Laboratory Diagnosis of Viral Infections affect the urinary and Genital Tract

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HERPES SIMPLEX VIRUS (HSV)

• HSV 1 infect the upper part of the body
  - mouth and the face
• HSV 2 infect the lower part of the body
  - genital infections
• There is little cross protection
VIRAL STRUCTURE

Core: Consists of a single linear molecule of dsDNA in the form of a torus

Capsid: Surrounding the core with a 100 nm diameter and 162 capsomeres

Tegument: Consists of viral enzymes

Envelope: Outer layer composed of altered host membrane and a dozen of viral glycoproteins
**Virus Structure**

Enveloped, slightly pleomorphic
Spherical
120 – 200 nm in diameter

**Capsid**
**Envelope**
**Tegument**
**Genome**
Double stranded DNA per virion
**Herpesviridae**

- **Characteristics**
  - large enveloped, *double stranded DNA viruses*
  - genome encodes for proteins which regulate viral m-RNA synthesis by the cell's DNA dependent RNA polymerase
  - DNA replication and assembly *occur in the nucleus*
  - virus buds through the *nuclear membrane*, and is released from the cell by exocytosis or by lysis
  - herpesviruses infections can result in lysis, *latent persistence*, and *oncogenesis* (immortalization)
    - as a group they have a significant tendency toward latent persistence in semi-permissive cells
  - viruses in this group *are very common*
Herpesvirus Cycle

- **Virus Cycle**
  - Viral glycoproteins (VAP’s) bind virus to host cell receptors
  - Virus fuses with the host cell membrane; this removes the envelope and releases then nucleocapsid into the cytoplasm
  - Nucleocapsid binds to the nuclear membrane and releases the genome into the nucleus of the host cell
  - Early proteins facilitate transcription of viral genome and include the DNA dependent DNA polymerase; viral genome is transcribed by the cellular DNA dependent RNA polymerase
  - Late proteins are structural and are synthesized after DNA is replicated
    - Viral genome replication requires viral DNA dependent DNA polymerase
  - Cells that promote latency restrict viral transcription of early and late proteins
  - Cells that complete early and late protein synthesis will die
  - Viruses are assembled in the nucleus and bud through the nuclear membrane
  - Viruses exit the cell via exocytosis or via cell lysis
<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Genus</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alphaherpesvirinae</strong></td>
<td>Simplexvirus</td>
<td>Herpes simplex virus - 1 (HSV-1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Herpes simplex virus - 2 (HSV-2)</td>
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<tr>
<td></td>
<td>Varicellovirus</td>
<td>Varicella-zoster virus (VZV)</td>
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<tr>
<td><strong>Betaherpesvirinae</strong></td>
<td>Cytomegalovirus</td>
<td>Cytomegalovirus (CMV)</td>
</tr>
<tr>
<td></td>
<td>Roseolovirus</td>
<td>Human herpesvirus 6 (HHV-6)</td>
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<td></td>
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<td>Human herpesvirus 7 (HHV-7)</td>
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<tr>
<td><strong>Gammaherpesvirinae</strong></td>
<td>Lymphocryptovirus</td>
<td>Epstein-Barr virus (EBV)</td>
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<tr>
<td></td>
<td>Rhadinovirus</td>
<td>Human herpesvirus 8 (HHV-8)</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Growth Cycle and Cytopathology</td>
<td>Latent Infections</td>
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<tr>
<td>---------------</td>
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<tr>
<td>Alpha</td>
<td>Short, cytolytic</td>
<td>Neurons</td>
</tr>
<tr>
<td>Beta</td>
<td>Long, cytomegalic</td>
<td>Glands, kidneys</td>
</tr>
<tr>
<td></td>
<td>Long, lymphoproliferative</td>
<td>Lymphoid tissue</td>
</tr>
<tr>
<td>Gamma</td>
<td>Variable, lymphoproliferative</td>
<td>Lymphoid tissue</td>
</tr>
</tbody>
</table>
• Primary infection - first contact with HSV

• Latent infection - persistent virus in root ganglia

• Reactivation - production of infective virus by latently infected cell

• Recurrence - clinically apparent disease produced by reactivation
Virus Latency

Herpesvirus enters the body
Herpesvirus lies dormant in the nerves
Herpesvirus is reactivated, causing another outbreak

