# VACCINE PRODUCTION TECHNIQUE, GROWING THE MICROORGANISMS IN MAXIMUM TITRE

B.VAIDHYANATHAN
3RD YEAR BIOTECHNOLOGY

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# PRINCIPLE OF VACCINE PRODUCTION

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- Standard manufacture uses a bacterial or viral antigen, e.g. bacterium or virus, which may be killed or may be living but attenuated.
- 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.
- Cell cultures are required for viral vaccines since viruses can replicate only inside the living cells.
- For example To produce the **Sabin polio vaccine**, attenuation was only achieved with high inocula and rapid passage in primary monkey kidney cells.
- **Inactivated vaccines** are produced by killing the disease-causing microorganism with chemicals or heat.

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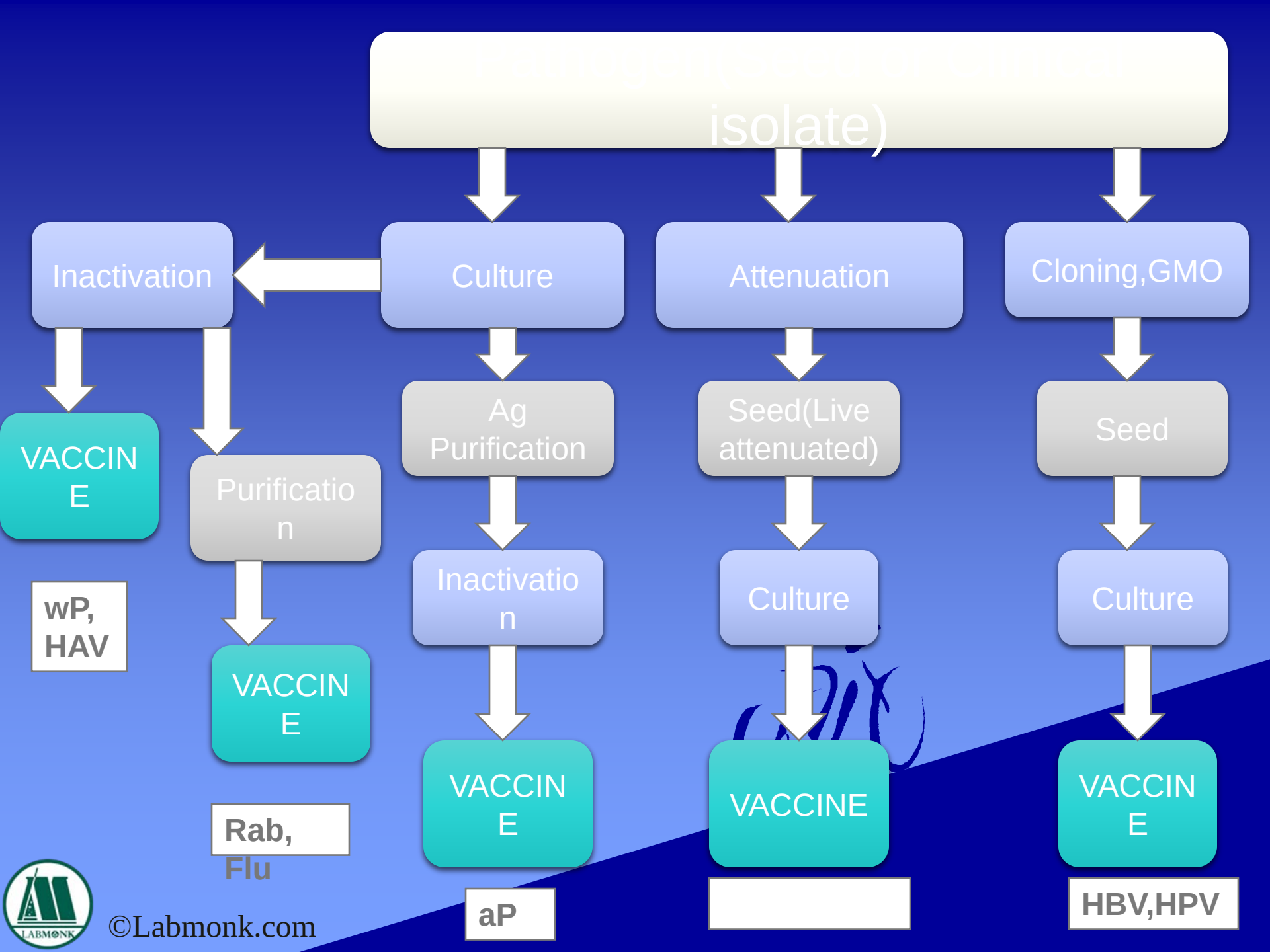
isolate)
Inactivation
Culture
Attenuation
Cloning,GMO
VACCINE
Purification
Ag Purification
Seed(Live attenuated)
Seed
wP, HAV
Inactivation
Culture
Culture
VACCINE
VACCINE
VACCINE
VACCINE
Rab, Flu
aP
HBV,HPV
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- Vaccines are currently produced by **gene techniques**, i.e. instead of using a virus or bacterium, A single gene (usually a surface glycoprotein of the virus) can be expressed in a foreign host **by Cloning**. (Expression vectors are used to make large amounts of antigen to be used as a vaccine. Most used vectors for expression are **Bacteria: Escherichia coli, Yeasts ,Baculovirus**.)
- This process induces the vector to produce an antigen, which is then purified.
- The purified antigen, when combined with an adjuvant results in a safe and very effective vaccine.
- Example: **Gardasil, an anti-human papilloma virus vaccine** that is very effective in preventing cervical cancer.
- The current **Hepatitis B vaccine** is also this type.

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# STEPS IN VACCINE PRODUCTION

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SELECTING THE STRAINS FOR VACCINE PRODUCTION

GROWING THE MICRO-ORGANISMS

UPSTREAM PROCESSING

ISOLATION & PURIFICATION OF MICROORGANISM

INACTIVATION OF ORGANISM

FORMULATION OF VACCINE

QUALITY CONTROL AND LOT RELEASE

DOWNSTREAM PROCESSING

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# Selecting the Strains for Vaccine Production

***The Seed(Strain) -***

- Manufacturing begins with small amounts of a specific virus (seed).
- Viruse  or Bacteria used in manufacture shall be derived from a Seed Lot System.

- A record of the origin, passage history (including purification and characterisation procedures) and storage conditions should be maintained for each Seed Lot.
- The virus must be free of impurities, including other similar viruses and even variations of the same type of virus.
- The seed must be kept under "ideal" conditions, usually frozen, that prevent the virus from becoming either stronger or weaker than desired.

Stored in small glass or plastic containers.

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## Selecting the seed(Strain) -

- The choice of the the seed is depends on a number of factors including the efficacy of the resulting vaccine, and its secondary effects.
- If possible, the bacterial strain or cell line should be obtained from a recognized culture collection with an established and documented provenance.
- Alternatively, if the chosen vaccine strain is an “in house” clinical isolate, it will be necessary to compile a complete history of the strain, including details of its isolation, identification, and maintenance for product registration.

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# GROWING THE MICROORGANISMS

## GROWING BACTERIA

- **BATCH CULTURE**
  - the microbe is grown in a closed vessel
  - typically in a test tube or flask
- **CONTINUOUS CULTURE**
  - the microbe is grown in vessel which has medium constantly added and spent medium constantly removed.
  - It is performed in a **chemostat.**

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# GROWING VIRUSES

- Methods used are :
  - **CELL (TISSUE) CULTURES** – cultured cells grow in sheets that support viral replication and permit observation for cytopathic effect.
  - **BIRD EMBRYOS** – incubating egg is an ideal system; virus is injected through the shell.
  - **LIVE ANIMAL INOCULATION** – occasionally used when necessary
  - **TRANSGENIC ANIMALS**

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# ISOLATION & PURIFICATION OF MICROORGANISM

- **Product isolation** is the removal of those components whose properties vary markedly from that of the desired product.
- **Purification** selectively separates and retains the desired product at the highest purity per its pre-determined specification. (Remove unwanted compounds)
- The most common method of vaccine production is based on an initial fermentation process followed by purification.

- CENTRIFUGATION
- FILTRATION
- CHROMATOGRAPHY

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# Centrifugation

- Centrifugation is a process by **which solid particles are sedimented and separated from a liquid** using centrifugal force as a driving force.
- Centrifugation is used to separation and purification of pathogenic viruse antigens and other agents used in the production of vaccine.
- Centrifugation is also used to remove dead cells, cell debris etc.
- Example : Influenza vaccine, rabies vaccine ,Hepatitis B vaccine, and Japanese encephalitis vaccine production.

