Laboratory Diagnosis of Viral Infections affect the Skin and Mucous Membrane

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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.
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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.

Figure 11A: Herpes Simplex Virus
© Phototake/Alamy Images

Figure 11B: Herpes sore on mouth
Courtesy of Dr. Hermann/CDC
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
• **Specimens**
  – aspirate from vesicle
  – scraping from base of ulcer
  – serum / CSF for antibody
Laboratory diagnosis of HSV

Direct staining

Tzanck test

Immunostaining

HSV isolation

Serology

PCR
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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
VARICELLA ZOSTER VIRUS
VZV

• Causes chicken pox -fever + characteristic rash
• variable incubation period 14-21 days
• usually mild in children and more severe in adults
• complications
  – secondary infection - uncommon
  – varicella pneumonia
  – secondary bacterial pneumonia S aureus & pneumococci
  – post-infetious encephalitis
  – generalised varicella (in immunocompromised patients)
  – congenital and neonatal varicella
Skin

• **Viral systemic infections**
  
  – Rashes
    
    • Viruses invade skin via blood vessels
      
      – Macules
      
      – Papules
      
      – Vesicles
      
      – Pustules
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
Prevention of Chickenpox

Susceptible population
- children
- adults living in close proximity

Immunize
live attenuated vaccine

Protect if contact with patient with chickenpox and at risk of severe disease
Zoster Immune Globulin (ZIG)
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 ≥ 4 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 + ....
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HUMAN HERPES VIRUSES-6

- **HHV6**
  - Worldwide
  - virus replicates in T and B cells
  - infection occurs in first 3 years of life
  - Clinical *Exanthem subitum*
  - *(roseola infantum)*
    - mild acute febrile illness
    - incubation period of 2 weeks
    - fever lasts several days
    - macular papular rash appears within 2 days of fever
  - 85% of adults carry virus in saliva
Roseola Infantum or exanthem subitum (6th disease)
HUMAN HERPES VIRUSE-7

• HHV7
  – isolated from CD4 positive cells
  – virus present in saliva of >75% of adults
  – role in disease unclear
  – Evidence of infection present (seroconversion)
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EPSTEIN BARR VIRUS
EBV

• Discovered in 1964 by Epstein & colleagues
• Definite association with malignancy
• is able to ‘transform’ cells resulting in ‘immortalization’ of cell
Infectious mononucleosis

- Affects adolescents and young adults
- worldwide distribution
- called ‘kissing’ disease
- IP - one month
- presents with fever,
  sore throat, rash & lymph nodes
EBV

Mononucleosis causes:

- Fever
- Fatigue
- Sore throat
- Swollen lymph glands
Hairy tongue - EBV
DIAGNOSIS:

raised WBC with >20% lymphocytes (Atypical Lymphocyte)

Paul-Bunnell test (heterophile antibodies) or monotest

Detection of Antigen

Serologic (IgM and IgG)

PCR
Atypical Lymphocyte
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**Human Herpesvirus 8**

| **Sarcoma tissue** |
HUMAN HERPES VIRUS E-8

- HHV8
- Kaposi sarcoma- associated Herpes Virus (KSHV)
  - detected in epithelial cells of Kaposi sarcoma
  - also present in semen
  - postulated as cause of Kaposi sarcoma
Kaposi’s Sarcoma
Kaposi sarcoma is an angiogenic tumor of the **blood vessel walls**.

- It is most commonly seen in **immunocompromised** individuals, such as AIDS patients.
- It is caused by HHV-8, forming dark or purple skin lesions.
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) **Coxsackie** viruses A1-A24 (no A23), B1-B6
  c) Echoviruses 1–34 (no 10 or 28)
  d) Enteroviruses 68-71

- **Rhinovirus** (rhinoviruses)

- **Hepatovirus** (hepatitis A virus)

- **Parechovirus** (parechoviruses)

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

- **Cardiovirus** (cardioviruses)
Skin and Mucosae

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
PicornaVirus - 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
Other Viral Diseases of the Skin

