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Practical virology
2023-2024

Tissue culture

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Tissue culture

Is the growth of tissues or cells in an artificial medium separate from the parent organism. This technique is also called micro propagation.

This is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or agar.

Tissue culture commonly refers to the culture of animal cells and tissues, with the more specific term plant tissue culture being used for plants.

The term "tissue culture" was coined by American pathologist Montrose Thomas Burrows. This is possible only in certain conditions. It also requires more attention. It can be done only in genetic labs with various chemicals.

Steinhardt and colleagues used tissue culture for the first time in diagnostic virology in 1913.

Tissue culture is an important tool for the study of the biology of cells from multicellular organisms. It provides an in vitro model of the tissue in a well defined environment which can be easily manipulated and analyzed.

In animal tissue culture, cells may be grown as two-dimensional monolayers (conventional culture) or within fibrous scaffolds or gels to attain more naturalistic three-dimensional tissue-like structures (3D culture).

Applications of animal cell culture

Animal cell culture is used for many research purposes and commercial business also as:

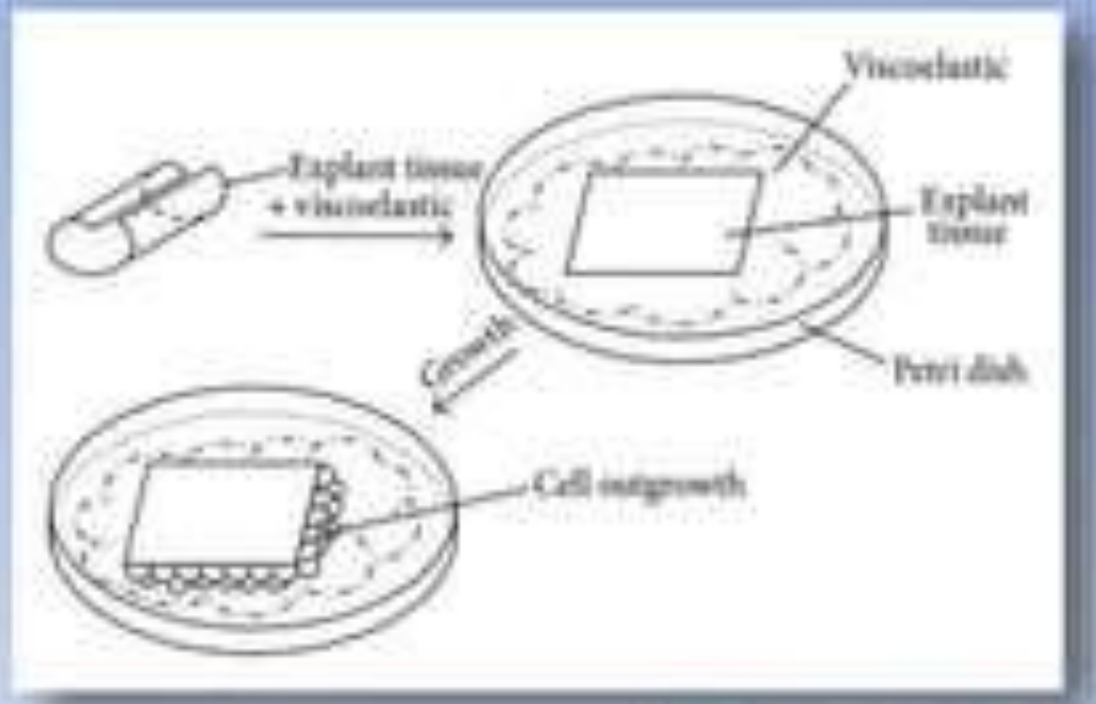
1-Vaccine production
2-Monoclonal antibody production
3-Enzymes and hormones production
4-In vitro skin and other tissues and organs by stem culturing
5-Viral cultivation.

Cell culture systems can facilitate virus growth and elucidate development and interactions with host cells at every stage of replication.

Methods of animal tissue culture

There are three common methods to establish cell culture from animals.
The first is organ culture; involves growing whole an organ from embryos or partial adult organs in a laboratory [in vitro], are mainly done for highly specialized parasites of certain organs e.g., tracheal ring culture is done for isolation of coronavirus.
where organs are used to initiate the organ culture .
These cells retain their differentiated character and functional activity in organ culture.

