Lab#7 Embryonated egg culture for viruses

Embryonated chicken egg inoculations were first used with propagation of virus by Roux and Marfey in 1911. In 1931 Woodruff and Goodpasture cultivated fowl poxvirus on the chorioallantoic membrane. Burnet used chicken embryo for cultivation of viruses very extensively. Since that time and until mid 1950 almost all research and testing with viruses depended on animal or embryonated eggs for source of living tissue. Later on there were replaced gradually by tissue culture which is now most commonly used for propagation of viruses. The embryonating egg is nevertheless still a convinient and easily manipulated source of living tissue for propagation and testing of virus.

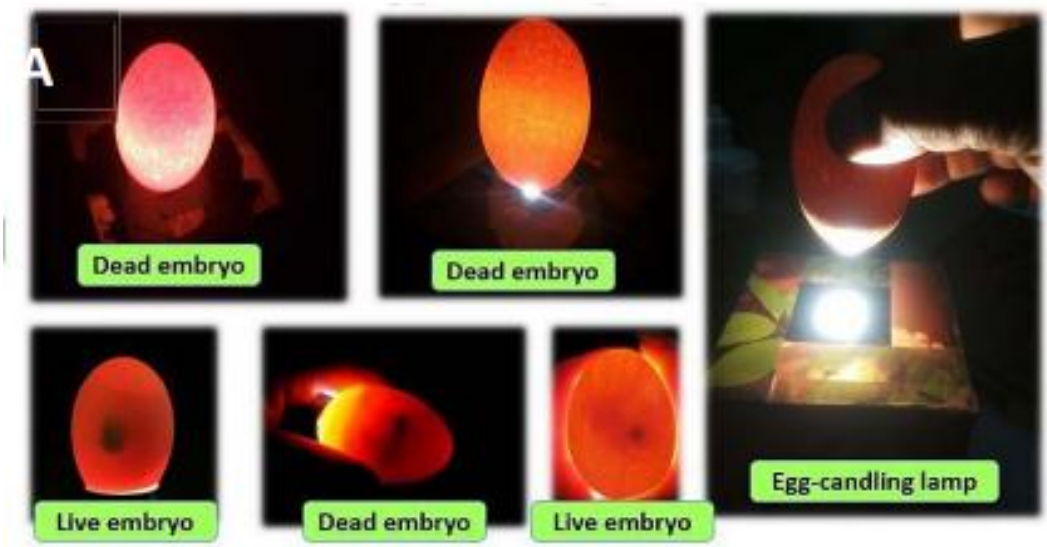
The use of fertile hen's egg (Chicken, duck, and turkey eggs) in diagnostic virology has a number of advantages:-

1. Readily available, cheap and easily maintained.
2. Sheltered from the natural diseases often observed in laboratory animals, and are relatively free from bacterial and many latent virus infection.
3. Easily manipulated under sterile conditions.
4. Generally free from natural factors of defence, specific or non-specific, that some time intervene and prevent passage in adult animals.
5. Sensitive to some virus that are harmless to the adult birds.
6. Easily identified and labeled with the details of the date, nature of the virus and the experimental procedure.

Cultivation of Virus in Eggs

1. **Determination of Viability/Egg:** The viability of embryo and the progress of embryo development is determined by candling. The egg at

interval during incubation candling consist of giving the egg against the constructed light source preferably in dark room so that the shadow of embryo and associated structure specially blood vessels are visible for routine purpose, the healthy embryo shows the characteristics movement will impart an orange yellow color to its egg because of blood circulating in vessel. In embryo which is dead or dies will have low, no; or sluggish movement and will be easily detached because of the diminish of vessel or their complete absence. If the embryo has grown large enough nearly to fill the egg lack of motion may be only due to death: An egg obtaining a small embryo (dead) will usually have a cleared yellow color. Any coloration tending to green or black is an indication of extreme contamination and such egg should be carefully removed and placed in a discard container. Candling also enables in selecting point of entry through the shell.



2. **Sterilization of egg** - Egg shell is sterilized with the swab tightly squeezed in tincture of iodine, taking care that not too much of the solution soak into the shell. Alternatively 70% ethyl alcohol may be used .

3- Marking the inoculation site:

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1. Hold the blunt end of the egg against the candling lamp and note the position of the head of the embryo.

