Medical laboratory techniques

(Lab 3-4)

Assist lect: Zahraa Abd AL_Ameer + Retaj aqeel dawood

Direct Examination

Diagnostic tests can be grouped into 3 categories:

(1) direct detection
(2) indirect examination (virus isolation)
(3) serology. In direct examination

Note : the clinical specimen is examined directly for the presence of virus particles, virus antigen or viral nucleic acids. In indirect examination, the specimen into cell culture, eggs or animals in an attempt to grow the virus: this is called virus isolation.

Direct Examination of Specimen

- Electron Microscopy morphology
- Light microscopy histological appearance - e.g. inclusion bodies
- Antigen detection immunofluorescence, ELISA etc.
- Molecular techniques for the direct detection of viral genomes

2) Indirect Examination

- Cell Culture - cytopathic effect
- Eggs pocks on CAM - haemagglutination, inclusion bodies
- Animals disease or death confirmation by neutralization

2) Serology

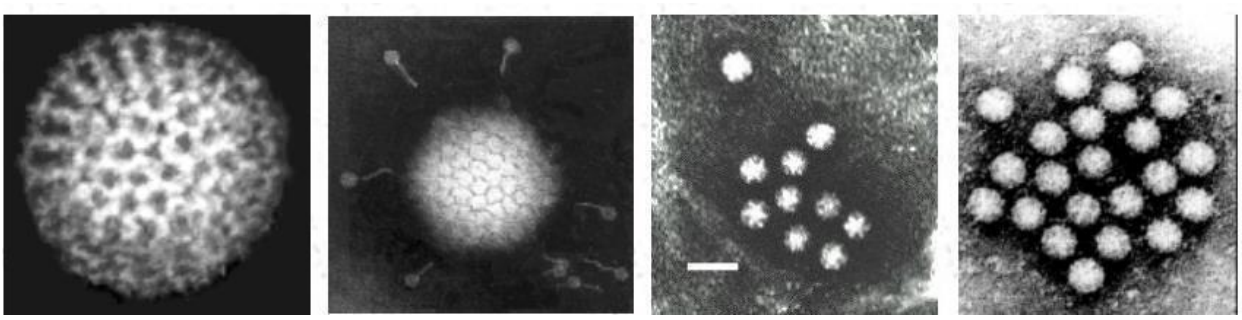
- Complement fixation tests (CFT)
- Enzyme linked immunosorbent assay
- Immunofluorescence techniques (IF)
- Particle agglutination

Electron Microscopy (EM)

1. Virus particles are detected and identified on the basis of morphology. A magnification of around 50,000 is normally used. EM is now mainly used for the diagnosis of viral gastroenteritis by detecting viruses in faeces e.g. rotavirus, adenovirus.
2. The sensitivity and specificity of EM may be enhanced by immune electron microscopy, whereby virus specific antibody is used to agglutinate virus particles together and thus making them easier to recognize, or to capture virus particles onto the EM grid.
3. The main problem with EM is the expense.

Types of electron microscope:

1- transmission EM (TEM): is used to image the interior of cells, the structure of protein molecules and he organization of molecules in viruses **2- scanning EM (SEM):** provides detailed images of the surfaces of cells of virus



Light Microscopy

1. Replicating virus often produce histological changes in infected cells. These changes may be characteristic or non-specific. Viral inclusion bodies are basically collections of replicating virus particles either in the nucleus or cytoplasm.
2. not sensitive or specific, serves as a useful adjunct in the diagnosis of certain viral infections.

Viral Genome Detection

Methods based on the detection of viral genome are also commonly known as molecular methods. It is the future direction of viral diagnosis. These techniques may allow for the quantification of DNA/RNA present in the specimen.

