بسم الله الرحمن الرحيم
Laboratory Diagnosis of Viral Ocular Infections

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important viral agents causing eye infections in human

- Adenoviruses
- Herpes simplex viruses
- Varicella zoster virus
- Cytomegalovirus
- Epstein-Barr virus
- Enterovirus type 70
- Coxsackie virus A24
- Influenza A
- Molluscum contagiosum virus
- Human Papilloma virus
Although treatment of viral infections is often non-specific, diagnosis assists the control of inappropriate treatment that could lead to more serious clinical sequela, e.g., the application of steroids during infection with HSV allows the virus to multiply more rapidly.

Specific therapy of ocular infections often requires etiological diagnosis that is a combined effect of observation of characteristic clinical features and laboratory diagnosis.
There are five major methods for identification of virus infections

- direct observation
- antigen detection
- Culture
- Serology
- molecular diagnostics
Adenovirus

- **Family:** Adenoviridae
- **Genus:** Mastadenovirus
- **Type:** Adenovirus
- ds DNA virus
- non-enveloped
- 51 serotypes are known
- classified into 6 subgenera: A to F
Clinical Syndromes

1. Pharyngitis 1, 2, 3, 5, 7
2. Pharyngoconjunctival fever 3, 7
3. Acute respiratory disease 4, 7, 14, 21
4. Pneumonia 1, 2, 3, 7
5. Follicular conjunctivitis 3, 4, 11
6. Epidemic keratoconjunctivitis 8, 19, 37
7. Pertussis-like syndrome 5
8. Acute haemorrhagic cystitis 11, 21
9. Acute infantile gastroenteritis 40, 41
10. Severe disease in immunocompromized patients 5, 34, 35
11. Meningitis 3, 7
Laboratory Diagnosis of Adenoviruses

The laboratory diagnosis of ocular adenovirus infection is a function of the onset of clinical symptoms.

The earlier the conjunctival samples are collected after clinical onset, the higher likelihood of a positive result.

The adenoviral load of viable virus and antigen decreases over time.

- Virus isolation
- Rapid immunodetection assays
- Paired serologic titers 2-3 weeks apart
- DNA based techniques

Specimens:
- Conjunctival swabs
Specimen Collection and processing

- Specimens are directly collected by vigorously swiping the exposed conjunctiva with a plastic soft-tipped applicator.
- Collected samples are placed in 2.0 ml of viral transport medium.
- Adenovirus is not a fastidious virus.
- It will remain viable under many conditions and collected samples should be easily transported through mail carriers.
Specimen Collection and processing

Specimen processing

- **All laboratory testing** can be processed from the 2.0 ml of transport medium

- The **swab should be agitated** to release maximum material into the virus transport medium.
Virus isolation

➢ Most serotypes of adenoviruses replicate readily in any of the following human cell lines HEK, HEp2, HeLa, and A549 cells with or without rolling.

➢ The "gold" standard for adenovirus laboratory testing is cell culture.

➢ CPE includes rounding, clustering of cells with refractile intranuclear inclusion bodies.
Cell culture

- The adenoviruses are among the easiest viruses to identify because they have a distinctive CPE and are unique in producing prodigious quantities of soluble antigens as they grow in cell culture, and these antigens possess many type and group specific properties that aid diagnosis.

- When samples are collected within one to three days of clinical onset, cell culture generally is positive within four to seven days.

- Samples collected after three days may take one to three weeks to produce cytopathic effect.

- Cell culture will confirm an adenovirus diagnosis but it may not provide timely results for immediate patient care.
Shell Vial Culture

- Shell vial is another cell culture test but the results are ready in three days.
- Vials of A549 cells are inoculated with collected samples and centrifuged.
- The vials are then incubated and stained at day three with immunofluorescent antibodies specific to Adenovirus.
- The cells infected with Adenovirus will light up with examination under a fluorescent microscope.
- Shell vial highly correlates with standard cell culture especially when samples are collected within seven days of clinical onset.
- Shell vial testing is highly recommend for diagnosing ocular adenovirus infection.
EIA (Adenoclonetm)

- Adenoclonetm is an enzyme immunoassay that can detect adenoviral antigen from collected ocular specimens.
- Positive results can be obtained within 75 minutes.

- Unfortunately, Adenoclonetm is only 40 to 50 percent sensitive in detecting adenoviral antigen from clinical specimens.

- A high load of antigen is necessary for a positive test and this correlates with collection within one to three days of clinical onset.

