Practical Virology_Lab. (5)

Inoculation of clinical sample in embryonated egg
Inoculation of Virus in Animals

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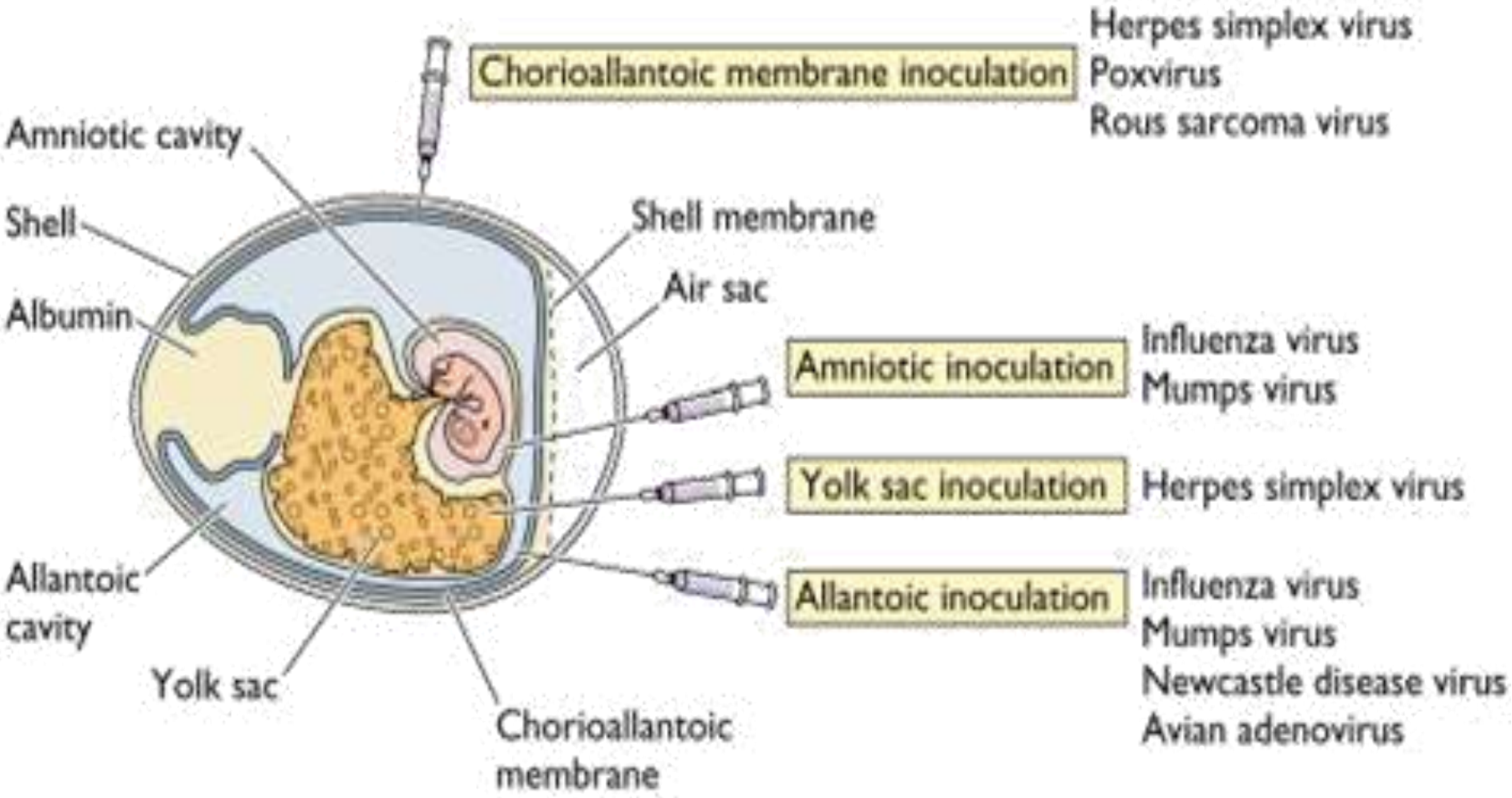
Factors affect the multiplication of viruses are:

