((Propagation of viruses))

- **Estimation of virus yields plaque assay.**
- **Preparation virus stocks and determination of mouse LD50.**
- **Routes of inoculations in embryonated**

Assays for quantitation virus particles:

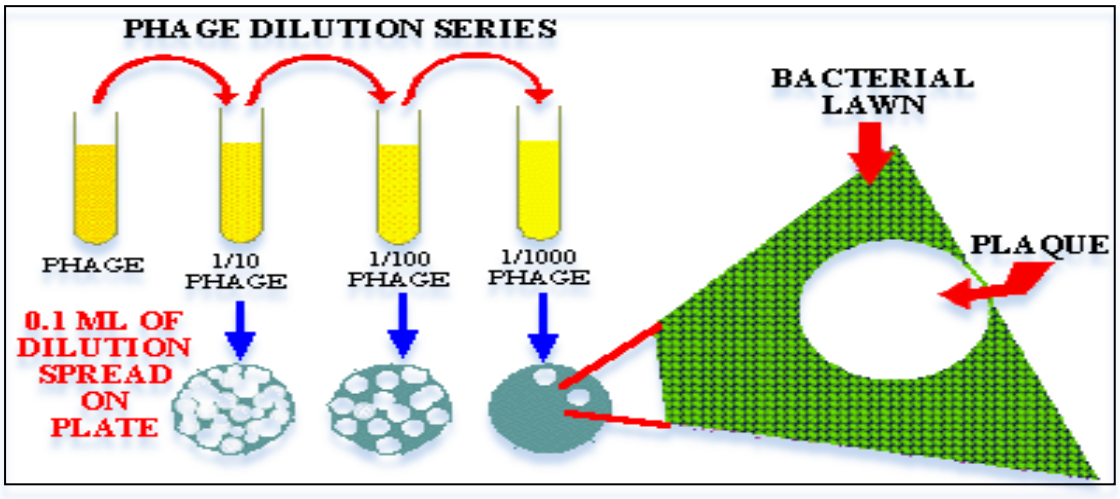
- Electron Microscopy
- Hemagglutination assay
- ELISA
- Quantitative PCR

Plaque assay

Titer-concentration of a virus in a sample

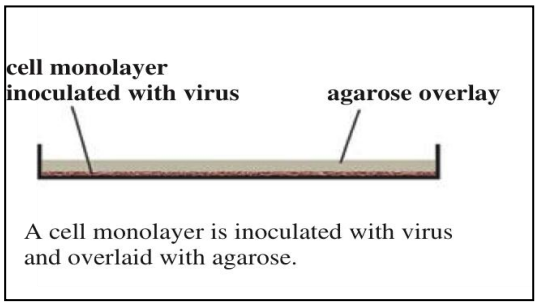
Plaque: circular zone of infected cells that results from a single infectious particle.

Plaques due to cell death.

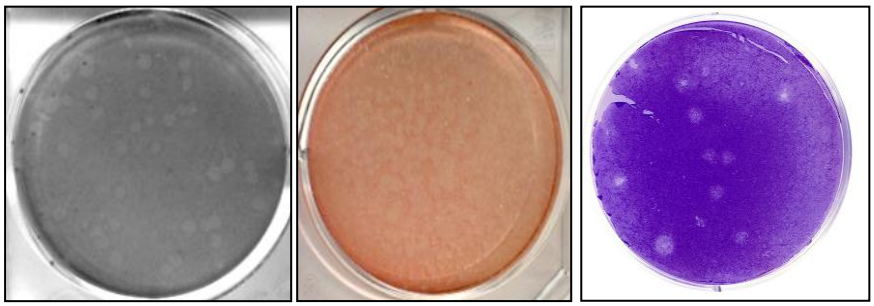


Note: Titer-quantitative measure of the infectious viral particles in a sample (PFU/ml)

- Serial dilution of sample
- Add diluted virus to cell monolayer
- Incubate cultures at 37^{o}C to allow adsorption of virus
- Remove inoculum
- Add overlay to culture (Culture media +agar or agarose)
- Plaque results from a single virus



A cell monolayer is inoculated with virus and overlaid with agarose.



Unstained Neutral red Crystal violet

Plaque forming unit (PFU)-virus particle able to initiation a productive infection

Lytic infection- Virus enters cell and usurps cellular machinery to rapidly multiply and in the process kill the cell (many flu and cold viruses).