Centrifugation methods used for purification are –

1. **Differential Centrifugation –**
2. **Density gradient Centrifugation -**
   a. **Rate- zonal**
   b. **Isopycnic**

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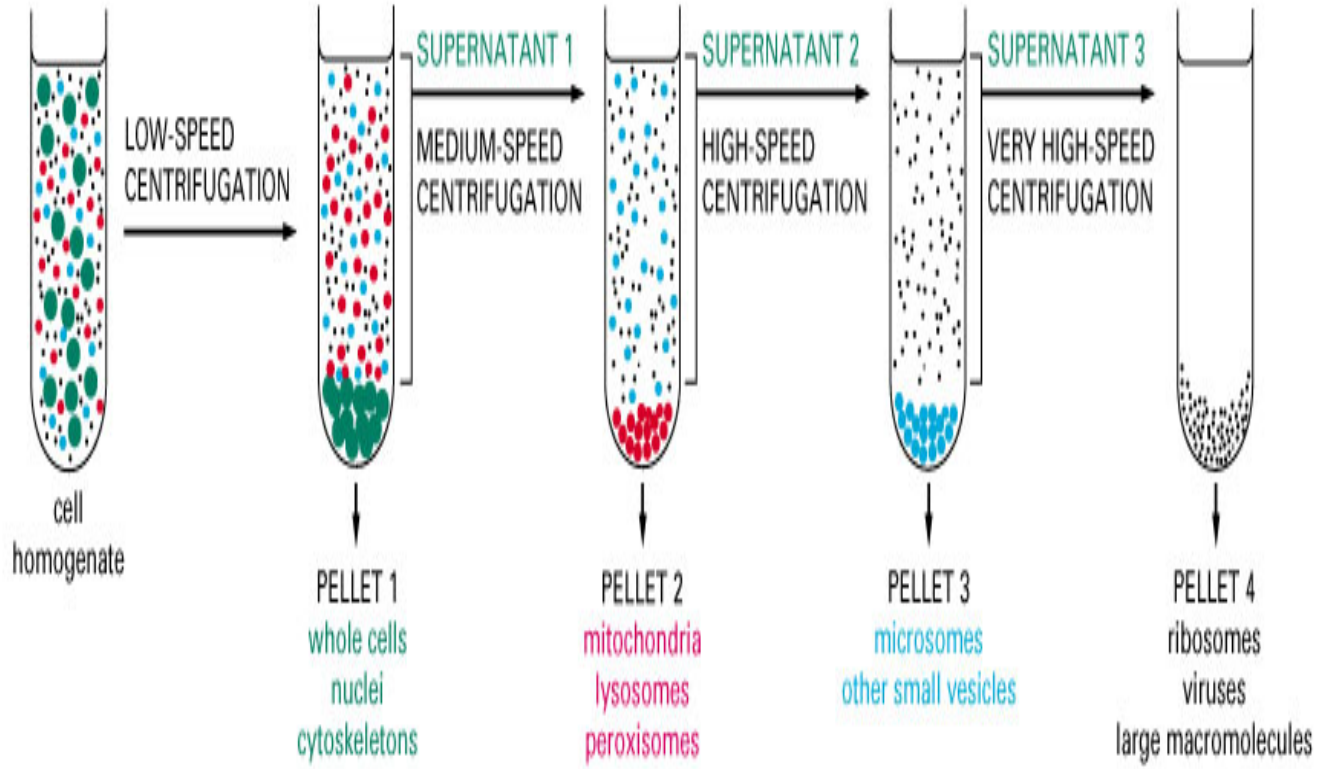
## Differential Centrifugation –

This technique is used **for separation of cell organelles** and involves **different speeds and at different times.**

Pellet and supernatant obtained as a result are subjected to different speeds at different times, further the supernatant is taken and the process is continued.

At low speeds some fractions get separated and small fragments remain in the supernatant.

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

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## Density gradient Centrifugation -

Density gradient centrifugation is a technique that allows the **separation of cells, organelles and macromolecules, depending on their size, shape and density.**

### a. Rate-zonal centrifugation -

used to separate molecules on the **basis of their size,shape and density.**

### b. Isopycnic centrifugation –

a technique used to separate molecules **on the basis of density.** (The word "isopycnic" means "equal density".)

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# Chromatography

- a group of physical separation techniques, which are characterized by the **separation of mixtures due to differences in the distribution coefficient of sample components** between two phases, one ***stationary*** and the other ***mobile phase***.

*Example :* Modified Vaccinia Ankara virus (Small pox vaccine)

## Column Chromatography:-

- **Ion exchange chromatography:-**
- **Affinity chromatography :-**

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# Column Chromatography

- Separates molecules by their **chemical and physical differences.**
- Most commonly used column chromatography are:

**Ion exchange chromatography:-**

Separation on the **basis of charge**.

- Cell culture-derived inactivated whole virus vaccines.

**Affinity chromatography :-**

Separation on the **basis of specific binding sites on the protein.**

- Recombinant human glycoproteins
- Cell culture-derived influenza virus particles.

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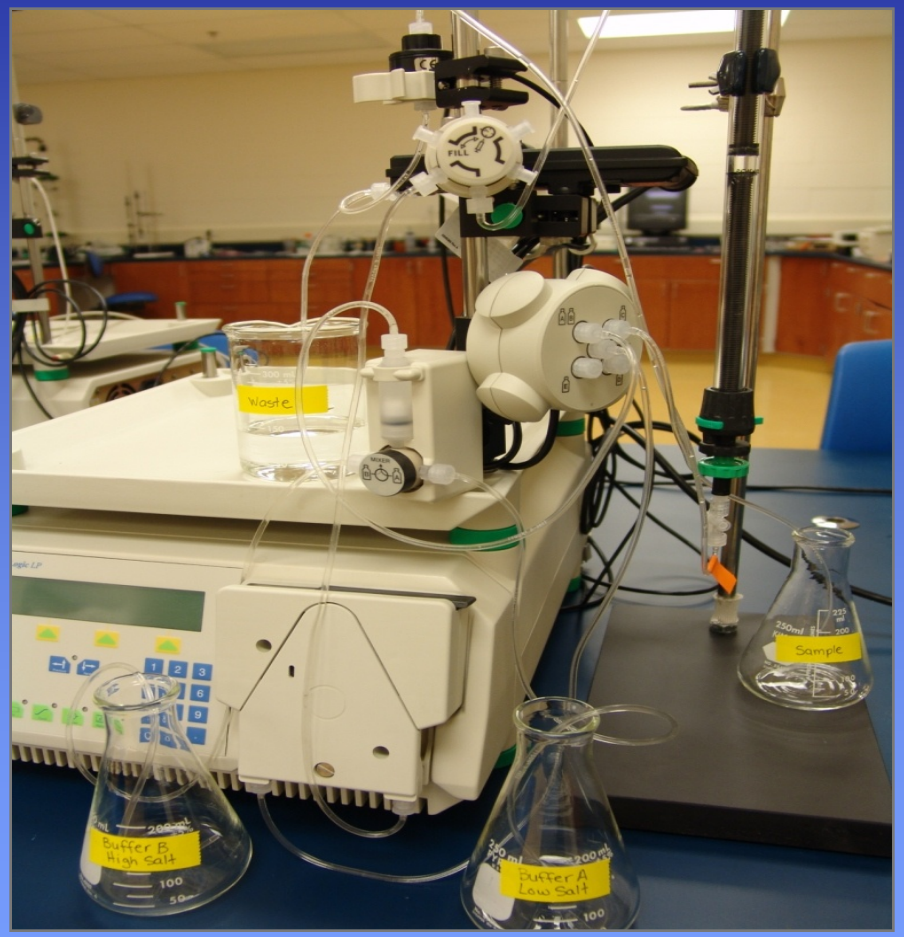
## Lab Scale Chromatography System




## Large Scale Chromatography System

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# Filtration

- **Separation of particles from liquid by applying a pressure to the solution to force the solution through a filter.**
- Filtration is classified in two ways.

1. Dead end filtration 2. Tangential filtration

**1. DEAD END FILTRATION :-**

- all the flows are directed through the membrane with material building up on the surface of filter. (Flow perpendicular to membrane surface )
- As these particles build up, flow through the filter is quickly reduced and finally it ceases completely. (Causes build up of filter cake on membrane )

