Virus Isolation
MIC 204

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Introduction

- Viruses are obligate intracellular parasites and therefore must be grown in living cells
- Cannot be grown in culture media
- Viruses are host specific therefore appropriate cells have to be used in which it can replicate
- Host: whole animal/ eggs, insect larvae or cell culture
- Majority can be adapted to foreign hosts by multiple passaging

Purpose of virus cultivation/propagation

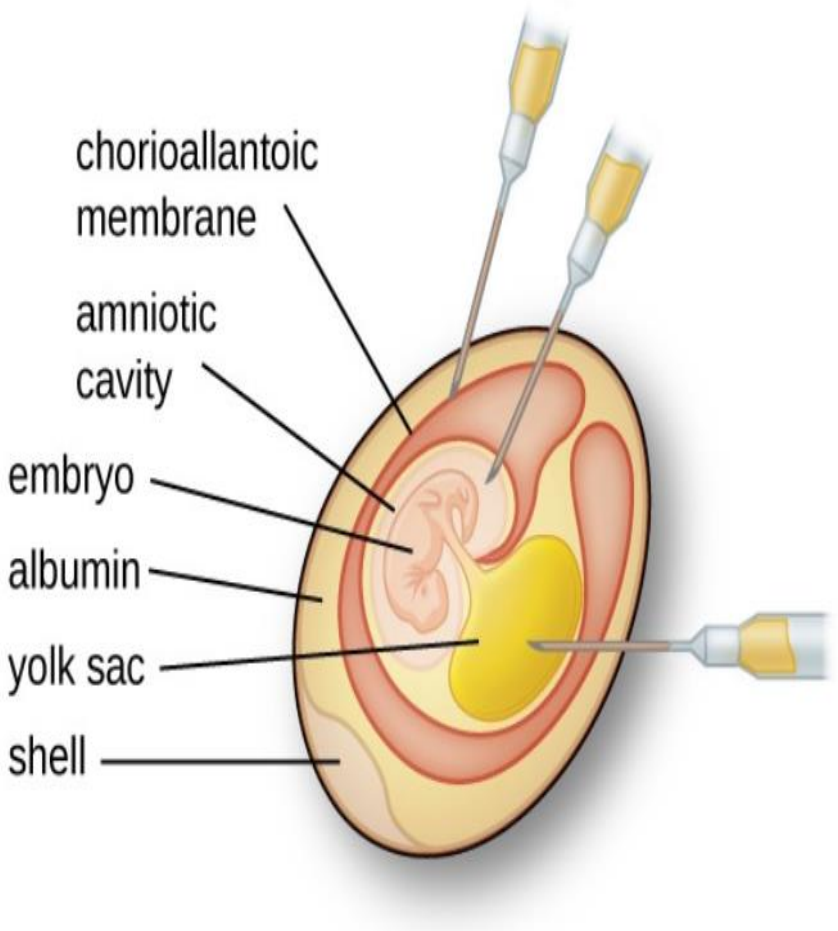
- To isolate and identify virus (infectious agent) in different specimen
- Prepare virus for vaccines
- To understand viral structure, life cycle, genetics and effects on host cells
- Biodiversity and ecological perspective

Virus Cultivation Systems

- Embryonated Eggs
- Whole systems
  - Natural hosts
  - Experimental animals/plants
  - Transgenic animals/plants
- Tissue Culture Systems

Embryonated Eggs

- 1931. Goodpasteur and Burnet used embryonated hen's eggs
- Eggs represent living cells
- Eggs have no developed immunologic function to hinder virus replication
- Naturally sterile and free from bacteria and latent virus
- Easily available, less cost, easy to maintain, less labor and infrastructure intensive
- Process of virus cultivation depends on eggs used
  - Chick embryo
  - Duck egg
  - Turkey
- Egg must be sterile and shell intact
- 7-14 days old



(a) (b)

Figure 3. (a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the egg, including the chorioallantoic membrane, the amniotic cavity, and the yolk sac. (credit a: modification of work by "Chung Hoang"/YouTube)

https://courses.lumenlearning.com/microbiology/chapter/isolation-culture-and-identification-of-viruses/

