

Virological Tests

Laboratory Diagnosis of Viral Diseases

- Description of virus-induced **cytopathologic effects (CPEs)** on cells
- Detection of viral particles
- Isolation and growth of the virus
- Detection and analysis of viral components (e.g., proteins (antigens), enzymes, genomes)
- Evaluation of the patient's immune response to the virus (**serology**)

Collection of specimens for virologic examination

- Swabs
- Washes
- CSF, urine, stool, anticoagulated blood, tissue samples (biopsy or necropsy)
- Transport of specimens
- Storage temperature
- Specimen inspection

Collection of specimens for virologic examination

- *Bone marrow*
- *Cervix specimen*
- *Eyes, conjunctival swab*
- *Fluid specimen*
- *Lesion swab*
- *Nasal wash (optimal for RSV)*
- *Nasopharyngeal swab*
- *Rectal swab*
- *Throat swab*
- *Urethral specimen*

Viral transport medium

- bovine albumin fraction
- gentamicin sulfate solution
- amphotericin

Diagnostic Methods in Virology

1. Direct Examination
2. Isolation of virus
3. Indirect Examination - Serology

Direct Examination

- 1. Antigen Detection** immunofluorescence, ELISA etc.
- 2. Electron Microscopy** morphology of virus particles
immune electron microscopy
- 3. Light Microscopy** histological appearance
inclusion bodies
- 4. Viral Genome Detection** hybridization with specific
nucleic acid probes
polymerase chain reaction (PCR)

direct Examination

1. Cell Culture

cytopathic effect (CPE)

haemabsorption

immunofluorescence

2. Eggs

haemagglutination

inclusion bodies

3. Animals

disease or death

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
5. Counter-immunoelectrophoresis

Newer Techniques

1. Radioimmunoassay (RIA)
 2. Enzyme linked immunosorbent assay (EIA)
 3. Particle agglutination
 4. Western Blot (WB)
 5. RIBA, Line immunoassay
-

Electron Microscopy

10^6 virus particles per ml required for visualization, 50,000 - 60,000 magnification normally used. Viruses may be detected in the following specimens.

Faeces

Rotavirus, Adenovirus
Norwalk like viruses
Astrovirus, Calicivirus

Vesicle Fluid

HSV
VZV

Skin scrapings

papillomavirus,
molluscum contagiosum

- ELECTRON MICROSCOPE
- uses a beam of [electrons](#) to illuminate a specimen and produce a magnified image

- A **Transmission Electron Microscope (TEM)** produces a **2D image** of a thin sample, and has a maximum resolution of **×500000**

- A **Scanning Electron Microscope (SEM)** produces a **3D image** of a sample by 'bouncing' electrons off and detecting them at multiple detectors. It has a maximum magnification of about **×100000**

VIRUS CULTIVATION SYSTEMS

- 1. BIOLOGICAL SYSTEM**
- 2. EMBRYONATED EGGS**
- 3. TISSUE CULTURE SYSTEM**

EMBRYONATED EGGS

ADVANTAGES

- Isolation and cultivation of many avian and few mammalian viruses
- Ideal receptacle for virus to grow
- Sterile & wide range of tissues and fluids
 - Free from bacteria and many latent viruses.
 - Free from specific and non specific factors of defence.
 - Sensitive to viruses which do not produce infection in adult birds
- Cost- much less
- Maintenance-easier
- Less labour
- Readily available

METHODS OF CULTIVATION

Various routes of inoculation

- a)Yolk sac**
- b) Allantoic sac**
 - c) Chorioallantoic membrane**
- d.) Amniotic cavity**
- e.) Intravenous**

Virus Isolation

Cell Cultures are most widely used for virus isolation, there are 3 types of cell cultures:

1. Primary cells - Monkey Kidney
2. Semi-continuous cells - Human embryonic kidney and skin fibroblasts
3. Continuous cells - HeLa, Vero, Hep2, LLC-MK2, MDCK

Cell Cultures

Growing virus may produce

1. Cytopathic Effect (CPE) - such as the ballooning of cells or syncytia formation, may be specific or non-specific.
2. Haemadsorption - cells acquire the ability to stick to mammalian red blood cells.

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

Cytopathic Effects :

- • Rounding
- • Detachment (plaques)
- • Clumping
- • Ballooning (Giant Cell)
- • Fusion (Syncytium Formation)
- • Inclusion Formation

Problems with cell culture

- Long period (up to 4 weeks) required for result.
- Often very poor sensitivity
- Susceptible to bacterial contamination.
- Susceptible to toxic substances which may be present in the specimen.
- Many viruses will not grow in cell culture e.g. Hepatitis B, Diarrhoeal viruses, parvovirus, papillomavirus.

Serology

Criteria for diagnosing Primary Infection

- 4 fold or more increase in titre of IgG or total antibody between acute and convalescent sera
- Presence of IgM
- Seroconversion
- A single high titre of IgG (or total antibody)

Criteria for diagnosing Reinfection

- fold or more increase in titre of IgG or total antibody between acute and convalescent sera
- Absence or slight increase in IgM
- Note that during reinfection, IgM may be absent or present at a low level transiently

Serological tests

- Virus neutralization test
- Complement fixation test (CFT)
- Hemagglutination inhibition assay (HI test)
- ELISA/ELISPOT
- Immunoprecipitation and Immunoblotting
- Immunofluorescence
- Line immune assay

Molecular Methods

- 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.
- However in practice, although the use of these methods is indeed increasing, the role played by molecular methods in a routine diagnostic virus laboratory is still small compared to conventional methods.
- It is certain though that the role of molecular methods will increase rapidly in the near future.

- <https://virology-online.com/general/Tests.htm>
- <https://www.jobilize.com/microbiology/test/detection-of-a-virus-isolation-culture-and-identification-by-openstax>
- <https://www.asmscience.org/content/education/imagegallery/image.2616>
- https://www.lgcstandards-atcc.org/support/faqs/d1f5b/General+protocol+for+the+Hemadsorption+Assay-234.aspx?geo_country=sk
- https://www.researchgate.net/figure/Egg-inoculation-into-the-allantoic-cavity-a-Drill-or-pierce-a-hole-in-the-egg-5-mm_fig1_272836131
- <https://microbeonline.com/hemagglutination-inhibition-test-hai-principle-procedure-result-interpretations/>
- <https://www.slideshare.net/TapeshwarYadav1/immunofluorescence-55934842>
- <https://theconversation.com/five-techniques-were-using-to-uncover-the-secrets-of-viruses-144363>