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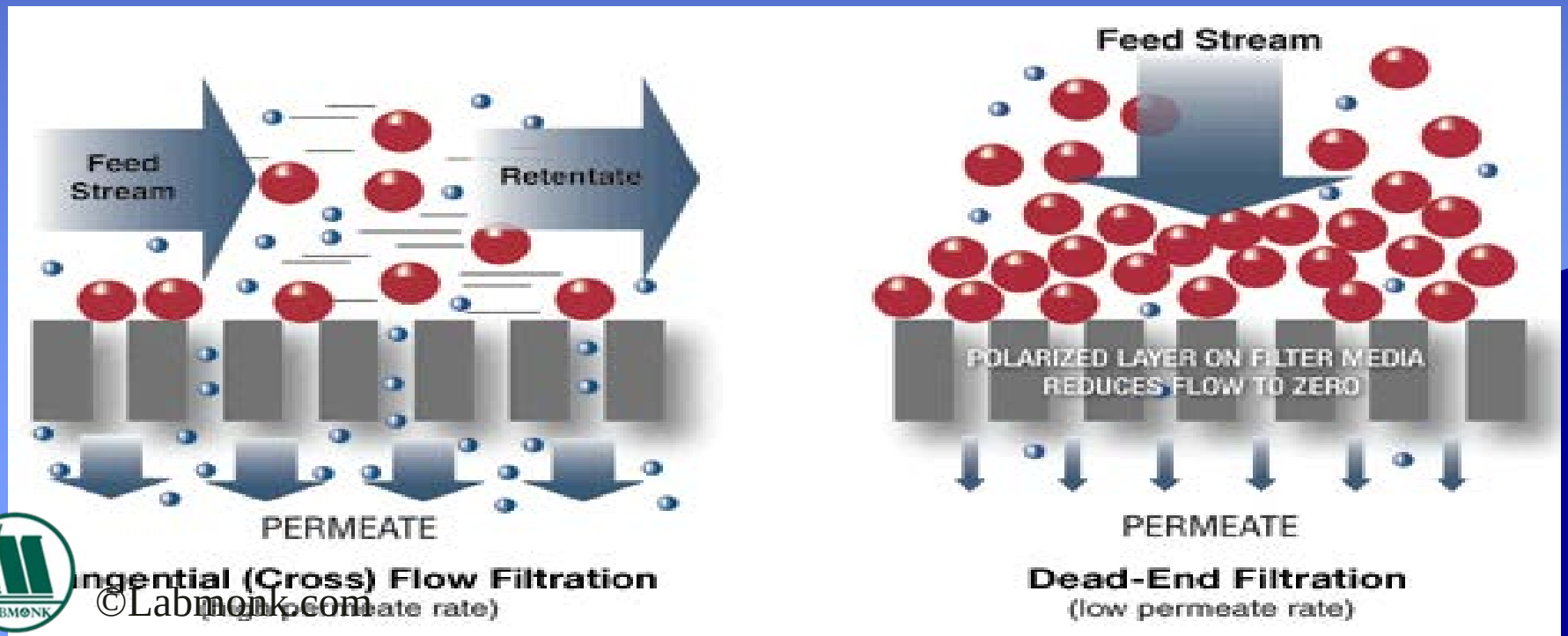
## 2. TANGENTIAL FLOW FILTRATION (CROSS FLOW TECHNOLOGY) :-

During CFF, culture fluid is re-circulated in tangential flow, parallel to the filter membrane.

Build-up of viral particles on the membrane is minimised by the recirculation of fluid over the surface, which also facilitates the concentration of particles present in the retained fluid.

**Mainly used in purifying inactivated Arboviral antigen.**

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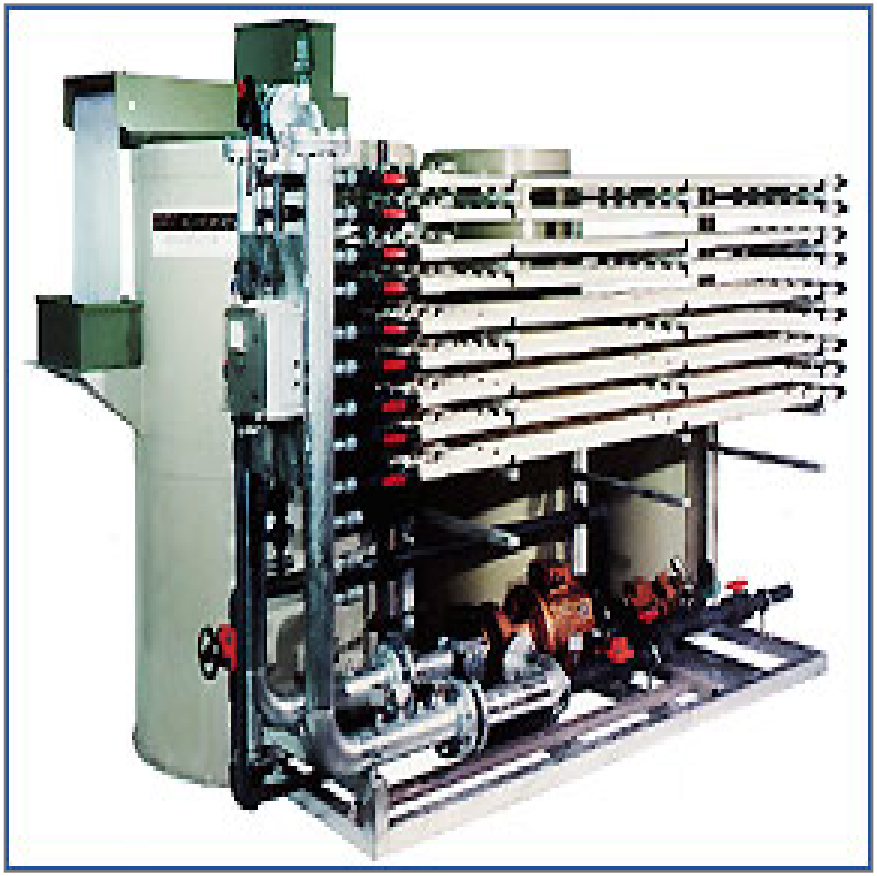
## Ultrafiltration:-

- A technique for **separating dissolved molecules in solution on the basis of size rating the particles will be retained at the surface of the membrane.**
- During this process the desired proteins and their allied products are separated by their molecular weight, and the volume is reduced thereby increasing the purity considerably compared to the starting volume.



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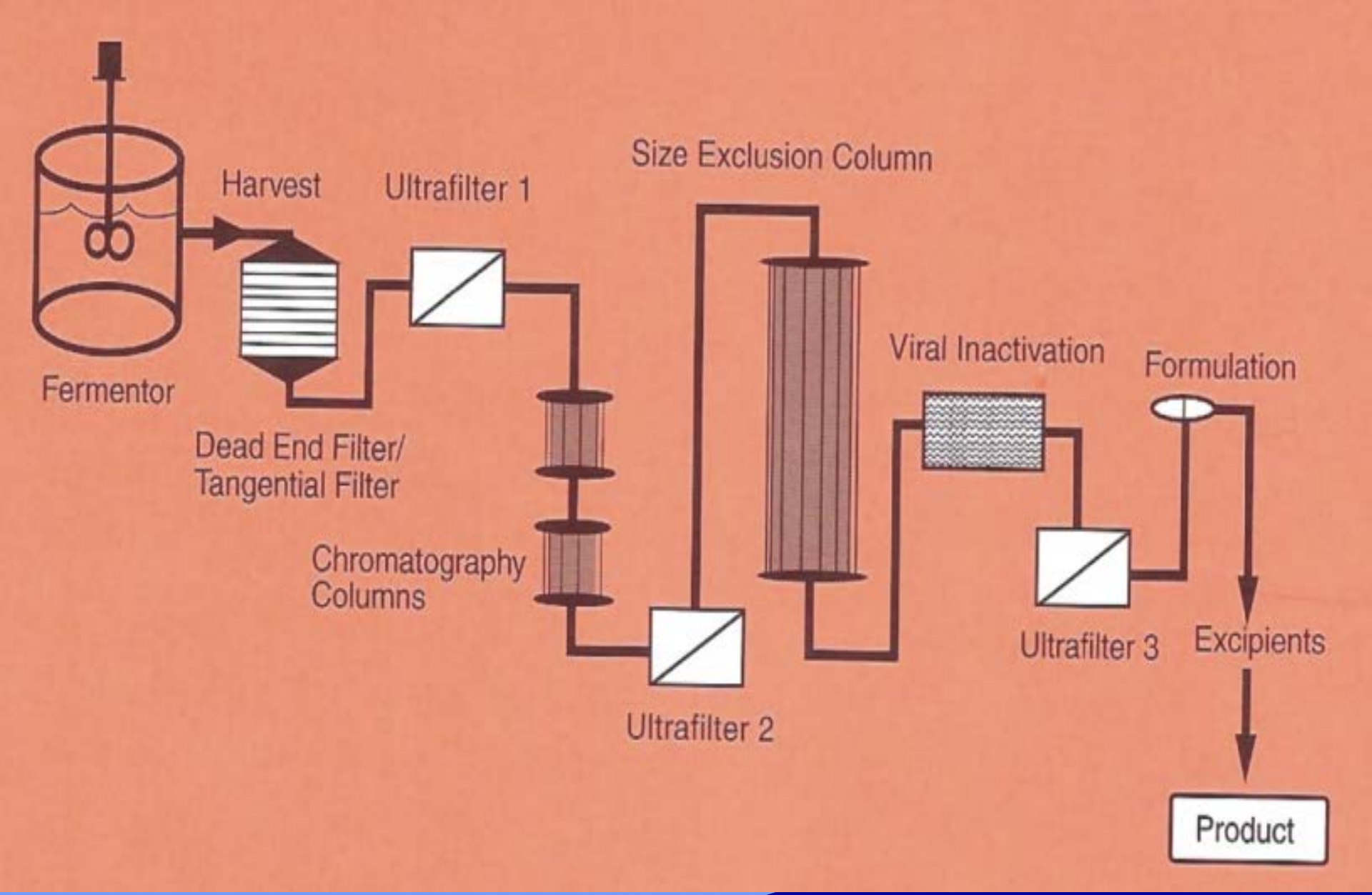


A General flow diagram of a purification train in the
ine production process (Paul K Ng. *et. al)*

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# INACTIVATION OF MICROORGANISM

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# KILLED/INACTIVATED VACCINE:

## VIRUS INACTIVATION:

Viruses can be lipid-coated(enveloped) or non-enveloped.