Procedure

- 7-10/11-12 days (influenza'virus) embryo eggs shell cleaned and sterilized. Check with ovoscope to see if alive
- Mark non veined area of allantoic cavity by placing egg in front of light source. Make a small nick
- Drill pin point hole at top of egg using Dremel motorized tool and inject the viral suspension with syringe at appropriate site of inoculation. Decreases air pressure to prevent inoculum leakage
- Automated machines used for making perforations
- Seal hole with paraffin wax
- Incubate eggs for 2-3 days at 36 deg C
- Check for sign of viral growth using ovoscope/candling
- Harvest embryo and check for viral growth

Virus Cultivation

- **Chorio Allantoic membrane (CAM):** Viruses produce visible foci or ‘pocks’, inclusion bodies, oedema or other abnormalities. Each infectious virus particle forms one pock. Viruses which can be grown include: Herpes viruses and poxviruses
- Used for POCK assay

- **Amniotic Cavity:** The virus is introduced directly into the amniotic fluid that bathes the developing embryo.
  The volume of fluid in the infected amniotic sac is small (1-2 ml). The amniotic route is recommended for the primary isolation of human viruses: mumps virus, and influenza A, B and C viruses

Virus Cultivation

- **Allantoic Cavity**: Many viruses such as Newcastle disease virus can grow readily. Other viruses such as influenza, may require repeated amniotic passages before becoming adapted to the egg and grown in the allantoic cavity. Allantoic inoculation is a quick and easy method that yields large amounts (8–15 ml) of virus-infected egg fluids.
- **Yolk sac:** It is also a simplest method for growth and multiplication of virus. Mostly mammalian viruses are isolated using this method. Immune interference mechanism can be detected in most of avian viruses. This method is also used for the cultivation of some bacteria like Chlamydiae and Rickettsiae.

Virus Harvesting

- Remove top part of egg shell covering the air sac
- Pierce the shell membrane and chrorio allantoic membrane
- Harvest part of egg used for virus cultivation
- Duck eggs used for vaccine production as larger- Yellow fever virus, Rabies virus, Influenza virus