Multiplicity of infection (MOI)-number of infectious units/cell. Ratio of input virus to the number of target cells in an infection. Usually used to describe the infection of a particular cell type grown on a plate or in culture

CFU: colony forming unit

- Some viruses can 'transform' cells i.e. allow them to grow when they otherwise would not
- Cells that grow form colonies
- CFU is a measure of viruses per volume of stock that can transform cells.

Animal model as purpose-bred animal……. Mouse model

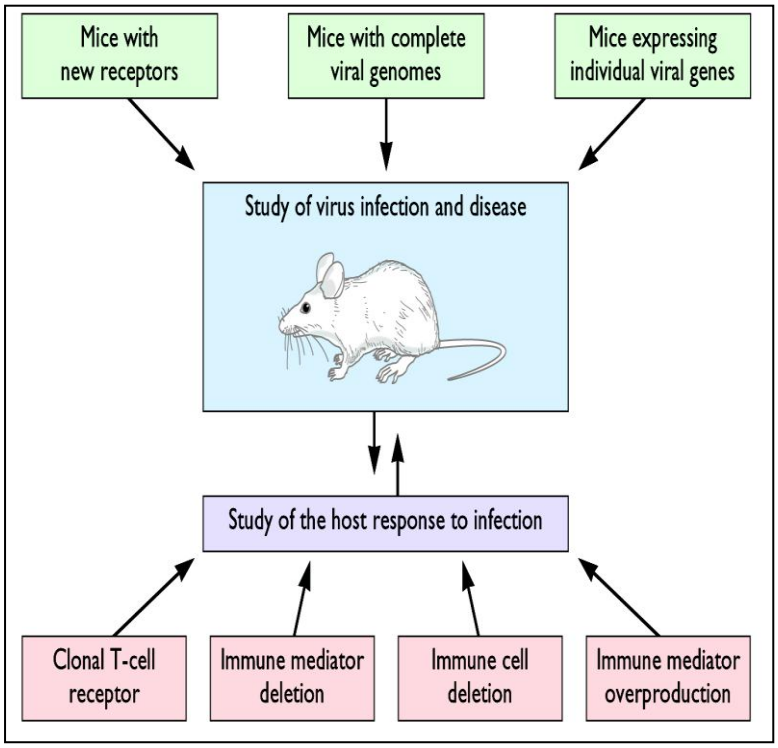
Advantages

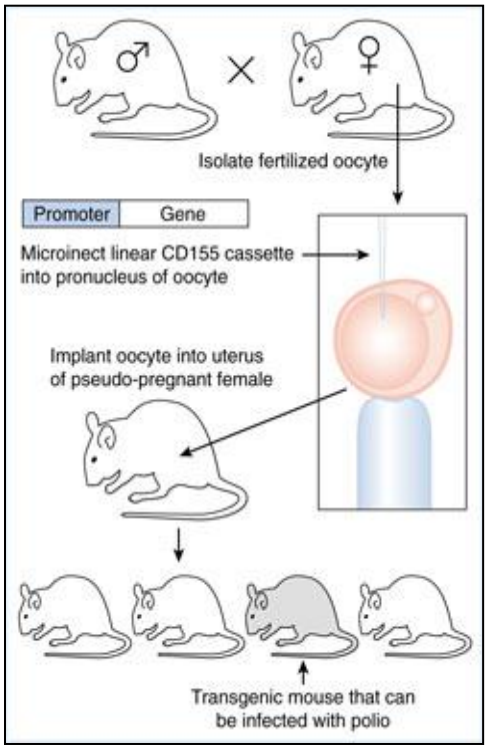
- in-breed strains reduce genetic variability
- genetics are well understood
- Introduce, mutate or inactivation specific genes thought to control the immune response.

Disadvantages

- Sometimes not infected-therefore virus has to be adapted or use a closely related surrogate virus
- Does not always cause same disease state
- Mice are not humans

"Transgenic mouse models"





VIRUS TITRATION:

After a virus is propagated in either cell culture or in a suitable animal, we need to know the infectivity titre of the virus material obtained. This can be determined in vivo by inoculating increasing dilutions of the virus material to a susceptible host animal such as laboratory mice and based on mortality seen in different dilutions, the infectivity titre which is the reciprocal of highest dilution showing 50% mortality in the inoculated mice and expressed as LD_{50}/ml.