**Virus inactivation involves dismantling a virus's ability to infect cells without actually eliminating the virus.**

Virus inactivation works by one of the following two mechanisms:

- By attacking the viral envelope or capsid and destroying its ability to infect or interact with cells.
- 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**

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## Solvent/detergent (S/D) inactivation -

- Effective with **lipid-coated viruses**.
- The detergents used in this method, Disrupts the interactions between molecules in the lipid coat , rendering the coat dysfunctional and impeding replication
- 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, it does not matter whether the virus has an envelope or not because the envelope alone cannot protect the virus from such high temperatures.**(at 60° C for 10 hours )**

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## Acidic pH inactivation (Low pH Treatment) –

- Most effective with **lipid-coated viruses**
- Acidic conditions deactivate virus.
- Incubation typically occurs **at a pH of 4** and lasts anywhere between 6 hours and 21 days.

## Ultraviolet (UV) inactivation -

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

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# Inactivation by Extraction

- **Nucleic acid —**

  **nucleic acid is obtained from collected and lysed cells.**

  The nucleic acid is purified by **solvent extraction** and **chromatographic** techniques and formulated for the final vaccine product.

  Nucleic acid vaccines can be regions of RNA or DNA that code for disease associated proteins.

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- **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**.

**Filtration** is employed to achieve clarification of the protein solution during this process.

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## Membrane extraction —

**vaccine products can be portions of bacterial or mammalian cell membrane structures.** These membrane structures are typically protein, but, can be lipid or carbohydrate molecules. The membrane components are usually associated with a disease state. 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.

The capsule extraction process usually requires multiple steps of **solvent extraction**, followed by **chromatographic separation** or other standard purification techniques.

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## LIVE WHOLE VACCINES:

Several methods have been used to attenuate viruses for vaccine production.

a) **Use of a related microorganism from another animal**
b) **Administration of pathogenic or partially attenuated microorganism by an unnatural route**
c) **Passage of the microorganism in an "unnatural host" or host cell**
d) **Development of temperature sensitive mutants**

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a) **Use of a related virus from another animal -**

the earliest example was the use of cowpox to prevent smallpox.

b) **Administration of pathogenic or partially attenuated virus by an unnatural route -**

the virulence of the virus is often reduced when administered by an unnatural route.

This principle is used in the immunization of military recruits against adult respiratory distress syndrome using enterically coated live adenovirus type 4, 7 and (21).

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## c) Passage of the virus in an "unnatural host" or host cell -

the major vaccines used in man and animals have all been derived this way.

After repeated passages, the virus is administered to the natural host.

The initial passages are made in healthy animals or in primary cell cultures.

There are several examples of this approach:

- the **17D strain of yellow fever** was developed by passage in mice and then in chick embryos.
- **Polioviruses** were passaged in monkey kidney cells and **measles** in chick embryo fibroblasts.

Human diploid cells are now widely used such as the

**WI-38 and MRC-5.**

## d) Development of temperature sensitive mutants -

This method may be used in conjunction with the above method.

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# FORMULATION OF VACCINE

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.

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**Preservatives and stabilizers** (the vaccine remain unchanged)

- 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.

Antibiotics that are used during vaccine manufacture include **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).**

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## Inactivating Agents-

- **Formaldehyde** is used to inactivate bacterial products for toxoid vaccines, (these are vaccines that use an inactive bacterial toxin to produce immunity.)
- It is also used to kill unwanted viruses and bacteria that might contaminate the vaccine during production.
- Most formaldehyde is removed from the vaccine before it is packaged.
- It is used to inactivate **influenza virus, poliovirus, and diphtheria and tetanus toxins.**
- **β-propiolactone**, which is used to inactivate **rabies virus**

- **Glutaraldehyde**, which is used to inactivate toxins contained in **acellular *pertussis* vaccines**.

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# Adjuvants enhance vaccine immunogenicity

**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)

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# QUALITY CONTROL AND LOT RELEASE

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# Quality control

**Table 1 - Schedule of final product testing for a veterinary vaccine**

| Test | Purpose of test |
|---|---|
| Sterility | Demonstrates that no live microorganisms are present in the product |
| Chemistry | Demonstrates that the product has the correct amount of adjuvant and preservative, and that the pH is correct |
| Safety | Demonstrates that an overdose of the product causes no harm |
| Residual toxicity | Demonstrates that the product contains no material that can cause harm |
| Efficacy | Demonstrates that each antigen in the product meets the recommended guideline level (or better) in internationally recognised tests |

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- **INCREASE IN VIRULENCE TESTS -**

With live vaccines, there is concern that the organism might be shed from the host and transmitted to contact animals, causing disease if it retains residual virulence or reverts to virulence.

**All live vaccines should be tested for virulence** by means of passage studies.

- **ASSESSING RISK TO THE ENVIRONMENT -**

The ability of each live vaccine to shed, to spread to contact target and non-target animals, and to persist in the environment must be evaluated to provide information for assessing the risk of the vaccine to the environment, taking into account human health.

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- **INTERFERENCE TESTS -**

  For products with two or more antigenic components, tests must confirm that there is no interference between individual components, that is, one component causing a decrease in the protective immunological response to another component.

- **CONSISTENCY OF PRODUCTION -**

  Prior to marketing approval of any new product, each establishment should produce in its facilities three consecutive production batches/serials of completed product to evaluate the consistency of production.

- **STABILITY TESTS -**

  Stability studies (based on an acceptable potency test) are required to establish the validity of the expiry date that appears on the product package.

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# Lot release

**BATCH/SERIAL RELEASE FOR DISTRIBUTION :**

- Prior to release, the manufacturer must test each batch/serial for purity, safety, and potency.

1. **Batch/serial purity test –**

- Purity is determined by testing for a variety of contaminants.
- Tests to detect contaminants are performed on: master seeds, primary cells, MCSs(Master cell stock), ingredients of animal origin if not subjected to sterilisation (e.g. fetal bovine serum, bovine albumin, or trypsin), and each batch of final product prior to release.

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## 2. Batch/serial safety test -

- Batches are considered satisfactory if local and systemic reactions to vaccination with the batch to be released are in line with those described in the registration dossier and product literature.

## 3. Batch/serial potency test -

- Batch/serial potency tests, required for each batch prior to release, are designed to correlate with the host animal vaccination–challenge efficacy studies.

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- **OTHER TESTS :-**

- Depending on the form of vaccine being produced, certain tests may be indicated.
- These tests may concern:
  - The level of moisture contained in desiccated products,
  - The level of residual inactivate in killed products,
  - The complete inactivation of killed products,
  - pH,
  - The level of preservatives and permitted antibiotics,
  - Physical stability of adjuvant,
  - Retention of vacuum in desiccated products,
  - A general physical examination of the final vaccine. .

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- **SAMPLING**

  Samples should be selected from each batch/serial of product.

  The selector should pick representative sample.

- **LABELLING**

  Standards for labelling products will vary from country to country.