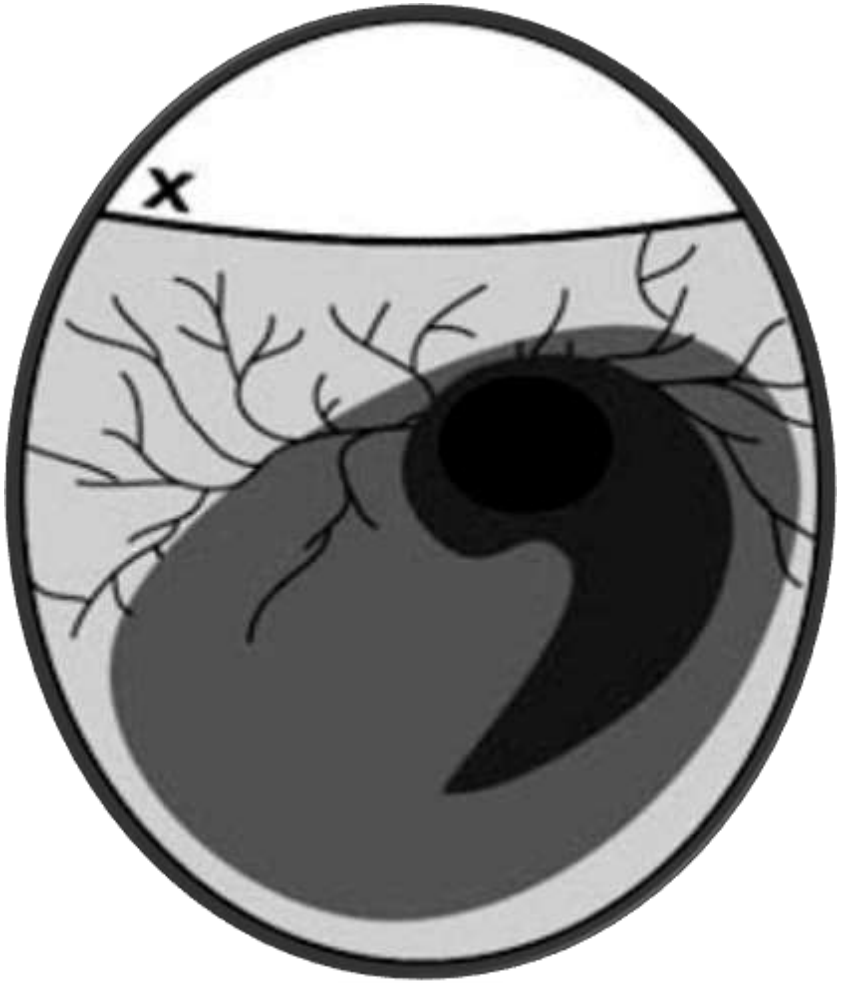
- Age of embryo (using 7-12 day)
- Route of inoculation
- Concentration and volume of inoculums
- Temperature of incubation
- Time of incubation after inoculation.

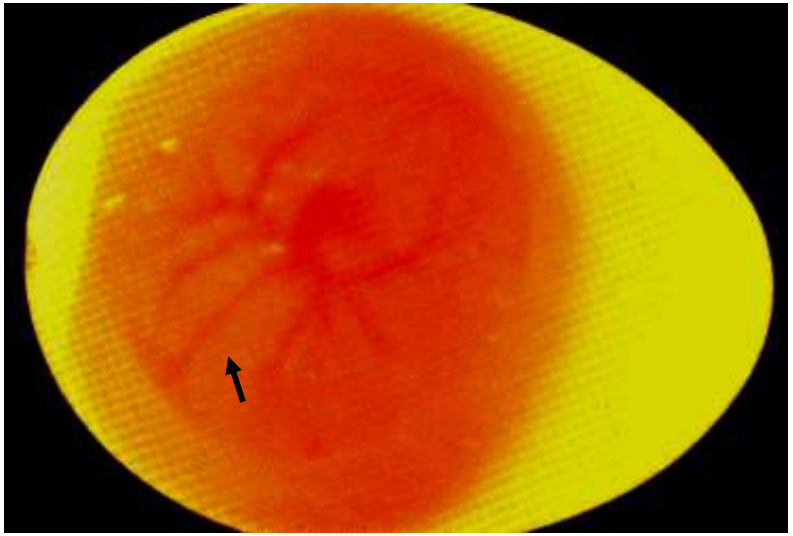
Structure of embryonated egg

- Selection of the routes depends upon the virus & its affinity to grow in certain tissues

