Techniken der Virologie
Diagnosis of viral diseases

- Clinical signs
- Virus detection
  - Visualization
  - Propagation
  - Isolation
  - Identification
  - Quantitation
- Serology

Require Specimens

Require Blood
SPECIMEN COLLECTION

- Specimens are to be collected from the freshest part of the lesion, where the most active and current virus replication is presumed to happen.
- Attempt to take the sample as sterile or as clear as possible.
- This is very important, as cell culture is ideal medium for bacterial growth.
- A contaminated sample negates VI as a valid diagnostic test.
- If the sample is definitely contaminated, antibiotics can be added.
Diagnostic Methods in Virology

1. Direct Examination
2. Virus Propagation Isolation
3. Virus Detection Methods
4. Serology
Direct Examination

1. Light Microscopy
   - histological appearance
   - inclusion bodies

2. Electron Microscopy
   - morphology of virus particles
   - immune electron microscopy
Microscope Comparison

[Diagram showing different types of microscopes: Light Microscope, Transmission Electron Microscope, Scanning Electron Microscope.]
Pox is the only virus that can be detected with the light microscope.
But aggregates of virus are visible

Cytoplasmic
Inclusion bodies
But aggregates of virus are visible

Negri bodies
e.g. Rhabdoviruses
Electron Microscopy

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

**Faeces**
- Rotavirus, Adenovirus
- Noroviruses
- Astrovirus, Calicivirus

**Vesicle Fluid**
- HSV
- VZV

**Skin scrapings**
- papillomavirus
Visualization of individual virus particles

- Most biological materials show little contrast with their surroundings unless they are stained.
- In the case of light microscopy, contrast can be enhanced by using coloured stains which selectively absorb certain wavelengths.
- The electrons in the electron microscope are absorbed very little by biological material and contrast is obtained mainly by electron scattering.
**Staining**

- To heighten the contrast between viruses and the background, use is made of electron-dense "stains".
- These are usually compounds of heavy metals of high atomic number, that serve to scatter the electrons from regions covered with the stain.
- If virus particles are coated with stain (positive staining), fine detail may be obscured.
Negative Staining

- **Negative staining** overcomes this problem by staining the background and leaving the virus relatively untouched.
  - The negative stain is moulded round the virus particle, outlining its structure, and is also able to penetrate between small surface projections and to delineate them.
  - If there are cavities within the virus particle that are accessible to the stain, these will be revealed and some of the internal structure of the virus may be disclosed.
Positive Staining

Negative Staining
Freeze fracture microscopy

(A) FRACTURE

the two fracture faces of the outer membrane of the nuclear envelope

knife

nucleus
cytoplasm

ice
Problems with Electron Microscopy

- Expensive equipment
- Expensive maintenance
- Require experienced observer
- Sensitivity often low
Diagnostic Methods in Virology

1. Direct Examination
2. Virus Propagation and Isolation
3. Virus Detection Methods
4. Serology
Virus propagation and isolation

- VIRUSES NEED HOST CELLS TO REPLICATE
Virus Isolation
VIRUS ISOLATION

● In the laboratory, solid samples are minced, homogenized, centrifuged at low speed to remove cellular debris that may be toxic to the cultured cells, and sterilized by 0.2 µm filter.

● A backup aliquot is stored at 4 or -70 C.

● Next step is the inoculation of a system supporting virus replication: cell cultures, embryonating eggs, organ cultures, laboratory animals or host animals.
<table>
<thead>
<tr>
<th>Method</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Animals</td>
<td>disease or death</td>
</tr>
<tr>
<td>Organ Culture</td>
<td>loss of function</td>
</tr>
<tr>
<td>Eggs</td>
<td>pocks on CAM</td>
</tr>
<tr>
<td>Cell Culture</td>
<td>cytopathic effect (CPE)</td>
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VIRUS REPLICATION

- Embryonated Egg
VIRUS REPLICATION

- Pseudorabies virus pocks on chorioallantoic membrane
Cell Culture

Cell Cultures are most widely used for virus isolation, there are 2 types of cell cultures:

1. Primary cells - Monkey Kidney
2. Continuous cells - HeLa, Vero, Hep2, LLC-MK2, MDCK

Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses. However, they are very expensive and it is often difficult to obtain a reliable supply.

Continuous cells are the most easy to handle but the range of viruses supported is often limited.
Adherent versus Suspension
Virus detection

Cultured cells → Cytopathic effect → Immunofluorescence (anti-herpesvirus antibody)
Problems with cell culture

• Long period (up to 4 weeks) required for result.
• Often very poor sensitivity, sensitivity depends on a large extent on the condition of the specimen.
• 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.
tissue culture cells

epithelial  epithelioid  fibroblastic

slides from CDC
epithelial cells - adenovirus (finally eosinophilic nuclear inclusions)

uninfected  early infection  late infection

slides from CDC
epithelial cells - respiratory syncytial virus (finally syncytia with cytoplasmic eosinophilic inclusions)

uninfected

respiratory syncytial virus

slides from CDC
SYNCYTIA

e.g. Respiratory Syncytial Virus
Mumps
Cytopathic Effect (4) - Plaques

Swine herpesvirus (pseudorabies virus) plaques in swine kidney cells
CPE - Plaques

Swine herpesvirus (pseudorabies virus) plaques in rabbit kidney cells
Virus quantitation (plaques)

Count plaques
(plaque forming unit/ml)
PLAQUE FORMING UNIT

P.F.U.

pfu
CYTOPATHIC EFFECT

- ANY DETECTABLE CHANGES IN THE HOST CELL
  - MORPHOLOGICAL CHANGES
  - DEATH
  - APOPTOSIS
  - INDEFINITE GROWTH
How to quantitate viruses?

