

VIROLOGY

Isolation & cultivation of Virus



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. Three :Stage

المرحلة الثالثة

...محاضرة رقم 4...

الجانب العملي

Lecture No.4 ..

Practical

تدريسي المادة : م. د. سيف علي

Isolation of Virus

The method used for isolation of virus depend upon the virus sought. In general they consist of inoculation into animals, eggs or tissue culture.

After that isolates are identified by neutralization or other suitable serological process. Isolation of virus from patient does not always means that it is the causative agent of the patients illness.

Many viruses like adeno viruses, entero virus etc. are frequently found in normal individuals. Thus it is imperative to interpret the isolation of virus in light of clinical data

Types of specimens that used for virus isolation

- ◆ **Blood** / used to detect CMV, enteroviruses and adenoviruses (5-10 ml) of anticoagulated blood collected tube.
- ◆ **Urine** / used to detect CMV, mumps, rubella, measles, polyomaviruses and adenoviruses can be detected in urine.
- ◆ **Rectal swabs and stool specimens** / used to detect rotavirus and enterovirus

Tissue / useful for detecting viruses that infect ♦ •
lung and gastrointestinal tract, specimens are
.collected during surgical procedures

Nasopharyngeal swab / for the detection of ♦ •
respiratory viruses such as influenza virus A and
.B or parainfluenza virus

Isolation and identification of viruses remains necessary **because** it allows the detection of multiple viruses and it is the only technique capable of providing a viable isolate . that can be used for antiviral susceptibility testing

Three methods are employed for the virus cultivation.

.Inoculation of virus into animals .

.Inoculation of virus into embryonated eggs .2

Tissue culture or cell culture .3



Inoculation of Virus in Animals Laboratory

Animals are widely used for routine cultivation of virus; they
.play an essential role in studies of viral pathogenesis

Live animals such as monkeys, mice, rabbits, guinea pigs,
ferrets are widely used for cultivating virus. Monkeys were
.used for the isolation of Poliovirus

But due to their risk to handlers, monkeys find only limited applications in Virology

.Mice are the most widely employed animals in virology

The different routes of inoculation in mice are intracerebral, subcutaneous, intraperitoneal or

.intranasal

After the animal is inoculated with the virus suspension, the animal is observed for signs of disease, visible lesions or is killed so that infected tissues can be examined for virus

Inoculation of Virus into Embryonated eggs

Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs

Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus. The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.

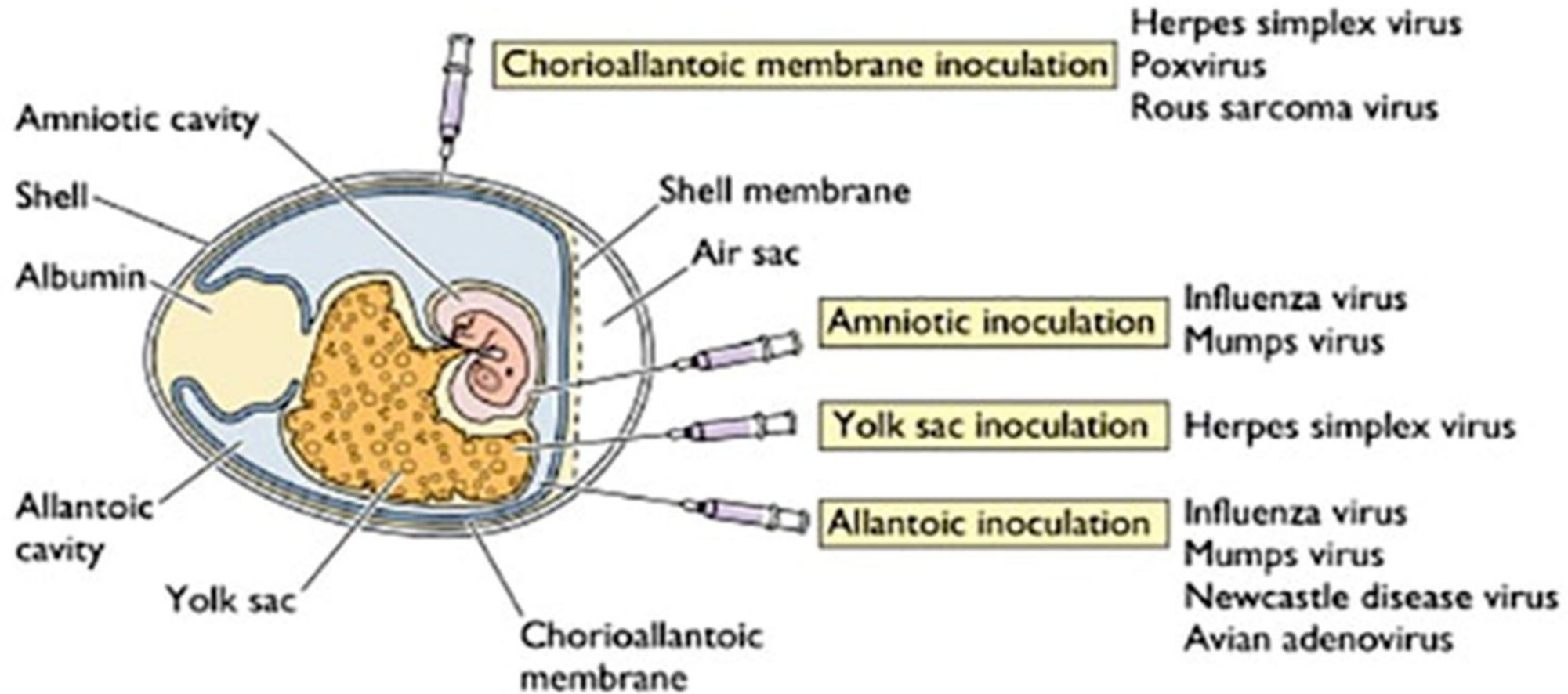
The egg used for cultivation must be sterile and the shell should be intact and healthy.