- **FIELD TESTS (SAFETY AND EFFICACY)**
- **PERFORMANCE MONITORING**

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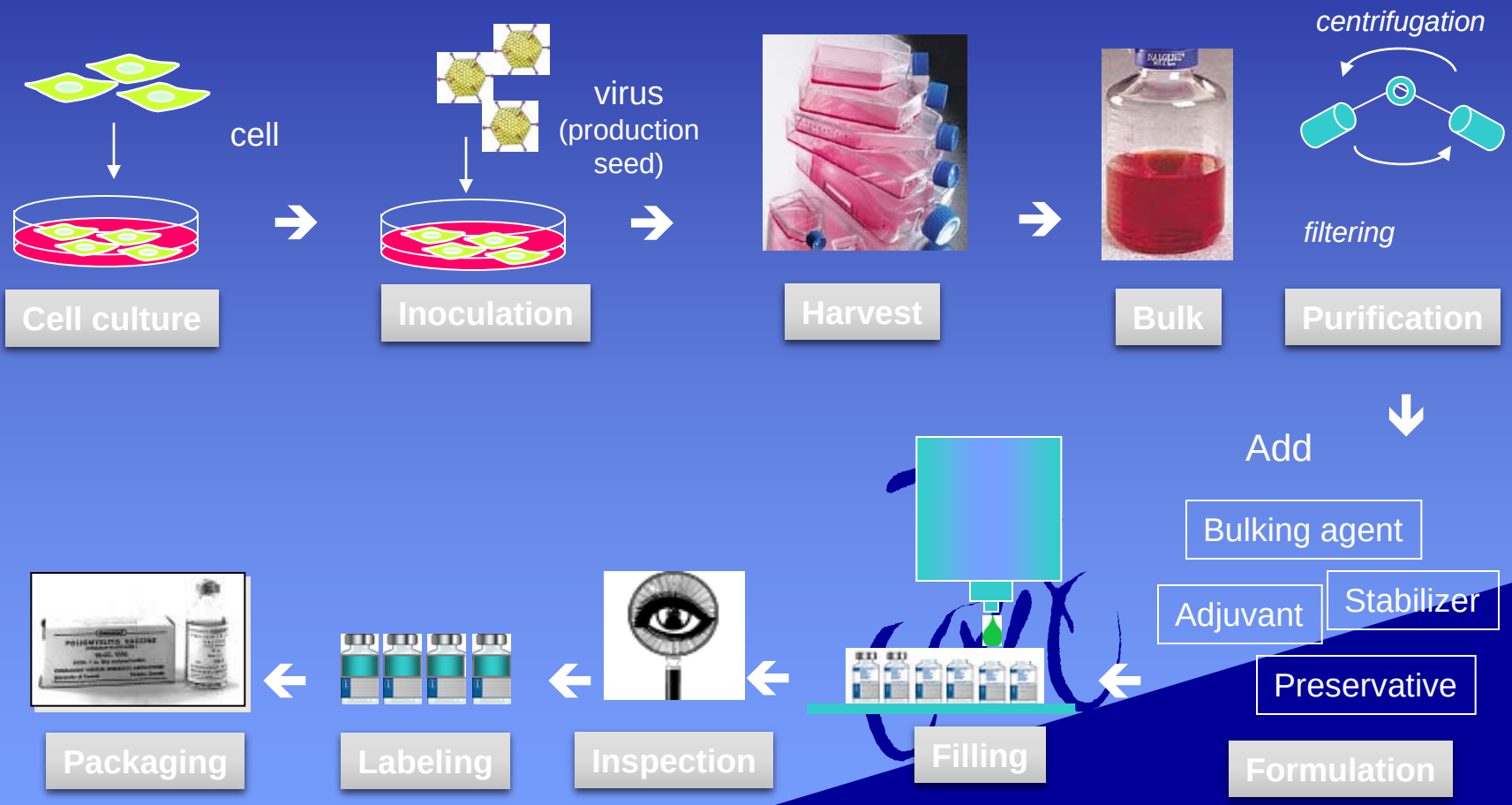
# How to produce Vaccine?



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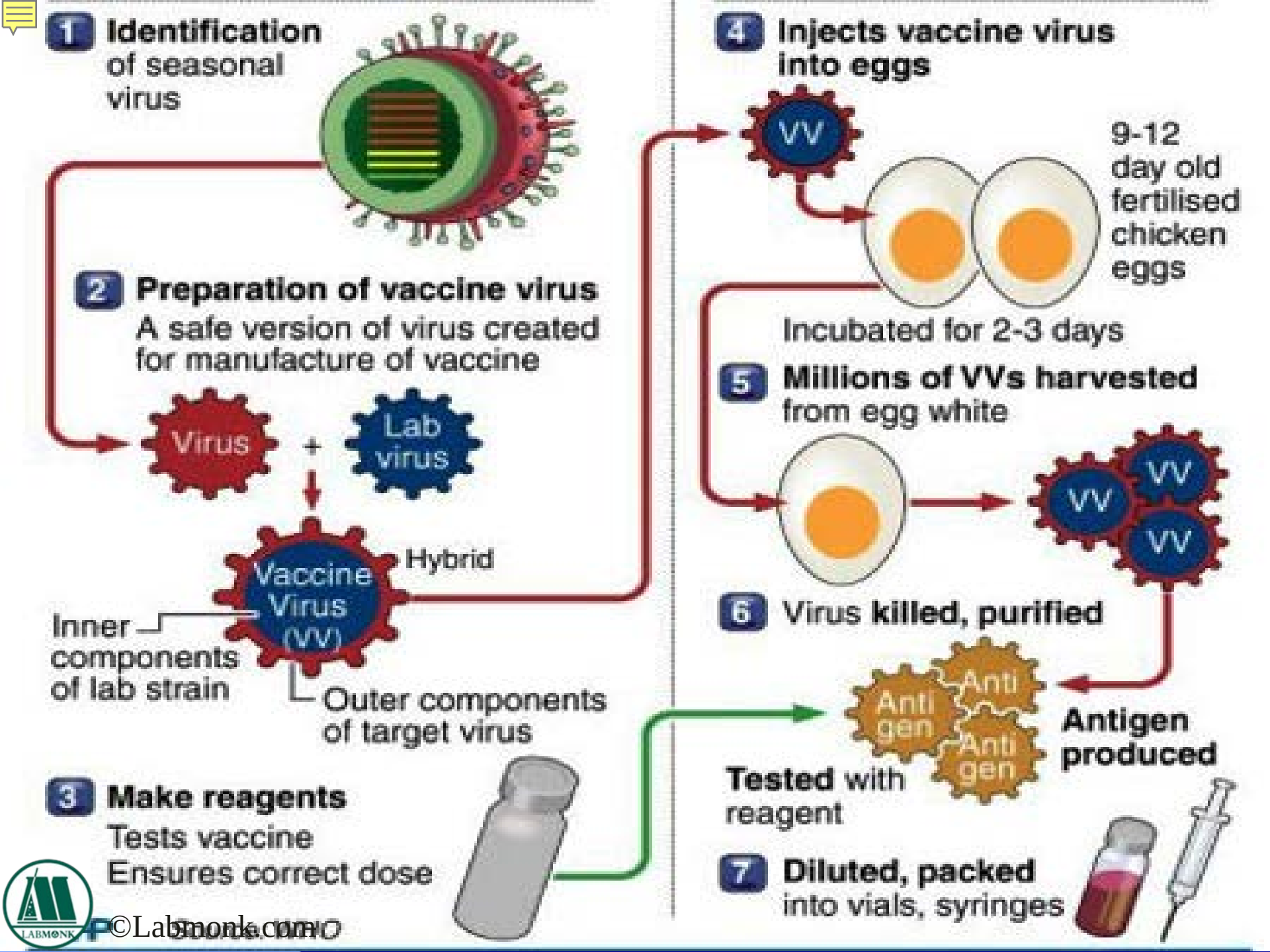
1 Identification of seasonal virus
2 Preparation of vaccine virus
A safe version of virus created for manufacture of vaccine
Virus
+
Lab virus
Vaccine Virus (VV)
Hybrid
Inner components of lab strain
Outer components of target virus
3 Make reagents
Tests vaccine
Ensures correct dose
4 Injects vaccine virus into eggs
VV
9-12 day old fertilised chicken eggs
Incubated for 2-3 days
5 Millions of VVs harvested from egg white
VV
VV
VV
6 Virus killed, purified
Anti gen
Anti gen
Anti gen
Antigen produced
Tested with reagent
7 Diluted, packed into vials, syringes

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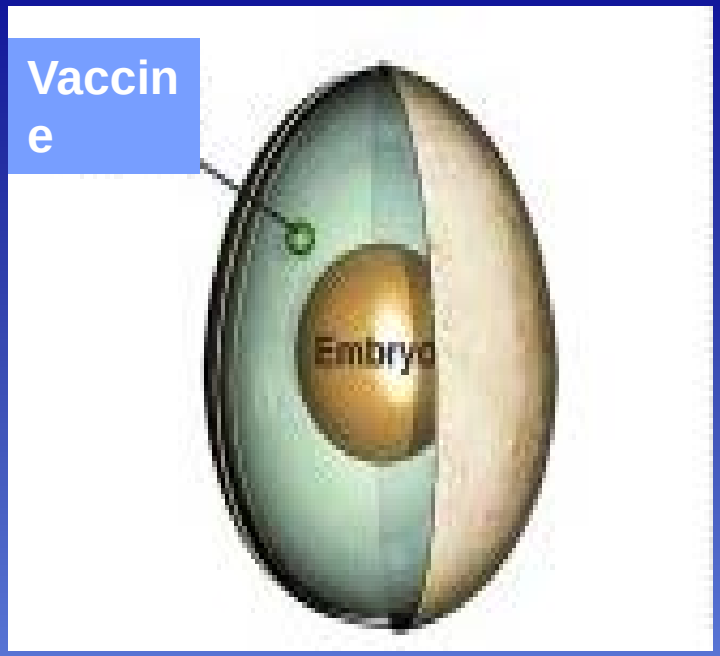


