Laboratory Diagnosis of Viral Skin Infections

M Parsania, Ph.D.
Tehran Medical Sciences Branch, Islamic Azad University
important viral agents causing skin and mucus membranes Infections in human

- viruses cause maculopapular rashes
  - rubella virus
  - Human parvovirus B19
  - Measles virus
  - Enteroviruses
  - Human herpes viruses types 6 and 7

- viruses cause vesicular skin rashes
  - Herpes simplex virus
  - Varicella-zoster virus
  - Enteroviruses
  - Pox viruses

- viruses cause wart-like lesions on the skin and mucus membranes
  - Human Papilloma viruses
  - Molluscum contagiosum virus
Diseases of the Skin Caused by Herpesviruses

The Relationships between the Human Herpesviruses
HSV Viral Structure

- Composed of a dsDNA (152kbp) nucleoprotein core
- Core is surrounded by an icosahedral protein capsid
- 100nm Capsid is enclosed in an outer envelope consisting of at least 8 glycoproteins.
- Envelope spikes ~8 nm long
- The virus requires a moist environment for survival.
<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>
</tr>
<tr>
<td></td>
<td>Varicellovirus</td>
<td>Varicella-zoster virus (VZV)</td>
</tr>
<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>
</tr>
<tr>
<td></td>
<td></td>
<td>Human herpesvirus 7 (HHV-7)</td>
</tr>
<tr>
<td><strong>Gammaherpesvirinae</strong></td>
<td>Lymphocryptovirus</td>
<td>Epstein-Barr virus (EBV)</td>
</tr>
<tr>
<td></td>
<td>Rhadinovirus</td>
<td>Human herpesvirus 8 (HHV-8)</td>
</tr>
</tbody>
</table>
Cold sores are contagious sores caused by HSV-1.

- After primary infection, the viruses become latent in sensory ganglia.
- Recurrence of cold sores occurs when viruses are reactivated and move to the epithelium.
ii. **Dermal** - mainly among the health care workers
   - **Herpetic whitlow**
     - painful
     - heals without treatment
     - no pus/is it necessary to do a stain
   - **Herpes gladiatorum** – among wrestlers
   - **Eczema herpeticum**
Herpetic whitlow
Herpes gladiatorum
Eczema herpeticum
• **Specimens**
  – aspirate from vesicle
  – scraping from base of ulcer
  – serum for antibody
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

**Multiplex primers:**
- cutaneous group; HSV, VZV
- lymphotrophic group; CMV,

**DNA extraction**

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

**Amplify 20-30 cycles**

**Detection:**
- gel electrophoresis
- dot blot hybridization
- *restriction fragment length polymorphism*
<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>
</tr>
<tr>
<td></td>
<td>Varicellovirus</td>
<td>Varicella-zoster virus (VZV)</td>
</tr>
<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>
</tr>
<tr>
<td></td>
<td></td>
<td>Human herpesvirus 7 (HHV-7)</td>
</tr>
<tr>
<td><strong>Gammaherpesvirinae</strong></td>
<td>Lymphocryptovirus</td>
<td>Epstein-Barr virus (EBV)</td>
</tr>
<tr>
<td></td>
<td>Rhadinovirus</td>
<td>Human herpesvirus 8 (HHV-8)</td>
</tr>
</tbody>
</table>
Varicella- Zoster Virus

- **Chickenpox**
  - VZV is extremely communicable
  - Reservoir = *infected humans* either symptomatic or asymptomatic
  - Primary Mode of Transmission = p-p, direct, *respiratory droplet*
  - Secondary Route = direct contact with active vesicles

- **Shingles**
  - Is a *reactivation disease*; resulting from previous VZV infection
  - Is generally not considered a communicable condition
  - Exception
    - There are a few documented cases of transmission from and adult with shingles to a young child
      - Child developed chickenpox
HERPES ZOSTER

- Reactivation of HVZ
- dermatomal distribution
- may recur
- can disseminate in immunocompromised patients
- complications
  - post herpetic pain
  - ophthalmic zoster - corneal scarring and loss of vision

**DIAGNOSIS**

**CLINICAL**
- EM of vesicle fluid

**SEROLOGY**
- IgM detection
DIAGNOSIS

- CLINICAL
- Isolation of virus
- EM of vesicle fluid
- SEROLOGY (IgM detection)
- PCR
Varicella-zoster virus (VZV) infection

Chickenpox  Zoster  →  Clinical diagnosis

Atypical clinical manifestation

Immunocompromised host

- Eye infection
- Brain infection
- Atypical skin rash
Laboratory diagnosis of VZV

Direct staining

- Samples → Infected cell scrape
- Tzanck test → ballooning cell with intranuclear inclusion, multinucleated cells
- Immunostaining: fluorescent staining
Tzanck test
Serological test of VZV