1. CANDLING

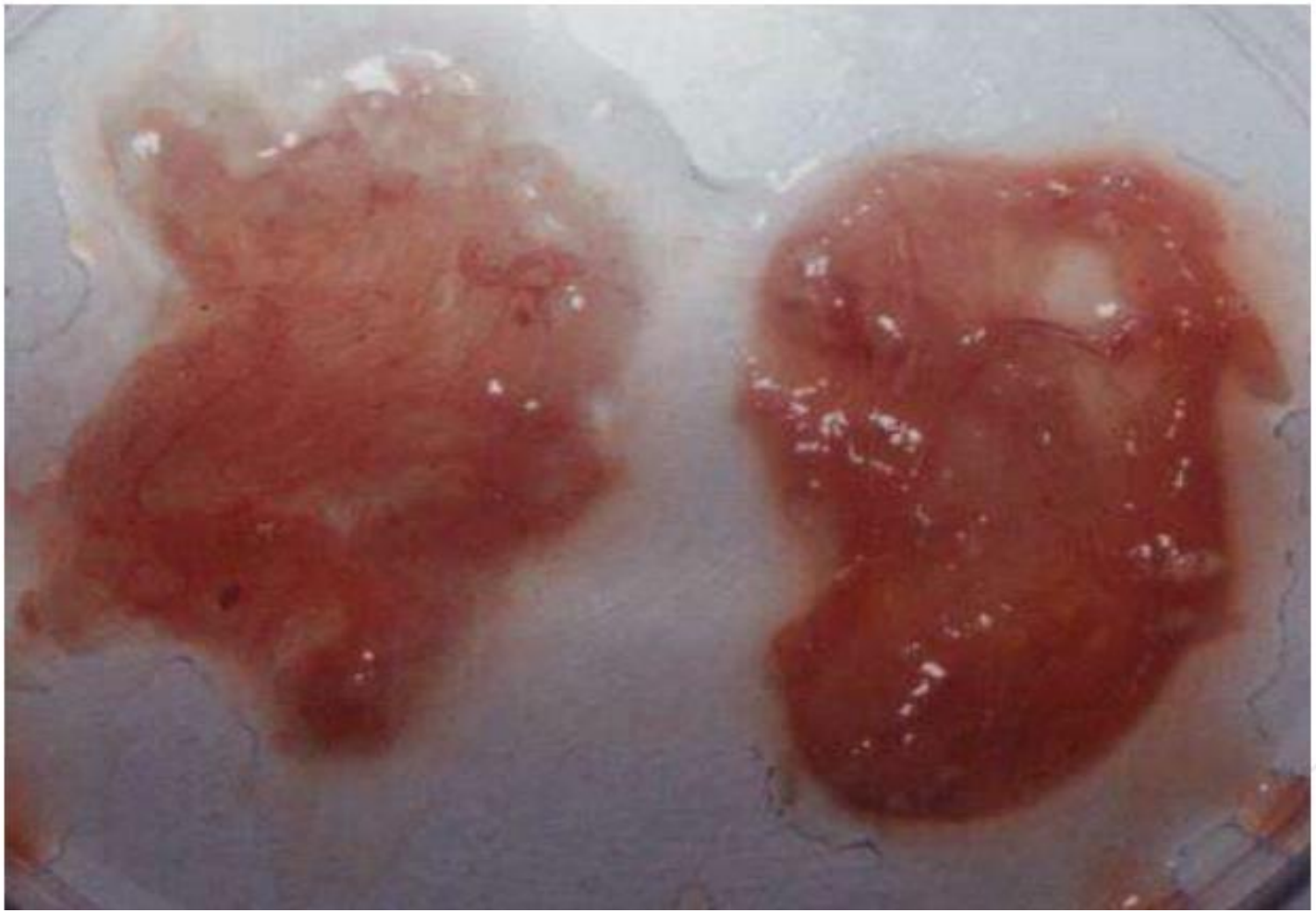
Handling of eggs in a influenza vaccine plant

Figure 2a & 2b: Candling of egg to observe embryo

Detection of Viral Growth

- Death of Embryo
- Defects in embryonic development
- Localized areas of damage/ cytopatheic effects- discrete opaque spots called pocks
- Embryonic fluid can be used for viral assays by electron microscopy, pock assay, heamagglutination assay

POCK LESIONS ON CAM



Disadvantage

- Site of inoculation varies with different viruses
- Compared to cell culture, higher variability
- Requires several eggs for vaccine production

Experimental Animals

- Largely replaced by cell culture except for viruses which are non permissive and use animals as natural hosts
- Include horses, chimpanzees, rabbits, bats, rodent family (mice, rats, guinea pigs etc)
- Healthy and free from communicable disease- germ free facility
- Transgenic animals permit human receptor expression and widely used
- Oncogenic viruses for tumor formation
- Endpoint assays, Vaccine production, polyclonal antibody production, viral immunopathological studies, tumor studies
- Dis advantage: expensive to maintain, cannot be used for vaccine, bioethics issues

Transgenic animals

- Syngeneic animals:genetically identical, or sufficiently identical and immunologically compatible as to allow for transplantation
- Mice are most widely used in virology

