The purpose is to diagnose viral diseases in the patient.

This can be achieved by:

**Directly** – detecting the virus or viral products

**Indirectly** – detecting an immunological response to the virus
Diagnostic Virology

Direct Methods

- Virus isolation
- Direct visualisation (EM)
- Direct antigen detection (Ab)
- DNA/RNA detection
Indirect Methods

- Antibody detection / Serology
- Lymphocyte activation
- Cytokine release
Virus isolation
Isolation of Virus

- Clinical Specimen
- Cell Culture
- Suckling mouse brain inoculation
- Egg inoculation
- Mosquito inoculation
Virus isolation 1

- 'Catch-all' cell culture-based
- Labour and resource intensive
- CPE and haemadsorption based
- Roller tubes, flasks or multi-well plates
Virus isolation 2

- Monolayers of primary, diploid and continuous cell lines
- pMK, pHEK
- MRC-5, WI-38
- Vero, RK13, HeLa
Virus isolation - Specimens

- Collect during acute phase
- Intact cells important
- Prompt delivery
- Refrigerate if stored less 24 h
- Seal well if stored on dry ice (pH change)
Virus isolation - processing

- Samples collected from contaminated sites placed in antibiotic transport medium
- Decontaminated by cent’gation, filtration and antibiotic usage.
- CSF, serum, mononuclear cells are inoculated directly
Virus isolation - processing

- Sample inoculated by either:
  - Inoculation of 0.2mL sample into tube with medium
  - Inoculation of 0.2mL sample onto decanted monolayer - incubated 1h - fresh medium
### Cells for virus isolation

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>MDCK</th>
<th>MK</th>
<th>LLC-MK2</th>
<th>VERO</th>
<th>HEP 2</th>
<th>HFF</th>
<th>RK-13</th>
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<tbody>
<tr>
<td>INFLUENZA</td>
<td>+*</td>
<td>+*</td>
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<tr>
<td>PARAINFLUENZA</td>
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<td>+</td>
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<tr>
<td>RSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
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<tr>
<td>AV</td>
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<td>+</td>
<td>+</td>
<td>+*</td>
<td>+</td>
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</tr>
<tr>
<td>EV</td>
<td>+*</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>MUMPS</td>
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<tr>
<td>HSV</td>
<td>+</td>
<td>+</td>
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<td>+*</td>
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<tr>
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<td>+*</td>
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<tr>
<td>MEASLES</td>
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<tr>
<td>RUBELLA</td>
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<tr>
<td>EMV</td>
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# Virus CPE

<table>
<thead>
<tr>
<th>Virus</th>
<th>pMK</th>
<th>MRC-5, WI-38</th>
<th>Continuous Vero, HeLa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>-</td>
<td>&lt; sensitive</td>
<td>enlarged, rounded grape-like clusters</td>
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<tr>
<td>Enterovirus</td>
<td>cells shrink, lyse,</td>
<td>&lt; sensitive</td>
<td>&lt; sensitive</td>
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<td></td>
<td>detach</td>
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<td></td>
</tr>
<tr>
<td>HSV-1/2</td>
<td>&lt; sensitive</td>
<td>enlarged-balloon</td>
<td>&lt; sensitive</td>
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<tr>
<td></td>
<td></td>
<td>ed cells</td>
<td></td>
</tr>
<tr>
<td>Measles</td>
<td>multinucleated giant</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>some CPE 5-10d</td>
<td>rounding &amp; refractility 2-7d</td>
<td>-</td>
</tr>
<tr>
<td>Influenza</td>
<td>CPE rare</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Virus isolation 3

- Presumptive based on CPE and time of CPE appearance in different cell lines
- Non-CPE producing cultures subjected to haemadsorption
Virus haemadsorption

- Performed on CPE-negative
- Medium decanted, GP RBCs added & incubated 30 min 4°C
- RBCs adhere to monolayer in clumps
- parainfluenza, influenza, measles, mumps
Haemadsorption

Haemagglutinin + RBC → Cell monolayer

haemadsorption
Isolation by egg inoculation

Arboviruses & Coxsachie A viruses
Culture Amplified DFA

- Virus is detected by centrifugal inoculation of clinical specimens onto cell monolayers in 96 well microtitre plates.
- After 48 hrs incubation cell layers are stained with monoclonal antibodies and fluorescent conjugate.
- Fluorescence is observed by use of an inverted fluorescent microscope.
Virus Identification

- Fluorescent foci-forming assays
- Neutralisation test
Virus Neutralisation Assays

- TCID$_{50}$
- Plaque reduction Neutralisation test (PRNT)
virus isolate mixed with dilutions of known antiserum

incubated 37°C 1 hour

Added to decanted cell monolayer & incubated 37°C 1 hour

1% Agarose over added, allowed to set and inverted, incubated at 37°C for 3-7 days
1% Agarose with neutral red added over existing agarose layer, allowed to set and inverted, incubated at 37°C overnight

Reduction/inhibition observed compared to a unrelated serum control
Electron Microscopy
Electron Microscopy

- Virus may be recovered direct from clinical sample
- Virus needs to be purified or concentrated
Density gradient centrifugation

Centrifugation in a solution whose density increases from the top of the tube to the bottom.