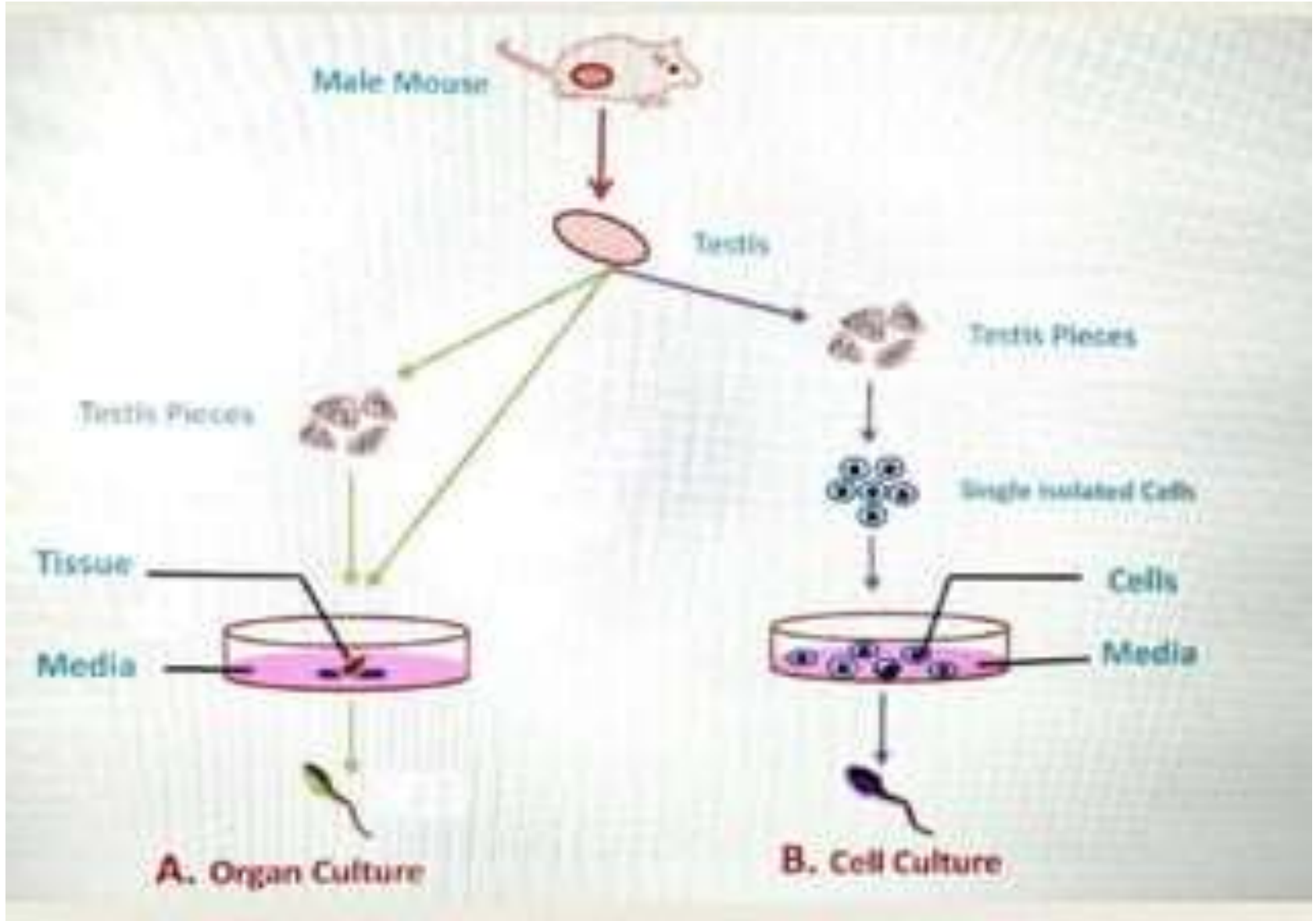
The second method is primary explant culture, in which fragments derived from animal tissue are attached to a surface using an extracellular matrix component (ECM), such as collagen or a plasma clot.
This culture is known as a primary explant, and migrating cells are known as outgrowth.
This has been used to analyze the growth characteristics of cancer cells in comparison to their normal counterparts.
Explant culture is rarely done.



explant culture

The third method is cell culture; involves growing cells in a laboratory, which there are three types:

(1) precursor cell culture, i.e. undifferentiated cells that are to be differentiate.
(2) differentiated cell culture, i.e. completely differentiated cells that have lost the capacity to further differentiate.
(3) stem cell culture, i.e. undifferentiated cells that can develop into any kind of cell.
Cell culture is mostly used for identification and cultivation of viruses.


Male Mouse
Testis
Testis Pieces
Testis Pieces
Single Isolated Cells
Tissue
Media
Cells
Media
A. Organ Culture
B. Cell Culture

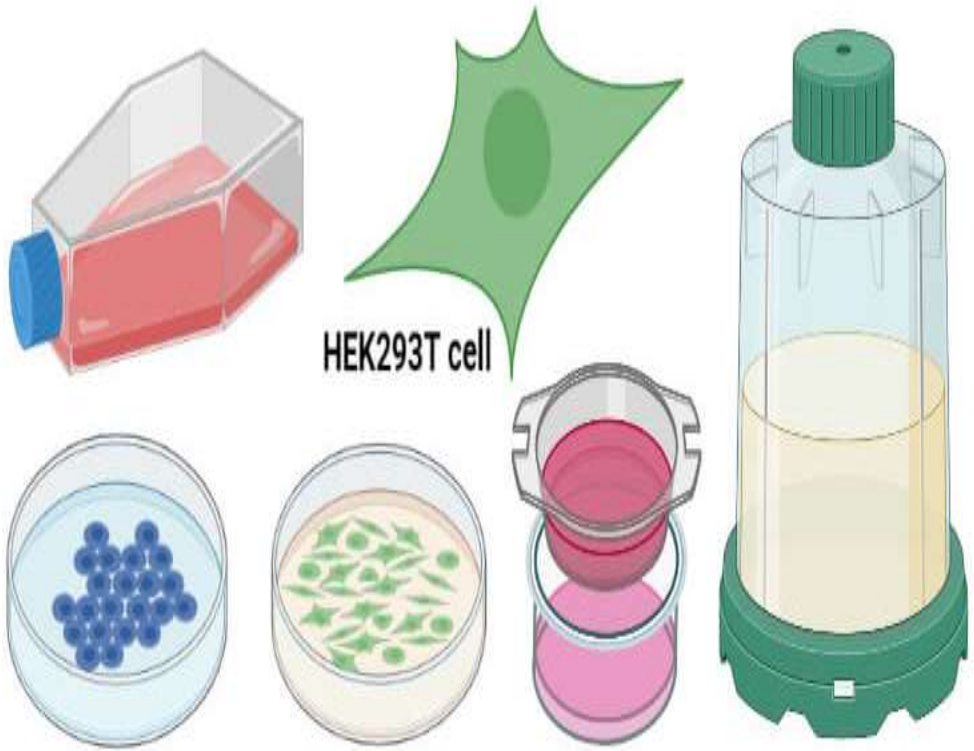
Cell culture is the process by which cells are grown under controlled conditions.

- Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.

At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.

- On incubation the cell divides and spread out on the glass surface to form a confluent monolayer.

The most important cell lines widely used for viral diagnosis are primary rhesus monkey kidney cells (RhMK), primary rabbit kidney cells, MRC-5, human foreskin fibroblasts, HEp-2, and A549


HEK293T cell

Types of 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.

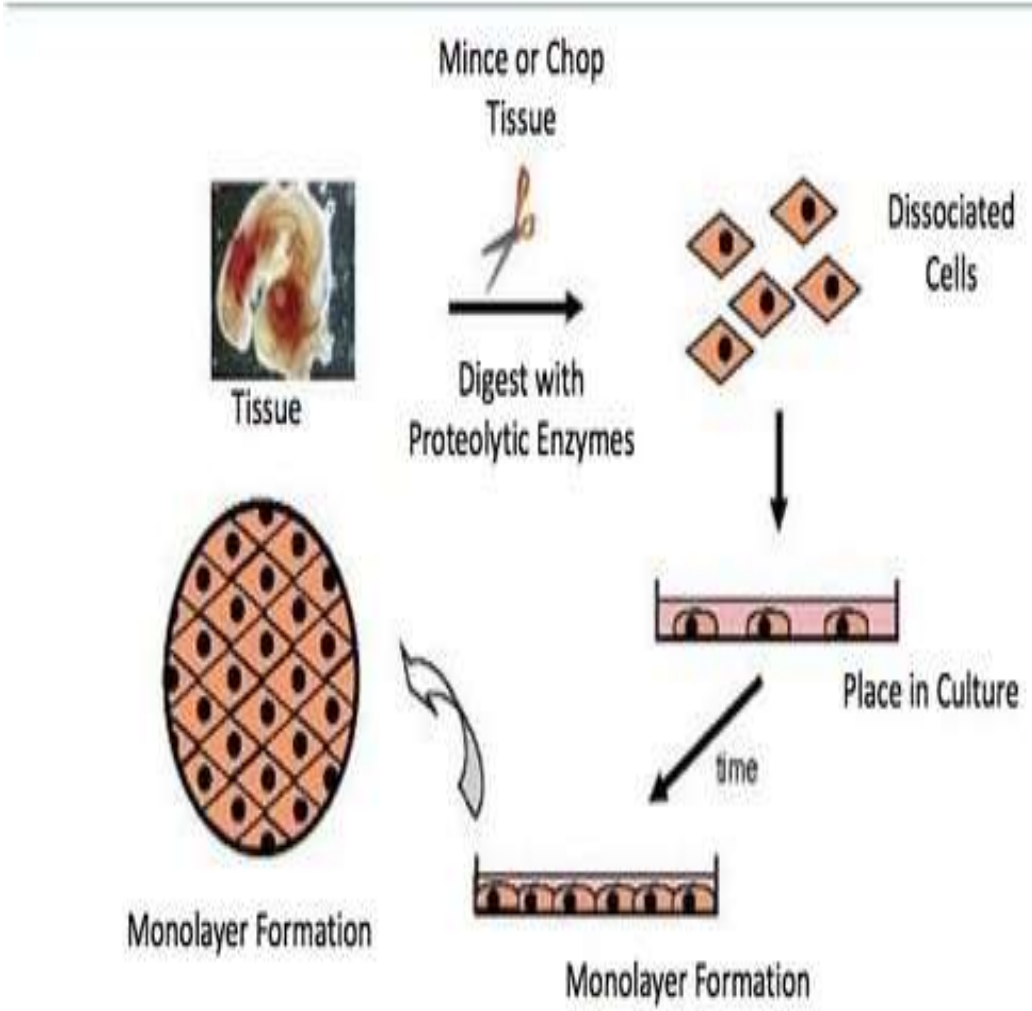
Examples: Monkey kidney cell culture, Human amnion cell culture