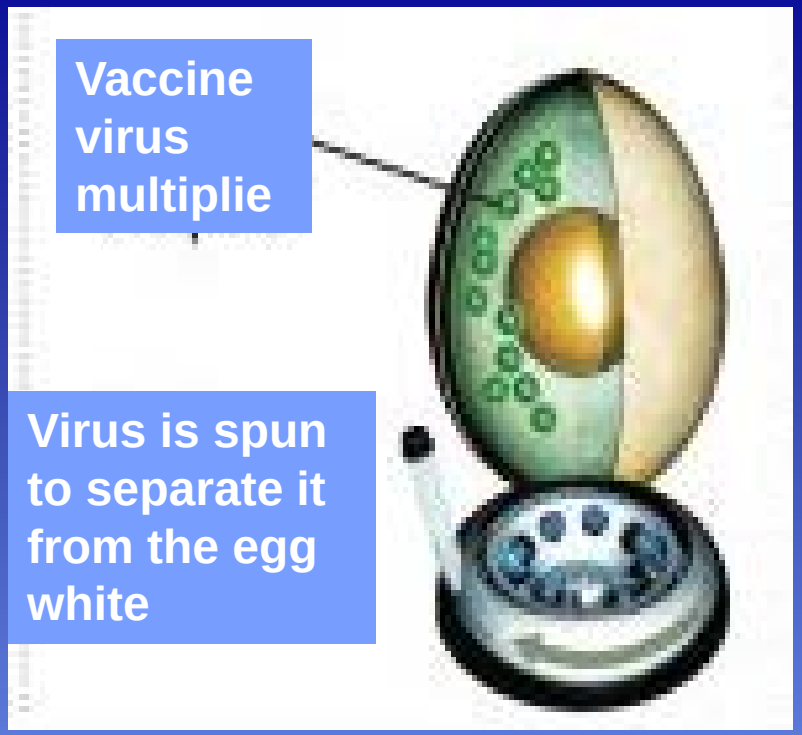


The vaccine virus is injected into a 9 to 12 day old fertilized egg and incubated for 2 to 3 days.(during this time the virus multiplies)

After incubation the egg white contains millions of vaccine viruses which are harvested and then separated from the egg white.

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## RECOMBINANT VACCINES:

- The vaccines are produced using **recombinant DNA technology or genetic engineering.**
- Recombinant vaccines are those in which genes for desired antigens of a microbe are inserted into a vector.

Different strategies are:

- **Using the engineered vector** (e.g., Vaccinia virus) that is expressing desired antigen as a vaccine
- **Introduction of a mutation by deleting a portion of DNA** such that they are unlikely to revert can create an attenuated live vaccine.

Live attenuated vaccines can also be **produced by reassortment of genomes** of virulent and avirulent strains.

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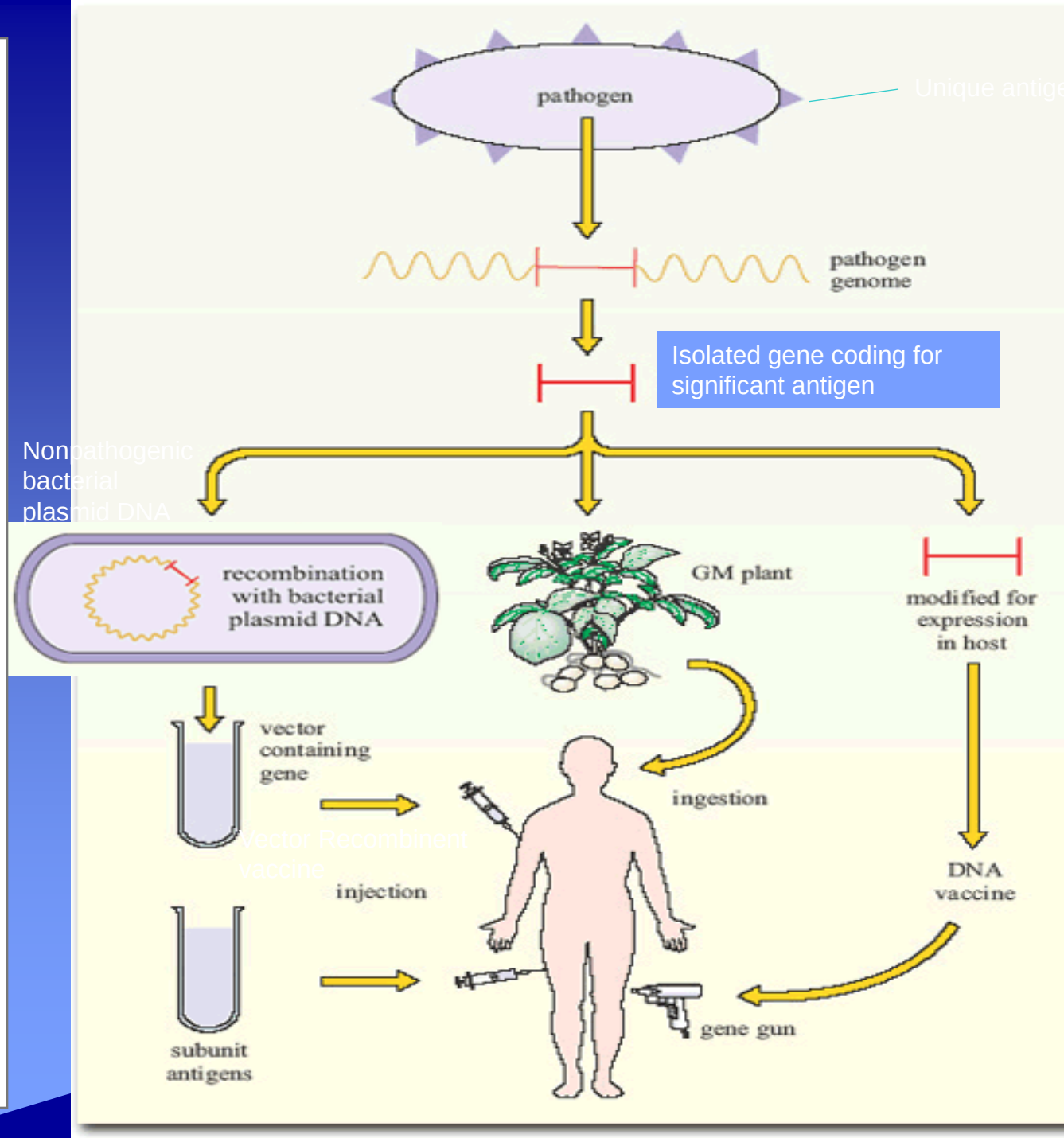
## Genetic approaches to vaccine development:

One or more genes encoding pathogen-specific antigens are isolated and
-recombined with a harmless or disabled vector for delivery by injection,
- or incorporated into food plants for ingestion,
-or modified for injection as naked DNA.
Subunit antigens can be produced by genetic engineering

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## 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.**

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Different expression systems used to express hepatitis B

surface antigen (HBsAg):

(A) banana fruits,

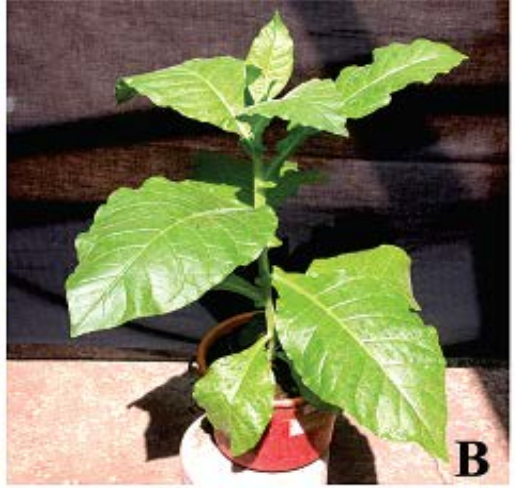
(B) tobacco plants,

(C)soybean callus,

(D) potato hairy roots,

(E) potato microtubers,

(F)tomato fruits.



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# GROWING THE MICROORGANISMS IN MAXIMUM TITRE

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Growing the microorganism in maximum are mainly classified in two category :

**GROWING BACTERIA :**

Bacteria are grown in bioreactors e.g.Haemophilus influenzae type b.

