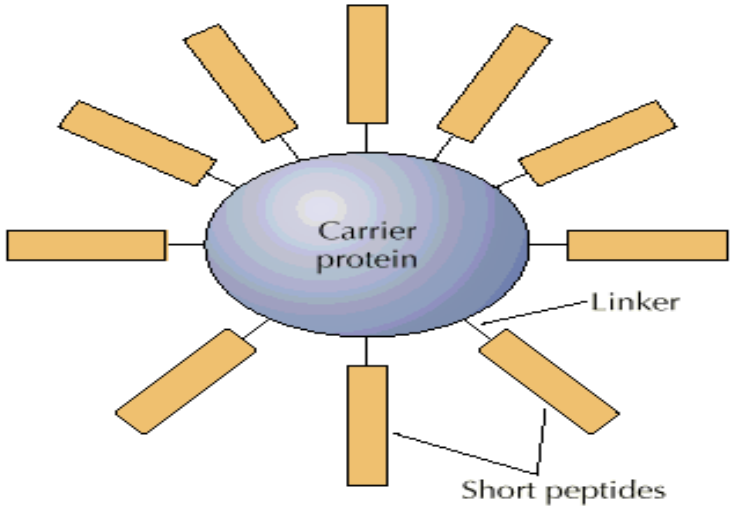
- Synthetic peptides,
- Carbohydrates, or
- Antigens
- The development of synthetic vaccine based on the identification of immunogenic sites.
- Their antigens are precisely defined and free from unnecessary components which may be associated with side effects.

# Selection & delivery of vaccine peptides:




Use discrete portion (domain) of a surface protein as Vaccine

These domains are 'epitopes' (antigenic determinants) -> are recognized by antibodies




**CARRIER PROTEINS**

Problem -> Small Peptides are often Digested
-> no strong immune response

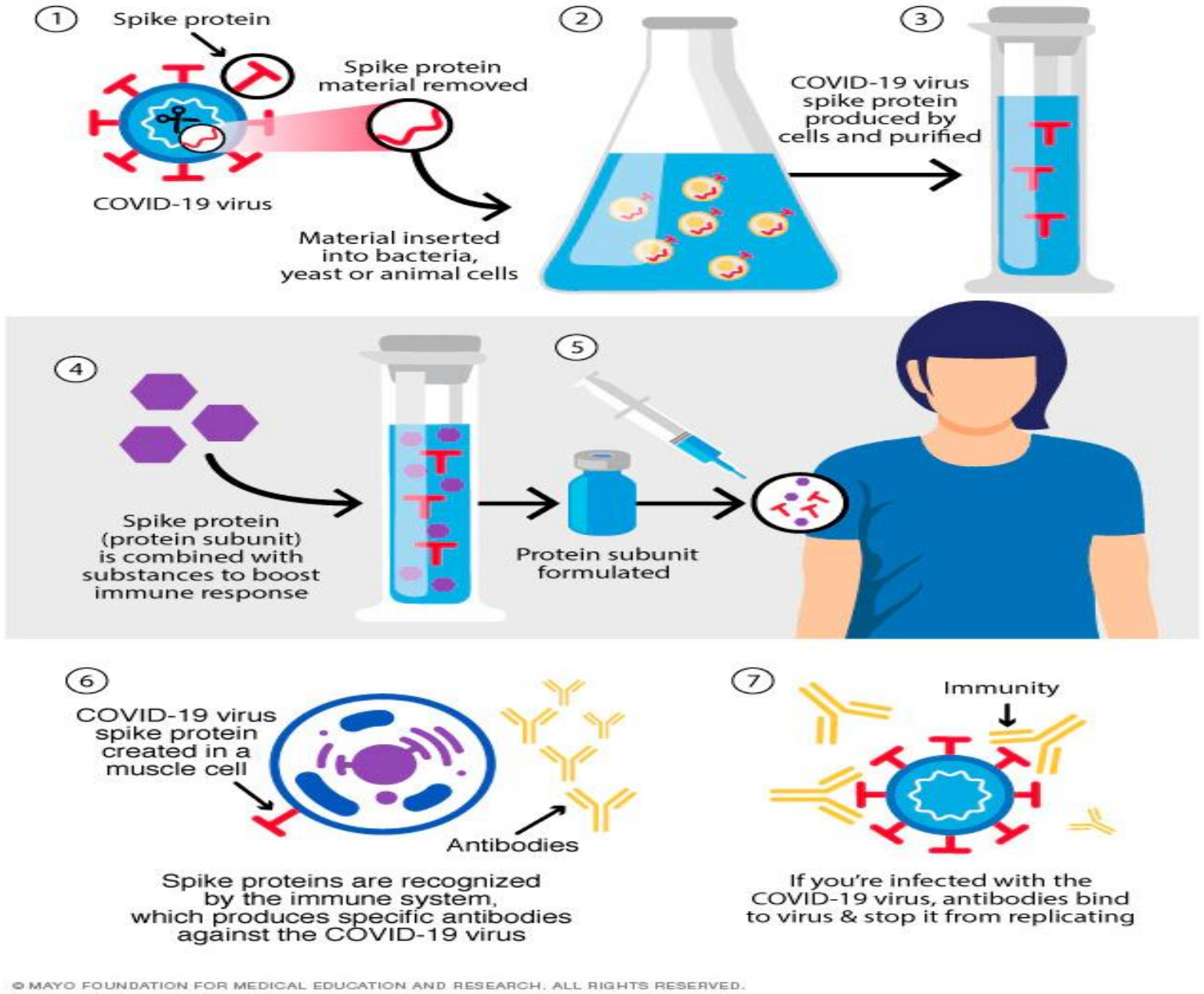
-> Carrier Proteins Make more Stable + stronger immune response