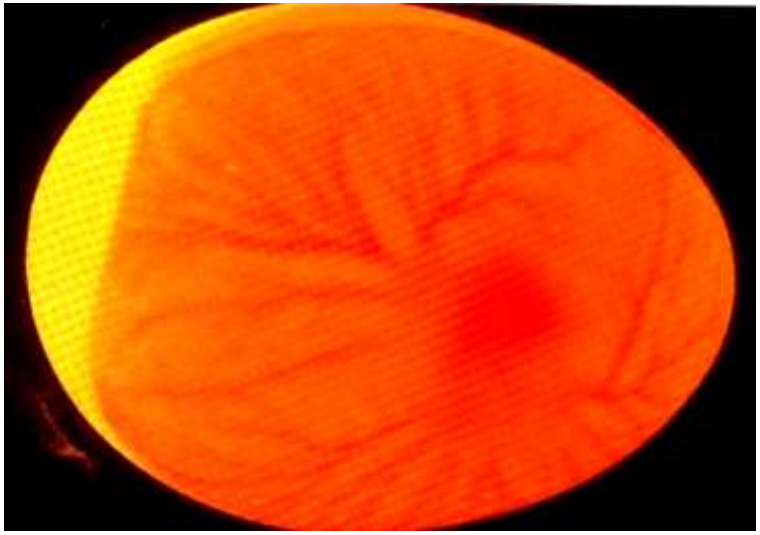


1 Candling Eggs

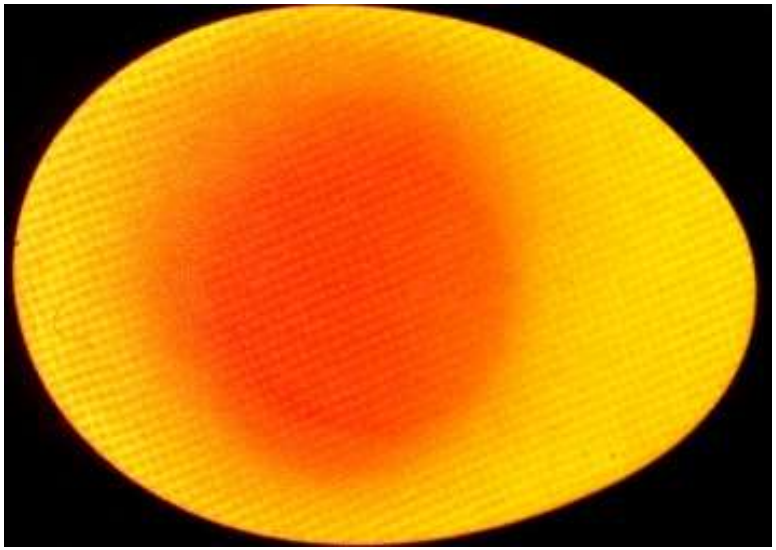




Three days aged embryo

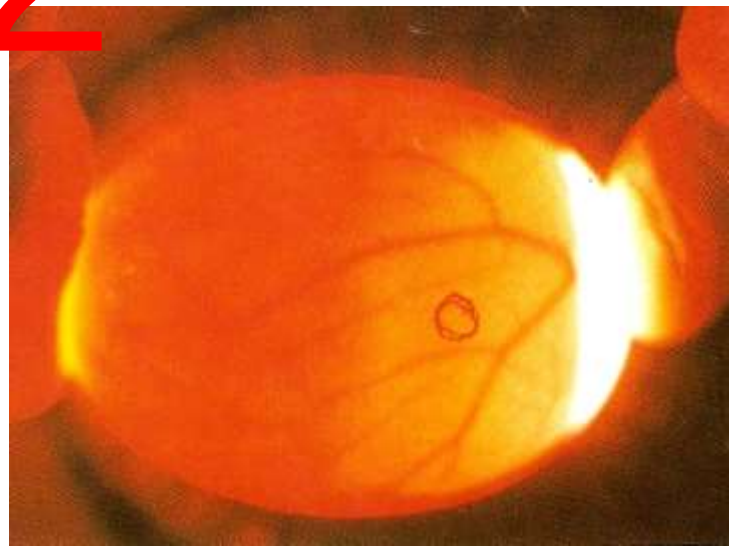


Five days aged embryo