- **BATCH CULTURE**
- **CONTINUOUS CULTURE**

**GROWING VIRUSES :**

- **CELL (TISSUE) CULTURES**
- **BIRD EMBRYOS**
- **LIVE ANIMAL INOCULATION**
- **TRANSGENIC ANIMALS**

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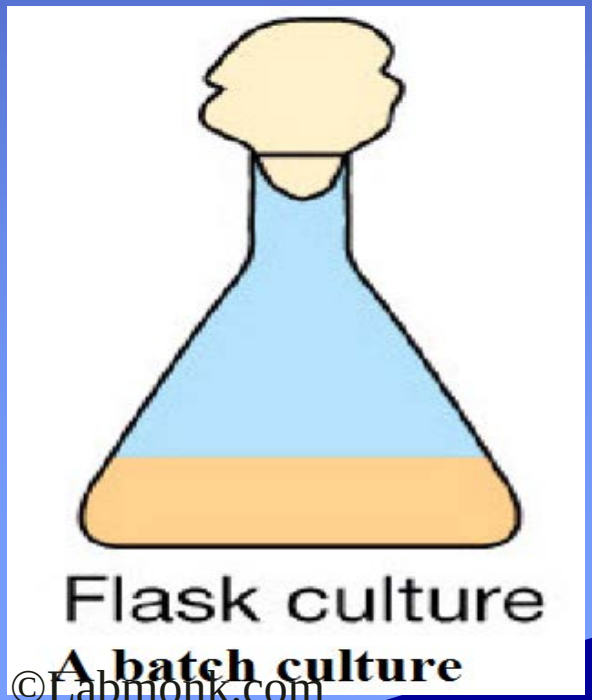
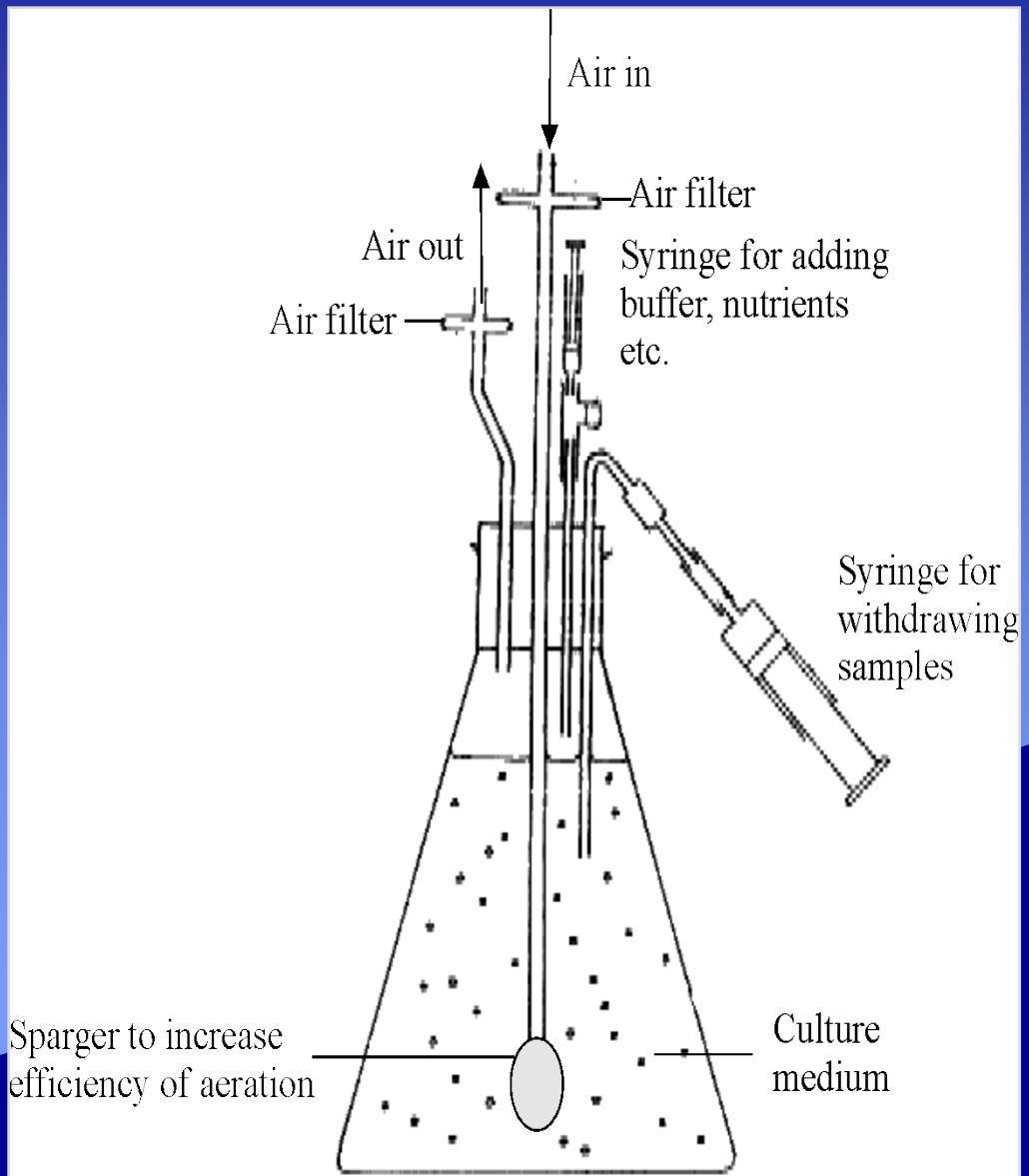
# Batch culture

- Culture incubated in a closed vessel with a single batch of medium.
- The fermenter shown here is set up for a **batch culture.**

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- Batch processing is a way of providing the best conditions for a micro-organism.
- All the raw materials are put in the fermenter at the start and then the micro-organism is added.
- The system is then left for a long time – possibly a week – until all the raw materials have been used up and there is loads of the product.
- The fermenter is then emptied and other processes are used to separate the product from the micro-organism.
- The fermenter is then cleaned out and the whole process begins again.

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# Continuous culture

- Growth in an open system
  - continual provision of nutrients
  - continual removal of wastes
- **Continuous culture** aims to keep a culture growing indefinitely. This can be done if:
  - fresh nutrients are continually supplied
  - Accumulated cells and waste products are removed at the same rate
  - Conditions such as temperature and pH are kept at their optimum values.

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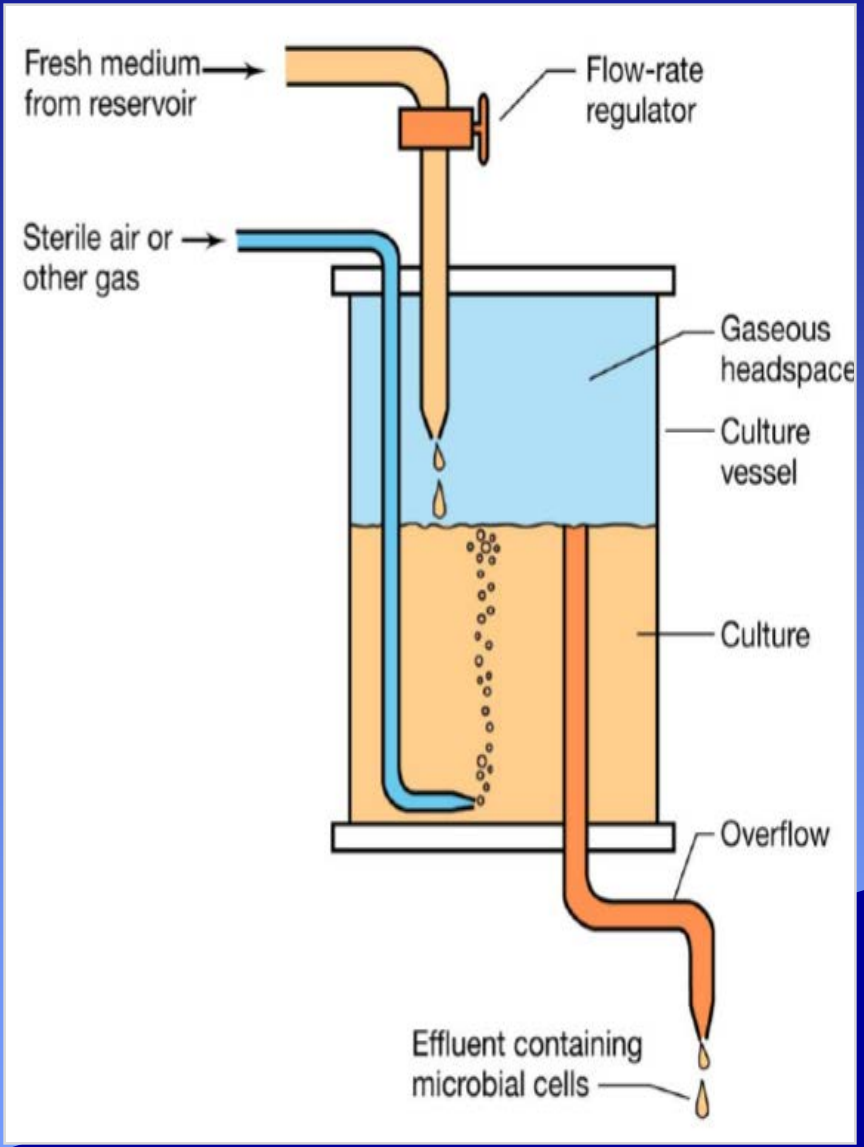
Here the raw materials are trickled in at the top of a column in which there are immobilised micro-organisms .

- The product flows out the bottom in a pure state.
- It does not need to be separated from the catalyst.
- However this process can only be used for reactions that are fast – possibly taking 10 minutes.
- A culture vessel designed for continuous culture is called a **chemostat.**

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Growing the microorganism in maximum are mainly classified in two category :

**GROWING BACTERIA :**

- **BATCH CULTURE**
- **CONTINUOUS CULTURE**

**GROWING VIRUSES :** Viruses are grown either on primary cells e.g.for influenza, or on continuous cell lines,e.g. for hepatitis A.

- **CELL (TISSUE) CULTURES**
- **BIRD EMBRYOS**
- **LIVE ANIMAL INOCULATION**
- **TRANSGENIC ANIMALS**

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# Cell culture

- **Cell culture** is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment.
- Cell cultures are separated into 3 types:-
- **Primary cell cultures, diploid cell strains or immortalized (continuous) cell lines.**

➢ ***Primary cell cultures -***

Cells that are cultured directly from animal or human tissues and can be subcultured only once or twice e.g. primary monkey or baboon kidney cell

- Commonly used cells double within 24 to 48 hours in appropriate media.