Make fusion protein of carrier + vaccine peptide
-> inert carrier or highly immunogenic carrier (hepatitis B core protein)

# Advantages Synthetic Peptide Vaccines?

- Chemically defined product
- Stable indefinitely at ambient temperature
- No infectious agent present - hence no problems with innocuity
- No large-scale production plant required
- No downstream processing required
- Can be designed to stimulate appropriate immune responses
- Provide the opportunity to use delayed-release mechanisms

# Protein subunit vaccine



# Advances in veterinary vaccines

| **Non-replicating recombinant antigen(s)-vaccine:** |
| --- |
| Feline Leukemia Vaccine, Killed Virus |
| Avian Influenza Vaccine, H5N3 Subtype, Killed Virus |
| Porcine Circovirus Vaccine, Type 1 -Type 2 Chimera, Killed Virus |
| Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector |
| *Escherichia Coli* Bacterin-Toxoid |
| *Borrelia Burgdorferi* Bacterial Extract |
| Feline Leukemia Virus Antigen |
| **Nucleic acid-mediated (not synthetic)-vaccine:** |
| West Nile Virus Vaccine, DNA |
| Canine Melanoma Vaccine, DNA |

# Advances in veterinary vaccines

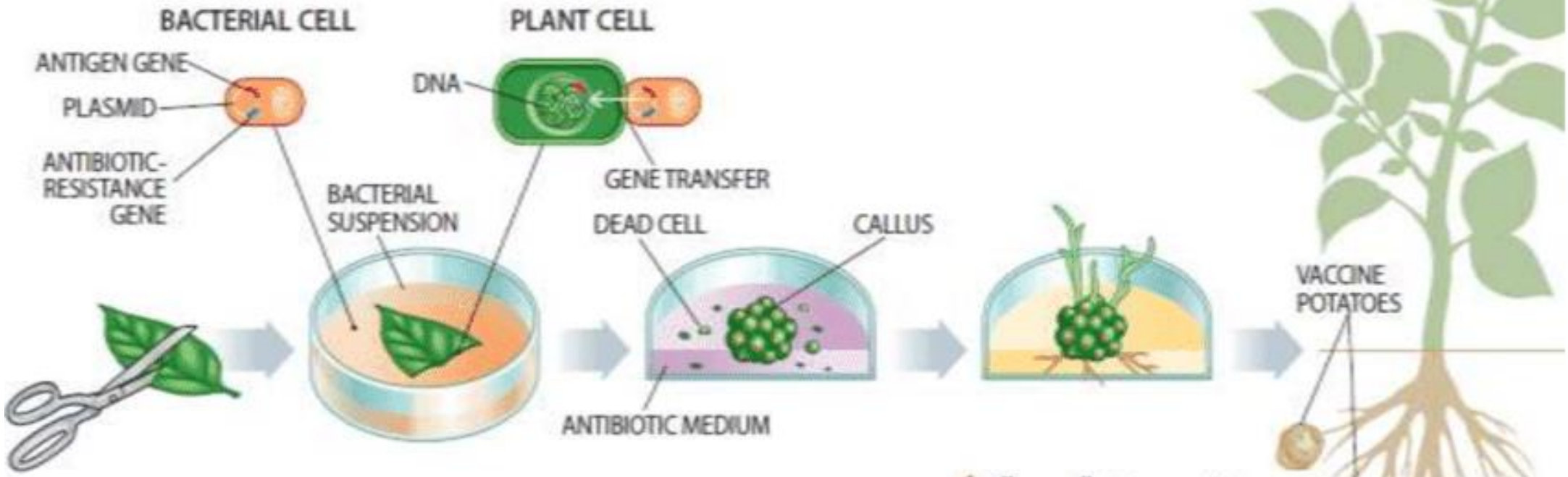
| **Live gene deleted:** |
| --- |
| *Escherichia Coli* Vaccine, Live Culture |
| *Salmonella Dublin* Vaccine, Live Culture |
| Pseudorabies Vaccine, Modified Live Virus |
| *Salmonella Typhimurium* Vaccine, Live Culture |
| **Live vectored:** |
| Marek's Disease Vaccine, Serotypes 1 & 3, Live Herpesvirus Chimera |
