

Direct examination of Viruses:

1. Electron Microscopy morphology / immune electron microscopy
2. Light microscopy histological appearance - e.g. inclusion bodies
3. Antigen detection immunofluorescence, ELISA etc.
4. Molecular techniques for the direct detection of viral genomes

Direct examination: are often also called rapid diagnostic methods because they can usually give a result either within the same or the next day, e.g. diagnosis of RSV infection in neonates, or severe CMV infections in immunocompromised patients. However, it is important to realize that not all direct examination methods are rapid, and conversely, virus isolation and serological methods may sometimes give a rapid result.

1. Antigen Detection

Examples of antigen detection include immunofluorescence testing of nasopharyngeal aspirates for respiratory viruses e.g. flu A, flu B, and adenoviruses, detection of rotavirus antigen in faeces, the detection of HSV and VZV in skin scrapings, and the detection of HBsAg in serum.

The main advantage of these assays is that they are rapid to perform with the result being available within a few hours. However, the technique is often tedious and time consuming, the result difficult to read and interpret, and the sensitivity and specificity poor

2. Electron Microscopy (EM)

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, **Occasionally it may be used for the detection of viruses in vesicles and other skin lesions**, such as herpesviruses and papillomaviruses. **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. The main problem with EM is the expense involved in purchasing and maintaining the facility.

3. Viral Genome Detection

Methods based on the detection of viral genome are also commonly known as molecular methods. It is often said that molecular methods is the future direction of viral diagnosis. Classical molecular techniques such as dot-blot and Southern-blot depend on the use of specific DNA/RNA probes for hybridization. The specificity of the reaction depends on the conditions used for hybridization. These techniques may allow for the quantification of DNA/RNA present in the specimen. However, it is often found that the sensitivity of these techniques is not better than conventional viral diagnostic methods.

Virus Isolation

Cell cultures, eggs, and animals may be used for isolation. However eggs and animals are difficult to handle and most viral diagnostic laboratories depend on cell culture only. There are 3 types of cell cultures:

1. **Primary cells: like** Monkey Kidney. These are essentially normal cells obtained from freshly killed adult animals. These cells can only be passaged once or twice.
2. **Semi-continuous cells: like** Human embryonic kidney and skin fibroblasts. These are cells taken from embryonic tissue, and may be passaged up to 50 times.
3. **Continuous cells: like** HeLa, Vero, Hep2, LLC-MK2, BGM. These are immortalized cells i.e. tumour cell lines and may be passaged indefinitely.

Identification of growing virus

The presence of growing virus is usually detected by:

1. **Cytopathic Effect (CPE)** - may be specific or non-specific e.g. HSV and CMV produces a specific CPE, whereas enteroviruses do not.
2. **Haemadsorption** - cells acquire the ability to stick to mammalian red blood cells. Haemadsorption is mainly used for the detection of influenza and parainfluenzaviruses.

Confirmation of the identity of the virus may be carried out using neutralization, haemadsorption- inhibition, immunofluorescence, or molecular tests

3. Problems with cell culture: The main issue with cell culture is the lengthy time required for a result (up to 4 weeks). Furthermore, sensitivity is frequently low and is affected by a variety of factors, including specimen condition and cell sheet condition. Cell cultures are also prone to bacterial contamination and toxic substances in the specimen. Finally, many viruses, for example, will not grow in cell culture. **Hepatitis B and C, diarrheal viruses, parvovirus**, and other infectious diseases

4 Rapid Culture Techniques: Rapid culture techniques are available for detecting viral antigens 2 to 4 days after inoculation. Shell vial cultures and the **CMV DEAFF test** are two examples of rapid culture techniques. The cell sheet is grown on individual cover slips in a plastic bottle for the CMV DEAFF test. After inoculation, the bottle is spun at a low speed for one hour (to accelerate virus adsorption) and then incubated for 2 to 4 days. The cover slip is then removed and immunofluorescence is used to check for the presence of CMV early antigens.