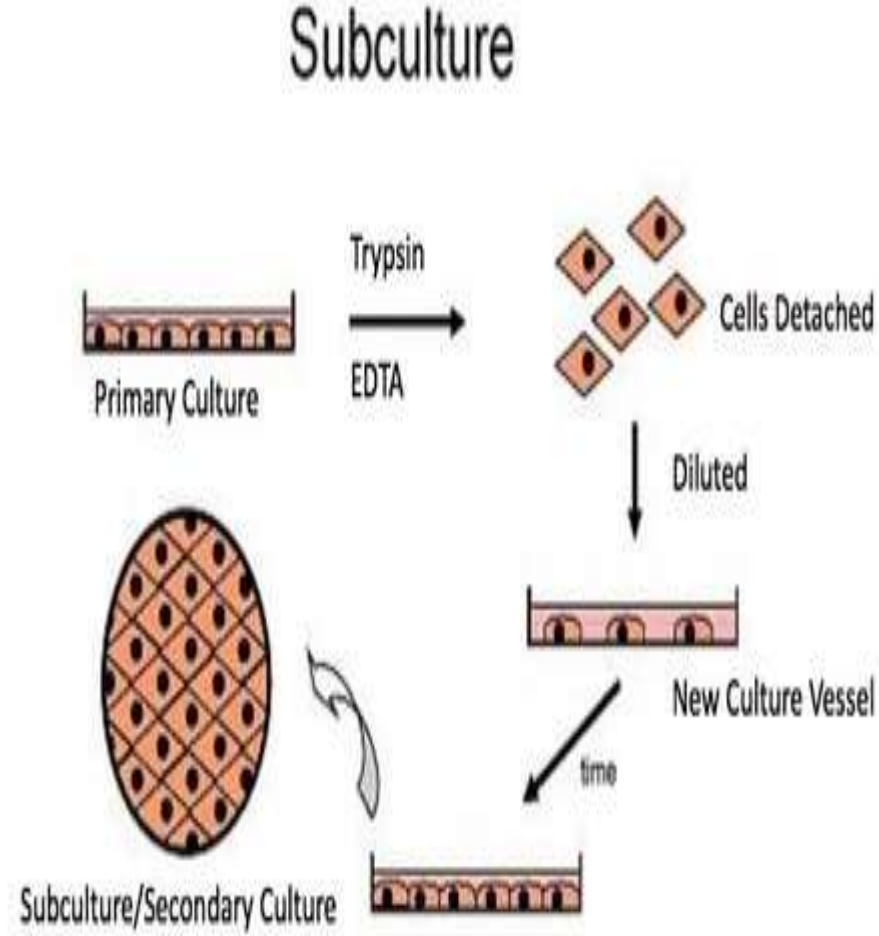
2. Diploid cell culture (Semi-continuous cell lines):

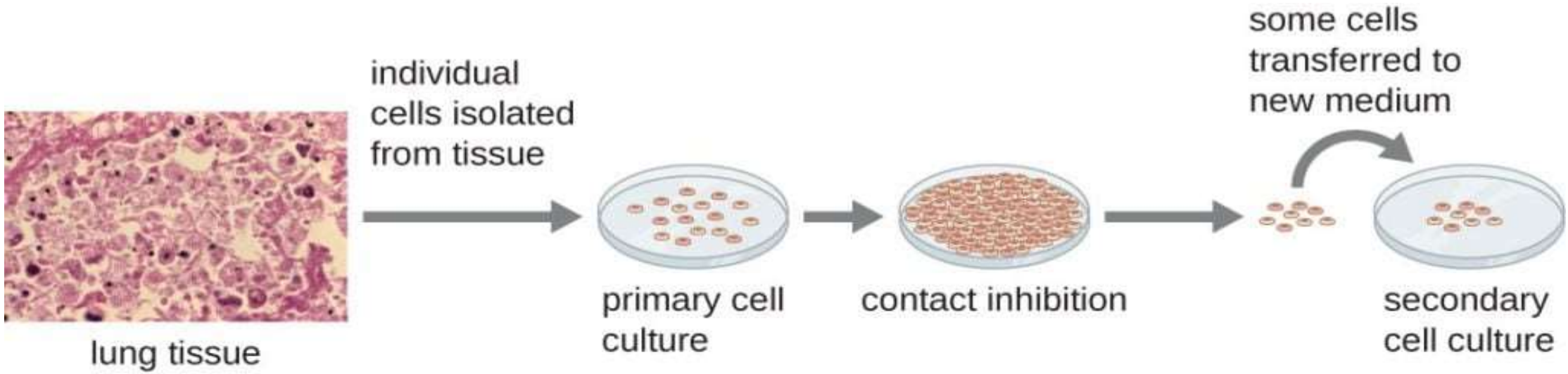
- They are diploid and contain the same number of chromosomes as the parent cells. •

They can be sub-cultured up to 50 times by serial transfer following senescence and the cell strain is lost.