| Avian Influenza-Fowl Pox Vaccine, H5 Subtype, Live Fowl Pox Vector |
| Distemper Vaccine, Live Canarypox Vector |
| Equine Influenza Vaccine, Live Canarypox Vector |
| Canine Distemper-Adenovirus Type 2-Parvovirus Vaccine, Modified Live Virus, Canarypox Vector |
| Canine Distemper-Adenovirus Type 2-Parainfluenza-Parvovirus Vaccine, Modified Live Virus, Canarypox Vector |
| Rabies Vaccine, Live Canarypox Vector |
| West Nile Virus Vaccine, Live Canarypox Vector |
| Feline Leukemia Vaccine, Live Canarypox Vector |
| Rabies Vaccine, Live Vaccinia Vector |
| Newcastle Disease-Fowl Pox Vaccine, Live Fowl Pox Vector |
| Fowl Pox-*Mycoplasma Gallisepticum* Vaccine, Live Fowl Pox Vector |
| Fowl Pox-Laryngotracheitis Vaccine, Live Fowl Pox Vector |
| Bursal Disease-Marek's Disease Vaccine, Serotype 3, Live Marek's Disease Vector |
| Marek's Disease-Newcastle Disease Vaccine, Serotype 3, Live Marek's Disease Vector |

# Edible vaccine

- Here edible plants are used as site of antigen production.
- Transgenic plants are developed expressing antigens derived from animal viruses / bacteria
- Here the antigenic gene carrying plasmid is introduced into the plant with the help of bacterium *Agrobacterium tumefaciens*.
- The first clinical trial conducted in 1997.
- Transgenic potatoes with a toxin of *E. coli* causing diarrhea.

# HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or bacterial "antigens"—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.