Materials :

1. Microbiological safety cabinet.
2. Sterile Pipettes, 10ml and 1ml
3. A tray with ice flasks. virus suspension (cell culture supernatant)
4. Mice (4-6 weeks old)
5. One ml syringe and needle for inoculation
6. Test tubes.
7. Sterile PBS pH 7.4
8. Sterile bovine or calf serum
9. Cages for housing mice.

Procedure:

1. Working in microbiological safety cabinet, prepare the diluting fluid which is PBS containing 2% serum and dispense 9ml in test tubes labeled 10^{-1} to 10^{-7} and keep the test tubes in rack immersed in plenty of ice.

2. To make 10 fold (log) dilutions of the virus material, dilute 1ml of virus in 9ml of diluent to get the initial dilution i.e. $10^{-1.}$ Subsequently transfer 1ml of previous virus dilution to next dilution by using at each step a fresh pipette, to achieve serial tenfold dilutions.

3.Inocultate 0.03 ml of each virus dilution intracerebrally, intradermally or intraperitonially according t viral infection into mice, starting from the highest dilution (in this case $10^{-7).}$ Use at least 6 mice per dilution and transfer these into cages appropriately labeled.

4.Observe the mice for 14 days. Any death occurring within first 5 days should be considered non-specific according of viral infection.

50 % Lethal Dose:

Infectious dose$_{50}$ (ID_{50})- Dose required to infect 50% of the inoculated animals. With most viruses several PFU are required to infect an animal.

Lethal dose$_{50}$ (LD50)-amount of virus required to kill 50% of animals & varies between:

1) Mouse strains

2) Route of inoculation

3) Animal models

Calculation of 50% endpoints

In any biological quantitation, the most desirable endpoint is one representing a situation in which half of the inoculated animals or cells show the reaction (death in the case of animals and in CPE case of cells) and the other half do not.

Routes of inoculations in embryonated eggs:

ADVANTAGES

- Isolation and cultivation of many avian and few mammalian viruses
- Ideal receptacle for virus to grow
- Sterile & wide range of tissues and fluids
- Cost- much less
- Maintenance-easier
- Less labour
- Readily available
- Free from bacteria and many latent viruses.
- Free from specific and non specific factors of defense.
- Sensitive to viruses which do not produce infection in adult birds.