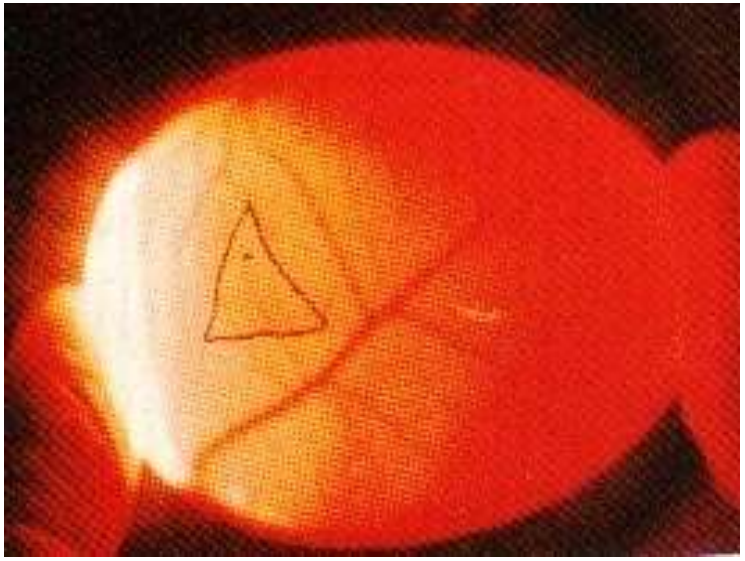


Died embryo

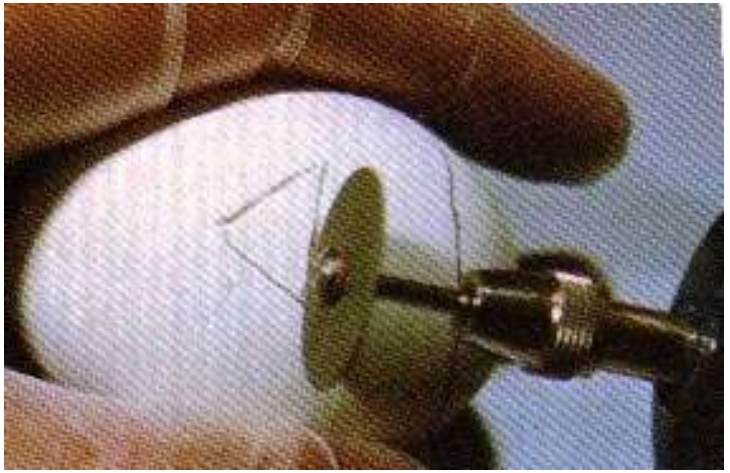
2 Drill a slit in the egg shell



Amniotic cavity
marked off with a pencil

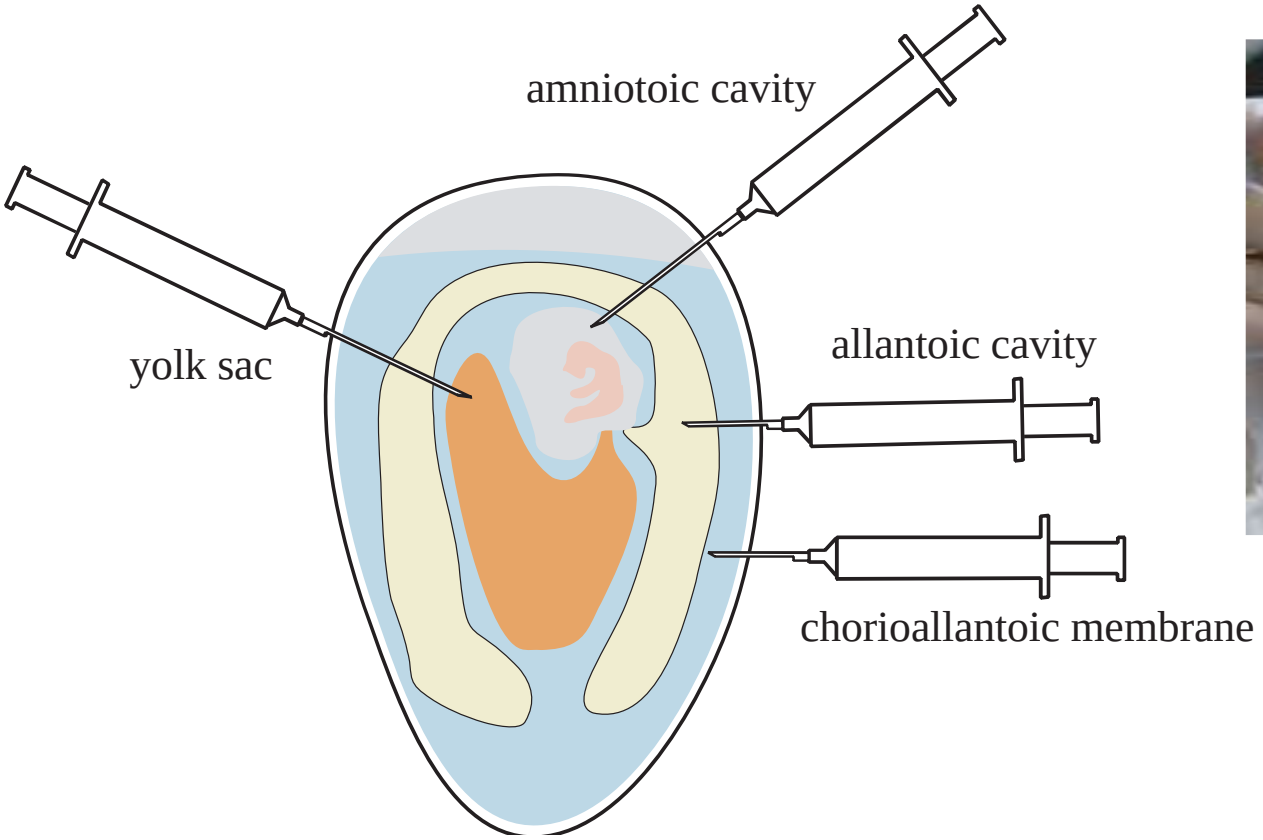