Herpesvirus enters body through skin or mucous membranes
After initial infection, herpesvirus settles in nerves near the spine
Herpesvirus travels along the nerves, back to the skin to form new blisters
HSV-2 infections

– Not synonymous with genital herpes however:
  • These are primarily infections of the genital tract
  • Neonates can be infected at the time of delivery
  • Extra genital lesions do occur

– Sexual transmission occurs during vaginal, anal and oral sex (although the latter are more often HSV1 viruses)

– The prevalence of HSV-2 antibodies in the population depends on the experience of the patient population.
Laboratory diagnosis of HSV

Direct staining

Tzanck test

Immunostaining

HSV isolation

Serology

PCR
Tzanck test

Cell scrape from base of the lesion
smear on slide

Staining
Wright-Giemsa, Giemsa

Ballooning cell with intranuclear inclusion
multinucleated cell
Tzanck test

Multinucleated cell
Immunofluorescent staining

Cell scrape, smear
  fix in cold acetone

  ↓

  rabbit anti-HSV Ig

  ↓

  goat anti-RaIg conjugated with fluorescein dye

  ↓

  mount with glycerine buffer
Specimen collection

Samples:
vesicle fluid, lesion swab

Transport media

Smear on slide
Transport media

Isotonic solution or culture media

Protein: bovine serum albumin, bovine serum

Antibiotics: streptomycin, penicillin, gentamycin

Anti-fungus: amphotericin B
Viral isolation

Specimens → Cell culture (human diploid cells, Vero cells, Hela cells)

→ Cytopathic effect (rounded, enlarged and multinucleated cell)

→ Identification or typing

*Immunofluorescent staining
HSV Cytopathic effect

Normal cells

CPE
Serological test for HSV infection

- Immunofluorescent staining
- Complement fixation test
- ELISA:
  - IgM capture test
  - IgG test
HSV serology

Primary infection

Pair serum: acute & convalescent serum

IgG assay *rising titer $\geq \alpha$ times

*seroconversion

Single serum: IgM assay

Recurrent infection

not useful; multiple reactivation
IgM capture ELISA

Substrate+chromogen

Enzyme labeled anti-viral antibody

HSV antigens

Tested sera (IgM)

Anti-m chain capture Ab
Polymerase chain reaction

Samples
infected cell, vesicle fluid, CSF

DNA extraction

PCR solution
(buffer, dNTP, Taq DNA pol, primers)

Amplify 20-30 cycles

Multiplex primers;
• cutaneous group; HSV, VZV
• lympphotropic group; CMV,

Detection:
• gel electrophoresis
• dot blot hybridization
• *restriction fragment length polymorphism
BK Virus - virology

- Polyomavirus (related to JC, SV40)
- small (30 to 45 nm), icosahedral, nonenveloped
- double-stranded, circular DNA, around 5000bp
- 6 viral genes

SV40 (www.steve.gb.com/images/science/sv40.jpg)
BKV genomic organization

- **Structural genes** (capsid proteins)
  - VP1, VP2, VP3
  - VP1 contains a hypervariable region (TR) useful for genotyping

- **regulatory proteins**
  - Early: large T, small t
  - Late: Agno

Pathogenesis

JC/BK viruses → Inoculation in respiratory tract → Multiplication in RT → Primary viremia

Immunocompetent (latent in kidney) ← Transit secondary viremia ← Multiplication in the kidney

Immunodeficient

Viruria, possible haemorrhagic cystitis

BK multiply in UTI ← Reactivation

JC virus viremia → CNS PML
Epidemiology

• Overall seroprevalence in humans of 60-90%
• BK primary infection usually occurs in childhood with chronic latent infection in kidney and urinary tract
• Clinical manifestations rare if immunocompetent - primary infection may manifest as childhood viral URI
• Clinically significant in the immunocompromised host
BK subtypes defined by VP1 typing region (TR)

- 4 major serologic/genotypic subtypes
- “phylogenetic analyses based on complete DNA sequences supported not only the subtype classification of BKV isolates [by TR sequence] but also the subclassification of subtype-I isolates.”

Geographic distribution of BK subtypes

Polyomavirus-associated nephropathy (PVAN)

- Affects 1-10% of renal transplant patients
- Incidence has increased in the last decade
- Most cases in first year after transplantation
- Risk of PVAN rises with intensity of immunosuppression
- Clinically resembles acute rejection
  - Rise in serum creatinine
Why measure BKV?