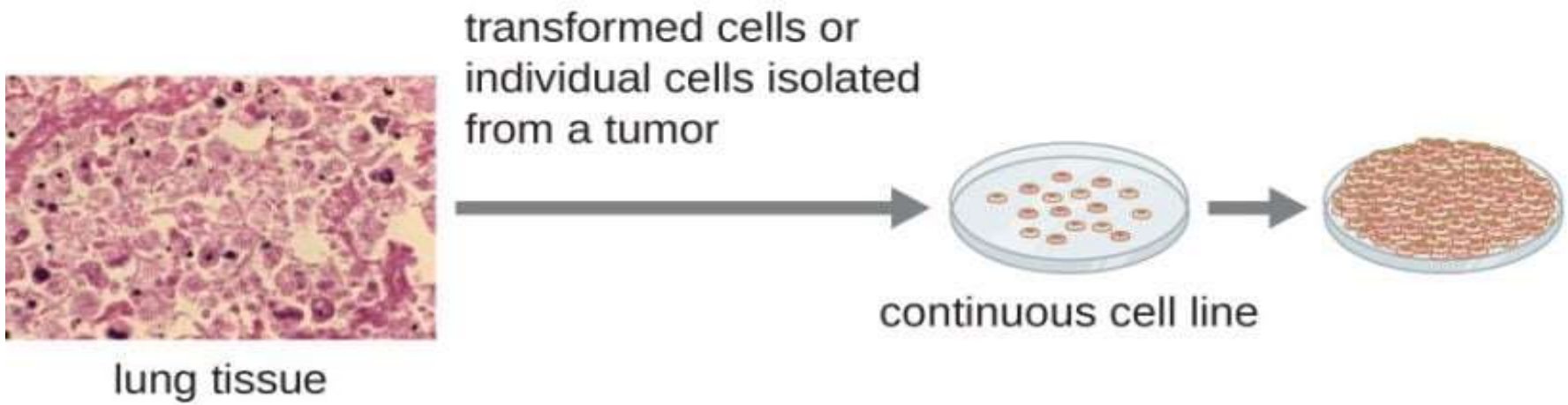
- They are used for the isolation of some fastidious viruses and production of viral vaccines. • Examples: Human embryonic lung strain, Rhesus embryo cell strain


Mince or Chop
Tissue
Tissue
Digest with
Proteolytic Enzymes
Dissociated
Cells
Place in Culture
time
Monolayer Formation
Monolayer Formation


Subculture
Trypsin
EDTA
Primary Culture
Cells Detached
Diluted
New Culture Vessel
time
Subculture/Secondary Culture


some cells
transferred to
new medium
individual
cells isolated
from tissue
lung tissue
primary cell
culture
contact inhibition
secondary
cell culture

(a)


transformed cells or
individual cells isolated
from a tumor
lung tissue
continuous cell line

(b)

3. Heteroploid cultures (Continuous cell lines):

- They are derived from cancer cells.
- They can be serially cultured indefinitely so named as continuous cell lines
- They can be maintained either by serial subculture or by storing in deep freeze at -70°c.
- 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).

Sample collection

The specimen processing protocol varies between laboratories, but the main steps followed are as follows:

First, the medium containing the sample is vortexed and the swap is discarded. The liquid medium is then centrifuged. The supernatant obtained is used in cell culture. In this method, fungi, cells, bacteria, and blood remain at the bottom of tube (pellet form), whereas viruses remain dispersed in the liquid.

Then, 0.2–0.3 mL of the liquid is added to the cell culture medium for absorption of the virus (inoculation). The cell culture tube containing the virus for absorption is then incubated at 35°C and 5% CO_2 for 90 minutes, following which the inoculum is discarded and substituted with fresh medium.

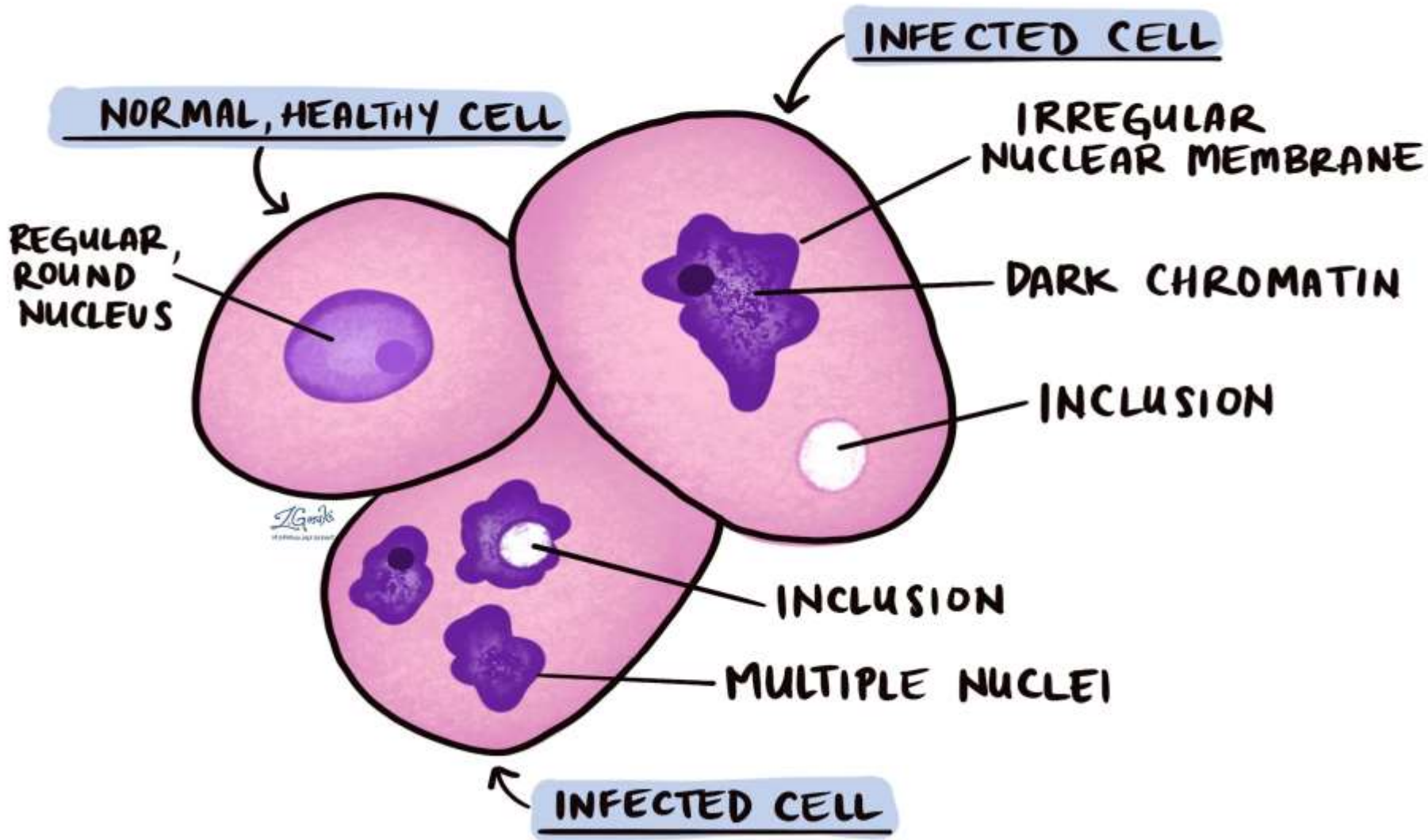
The cell culture tube is incubated until the virus begins to grow.
This process may take 1 day to several weeks depending on the type of virus. The cell culture tube is examined everyday using an inverted microscope.
The standard protocol applied for estimating the proliferation of the virus on monolayer cells involves examination of unstained cells on monolayer cells.
Changes in monolayer cells (e.g., swelling,shrinking, syncytium formation) indicate the presence of viruses. These changes in cell culture are defined as the cytopathic effect (CPE), which is due to the presence of the virus .