ELISA with VZV specific antigen

**IgG**
- seroconversion
- rising Ab titer $\geq \alpha$ times

**IgM**
- detected both
- chickenpox & zoster

**Limitation:** sharing some Ag with HSV
Isolation of VZV

Nasal/throat washing vesicle fluid

Inoculate promptly

Human diploid cell culture

CPE
ballooning, multinucleated cell

Identification: IF
Polymerase Chain Reaction

Single/Nested PCR

using primer common with HSV

detected both VZV & HSV

Multiplex PCR

using mix primers

HSV + VZV + ....
Picornaviruses

Characteristics

• *pico* = small, *rna* = RNA Viruses
  • icosahedral 30 nm

• naked nucleocapsid = Nonenveloped

• *plus strand* (+) RNA m-RNA
  • single stranded and capped for infectivity and packaging
  • this genome is infectious (should it be introduced into a cell)

• vertices of capsid creates canyon-like depressions which contain the VAP’s, VAP -1, VAP -2, VAP -3
  • most VAP bind to intracellular adhesion molecule -1 (ICAM-1) expressed on epithelial cells, fibroblasts, and endothelial cells
Picornavirus Capsid Structure

Capsid is a pseudo T=3 icosahedron consisting of 60 identical asymmetric protomers arranged as 12 pentamers.

Each protomer is composed of a single copy of each of the four capsid proteins, VP1, VP2, VP3 and VP4.

VP4 is located on the inner surface of the protein shell formed by VP1, VP2 and VP3.
Classification

- **Enterovirus** (enteroviruses)
  a) Polioviruses types 1, 2 and 3
  b) Coxsackieviruses A1-A24 (no A23), B1-B6
  c) Echoviruses 1–34 (no 10 or 28)
  d) Enteroviruses 68 -71

- **Rhinovirus** (rhinoviruses)

- **Hepatovirus** (hepatitis A virus)

- **Parechoovirus** (parechooviruses)

- **Aphthovirus** (foot-and-mouth disease viruses)

- **Cardiovirus** (cardioviruses)
Skin and mucus membrane

1. Herpangina
   a) Coxsackievirus A

2. Hand-foot-and-mouth disease
   a) Coxsackievirus A16
Herpangia = fever, sore throat with painful swallowing, anorexia and vomiting

vesicular ulcerated lesion on the soft palate and uvula

etiological agent is *Coxsackie virus A*, an enterovirus

*virus is shed from* the lesions, respiratory droplets and in the feces (fecal-oral)
Herpangina
Hand-Foot-Mouth Disease (vesicular exanthem)

vesicular lesions on the hands, feet, mouth, tongue accompanied by mild fever

etiological agent: *Coxsackie virus A16*

virus is shed/transmitted from lesions and is also shed in the feces(fecal-oral)
Coxsackie Virus and Hand, Foot and Mouth Disease

Hand, foot, and mouth disease (HFMD) is a common viral illness that primarily affects infants and children. It's caused by the coxsackie virus, and its main symptoms are blisters in and around the mouth and on the hands and feet. HFMD is moderately contagious and is spread through direct contact with fluids from the blisters, sneezing, coughing and saliva.
Hand-Foot-Mouth Disease
Picornaviruses - Diagnosis

• Enteroviruses
  • Laboratory
    • Serology
      • detection of specific viral antibody in IgM fraction
      • four fold increase in IgG from acute to convalescence
    – Culture performed only for epidemiological confirmation
      • coxsackie or echoviruses from throat or feces
        • monkey kidney tissue culture
        • human embryo kidney tissue culture
      • culture virus is specifically identified with antibody assays
  - RT-PCR
• 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
Poxviruses (continued)

Figure 1. Structure of the variola 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>
</tr>
<tr>
<td></td>
<td>Vaccinia virus man</td>
</tr>
<tr>
<td></td>
<td>Cowpox virus cattle, cats</td>
</tr>
<tr>
<td>Parapoxvirus</td>
<td>Pseudocowpox virus</td>
</tr>
<tr>
<td></td>
<td>Orf virus (milker’s nodules)</td>
</tr>
<tr>
<td>Leporipoxvirus</td>
<td>Not important to man</td>
</tr>
<tr>
<td>Avipoxvirus</td>
<td></td>
</tr>
<tr>
<td>Capripoxvirus</td>
<td></td>
</tr>
<tr>
<td>Suipoxvirus</td>
<td></td>
</tr>
<tr>
<td>Molluscipoxvirus</td>
<td>Molluscum contagiosum virus</td>
</tr>
<tr>
<td>Yatapoxvirus</td>
<td>Yaba monkey tumor virus</td>
</tr>
</tbody>
</table>
Smallpox