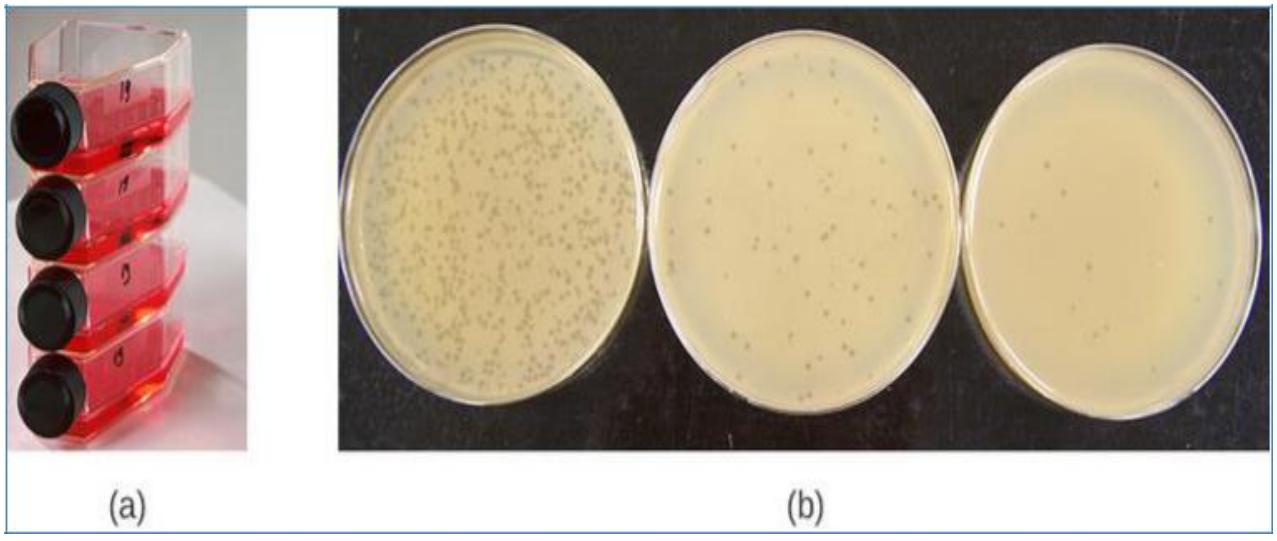
Isolation of Viruses:

Unlike bacteria, many of which can be grown on an artificial nutrient medium, viruses require a living host cell for replication. Infected host cells (eukaryotic or prokaryotic) can be cultured and grown, and then the growth medium can be harvested as a source of the virus.

Virions are capsid-encapsulated viruses with DNA or RNA molecules. in the liquid medium can be separated from the host cells by either centrifugation or filtration. Filters can physically remove anything present in the solution that is larger than the virions; the viruses can then be collected in the filtrate.

Cultivation of Viruses

Viruses can be grown in vivo (within a whole living organism, plant, or animal) or in vitro (outside a living organism in cells in an artificial environment, such as a test tube, cell culture flask, or agar plate). Bacteriophages; viruses that infect and replicate only in bacterial cells bacteriophage can be grown in the presence of a dense layer of bacteria (also called a bacterial lawn) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask (Figure a). For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a clear zone called a plaque is detected (Figure b). As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.



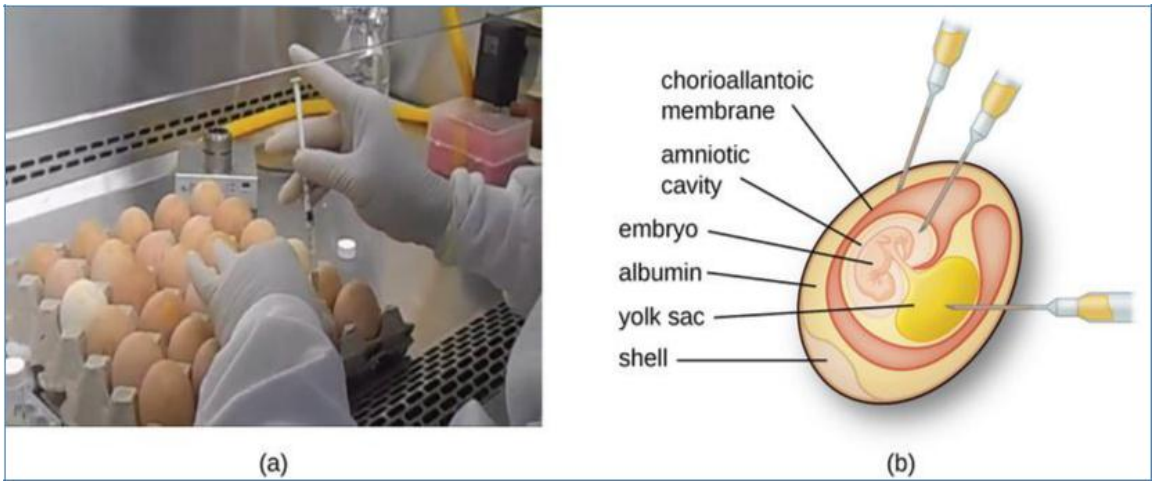
Animal viruses require cells within a host animal or tissue-culture cells derived from an animal. Animal virus cultivation is important for:

