Laboratory Diagnosis of Viral Infections

G. Jamjoom 2005
Five Main Techniques:

- Virus Culture and Isolation
- Serology
- Rapid Detection of Viral Antigens
- Detection of Viral Nucleic Acid
- Electron Microscopy
Virus Culture and Isolation

- Which cell lines
- How many cell lines
Virus Culture and Isolation

- Cytopathic Effects (CPE)
Cell Lines

- Primary
- Continuous
Examples of Commonly Used Cell Lines:

• Primary Cell Lines:
  - monkey kidney cells
  - human foreskin fibroblasts
Examples of Commonly Used Cell Lines:

- Continuous Cell Lines:
  - VERO
  - HEP-2
  - MDCK
tissue culture cells

epithelial

epithelioid

fibroblastic

slides from CDC
epithelial cells - adenovirus

uninfected  early infection  late infection

slides from CDC
fibroblastic cells - poliovirus

uninfected  early infection  late infection

slides from CDC
Diluted 10 fold  
Diluted 100 fold  
Diluted 1000 fold
PLAQUE FORMING UNIT

P.F.U.

pfu
Cytopathic Effects:

- Rounding
- Detachment (plaques)
- Clumping
- Ballooning (Giant Cell)
- Fusion (Syncytium Formation)
- Inclusion Formation
Cytopathic Effect of HSV in cell culture:

(Note the ballooning of cells).
Cytopathic Effect of CMV

(Courtesy of Linda Stannard, University of Cape Town, S.A.)
Cytopathic Effect of VZV
ADENOVIRAL INCLUSION BODIES
Virus Culture and Isolation

- Shell Vial
DEAFFF test for CMV

Fig. 2, CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

(Virology Laboratory, New-Yale Haven Hospital)
Rapid Detection of Viral Antigens
CMV pp65 antigenaemia test

Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

(Virology Laboratory, New-Yale Haven Hospital)
Positive immunofluorescence test for HSV antigen in epithelial cell.
Serology:

- Hemagglutination Inhibition
hemagglutination
Serology:

- No rise

Acute Sample

Convalescent Sample
Serology:

- Two-fold rise (not significant)
Serology:

- Four-fold rise (significant)
Serology:

- Hemadsorption
Hemadsorption - influenza virus infected cells

MURRAY, 2nd Ed., Fig 53.5
Serology:

- Immunofluorescence
Serology:

- Enzyme Linked Immuno Sorbent Assay (ELISA) (EIA)
Serological tests for HIV infection-2

**ELISA**

The viral proteins can be derived from:
- Gp160
- Gp120
- Gp24
- Gp17/18

Synthetic peptides corresponding to viral proteins are also commonly used in ELISA
• ELISA
Hepatitis A Infection
Typical Serological Course

Fecal HAV
Symptoms

ALT

IgM anti-HAV

Total anti-HAV

Months after exposure

0 1 2 3 4 5 6 1 2 3 4

Titre
Acute Hepatitis B Virus Infection with Recovery Typical Serologic Course

Symptoms

- HBsAg
- HBeAg
- anti-HBe
- Total anti-HBc
- IgM anti-HBc
- anti-HBs

Weeks after Exposure

Titre

0 4 8 12 16 20 24 28 32 36 52 100

0 4 8 12 16 20 24 28 32 36 52 100

Acute Hepatitis B Virus Infection with Recovery Typical Serologic Course
Progression to Chronic Hepatitis B Virus Infection

Typical Serologic Course

Acute (6 months)

Chronic (Years)

HBsAg

HBcAg

anti-HBe

IgM anti-HBc

Total anti-HBc

Weeks after Exposure

Years

0 4 8 12 16 20 24 28 32 36 52
Serologic Pattern of Acute HCV Infection with Progression to Chronic Infection

- Titer
- Symptoms +/-
- HCV RNA
- ALT
- Anti-HCV

Time after Exposure

Symbols:
- Normal
Serology:

- Western Blot
The Western Blot Confirmatory Test (HIV)
How to prepare strip for the Western blot
Western blot for HIV antibody

There are different criteria for the interpretation of HIV Western blot results e.g. CDC, WHO, American Red Cross.

The most important antibodies are those against the envelope glycoproteins gp120, gp160, and gp41.

p24 antibody is usually present but may be absent in the later stages of HIV infection.
Detection of Viral Nucleic Acid

- Polymerase Chain Reaction (PCR)
The Polymerase Chain Reaction (PCR)
Thermal Cyclers for PCR
DNA sequencing
Electron Microscopy:

- Used to be very important before virus isolation and identification was developed.
- Still important with viruses that cannot be cultured and with new viruses.
Electron Microscopy:

- Examples:
  - Adenovirus
  - Papillomaviruses
  - Enterovirus
  - Rotavirus
Hepatitis B Virus
Hepatitis A Virus
HIV budding and maturation

Hsiung, GD et al., Diagnostic Virology 1994 p204 (D. Medina)
ROTAVIRUS

Erskine Palmer, CDC
smallpox virus cytoplasmic assembly and maturation

F. A. Murphy, School of Veterinary Medicine, University of California, Davis.
http://www.vetnet.ucdavis.edu/fam_graphics/download.html
Adenovirus
Influenza Virus
Rotavirus
Rift Valley Fever Virus
Papilloma virus
Enterovirus
Paramyxovirus
<table>
<thead>
<tr>
<th>Time after Exposure</th>
<th>Titer</th>
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<tr>
<td>Jaundice</td>
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<td>Symptoms</td>
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<tr>
<td>ALT</td>
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<tr>
<td>HDV RNA</td>
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<td>HBsAg</td>
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<td>IgM anti-HDV</td>
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**HBV - HDV Superinfection**

**Typical Serologic Course**

- **ALT**: Increases during jaundice and symptoms.
- **HDV RNA**: Persistent during the course.
- **HBsAg**: Presence of the hepatitis B surface antigen.
- **IgM anti-HDV**: Detects the presence of IgM antibodies against HDV.
- **Total anti-HDV**: Detects the presence of total antibodies against HDV.
Hepatitis E Virus Infection
Typical Serologic Course

<table>
<thead>
<tr>
<th>Weeks after Exposure</th>
<th>Titer</th>
<th>Symptoms</th>
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<td>Virus in stool</td>
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ALT, IgG anti-HEV, IgM anti-HEV