Gradient with low MW compound sucrose, potassium tartrate.
Sucrose gradient centrifugation

- Separation on density differences
- Particles sediment or float until they reach point where density of gradient is equal to their buoyant density
Sucrose gradient centrifugation

- Gradient plays a critical role
- Sucrose adequate for enveloped viruses with densities 1.1-1.2 g/cm³ - cesium chloride
Centrifuge in an ultracentrifuge @ 100,000 g for 4 – 12 hours
Bottom of tube is punctured

Fractions analysed
By EM

0.5 mL fractions collected
Electron Microscopy

- Selective tool to be applied in specific situations
- Immuno-EM increase sensitivity
- Negative stain
- Thin section
Electron Microscopy

- Resolution of < 0.5 nm
- 1933 EM 'invented'
- 1948 First used to identify smallpox virus in clinical sample
- 1959 Negative staining
Negative staining

Phosphotungstic acid PTA
Virus Sizes

- **Monomorphic**
  - Picornaviruses: 25 nm
  - Herpesviruses: 100 nm
  - Poxviruses: 250 nm

- **Pleomorphic**
  - Paramyxoviruses: 120 nm
  - Coronaviruses: 90 nm
Electron Microscopy

- 1 Angstrom (Å)  \(10^{-10}m\)
- 1 Nanometre (nm)  \(10^{-9}m\)
- 1 Micron (µm)  \(10^{-6}m\)
- 1 Millimetre (mm)  \(10^{-3}m\)
Direct Antigen Detection
Direct Antigen Detection

- Viral antigen > abundance than virions
- Specific approach / yes-no
- Tests include:
  - Immunofluorescence (IFA)
  - Enzyme Immuno Assays (EIA)
  - Agglutination
Immunofluorescence

- Requires infected cells
- Most common fluorochrome
  FITC (Rhodamine, Texas Red)
- Polyclonal or monoclonal Ab
Immunofluorescence 2

- Collection of specimens critical
- Tissue scrapings / NPA
- Antigenicity rapidly lost at RT
- Smears prepared & fixed at collection
- Acetone Fixation
Direct IFA

- Apply specimen to slide
- Acetone / Methanol fix
- Add conjugated Ab, incubate
- Wash 3x
- Dry and examine
Direct IFA Ag Detection

Sample applied → Sample fixed

Conjugated Ab Added

UV
Immunofluorescence 3

- Used for herpesviruses and respiratory specimens
- A pool of Mabs used 1st
  - Positives retested using panel of individual, virus-specific Mabs
Shell Vial Test

- Cell monolayers on coverslips
- Medium decanted, clinical material added directly
- Centrifuged at 500-1000 xg 1h
- Fresh media added 24-48 h
- Stained using direct IFA
Solid-Phase immunoassay

- Enzyme immunoassay (EIA)
- Radioimmunoassay (RIA)
Solid-Phase immunoassay

- No special requirement for transport
- Suited to semi-automation
- More easily standardised
- Automatic result interpretation (less subjective)
Solid-Phase immunoassay

- Specimen incubated with immobilised solid-phase Ab
- Washed away
- Labelled Ab (enzyme or radioactive)
- Washed away
- Detected
Enzyme immunoassay (EIA)

- Most widely used for viral antigen
- Minimal variation
- Tests available for most common viral pathogens
ELISA (Direct)
Enzyme-Linked Immuno-Sorbent Assay

Antigen is captured

Antigen

Solid-phase

antibody

Ag-specific antibody-conjugate

Substrate
Radioimmunoassay

- Highest reproducibility
- Least daily variation
- Hazardous radioactive material
- Disposal problems
Latex Agglutination Tests

- Lower sensitivities
- Read macroscopically
- Very rapid
Recombinant Cell lines

- Transgenic cell lines using a reporter gene under the transcriptional control of viral specific promoter
- Herpes & Alphaviruses
- β-galactosidase, GFP?
- Lower cost, more rapid & more general approach
Recombinant Cell lines

HSV viral protein

Transfected cell genome

GFP gene

HSV promotor

GFP