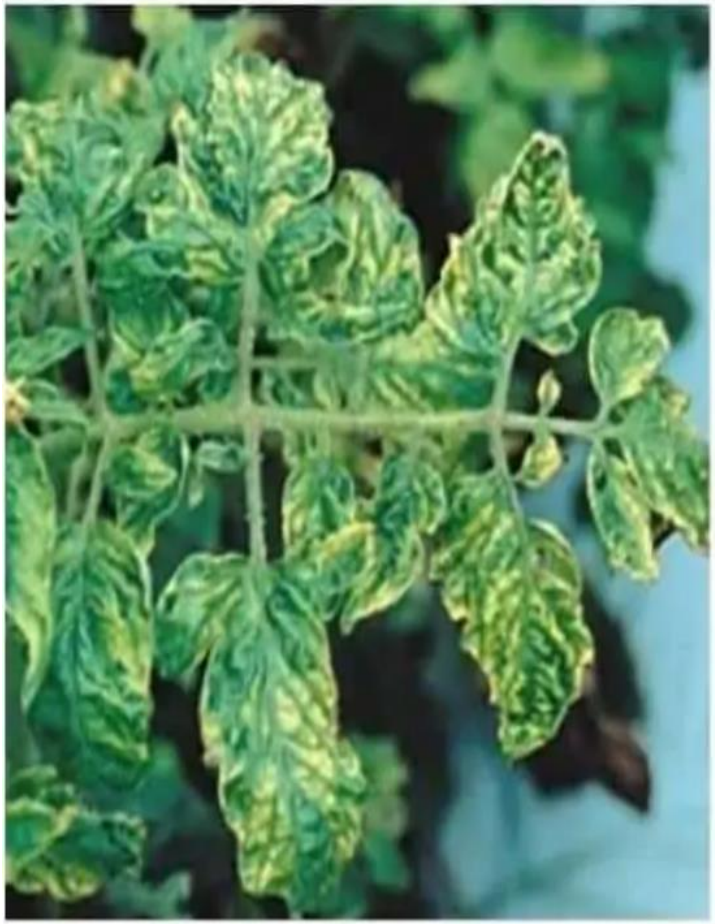
Plant systems

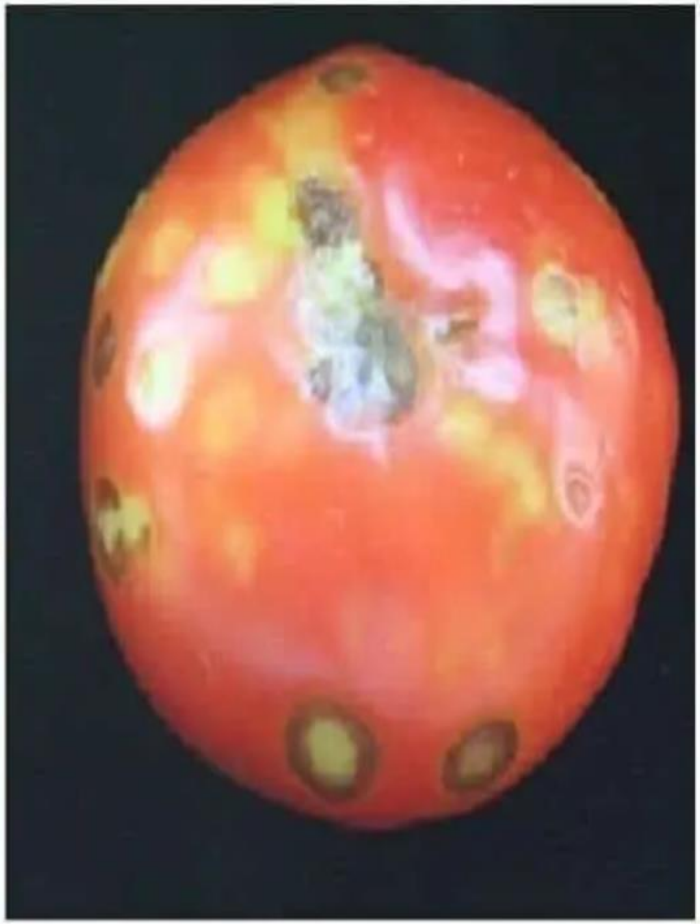
- Plant viruses are propagated in host plants, which are usually grown in glasshouses, screen houses, or growth cabinets.
- In most cases, the plants are grown from seed; in some cases, they are propagated as cuttings.
- Transgenic plants