• **BK virus nephropathy** (BKVN) in renal transplant recipients
  – screening
  – adjunct to diagnosis
  – monitor success of intervention

• **Hemorrhagic cystitis** (Hematopoietic Stem Cell Transplant recipients)
  – may play a diagnostic and/or prognostic role
BK detection methods

- **Culture** - rarely used clinically
- **Urine cytology**
  - “decoy cells” = enlarged nucleus with a single large basophilic intranuclear inclusion
  - good sensitivity, poor specificity
- **Serology**
  - high seroprevalence in adults; not diagnostically useful
  - may play a role in pediatric transplantation
- **Renal biopsy**
  - Indicated in setting of suspected BK-nephropathy versus graft rejection
  - Histology/IHC/in situ hybridization/tissue PCR/EM
- **PCR**
  - Urine
  - Serum
Adenovirus

First isolated from adenoidal tissues in 1953
PROPERTIES

- Naked double stranded DNA virus
- Icosahedral symmetry
- 80 nm in diameter
- Capsid consist of 252 capsomeres (240 hexons, 12 pentons)
  Pentons make up the apices and possess projecting fibers.
- The fibers possess hemagglutinating activity and mediate the attachment of the virus to cellular receptors.
51 human adenoviruses in groups A - F
based upon DNA homology, disease tropism, and fiber antigens

- Mastadenovirus
- Aviadaenovirus
viral genome encodes many viral proteins. Early proteins promote the growth of the infected cell. E1A/E1B viral proteins bind and inactivate cellular p53 and pRB, thus stimulating cells' growth.

Virus also provides its own DNA-dependent DNA polymerase. Some viral proteins suppress the host immune response, including inflammation. Late proteins provide structural proteins and those carried in mature virion.

Virus enters the cell by endocytosis, lysing the endosomal vesicle, and capsid is removed as it delivers the DNA to the nucleus.
Adenoviruses cause diseases that involve respiratory, urinary, gastrointestinal tracts and eye.

- **Permissive cells** will ultimately exhibit lysis & death

- **Non-permissive cells** – exhibit latency
  - Mostly in lymphoid tissue – tonsils, adenoids, Peyer’s Patches

- Replication via general model for DNA viruses
  - Attachment to host cell receptor via knobs on tips of the viral fibers
  - Entry into cell by receptor-mediated endocytosis
  - Uncoating of viral genome, transported to nucleus of host cell
  - Transcription of viral genes, genome replication, & assembly all occur w/in Nucleus

- Virus may be introduced through contact, respiratory droplets or ingestion.

- The association of particular types with specific disease syndromes is striking.
# Diseases caused by Adenoviruses

<table>
<thead>
<tr>
<th>Group Affected</th>
<th>Syndromes</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td>Fatal disseminated infection</td>
<td>3, 7, 21, 30</td>
</tr>
<tr>
<td>Infants</td>
<td>Coryza, pharyngitis</td>
<td>1, 2, 5 (C)</td>
</tr>
<tr>
<td>Children</td>
<td>Upper respiratory disease</td>
<td>1, 2, 4-6</td>
</tr>
<tr>
<td></td>
<td>Pharyngoconjunctival fever</td>
<td>3, 7 (B)</td>
</tr>
<tr>
<td></td>
<td>Hemorrhagic cystitis</td>
<td>7, 11, 21 (B)</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>2, 3, 5, 40, 41 (F)</td>
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<tr>
<td></td>
<td>Intussusception</td>
<td>1, 2, 4, 5</td>
</tr>
<tr>
<td></td>
<td>Meningoencephalitis</td>
<td>2, 6, 7, 12</td>
</tr>
<tr>
<td>Young adults</td>
<td>Acute respiratory disease and PNA</td>
<td>3, 4, 7</td>
</tr>
<tr>
<td>Adults</td>
<td>Epidemic keratoconjunctivitis</td>
<td>8, 19, 37 (D)</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>PNA with dissemination</td>
<td>5, 31, 34</td>
</tr>
<tr>
<td>patients</td>
<td>Liver infection</td>
<td>1, 2, 5 (C)</td>
</tr>
<tr>
<td></td>
<td>Urinary Tract Infection</td>
<td>35, 39</td>
</tr>
<tr>
<td></td>
<td>Intestinal Infection</td>
<td>42-51 (D)</td>
</tr>
<tr>
<td></td>
<td>CNS disease including encephalitis</td>
<td>7, 12, 32</td>
</tr>
</tbody>
</table>
# Diseases caused by Adenoviruses