- Smallpox was transmitted by respiratory route from lesions in the respiratory tract of patients in the early stage of the disease.
- During the 12 day incubation period, the virus was distributed initially to the internal organs and then to the skin. Variola major caused severe infections with 20-50% mortality, variola minor with <1% mortality. Management of outbreaks depended on the isolation of infected individuals and the vaccination of close contacts.
- The vaccine was highly effective. If given during the incubation period, it either prevented or reduced the severity of clinical symptoms. The origin of the vaccine strain is not known.
Smallpox
Smallpox
The Eradication of Smallpox

- Smallpox was eradicated from most countries in Europe and the US by 1940s. By the 1960s, smallpox remained a serious problem in the Indian subcontinent, Indonesia and much of Africa. The WHO listed smallpox as the top on the list for eradication in 1967. The WHO smallpox eradication unit was set up in 1967.
- Smallpox was officially declared eliminated in 1980.
Monkeypox

- Although Monkeypox was first isolated from monkeys, there is no evidence that African monkeys act as the reservoir.

- The most likely candidate for reservoir is the African squirrel.

- One important difference between human Monkeypox and smallpox is the lower capacity for human spread.
Monkeypox Virus
COWPOX

- Infection has been described in humans, cows and cats.
- Infection in humans usually remain localized, often producing a lesion which is similar to that caused by vaccination, although the inflammatory response is greater and general constitutional symptoms such as fever and myalgia may be present in some cases. In humans, lesions are usually restricted to the hands, but may also be transferred to the face.
- EM is generally used for the diagnosis of infection. The virus will also grow well on CAM.
- Although cowpox was first isolated form cattle and farm workers. There is no evidence that cattle serve as the reservoir. In fact, cowpox is very rare in cattle. It has been suggested that the reservoir is actually a small rodent but this is not proven.
Cowpox virus
PARAPOXVIRUSES

- The laboratory diagnosis is usually made by EM. The virus may also be isolated in human, bovine and ovine cells but such investigations are not part of routine diagnostic virology.
- Parapoxvirus infections occur worldwide, and are of considerable importance.
- The lesions are surprisingly painless and thus there is probably substantial under-reporting.
- Idoxuridine had occasionally been prescribed for treatment but no trials have been carried out to prove the efficacy of treatment. Prevention of human infection is difficult. Reasonable precautions should be undertaken when handling infected animals.
A scabby sore on a human hand caused by **orf**

A thumb with two denuded **orf** lesions

**Orf** Virus in Sheep

A sheep infected with **Orf disease**
milker's nodes in Human
Lab Methods for Confirmation of poxvirus Diagnosis

- PCR related methods for DNA identification, (e.g., real-time PCR)
- Electron microscopy
- Culture
- Antigen detection (IFA, EIA Ag capture)
- Serology
  - IgM capture
  - Neutralization Test
  - IgG ELISA
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
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)
**HPV Infections/ Lesions**

- **Skins Warts**
  - Hands and Feet: HPV 1 - 4
  - Most common type
- **Head and Neck Tumors**
  - Oral papillomas: benign epithelial tumors of the oral cavity
  - Laryngeal papilloma: HPV 6/11 benign epithelial tumors
- **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
  - Koilocytic cells observed in Papanicolaou-stained cervical smears
    - Perinuclear cytoplasmic vacuolization
Replication

- Papillomavirus-cell interactions can be classified into three main groups: permissive, non-permissive transformable, and non-permissive non-transformable depending on the particular virus and cell\(^8\). Sarcoid cells are non-permissive to BPV replication and propagation\(^7\).
- BPV targets basal cells\(^5\).

Transcriptional states are regulated by the differentiation of the squamous epithelium\(^9\). Maturation requires viral transport from the basal layer to the surface epithelium. During this movement, the differentiating keratinocyte undergoes complex changes to provide a correct intracellular environment for viral replication\(^8\).

www.gsbs.utmb.edu
### 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
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*
KOILOCYTE

• Hallmark of HPV infection in an epithelium
  • “Raisinoid” nucleus (enlarged, hyperchromatic, irregular), perinuclear halo and cytoplasmic thickening
• Upper epithelial layer
Laboratory Diagnosis of HPV Infections

- **Polymerase chain reaction** detects viral nucleic acid
- **Southern Blot Hybridization** detects viral nucleic acid
- **Immunofluorescence** detects structural viral antigens
- **Electron Microscopy** detects intact virus
- **Culture**: not useful
<table>
<thead>
<tr>
<th>Lesion</th>
<th>Associated HPV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-malignant lesions</td>
<td>Common warts</td>
</tr>
<tr>
<td></td>
<td>Flate warts</td>
</tr>
<tr>
<td></td>
<td>Genital warts</td>
</tr>
<tr>
<td></td>
<td>Laryngeal papilloma</td>
</tr>
<tr>
<td>Premalignant lesion</td>
<td>Epidermodysplasia verruiformis</td>
</tr>
<tr>
<td>Malignant lesion</td>
<td>Cervical cancer</td>
</tr>
</tbody>
</table>
Rapid Genotyping of Human Papillomavirus by Post-PCR Hybridization