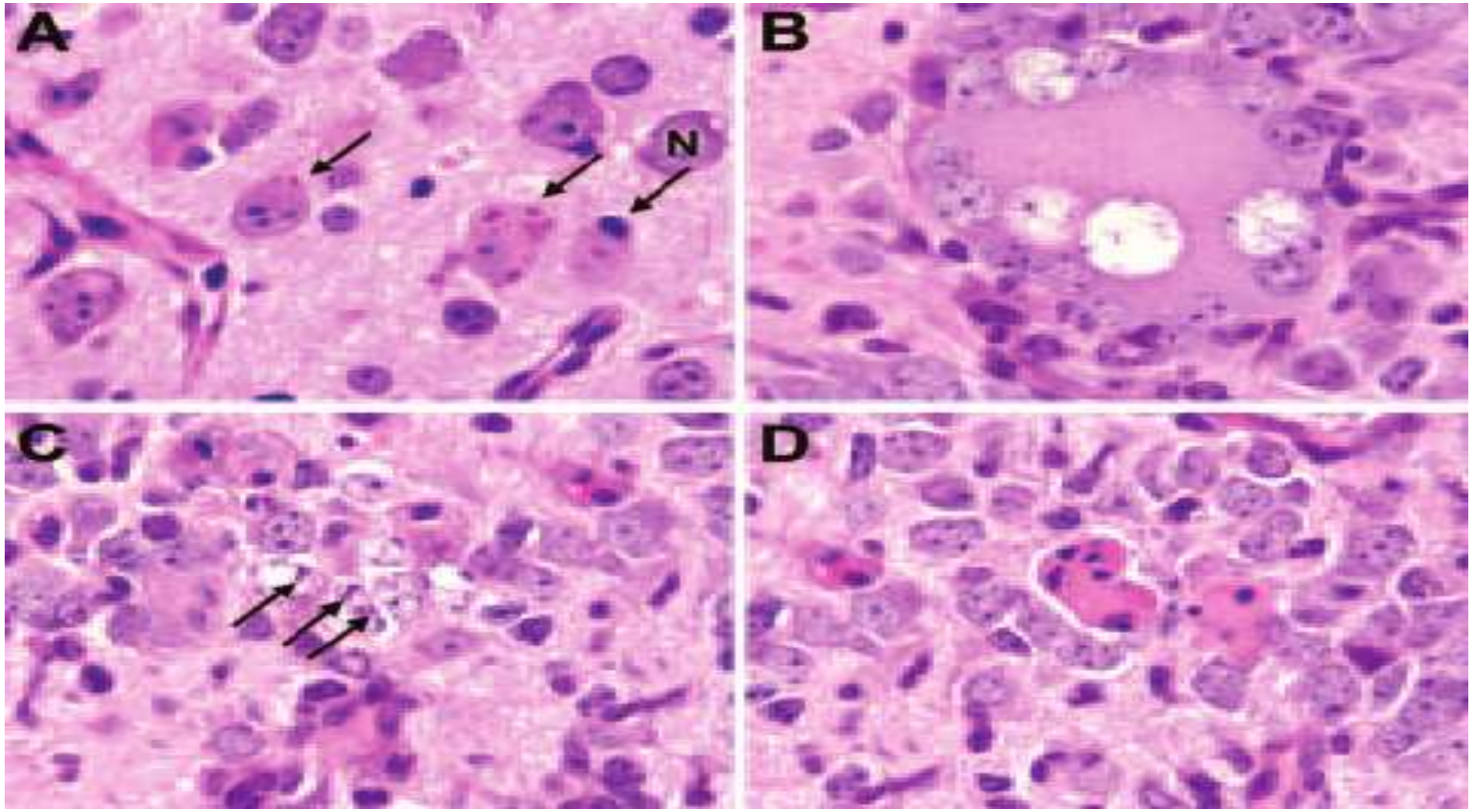
In most cases, the CPE appears after 5–10 days of incubation; however, an exception is herpes simplex virus (HSV) in which the CPE is observed after just 24 hours.