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<tr>
<th>Groups Affected</th>
<th>Diseases</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>hematopoietic stem cell transplant</td>
<td>Gastroenteritis, pneumonitis, hepatitis, hemorrhagic cystitis, encephalitis, disseminated disease</td>
<td>1-3, 5, 7, 11, 31</td>
</tr>
<tr>
<td>(HSCT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver transplant</td>
<td>Gastroenteritis, hepatitis pneumonitis, disseminated disease</td>
<td>1, 2, 5, 7, 31</td>
</tr>
<tr>
<td>Renal transplant</td>
<td>Hemorrhagic cystitis, pneumonitis, Gl disease</td>
<td>11, 34, 35</td>
</tr>
<tr>
<td>Lung transplant</td>
<td>Pneumonitis, disseminated diseases</td>
<td>2, 5</td>
</tr>
</tbody>
</table>
• **Acute Hemorrhagic Cystitis**
  – Occurs *predominantly in boys (6 – 15 years)*
  – Acute dysuria
  – Haematuria
  – Adenovirus *serotype 11*
LABORATORY DIAGNOSIS

• **Virus Isolation**
  – Adenovirus may be isolated from most body fluids and secretions; eye swabs, NPA, throat swabs, **urine**, faeces, and CSF.
  – Human embryonic kidney cells Hep-2 cells
  – Primary monkey kidney cells 293 cells
  – **CPE includes rounding, clustering of cells with refractile intranuclear inclusion bodies**

• **Detection of antigen by Immunofluorescence (IF)**

• **Serology**
  – Infection of humans with any adenovirus type stimulates a rise in complement-fixing antibodies to adenovirus group antigens shared by all types. A **four-fold or greater rise** in these antibodies between acute phase and convalescent phase sera indicates recent infection.
  – **The fastidious (no growth on cell cultures) enteric adenoviruses can be detected by direct examination of fecal samples by ELISA or latex agglutination tests.**

• **Polymerase Chain Reaction (PCR)**
Adenovirus CPE in RMK

infected RMK  Un-infected RMK
Antigen detection by Immunofluorescence

- Rapid
- Relatively insensitive
- Not suitable for all specimen types
MOLLUSCUM CONTAGIOSUM VIRUS
• Poxviridae

– Brick-shaped or ovoid
– Size: 220-450nm long x 140-260nm wide x 140-260nm thick
– Enveloped
– ds DNA
– Genome size: 130-375kbs (large!)
– Produce skin lesions eg. Small pox and vaccina virus
<table>
<thead>
<tr>
<th>Genera</th>
<th>Characteristic Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthopoxvirus</td>
<td>Variola Major (Smallpox virus) man</td>
</tr>
<tr>
<td></td>
<td>Variola Minor (Alastrim virus)</td>
</tr>
<tr>
<td></td>
<td>Monkeypox</td>
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<tr>
<td></td>
<td>Vaccinia virus man</td>
</tr>
<tr>
<td></td>
<td>Cowpox virus cattle, cats</td>
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<tr>
<td>Parapoxvirus</td>
<td>Pseudocowpox virus</td>
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<tr>
<td></td>
<td>Orf virus (milker’s nodules)</td>
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<tr>
<td>Leporipoxvirus</td>
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<tr>
<td>Avipoxvirus</td>
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<tr>
<td>Capripoxvirus</td>
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<tr>
<td>Suipoxvirus</td>
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<tr>
<td>Molluscipoxvirus</td>
<td>Molluscum contagiosum virus</td>
</tr>
<tr>
<td>Yatapoxvirus</td>
<td>Yaba monkey tumor virus</td>
</tr>
</tbody>
</table>
Molluscum contagiosum virus

• Molluscum contagiosum is a specifically human disease of worldwide distribution.

• The incubation period varies from 1 week to 6 months. The lesion begins as a small papule and gradually grows into a discrete, waxy, smooth, dome-shaped, pearly or flesh-coloured nodule.

