

Laboratory Diagnosis:

Specimen Selection and Collection:

Specimen selection depends on the specific disease syndrome, viral etiologies suspected and time of year.

1- Throat, nasopharyngeal swab or aspirate: Throat swabs are acceptable for recovering enteroviruses, adenoviruses and HSV, whereas nasopharyngeal swab or aspirate specimens are preferred for the detection of influenza and parainfluenza viruses. Throat specimens are collected by rubbing inflamed, vesiculated, or purulent areas of the posterior pharynx with a dry, sterile swab. Nasopharyngeal secretion specimens are collected by inserting a swab with flexible shaft through the nostril to the nasopharynx.

2- Rectal swabs and stool specimens: Used to detect rotavirus, enteric adenoviruses and enteroviruses. Rectal swabs are collected by inserting a swab (3-5) cm into the rectum to obtain feces.

3- Urine: CMV, mumps, rubella, measles, polymaviruses and adenoviruses can be detected in urine. Virus recovery may be increased by processing multiple (2-3) specimens because virus can be shed intermittently or in low numbers. The best specimen is at least (10 ml) of a clean-voided first-morning urine.

4- Blood: Used to detect CMV, HSV, enteroviruses and adenoviruses. (5-10 ml) of anticoagulated blood collected in a tube is needed. Heparinized, citrated or EDTA anticoagulated blood is acceptable for detection.

5- Tissue: Useful for detecting viruses that infect lung (CMV, influenza virus, adenovirus), brain (HSV), and gastrointestinal tract (CMV). Specimens are collected during surgical procedures.

Specimen transport and storage:

All specimens collected for detection of viruses should be processed by the laboratory immediately. Specimens for viral isolation should not be allowed to sit at room or higher temperature. Specimens should be placed in ice and transported to the laboratory at once. Under unusual circumstances, specimens may need to be held for days before processing. For storage up to 5 days, hold specimen at 4°C. storage for 6 or more days should be at – 20°C or preferably at – 70°C.

Viral transport media are used to transport small volumes of fluid specimens, small tissues and scraping, and swab specimens, especially when contamination with microbial flora is expected. Examples of successful transport media include Stuart's medium, Amie's medium, Hanks balanced salt solution and Eagle tissue culture medium.

Virus Detection Methods:

A- Virus isolation:

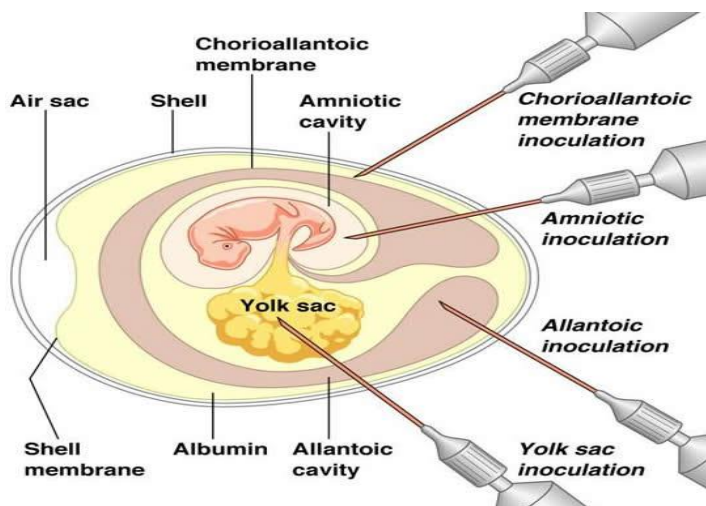
1- Embryonated eggs:

In order to cultivate viruses in eggs, the procedure adopted should be very simple. The eggs are kept in incubator and embryos of (7-12) days old are used. The egg containing embryo usually has an air space at the larger end. The position of this sac is first determined. The shell over the air sac is then cut off and removed. The membrane adjacent to the shell is then pierced. Usually the hypodermic syringe is used for piercing the shell. At this stage, the embryonic fluid may ooze out. The embryo then gets exposed and ready for use.

Virus suspension to be cultivated is taken in dropper and gently spread over the exposed embryo. After inoculation is thus completed, the open area of the shell is sealed eggs are incubated for one week as in hatching. The virus particles infect the membrane at random and create pock marked appearance against the transparent background. This indicate viral basis.

The embryonated offers several sites for the cultivation of viruses:

1. Chorioallantoic membrane.
2. Allantoic cavity.
3. Amniotic cavity.
3. Yolk sac.



2- laboratory animals:

Mice are still most widely used animals in virology. Mice can be inoculated through several routes, i.e. intracerebral, subcutaneous, intraperitoneal, intranasal. Other animals such as rabbits and ferrets are also used. The growth of virus in inoculated animals is indicated by death, disease or visible lesions.