Bacteria as hosts for bacteriophages

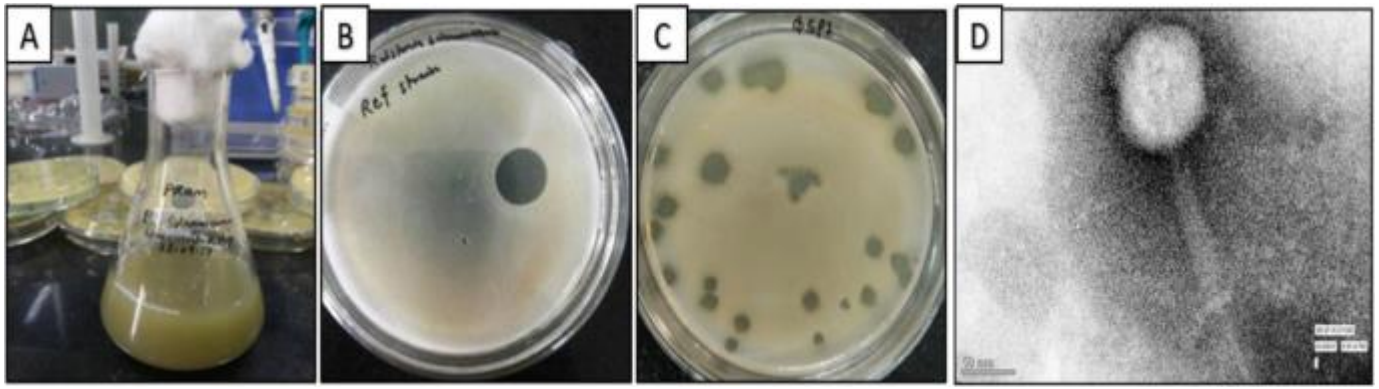
- Obtain sample containing phage and add to bacterial host
- Amplification: Incubate to allow phage to multiply
- Centrifuge to remove bacterial debri, filter to obtain phage lysate
- Purification by ultracentrifugation
- Assay using Plaque assay