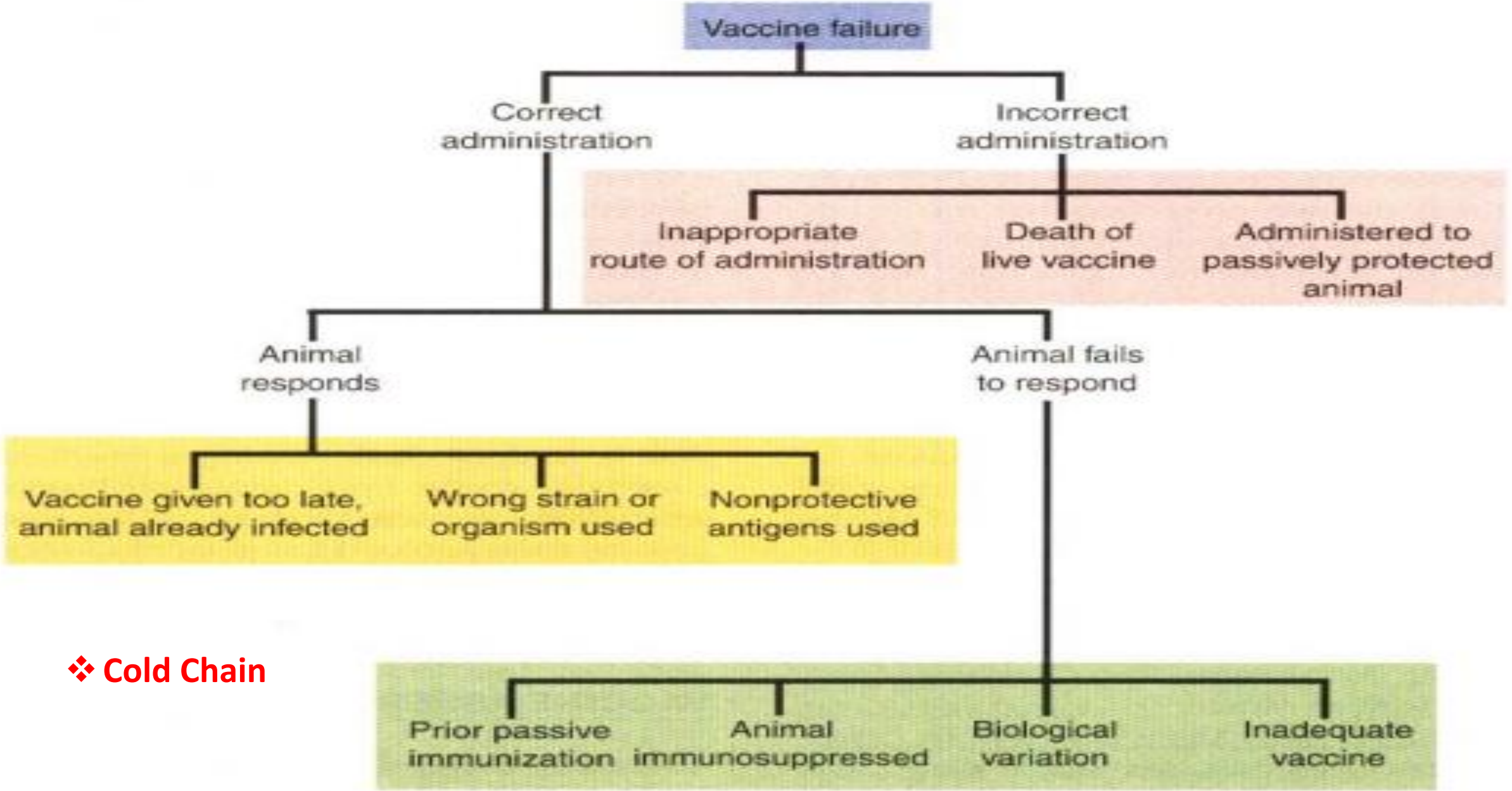

1 Cut leaf.

2 Expose leaf to bacteria carrying an antigen gene and an antibiotic-resistance gene. Allow bacteria to deliver the genes into leaf cells.

3 Expose leaf to an antibiotic to kill cells that lack the new genes. Wait for surviving (gene-altered) cells to multiply and form a clump (callus).

4 Allow callus to sprout shoots and roots.

5 Put in soil. Within three months, the plantlets will grow into plants bearing antigen-laden vaccine potatoes.




❖ **Cold Chain**

**Figure 22-1.** A classification of the causes of vaccine failure.

# Thank You

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