- Plaque Forming Units
- Tissue Culture Infectious Dose
- Colony Forming Units

Virus Titration
Virus quantitation (TCID50)

10\(^{-2}\)  10\(^{-3}\)  10\(^{-4}\)  10\(^{-5}\)  10\(^{-6}\)

\[ \text{TCID}_{50} = 10^4 \]

50% Tissue culture infectious dose
SOME POINTS TO REMEMBER

- **INFECTIVITY**
  - **NOT EVERY RELEASED PARTICLE IS INFECTIOUS**

- **ASSAYS**
  - every particle (e.g. electron microscope)
  - infectious particles (e.g. plaque assay)
Tests to detect virus (viral antigen)

- Haemagglutination
Haemagglutination (HA)

virus

No virus
**Haemagglutination**

### Dilution

<table>
<thead>
<tr>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
<th>1024</th>
</tr>
</thead>
</table>

No virus

![Image of haemagglutination assay](image)

↓ titre
Tests to detect virus (viral antigen)

- Haemagglutination
- ELISA
VIRUS ANTIGEN DETECTION

- **ELISA**

  - Antibody binds to antigen;
  - enzyme-labeled anti-IgG binds to antibody;
  - substrate changes color
Methods of ELISA testing (1)

(a) Direct Antibody Sandwich Method

Antibody is adsorbed to well.

Test antigen is added; if complementary, antigen binds to antibody.

Enzyme-linked antibody specific for test antigen then binds to antigen, forming sandwich.
Methods of ELISA testing (2)

Enzyme's substrate (□) is added, and reaction produces a visible color change (●).
Enzyme linked immunabsorbant assay (ELISA)

Sample to be tested

virus

Capturing antibody

Enzyme -> colour

Detecting antibody
ELISA – Enzyme-Linked Immunosorbent Assay
ELISA Machine
Competitive ELISA
Tests to detect virus (viral antigen)

- Haemagglutination
- ELISA
  - Immunofluorescence
VIRUS ANTIGEN DETECTION

- **IMMUNOFLUORESCENCE**
  - Antibody binds to antigen in fixed cells;
  - fluorescein-labeled anti-IgG binds;
  - fluorescens by UV microscopy
Immunological detection

Immunohistochemistry

Bovine herpesvirus antigens in endothelial cells

Immunofluorescence

BHV-1 antigens in neuron in trigeminal ganglion
Indirect Immunofluorescence

- Sensitivity can be increased by using indirect immunofluorescence
  - the antibody specific for the antigen is unlabeled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome.

Multiple fluorochrome labeled antibodies can bind one unlabeled specific antibody
Diagnosis of viral diseases

• Detection of exposure (Serology)
  – Virus neutralization
  – Haemagglutination inhibition
  – ELISA
  – Limitations
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) - very unreliable

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
SEROLOGY

- Serum Neutralization

- Antibody neutralizes infectivity of virion; inhibits cytopathology, reduces plaques, or protects animals
• **Serum neutralization**

Serially dilute serum

\[
\frac{1}{2} \quad \frac{1}{4} \quad \frac{1}{8} \quad \frac{1}{16} \ldots \ldots \frac{1}{512}
\]

Add equal amount of virus (100 plaque forming units) to each tube

Infect cultured cells

Last dilution that can prevent plaque formation is titre
Usefulness of Serological Results

- How useful a serological result is depends on the individual virus.
- E.g. for viruses such as rubella and hepatitis A, onset of clinical symptoms coincide with production of antibodies. Detection of IgM or rising titres of IgG in the patient’s serum indicates active disease.
- However, many viruses often produce clinical disease before the appearance of antibodies such as respiratory and diarrhoeal viruses. So in this case, any serological diagnosis would be retrospective and therefore will not be that useful.
- There are also viruses which produce clinical disease months or years after seroconversion e.g. HIV and rabies. For these viruses, the mere presence of antibody is sufficient to make a definitive diagnosis.
Problems with Serology

• Long period of time required for diagnosis for paired acute and convalescent sera.

• Mild local infections such as HSV genitalis may not produce a detectable humoral immune response.

• Antigenic cross-reactivity between related viruses e.g. HSV and VZV, Japanese B encephalitis and Dengue, may lead to false positive results.

• Immunocompromised patients often give a reduced or absent humoral immune response.

• Patients with infectious mononucleosis SLE may react non-specifically giving a false positive result.

• Patients given blood or blood products may give a false positive result due to the transfer of antibody.