• Usually 1-20 lesions but occasionally they may be present in hundreds.
Molluscum contagiosum virus

• The disease occurs world-wide and is spread by direct contact.
• In general it tends to occur in children.
• MC is transmitted by close personal contact including sexual contact.
Diagnosis

• Diagnosis is usually done on clinical grounds alone by the typical appearance of the lesions.

• Expression of materials stained with Giemsa, Wright or Gram stain reveals molluscum bodies.

• Biopsy, which shows characteristic features of epidermal hyperplasia.

• Polymerase Chain Reaction

• The diagnosis can be supported by EM.

• Unlike other poxviruses, molluscum have not been demonstrated to grow in cell culture.
• Papillomaviridae
  – Similar to polyomaviruses
  – Diameter: 55nm
  – Genome size: 6.8 - 8.4kbs
    (larger than polyomaviruses)
  – In humans: May cause warts and genital cancers.
  – Eg. Human Papillomavirus (HPV)
Papova*viridae*

**Papilloma viruses**
- 100 genotype
- Oncogenic

**Polyoma viruses**
- Polyoma virus homonis 1&2 (BK&JC)
  - Asymptomatic infection but are associated with renal disease and progressive multifocal

*Envelop Protein E6&E7*--------cancer by oncogenic HPV genotypes

 cause **warts**, and several genotypes are associated with human cancer (e.g., **cervical carcinoma**).
Human Papilloma Virus Infections

- HPV 16/18 cause cervical papillomas and dysplasia in which the virus DNA is integrated into the genome rather than acting as a plasmid
  - E6/E7 genes are oncogenes which produce proteins that bind to and inactivate cellular growth-suppressor proteins, p53 and pRb
    - unsuppressed cells are more prone to mutations and transformation
- infected cells exhibit nuclear changes with large perinuclear vacuoles
  - kiliocytosis
  - cause both benign and malignant lesions
HPV Infections/ Lesions

• **Anogenital Warts**
  – genital warts **HPV 6/11** exclusively on the squamous epithelium of the external genitalia and perianal areas rarely malignant

• **Cervical dysplasia and neoplasia**
  – malignant changes caused by **HPV 16/18** is an intraepithelial cervical dysplasia
  – koilocytotic cells observed in Papanicolaou-stained cervical smears
    • perinuclear cytoplasmic vacuolization
<table>
<thead>
<tr>
<th>Lesion</th>
<th>Associated HPV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-malignant lesions</td>
<td></td>
</tr>
<tr>
<td>Common warts</td>
<td>1,2,4</td>
</tr>
<tr>
<td>Flat warts</td>
<td>3</td>
</tr>
<tr>
<td>Genital warts</td>
<td>6,11</td>
</tr>
<tr>
<td>Laryngeal papilloma</td>
<td>6.11</td>
</tr>
<tr>
<td>Premalignant lesion</td>
<td></td>
</tr>
<tr>
<td>Epidermodysplasia verruciformis</td>
<td>2,3 and other</td>
</tr>
<tr>
<td>Malignant lesion</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>16,18,31,33,35, and others</td>
</tr>
</tbody>
</table>
Laboratory Diagnosis of HPV Infections

- **Cytology** detects koilocytic cells
- Warts are characterized by hyperplasia of the prickle cells and increased keratin production known as hyperkeratosis
- **Koilocytosis** of squamous epithelia cells which are rounded and clumped
  - as observed in a Papanicolaou smear
KOILOCYTES

• Hallmark of HPV infection in an epithelium
• “Raisinoid” nucleus (enlarged, hyperchromatic, irregular), perinuclear halo and cytoplasmic thickening
• Upper epithelial layer
Kiolocytosis. This screening power magnification shows scattered mature squamus with well-defined clearing of cytoplasm around the nuclei and slight nuclear enlargement (MP)
Laboratory Diagnosis of HPV Infections

- In situ DNA probe analysis detects viral nucleic acid method of choice
- Polymerase chain reaction detects viral nucleic acid method of choice
- Southern Blot Hybridization detects viral nucleic acid
- Immunofluorescence detects structural viral antigens
- Electron Microscopy detects intact virus
- Culture: not useful
A new HPV recently vaccine Gardasil vaccine which offer protection aganist HPV6, 11, 16 and 18, recommended for girls ages 9-26 y, given as a series of 3 injections over a 6-month period. The second dose is given two months after the first dose, followed four months later by the third dose.