Chorioallantoic CAM
marked off with a pencil



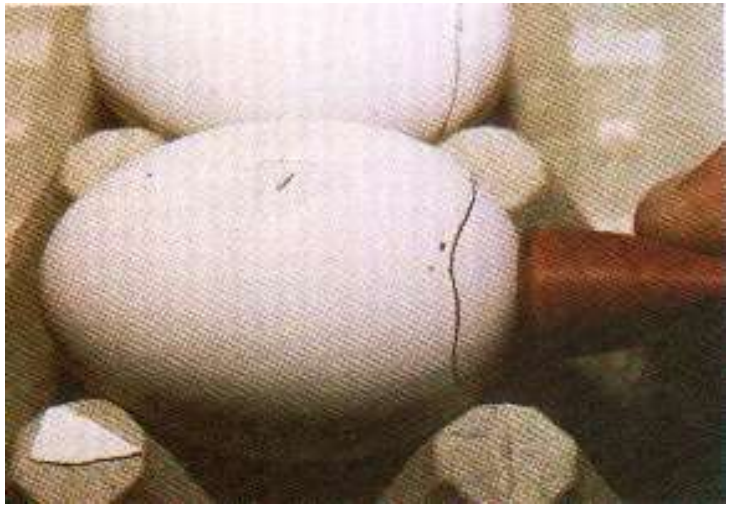
Routes of Injecting the Embryonated Eggs

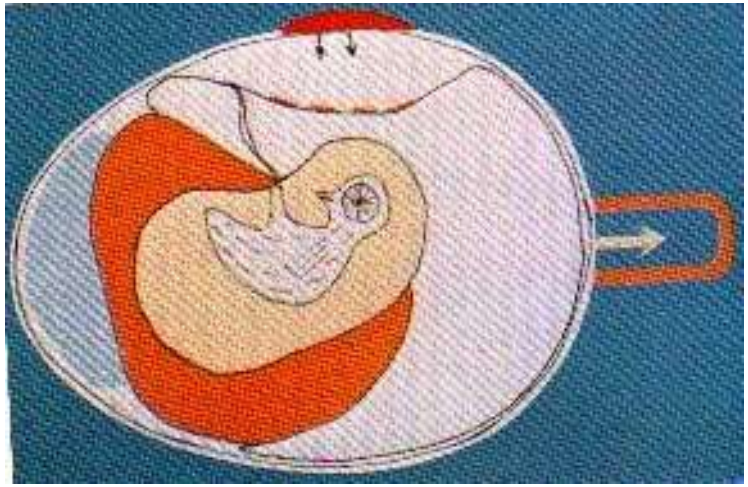
Sites into which viruses can be inoculated