ToMV





Plaque assay



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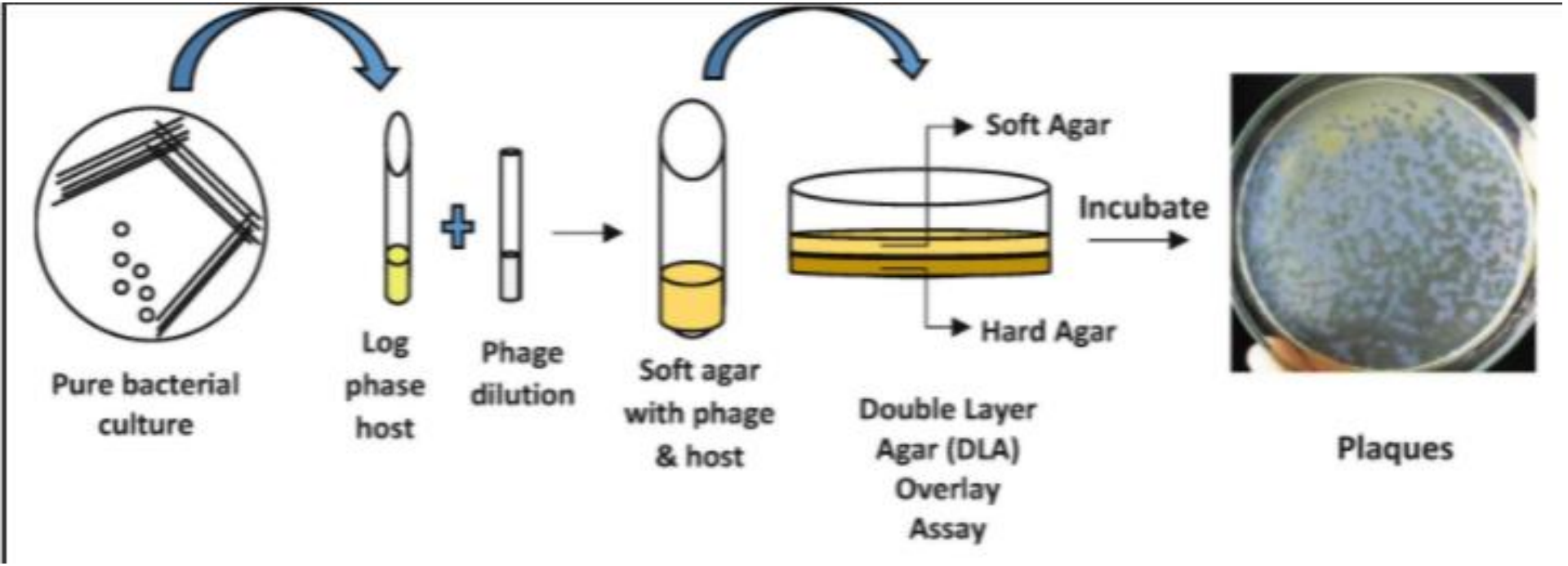
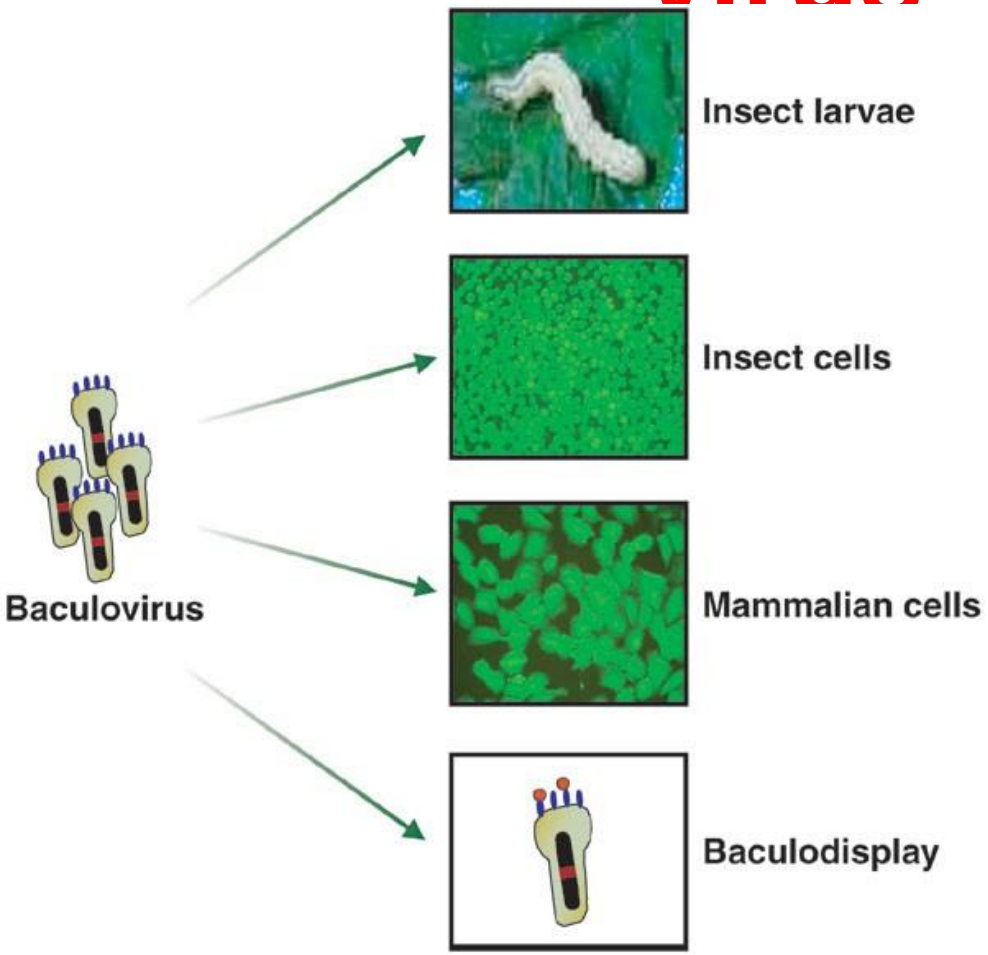


Insect larvae for growing insect viruses: eg Baculo virus



End Point Assay

- Infect animal with different doses of virus and check the end point of the virus infection
- Lethal Dose: Death
- Infectivity Dose: Infection symptoms
- Tumor development