A hole is drilled in the shell of the embryonated egg, and a viral suspension or suspected virus- containing tissue is injected into the fluid of the egg.

Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes.

An embryonated egg offers various sites for the cultivation of viruses.

Inoculation into embryonated egg :-



The different sites of viral inoculation in embryonated eggs are

.Chorioallantoic membrane(CAM)

- .Amniotic Cavity
- .Allantoic Cavity
- .Yolk sac

..Embryo

.Air sac.

- **Chorioallantoic Membrane(CAM)**

Is mainly employed in the growth of poxvirus. Virus growth and replication in the CAM is indicated by visible lesions (pocks); grey white area in transparent CAM. Herpes simplex virus is also grown.

Amniotic Cavity

The amniotic sac is employed inoculated for primary isolation of influenza a virus and the mumps virus. Growth and replication of virus in egg embryo can be detected by haemagglutination assay.

Allantoic Cavity

Is the most popular and simple method for viral inoculation. Allantoic inoculation is employed for the growth and replication of the influenza virus for vaccine production.

This will provide a rich yield of influenza and some paramyxoviruses. Other allantoic vaccines include Yellow fever and rabies vaccines.

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.

Cell culture(Tissue Culture)

is the process by which cells are grown under controlled conditions, generally outside of their natural environment.

Cell culture conditions can vary for each cell type, but artificial environments consist of a suitable vessel with substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (CO₂, O₂), and regulates the physio-chemical environment (pH buffer, osmotic pressure, temperature).

Tissue culture

Tissue culture is often a generic term. Three types of tissue cultures are available

a. **Organ culture**

Small bits of organs are maintained in vitro for days & weeks. They are used for cultivation of viruses which can be grown only in specific organ.

b. **Explants culture**

Fragments of minced tissues is grown as explants embedded in plasma clot. Eg. Adeno tissue explants for adenovirus.

c. **Cell culture**

It is routinely used based on their origin. Chromosomal characters & the number of generation through which they can be maintained, cell cultures are classified into 3 types

Cell culture

1. Primary cell culture

- These are normal cells derived from animal or human cells.

They are able to grow only for limited time and cannot be maintained in serial culture.

They are used for the primary isolation of viruses and production of vaccine.

- Examples: Monkey kidney cell culture, Human amnion cell culture human cells.