• Paramyxovirus infections can cause typical childhood diseases.
  – **Measles** (rubeola) is a highly contagious disease caused by a member of the *Paramyxoviridae* family.
    • Transmission occurs through respiratory droplets.
    • **Koplik spots** are red patches with white grain-like centers that appear along the gum line.
    • The characteristic red rash begins as *maculopapules* at the hairline, spreading to the face, trunk and extremities.
    • Vaccine is given in the measles-mumps-rubella (MMR) inoculation.
Figure 16A: Koplik Spots

© Medical-on-Line/Alamy Images

Figure 16B: Measles

Courtesy of CDC
DIAGNOSIS

- CLINICAL
- Isolation of virus
- Antigen Detection
- SEROLOGY (IgM & IgG detection)
- PCR
Parvoviridae
Properties of Parvoviruses

• **Structure**
  – Icosahedral
  – 18-26 nm diameter
  – Single-stranded DNA, 5.6 kb
  – Two proteins
  – Nonenveloped

• **Classification**
  – Parvoviridae (vertebrates)
    • *Parvovirus*
    • *Erythrovirus*
    • *Dependovirus* (requires helper virus, such as an adenovirus)
  – Densovirinae (insects)
Parvovirus Infections in Humans

• Diseases
  – **Fifth disease (cutaneous rash)**
  – **Transient aplastic crisis (severe acute anemia)**
  – **Pure red cell aplasia (chronic anemia)**
  – **Hydrops fetalis (fatal fetal anemia)**
  – **B19 virus most common**

• Fifth Disease
  – **Targets red blood cell progenitors**
  – **Pain in joints**
  – **Results in lysis of cells, thus depleting source of mature red cells**
  – **Anemia ensues**
  – **Rarely fatal and without complications**
Parvovirus Infections in Humans

- **Transient aplastic crisis**
  - B19 infection of those with other hemolytic anemias
    - Sickle cell disease
    - Thalassemias
  - Can complicate crises
  - Sometimes fatal

- **Infection of immunodeficient patients**
  - Can cause *persistent infection* in bone marrow
  - Suppress red cell maturation
  - Leads to anemia

- **Infection during pregnancy**
  - Can cause *fetal anemia*
  - Usually not fatal to fetus
Laboratory Diagnosis

• **PCR is most sensitive**
  – Most useful during *viremia*
  – Otherwise, requires tissue biopsy or bone marrow tap

• Serological testing **for IgM**
  – Determines recent infection
Togaviridae : Genus Rubivirus

• 1 member of genus = Rubella Virus
  – Etiological agent for Rubella (German Measles or “3-day Measles”)
    • mild respiratory disease in children
    • Consists of
      1. Mild fever & malaise
      2. Swollen glands (lymphadenopathy)
      3. Viremia
      4. 3-day rash; whole body exanthem in children
    • More severe in adults, causing bone & joint pain
    • Only a respiratory disease; does not cause readily detected cytopathic effects
Rubella
Genus Rubivirus

• Rubella Virus
  – Major cause of Teratogenesis
  • Occurrence in pregnant ♀’s – “Congenital Rubella Syndrome”
    – If ♀ NOT immune & gets infected → viremia → placenta → viral infection of fetus
    – In absence of Ab during pregnancy, virus replicates in placenta, spreads to fetal circulation and infects most of the tissues in developing fetus
    – Virus alters normal cellular growth, alters rates of mitosis, and alters chromosomal structures (teratogenesis)
Diagnosis of Rubella Virus

Isolate and culture the virus
- Nasopharyngeal/Throat swabs
- Viral Ag detection by Immunofluorescence
- Serology
  - ELISA Test for detection of IgM Ab’s
  - Detection of IgG = evidence of immunity (there is only 1 serotype)