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## ➢ *Diploid cell strains(Secondary) -*

are derived from human fetal tissue and can be subcultured 20 to 50 times e.g. human diploid fibroblasts such as MRC-5

## ➢ *Immortalized (continuous) cell lines –*

- a single cell type that can propagated indefinitely in culture.
- They are derived from tumors or by treating primary cell cultures or diploid strain cell with a mutagenic chemical or tumor virus. e.g. Vero, Hep2

## ➢ *Suspension cultures -*

- In contrast to cells that grow in plastic dishes to form a monolayer, other cells are non-adherent and can be maintained as suspension cultures.
- These cells are maintained by continuous stirring with a magnet and can be grown in large numbers in a relatively small volume. ( a culture in which cells multiply while suspended in a suitable medium.)

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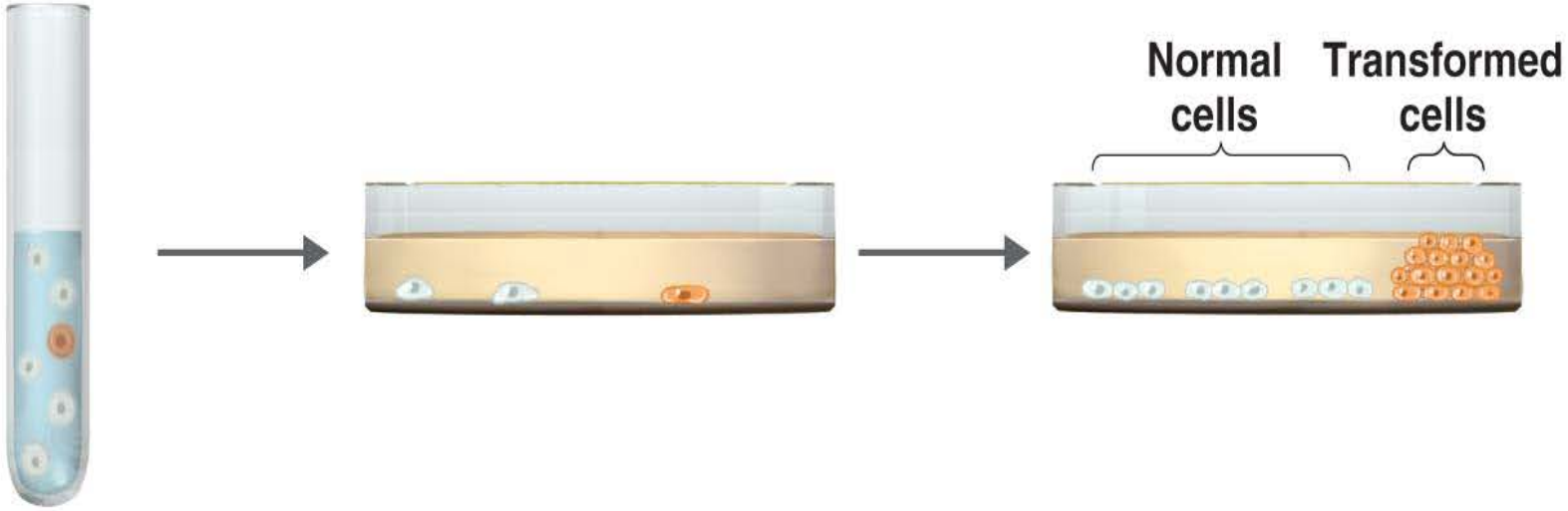
Animal viruses may be grown in Continuous cell culture.

Cell lines may be maintained indefinitely.




1 A tissue is treated with enzymes to separate the cells.

2 Cells are suspended in culture medium.

3 Normal cells or primary cells grow in a monolayer across the glass or plastic container. Transformed cells or continuous cell cultures do not grow in a monolayer.

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Figure 13.8

# Embryonated Eggs

- Many viruses can be propagated in embryonated chicken eggs but the method is now only used for **Influenza viruses.**
- **At 5 to 14 days after fertilization**, a hole is drilled in the shell and virus injected into the site appropriate for its replication (yolk sac, chorioallantoic membrane, amniotic cavity, allantoic cavity).

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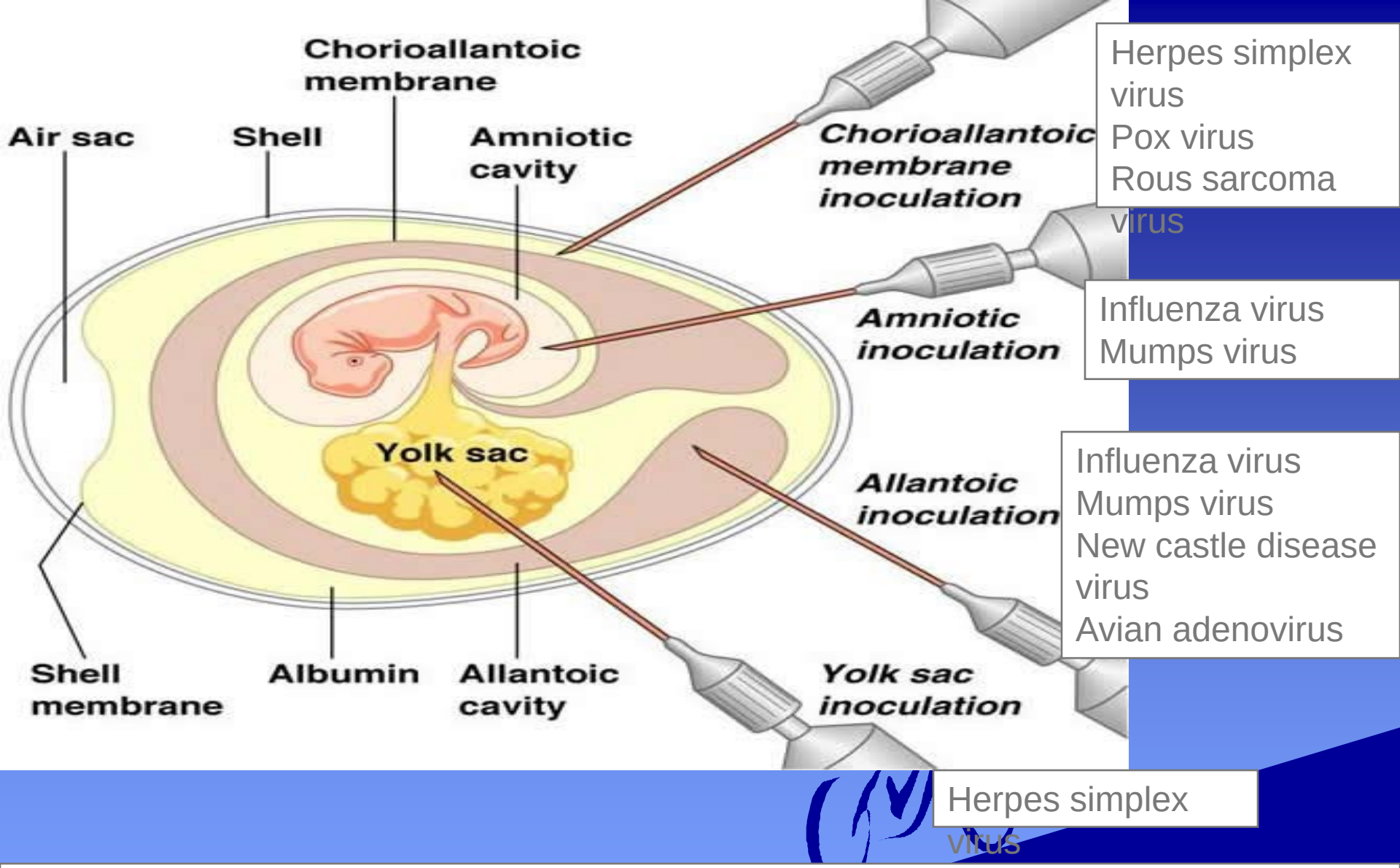


**GROWTH OF VIRUSES IN EMBRYONATED EGG –**

An embryonated chicken egg showing the different compartments in which viruses may grow. The different routes by which viruses are inoculated into eggs are indicated.

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# Live Animal inoculation

- Experimental animal are obligatory for studying ***virus pathogenesis***, which is the processes by which viruses can cause disease.
- Mice are the most widely used experimental animal.

# Transgenic Animals

- In recent years, a new technology involving insertion of the DNA of the whole or part of the virus genome, resulting in expression in somatic cells of virus mRNA and proteins has been developed.
- A modification of this technique allows the targeting of these genes to specific cells, e.g. hepatocytes, neurons, etc. by using cell-specific promoters in the transgene construction.
- The best example is the creation of **transgenic mice expressing Hepatitis B virus.**

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# THANK YOU

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