2. Diploid cell culture

- They are diploid and contain the same number of chromosomes as the parent cells

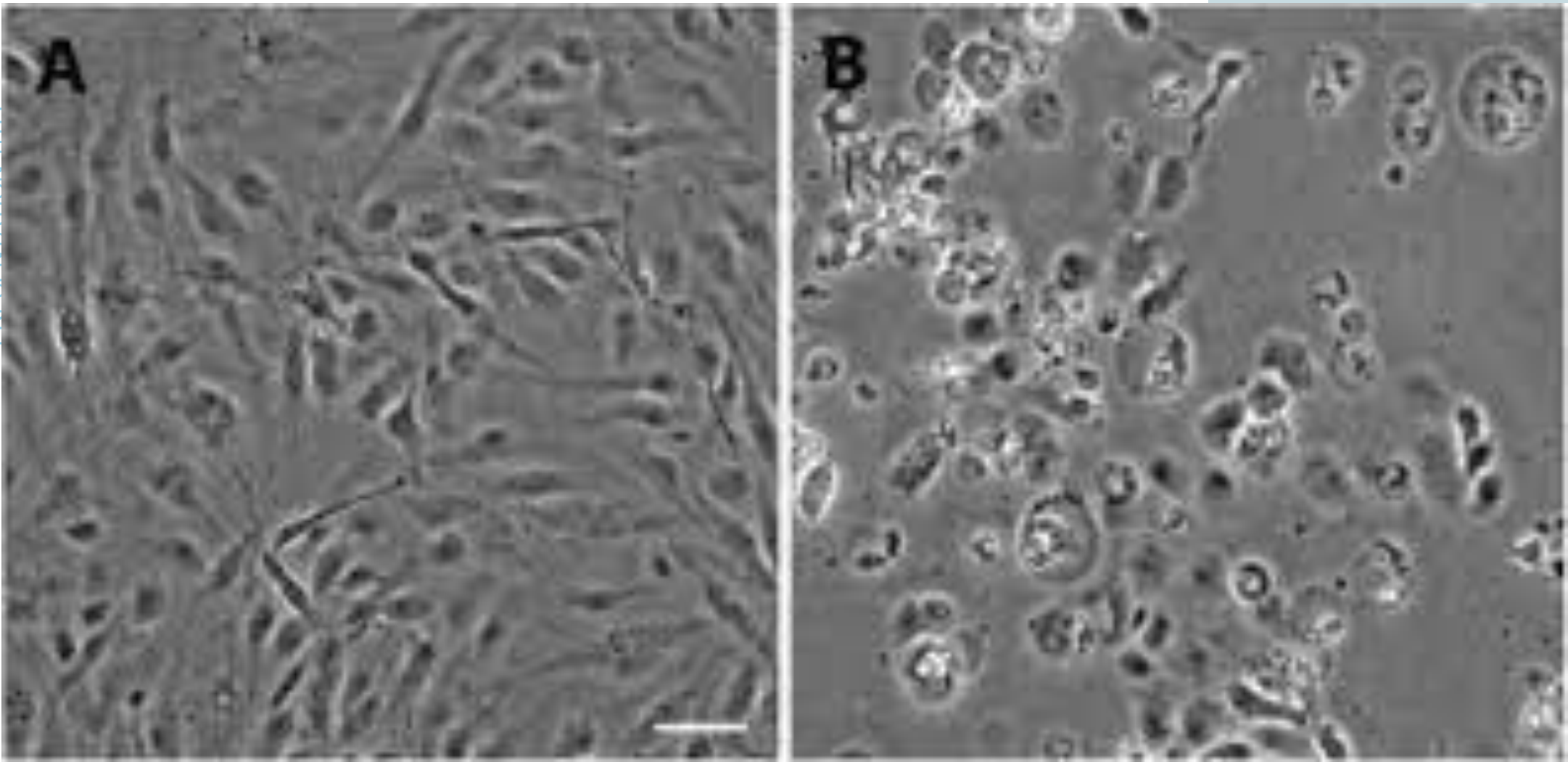
They are used for the isolation of some fastidious viruses and production of viral vaccines. Examples: Human embryonic lung strain,

3. Heteroploid cultures (Continuous cell lines):

. They are derived from cancer cells. They can be serially cultured indefinitely so named as continuous cell lines. These cell lines may be maintained by serial sub-cultivation or stored in the cold (-70°C) for use when necessary.

Due to derivation from cancer cells they are not useful for vaccine production.

. Examples: HeLa (Human Carcinoma of cervix cell line), HEP-2 (Human Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).



Cytopathic effect: The cytopathic effect was apparent as refractile, rounded, swollen, and semiattached cells, and areas of clearance were compared to those of the uninfected control cells

Any questions?

*Thank
You*