Various routes of inoculation

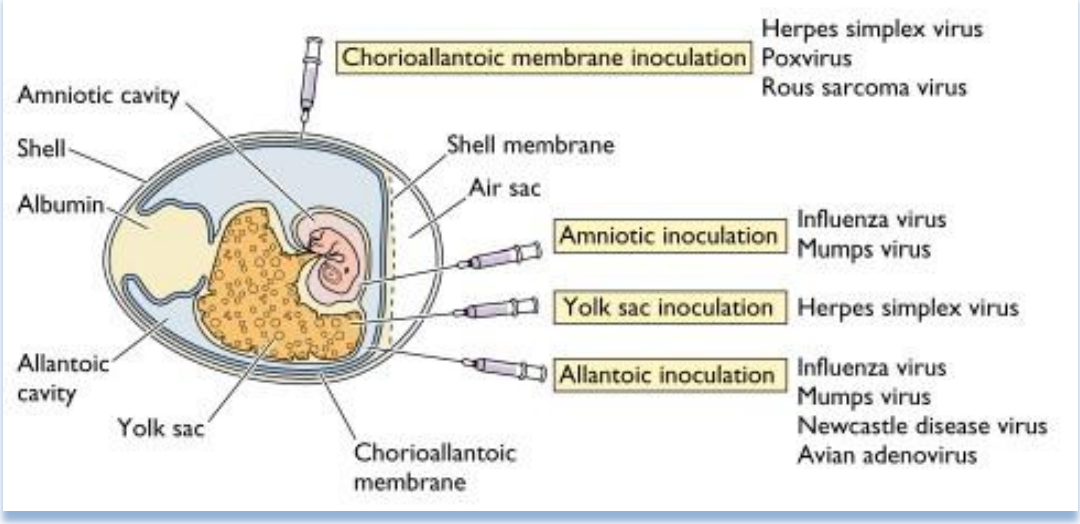
a)Yolk sac

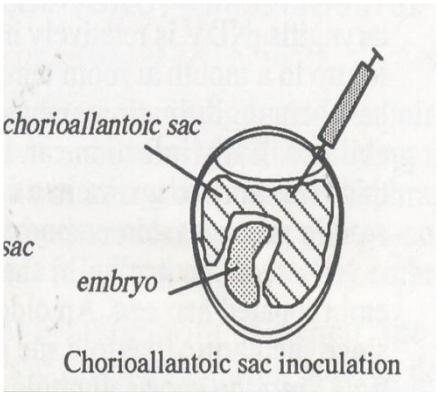
b) Allantoic sac

c) Chorioallantoic membrane

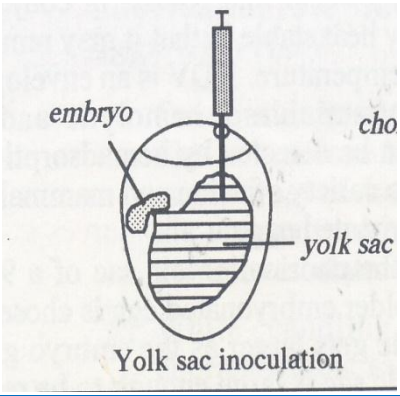
d) Amniotic cavity

e) Intravenous

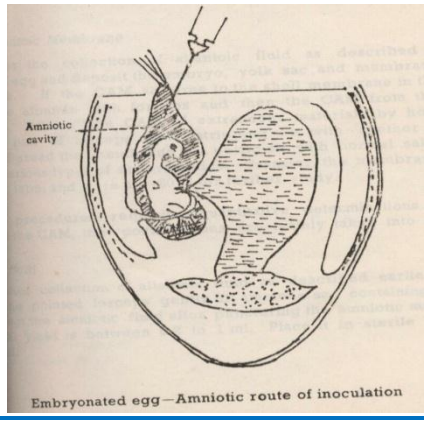




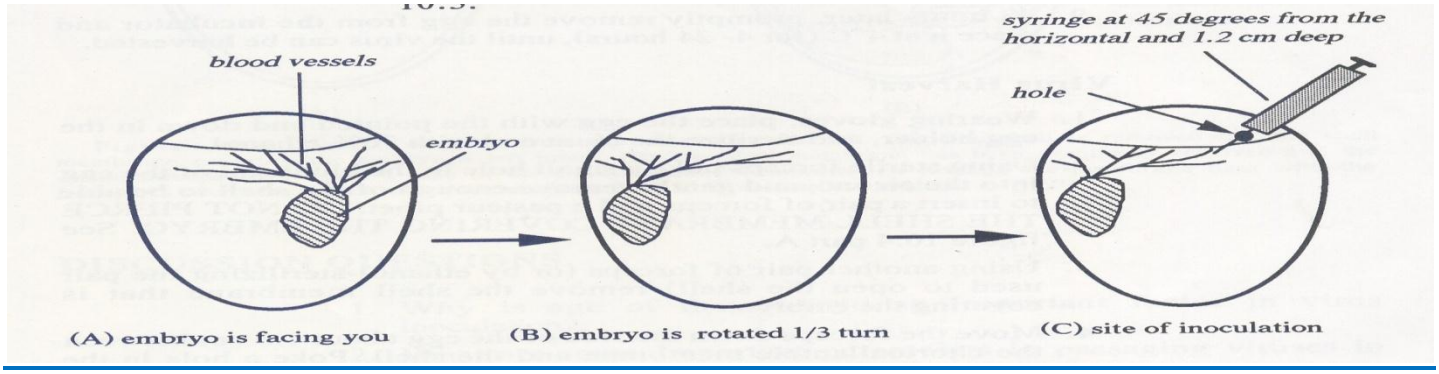
Chorioallantoic sac inoculation



Yolk sac inoculation



Embryonated egg—Amniotic route of inoculation



ALLANTOIC ROUTE

Yolk sac route:

Advantages	Disadvantages
-Simplest method	- Not suited for avian viruses
-Mostly mammalian viruses	
-Immune interference for most of avian viruses	

Allantoic route:

- Most popular
- Most of avian viruses
- High titered virus
- Simple technique

Chorioallantoic membrane route:

- Pox and Herpes viruses.
- 'Pock Lesions'
- Suitable for plaque studies

Amniotic route:

- Primary isolation of influenza and mumps viruses
- Growth of virus detected by haemagglutination.