Inoculation of an egg

Inoculation into chorioallantoic membrane of the chick embryo





Seal the opening and rotate the egg

Preparation of sealing mixture:

2 parts of paraffin (melting point ± 54˚C)

+

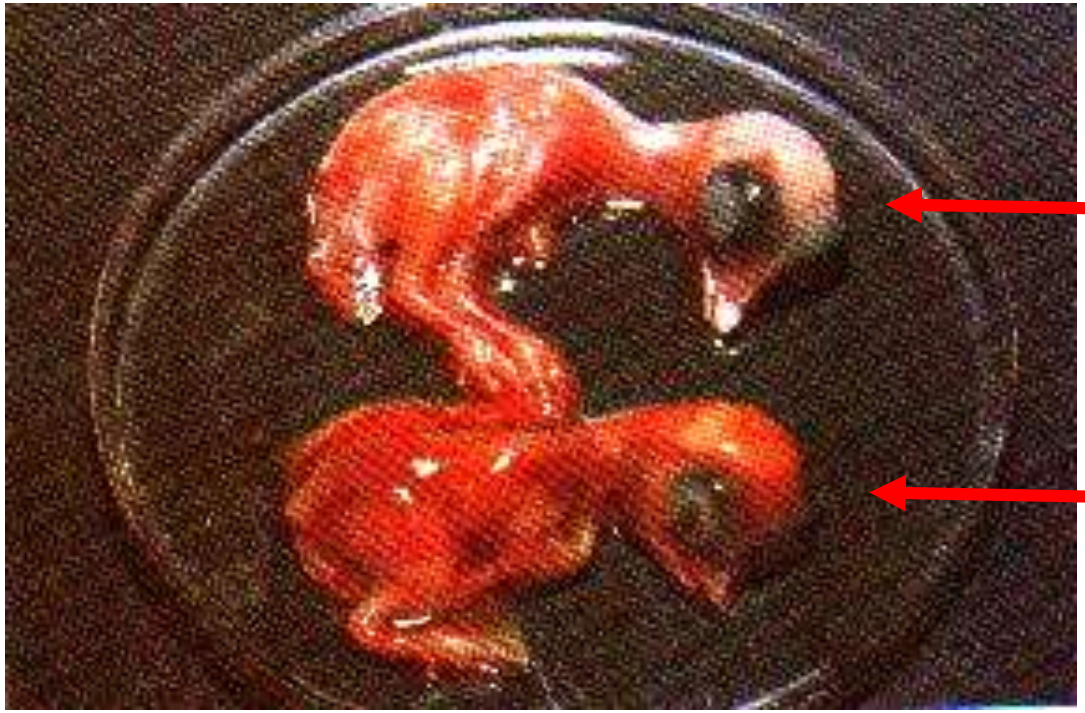
1 part of Vaseline

Incubate the egg at 37˚C

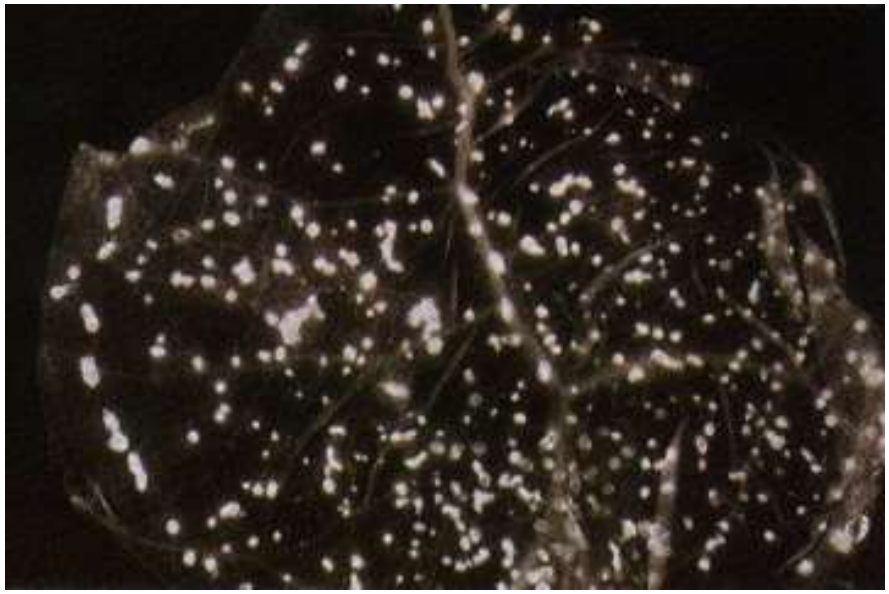
Harvest the egg and recognition

- Death of embryos.
- Curling & dwarfing of embryos.
- Hemorrhages of subcutaneous tissues.
- Pock lesions & thickening of Chorioallantoic membrane.
- Development of inclusion bodies in the cytoplasm or nucleus of infected cells.

Death of the embryo



13 days old embryo



Pocks on CAM (chorioallantoic membrane): chick embryo infected with HSV-1 show pocks on CAM.

Pocks on CAM (chorioallantoic membrane): chick embryo infected with HSV-2 show pocks on CAM.

Identification:

- Neutralization test (NT)
- Hemagglutination inhibition (HAI)
- Detection of Antigen
- Detection of nucleic acid

Advantages:

1. **Fertile eggs can be had anywhere in the world.**
2. **The different structures of the embryonated egg can support a number of viruses.**
3. **The inoculation of eggs can be performed aseptically, even in primitive laboratories.**
4. **Fertilized eggs are free from specific and nonspecific factors of defense.**
5. **The production of virus is high so that this method is suitable for the production of vaccine and of antigen for diagnosis purpose**

Disadvantage:

Does the chick embryo produce Abs?

The eggs contain a fraction of maternal Abs. disadvantages when the inculcated eggs are to be used for cultivation of avian viruses

Egg Allergies & Vaccines

- Inoculation into the Allantoic cavity provides a rich yield of influenza. Other Allantoic vaccines include Yellow fever (17D strain).
- Flu viruses are injected into chicken egg embryos, where they multiply. After several days of incubation a machine opens the egg and harvests the virus, which is then purified and chemically killed.