- PCR
Genus Rubivirus

- **Rubella Virus**
  - **Treatment & Prevention**
    - No specific tx is indicated b/c it is a mild, self-limiting illness
    - Attenuated, live Rubella vaccines – available since 1969; single Ag or combined w/ Measles and Mumps (“R” in MMR vaccine)
    - **purpose of Rubella Vaccine is to prevent congenital Rubella infections** – by decreasing # of susceptible people in population, esp. children → therefore, there are fewer seroneg. mothers and thus smaller chance that they will be exposed to virus from contact with children
    - Vaccine virus multiplies in body and is shed in small amounts, but does not spread to contacts
      - Vaccinated children pose no threat to mothers who are susceptible and pregnant
      - Vaccination in US has decreased incidence of Rubella from ~70,000 cases in 1969 to only a few 100 today
      - Live Rubella vaccine usu. administered as MMR @ 12-24 months of age – promotes both Humoral & Cellular immunity
• **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)
Human Papilloma Virus Infections

- viruses infect and are replicated by squamous epithelial cells of the skin and mucous membranes
  - gives rise to Warts in the skin
  - gives rise to Papillomas on mucous membranes
- infected cells exhibit nuclear changes with large perinuclear vacuoles
  - kiliocytosis
  - cause both benign and malignant lesions
- 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
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
  – koilocytotic 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. Sarcoid cells are non-permissive to BPV replication and propagation.
- BPV targets basal cells.
- Transcriptional states are regulated by the differentiation of the squamous epithelium. 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.

www.gsbs.utmb.edu
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
• 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
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<th>Characteristic Members</th>
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<td>Variola Minor (Alasstrim virus)</td>
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<td>Monkeypox</td>
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<td></td>
<td>Vaccinia virus man</td>
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<td>Cowpox virus cattle, cats</td>
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<tr>
<td>Parapoxvirus</td>
<td>Pseudocowpox virus</td>
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<td>Orf virus (milker’s nodules)</td>
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<td>Leporipoxvirus</td>
<td>Not important to man</td>
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<td>Yatapoxvirus</td>
<td>Yaba monkey tumor virus</td>
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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
Variola Major

Ordinary Type
Variola Major

Ordinary Type
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.
Features Which Made Smallpox an Eradicable Disease

1. A severe disease with morbidity and mortality
2. Considerable savings to developed non-endemic countries
3. Eradication from developed countries demonstrated its feasibility
4. No cultural or social barriers to case tracing and control
5. Long incubation period
6. Infectious only after incubation period
7. Low communicability
8. No carrier state
9. Sub-clinical infections not a source of infection
10. Easily diagnosed
11. No animal reservoir
12. Infection confers long-term immunity
13. One stable serotype
14. Effective vaccine available
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
VACCINIA

- Vaccination with vaccinia was associated with certain risks.
- Complications ranged from mild reactions and fatal encephalitis. *The overall incidence of complications was around 1/800* although the more severe forms occurred only in 15 per million vaccinees.
- Recent interest has focused on the possible usage of vaccinia as a vector for immunization against other viruses. It is possible that certain changes can be made to the vaccinia genome which makes it less likely to develop side effects.
Vaccinia lesion on foot
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 from 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
Edward Jenner inoculates a boy with cowpox vaccine
Parapoxvirus infections are widespread in sheep, goats and cattle and relatively unimportant but common human infections occur.

Infections in cattle and humans are usually referred to as pseudocowpox, paravaccinia or milker's nodes. Those in sheep and goats as orf. The viruses are closely related and the nomenclature of the human disease is based on the identity of the host form which the infection was acquired. (orf from sheep and pseudocowpox from cattle).

Infection occurs via small cuts and abrasions in all hosts and is usually localized. Although the lesions are similar to the early lesions of cowpox and vaccinia, true macrovesicles do not form. In humans, lesions usually occur on the hand but may be transferred to the face.
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
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.
Molluscipoxvirus

- **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
Molluscipoxvirus

• The disease **occurs world-wide** and is spread by direct contact.

• In general it tends to occur in children. The disease by may transmitted from skin to skin after **sexual intercourse**.

• A diagnosis can usually be made on clinical appearance alone.

• The **diagnosis can be supported by EM**. Unlike other poxviruses, molluscum **have not been demonstrated to grow in cell culture**.
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 antibodies
  – IgG ELISA