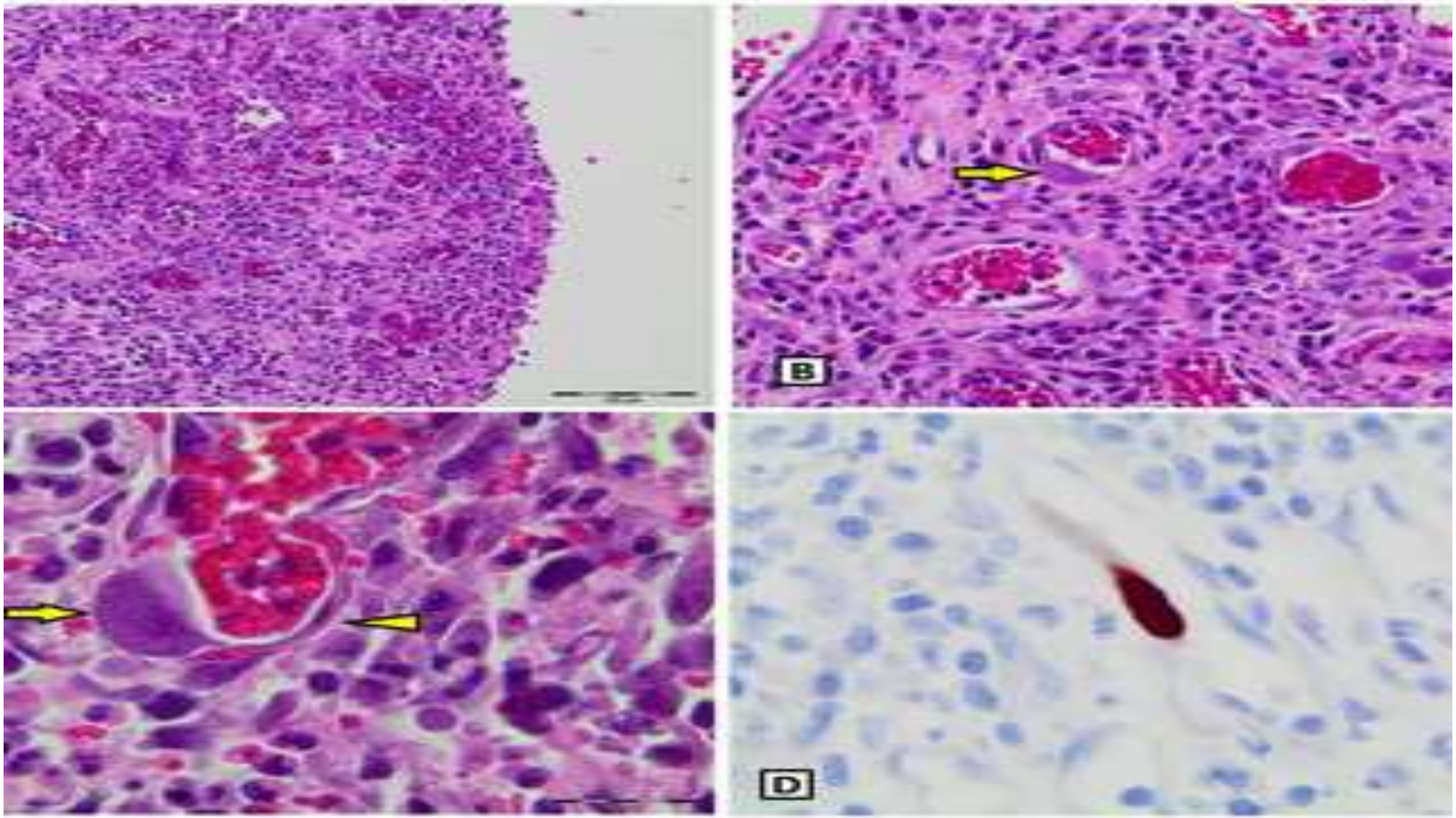
In some viruses, including cytomegalovirus (CMV), 10–30 days are needed after first incubation for CPE observation.

