

Viral Specimens 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, polyomaviruses 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, enterovirus and adenoviruses.

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:

1-All specimens for detection of viruses should be collected with aseptic technique and transported immediately to the laboratory by using transport media (in screw capped tube) and should be placed in ice, specimens for viral isolation should not be kept at room or higher temperature. 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.

2-Some antibiotics (e.g penicillin, streptomycin, nystatine) should be added to viral transport media especially when contamination with microbial flora is expected. Examples of successful transport media include Stuart's medium, Amie's medium.

3-Centrifuge under refrigeration, the supernatant will contain the viruses.

4-Filter the supernatant to give crude liquid of viruses.

5-Detection of viruses in this crude liquid of viruses by 3 methods

Three methods for virus detection

A. Direct method by microscopic examination:

1. Light microscope (L.M): viruses cannot be seen in L.M. but it used for demonstration of inclusion bodies.
2. Electron microscope (E.M): more useful for seeing structure of viruses, symmetry and No. of capsomeres.

3. Fluorescence microscope (F.M): used for detection of fluorescent Ab.

B. Isolation: by three methods

1. Chicken embryonated eggs:

- Sterilize the shell of eggs by iodine solution and embryos of (7-12) days old are used. The egg containing embryo usually has an air space at the larger end.
- Make hole in the shell in the air sac. Usually the hypodermic syringe is used. Virus suspension to be cultivated is taken in dropper and gently spread over the exposed embryo then incubated for one week as in hatching.
- Examine the egg by candling device and note the embryo alive.
- Inject 0.5 ml of viral dilution into site of inoculation and seal the hole with tape and incubate at 37°C for 3 days.
- After incubation, remove the tape and examine the embryo.

***Signs of viral infection in embryonated egg:**

- a- Death of embryo as in herpes, mumps.
- b- Demonstration of haemagglutination as in influenza, mumps viruses.
- c- Demonstration of local lesion called pocks as in vaccinia and small pox.
- d- Finding of virus particles in embryo materials by using microscope.

2. Laboratory animals:

Mice are still most widely used animals in virology. Suckling 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. Animal inoculation has a disadvantage that immunity may interfere with viral growth and that animals often harbor latent viruses.

3. Cell culture:

Cell cultures are cells from man or animal origin and grow into a monolayer on the sides of glass or plastic test tubes. Cells are kept moist and supplied with nutrient by keeping them continuously immersed in cell culture medium. There are two kinds of media:

Growth medium and maintenance medium, are used for cell culture. Growth medium – Minimum Essential Medium (MEM): essential amino acids, vitamins, salts, glucose & sodium bicarbonate, antibiotics & phenol red indicator. Usual antimicrobials added are vancomycin (10 µg/ml), gentamicin (20 µg/ml) and amphotericin (2.5 µg/ml). 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 in a roller drum that holds cell culture test tubes tilted (5-7) degrees while they slowly revolve at 35 to 37°C.

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 cell culture becomes a cell line once it has been passed or subcultured *in vitro*.

Cell line are classified as:

1. Primary cell lines: normal cells freshly taken from body prepared by dispersing cells with trypsin from tissue fragments, there is little cell division during growth in cell cultures and after 2-3 wks will degenerate and discarded ex. Primary monkey kidney cell.

2. Low passage (semi-continuous) Cell lines: cells of single type have diploid No. of chromosome, usually (fibroblast cells) that can be subcultivated for limited number of times, mostly 50 passages in culture ex. Lung fibroblast.

3. Continuous cell lines: usually derived from cancer cell, the chromosomes are herteroploid, there is rapid growth rate and cells can be subcultured indefinitely ex. Human epidermoid carcinoma cells.

Detection of virus growth in cell cultures

1. Cytopathic effects (CPE) – morphological changes in cultured cells, seen under microscope, characteristic CPE for different groups of viruses.

2. Immunofluorescence – test for viral Ag in cells from viral infected cultures.

C- Serology

Detection of rising titres of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection.

Classical Techniques

1. Complement fixation tests (CFT)
2. Haemagglutination inhibition tests
3. Immunofluorescence techniques (IF)
4. Neutralization tests

Newer Techniques

1. Radioimmunoassay (RIA)
2. Enzyme linked immunosorbent assay (EIA)
3. Particle agglutination
4. Western Blot (WB)