(1) identification and diagnosis of pathogenic viruses in clinical specimens,

(2) production of vaccines

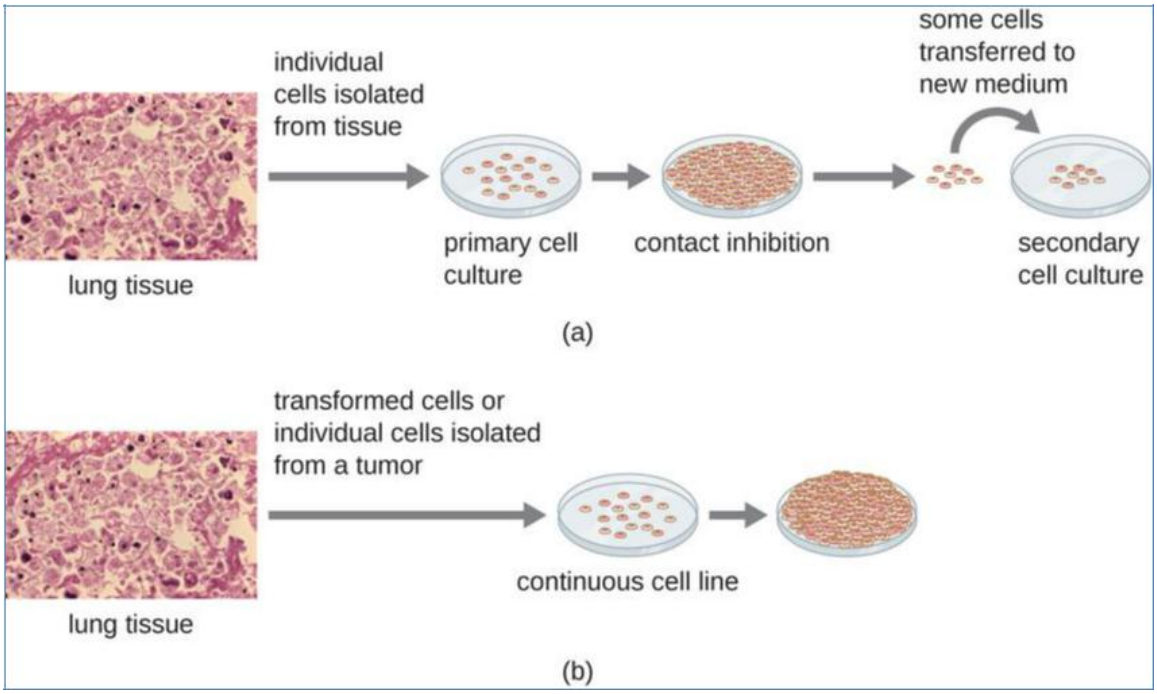
(3) basic research studies.

In vivo host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. The embryo or host animal serves as an incubator for viral replication.



For in vitro studies, various types of cells can be used to support the growth of viruses. Primary cell culture is freshly prepared from animal organs or tissues. Cells are extracted from tissues by mechanical scraping or mincing. To prevent contact inhibition, cells from the primary cell culture must be transferred to another vessel with a fresh growth medium. This is called secondary cell culture.

Periodically, cell density must be reduced by pouring off some cells and adding fresh medium to provide space and nutrients to maintain cell growth. In contrast to primary cell cultures, continuous cell lines, usually derived from transformed cells or tumours, are often able to be subcultured many times or even grown indefinitely (in which case they are called immortal).


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
continuous cell line
lung tissue
(b)