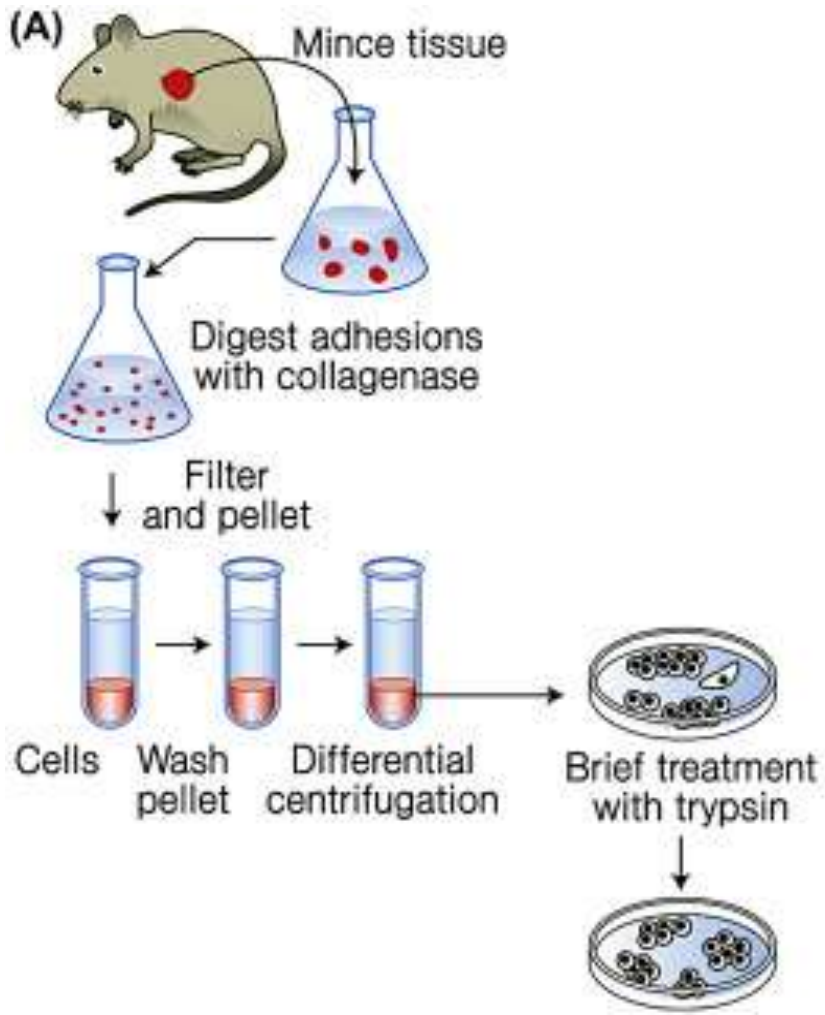
According to the type of cell line used for cell culture, type of specimen, the incubation period, and form of the CPE, the type of virus can be predicted; however, confirmatory testing such as immunofluorescence (IF) assay is needed for better diagnosis. This assay is based on the reaction between the antibody and viral antigen. Figure 2 shows the CPE formation by different types of viruses.

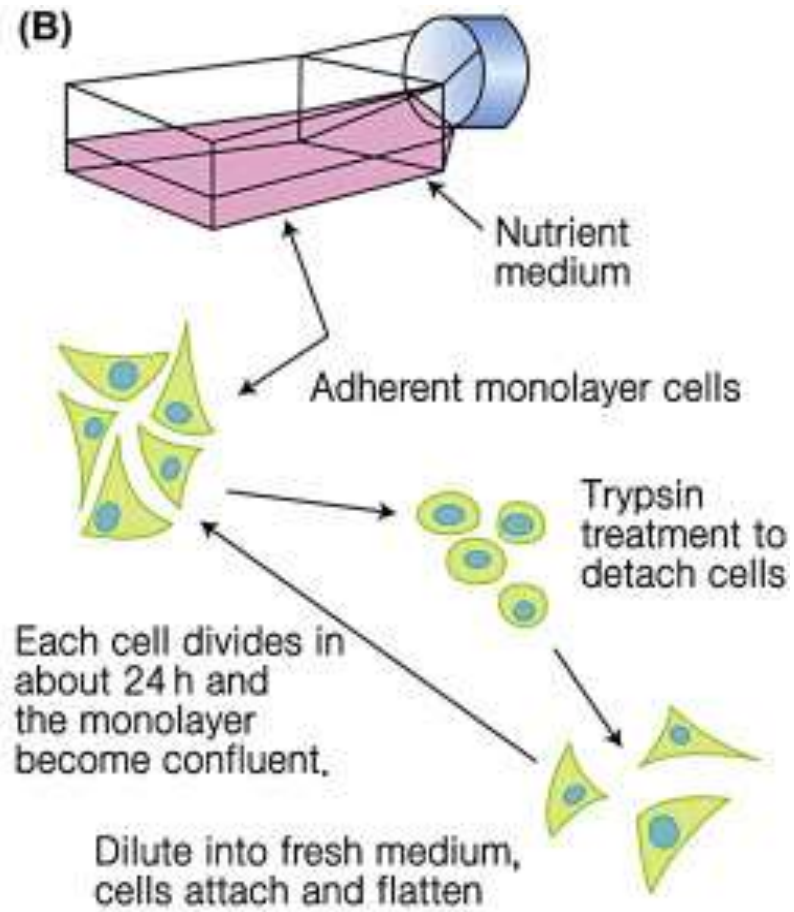
VIRAL CYTOPATHIC EFFECTS




A
N
B
C
D


B
D


(A)
Mince tissue
Digest adhesions with collagenase
Filter and pellet
Cells
Wash pellet
Differential centrifugation
Brief treatment with trypsin


(B)
Nutrient medium
Adherent monolayer cells
Trypsin treatment to detach cells
Each cell divides in about 24 h and the monolayer become confluent.
Dilute into fresh medium, cells attach and flatten