2. Turn the egg a quarter turn away from the head.

3. Draw a line on the shell marking the edge of the air sac.

4. Draw an X approximately 2 mm above this line.

5. The X marks the inoculation site.

4- Piercing a hole in the egg shell by using Egg shell punch or with needle

5- Injecting Infective virus with needle (Routes of Egg Inoculation)

An embryonated egg offers various route for the inoculation/ cultivation of viruses;

1. Chorioallantoic membrane(CAM) ; age of embryo (10-12) day
2. Amniotic Cavity; age of embryo (9- 12) day
3. Allantoic Cavity; age of embryo (7-15) day
4. Yolk sac ; age of embryo (5-8) day

The volume of inoculum in these four route is 0.1 ml

1. Chorioallantoic membrane (CAM): Many viruses grow readily or can be adapted to grow on the 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

2. 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. Newly isolated influenza viruses may require several passages before they adapt to growth by other routes, such as allantoic.

3. 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.

4. 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.

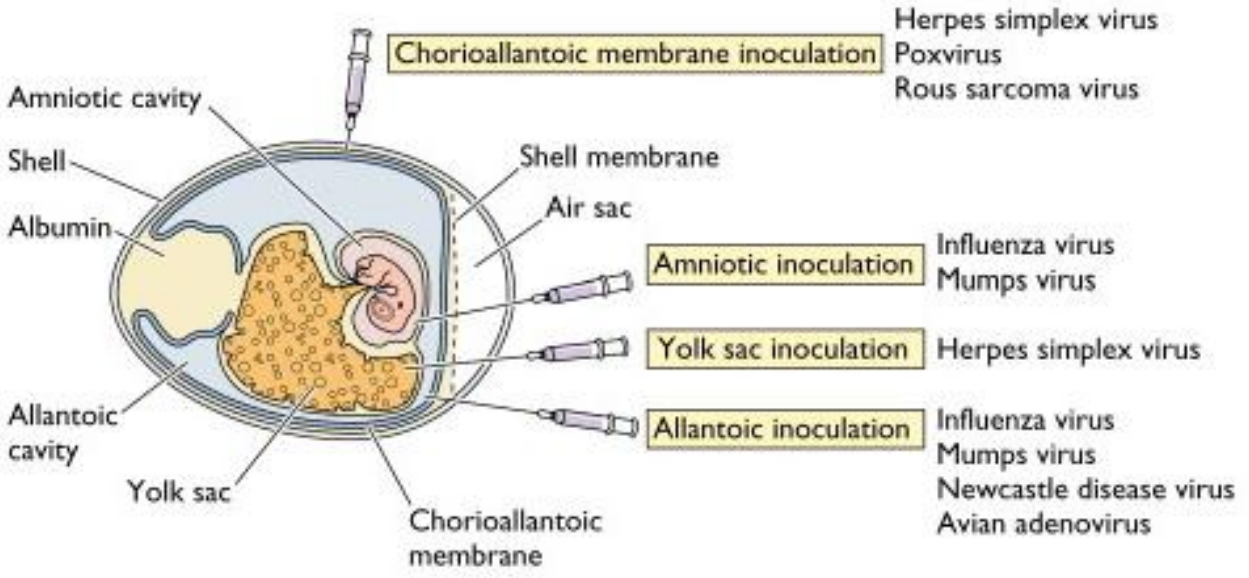
6- Overview of Inoculating Sites for Detecting of viral growth:

Viruses multiplying in embryos may or may not cause effects visible to the naked eye. The signs of viral growth include:

- Death of the embryo
- Defects in embryonic development
- and localized areas of damage in the membranes, resulting in discrete opaque spots called pocks

If a virus does not produce obvious changes in the developing embryonic tissue, virologists have other methods of detection. Embryonic fluids and tissues can be prepared for direct examination with an electron microscope. Certain viruses can also be detected by:

- their ability to agglutinate red blood cells
- or by their reaction with an antibody of known specificity



Materials Needed for Egg Inoculation with influenza virus
Eggs:11-day old or 12-day old embryonated eggs.

Cotton wool. A 70 percent alcohol solution in water.

Candle the eggs and mark the inoculation sites.(a non-veined area of the allantoic cavity just below the air sac)

Egg shell punch or using syringe

Needles preferably a 1 ml syringe fitted with a 1/2 inch, 27 gauge needle.

Stationery tape (also called cello or sticky tape) or melted wax to seal the inoculation site.

Inoculum. This must be free of microbial contamination.

Discard tray.

Eggs as Tools for Developing Influenza Vaccines

Influenza vaccine is manufacture in eggs. Fertilized chicken eggs can be used to produce vaccines against influenza viruses. 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. So, people with egg allergies cannot tolerate the influenza vaccines.