3- Cell culture:

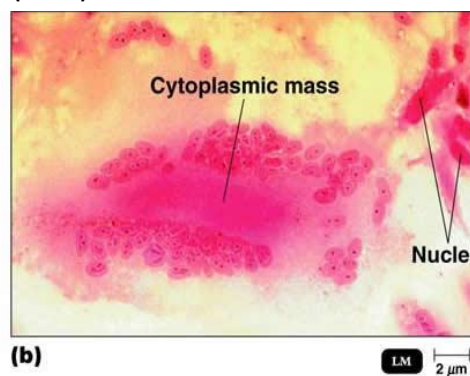
To detect virus using living cells, suitable host cells, cell culture media and techniques in cell culture maintenance are necessary.

Host cells referred to as cell cultures originate as a few cells and grow into a monolayer on the sides of glass or plastic test tubes. Cells are kept moist and supplied with nutrients by keeping them continuously immersed in cell culture medium.

Cell cultures are incubated in a roller drum that holds cell culture test tubes at 35 to 37°C. Incubation of cell culture tubes in a stationary rack can be used in place of a roller drum. To counteract the pH decrease, a bicarbonate buffering system is used in the culture medium to keep the cells at physiologic pH (pH 7.2). Phenol red a pH indicator that is red at physiologic, yellow at acidic and purple at alkaline once inoculated with specimen, cell cultures are incubated for (1-4) weeks depending on the viruses suspected. Periodically the cells are inspected microscopically for the presence of virus, indicated by areas of dead or dying cells called cytopathic effect (CPE).



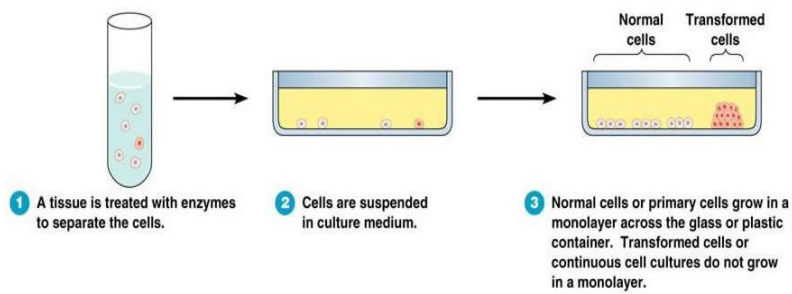
(a) Cytoplasmic inclusion body caused by rabies virus in brain tissue.



(b) cell fusion due to infection by measles virus.

Two kinds of media, growth medium and maintenance medium, are used for cell culture. Usual antimicrobials added are vancomycin (10 $\mu\text{g/ml}$), gentamicin (20 $\mu\text{g/ml}$) and amphotericin (2.5 $\mu\text{g/ml}$). A cell culture becomes a cell line once it has been passed or subcultured *in vitro*. Cell line are classified as:

- 1.Primary cell lines, ex. Primary monkey kidney cell.
- 2.Low passage (semi-continuous) Cell lines, ex. Lung fibroblast.
- 3.Continuous cell lines, ex. Human epidermoid carcinoma cells.



If the virus does not produce a CPE, its presence can be detected by several other techniques:

(1) **Hemadsorption** (i.e., attachment of erythrocytes to the surface of virus-infected cells). This technique is limited to viruses with a hemagglutinin protein on their envelope, such as mumps, parainfluenza, and influenza viruses.

2) **Interference** with the formation of a CPE by a second virus. For example, rubella virus, which does not cause a CPE, can be detected by interference with the formation of a CPE by certain enteroviruses, such as echovirus or Coxsackie virus.

(3) **A decrease in acid production by infected**, dying cells. This can be detected visually by a color change in the phenol red (a pH indicator) in the culture medium. The indicator remains red in the presence of virus infected cells but turns yellow in the presence of metabolizing normal cells as a result of the acid produced. This technique can be used to detect certain enteroviruses.

4. **A definitive identification** of the virus grown in cell culture is made by using known antibody in one of several tests. Complement fixation, hemagglutination inhibition, and neutralization of the CPE are the most frequently used tests.

Other procedures such as fluorescent antibody, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and immunoelectron microscopy are also used in special instances.

MICROSCOPIC IDENTIFICATION

Viruses can be detected and identified by direct microscopic examination of clinical specimens such as biopsy material or skin lesions. Three different procedures can be used. (1) Light microscopy can reveal characteristic inclusion bodies or multinucleated giant cells. The Tzanck smear, which shows herpesvirus-induced multinucleated giant cells in vesicular skin lesions, is a good example.

(2) UV microscopy is used for fluorescent antibody staining of the virus in infected cells.

(3) Electron microscopy detects virus particles, which can be characterized by their size and morphology.