- The egg is inoculated with a mixture of the epidemic influenza virus strain & a standard strain that can replicate in chicken eggs. Both strains replicate themselves, but as they do so their genetic material becomes mixed, producing hybrid viruses known as reassortants. The reassortants are analyzed, and those which have the epidemic strain surface proteins but other genes of the standard strain will be selected. These are injected into different eggs to replicate before harvesting.

Inoculation of Virus in Animals

Laboratory animals used in:

- Isolation of coxsackie viruses A & B by inoculation in white suckling mice.
- Rabies virus inoculation in mice.

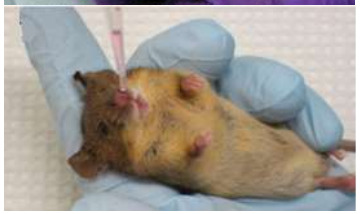
Laboratory animals include:

- Mice, hamster, rabbit, guinea pigs, monkeys and ferrets are widely used for cultivation virus.
- Mice are the most widely employed animals in virology.

Inoculation of Virus in Animals

The different routes of inoculation in mice are:

- Intracerebral injection
- Intramuscular injection
- Subcutaneous injection
- Intravenous injection
- Intraperitoneal injection
- Or intranasal infection.



- **Recognition:** After the animal is inoculated with the virus suspension, the animal is:
  - Observed for signs of disease
  - Visible lesions
  - Paralysis (flaccid or spastic)
  - Hemorrhage
  - Death or killed the animal so that infected tissues can be examined for virus.

Flaccid paralysis

Spastic paralysis

- **Identification:** immunoassay

Advantages of using animal systems

- Some viruses cannot be propagated in vitro.
- Gives unique insight into viral-host relation
- To study the pathogenesis.
- To study vaccine safety

Disadvantages of using animal systems

- Expensive, maintenance and time consuming.
- Whole animal is a complex system.
- Interference of immune system
- Results are not always reproducible due to host individual variation.
- Difficulty in choosing of animals for particular virus.
- Leads to generation of escape mutants.
- Animal welfare issues.
- Virus isolation in animals is inferior to the molecular techniques like PCR.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