- The power of this test is that it does provide rapid results when tests are positive but negative tests need to be confirmed with cell culture or shell vial.
PCR

- Polymerase Chain Reaction (PCR) is a molecular test that amplifies specific adenoviral DNA sequences from clinical samples and then identifies the amplified products with gel techniques.

- PCR is a highly sensitive and specific test that can detect adenoviral DNA from clinical samples.

- Results can now be obtained within one to three days.
# Herpesviridae

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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.
Complications

• **Meningitis**—infection of the sheaths and membranes (meninges) covering the brain and the spinal cord.

• **Encephalitis**—acute inflammation of the brain, commonly caused by a viral infection by insect bites or food and drink.

• **Eczema herpetiform**—widespread herpes across the skin

• **Keratitis**—Corneal infection, irritations, and inflammations

• **Keratoconjunctivitis**—with dendritic ulcers
Laboratory diagnosis of HSV

The detection of Herpes Simplex (HSV) from ocular specimens is essential for prompt and accurate therapy.

- Tzanck test
- Immunostaining
- HSV isolation
- Serology
- PCR
**Specimens:**

- Vesicles can be opened with a needle, and *vesicular fluid cultured*

- **Scrapings from the vesicle** base can be tested by cytology or for the presence of HSV antigen

- **Conjunctival scrapings** or impression cytology specimens can be similarly analyzed
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

\[\downarrow\]

rabbit anti-HSV Ig

\[\downarrow\]

goat anti-RaIg conjugated with fluorescein dye

\[\downarrow\]

mount with glycerine buffer
Viral isolation

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

Cytopathic effect (rounded, enlarged and multinucleated cell)

Identification or typing

*Immunofluorescent staining

The "gold" standard for HSV laboratory testing is cell culture.
HSV Cytopathic effect

Normal cells  CPE
HSV serology

Primary infection

Pair serum: acute & convalescent serum

IgG assay  *rising titer  $\rightarrow$  $\geq 4$ times

*séroconversion

Single serum: IgM assay

Recurrent infection

not useful; multiple reactivation
Polymerase chain reaction

Samples
infected cell, Conjunctival scrapings
  ↓
DNA extraction
  ↓
PCR solution
  (buffer, dNTP, Taq DNA pol, primers)
  ↓
Amplify 20-30 cycles

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

Detection:
• gel electrophoresis
• dot blot hybridization
• *restriction fragment length polymorphism
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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

  • Disease association:
    • Dermatoblepharitis
    • conjunctivitis
    • Keratitis
Laboratory diagnosis of VZV

- Tzanck test
- Immunostaining
- VZV isolation
- Serology
- PCR
• Specimens:

• As with HSV, scrapings from a vesicle base can be tested by cytology, PCR, or culture, or for the presence of VZV antigen.

• Conjunctival scrapings or corneal impression cytology specimens can be similarly analyzed.
Isolation of VZV

scrapings from a vesicle base or Conjunctival scrapings

Inoculate promptly

Human diploid cell culture

CPE
ballooning, multinucleated cell

Identification: IF
Serological test of VZV

ELISA with VZV specific antigen

**IgG**  
seroconversion  
rising Ab titer ≥ 4 times

**IgM**  
detected both  
chickenpox & zoster
Polymerase Chain Reaction

**Single/Nested PCR**

using primer common with HSV

detected both VZV & HSV

**Multiplex PCR**

using mix primers HSV + VZV + ....
Cytomegalovirus infections

- most populations - infections in early childhood
- often asymptomatic
- Latency
- Clinical disease increasing due to increasing number of immunocompromised patients

**Disease association:**
- Retinitis in immunocompromised patients

CMV retinitis
# Herpesviridae

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Cytomegalovirus

Laboratory diagnosis:
- Virus isolation
- PCR
- Ag detection

CMV retinitis can be identified by an ophthalmologist, who examines internal eye structures to check for characteristic abnormalities using an ophthalmoscope.

Vitreous sampling for culture or PCR is sometimes useful, but this procedure is not without risk.
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Epstein-Barr Virus

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

Disease association:
• dacryoadenitis,
• conjunctivitis,
• Keratitis
Replication and induction of antigens

Infection → EBNA → EMA → Persistent infection

Late MA → VCA → EA

LYSIS

EBNA - EB nuclear antigen
MA - membrane antigen
VCA - Viral capsid antigen
EA - Early antigen
 Epstein-Barr Virus

Specimens: Serum and eye fluid samples

Laboratory diagnosis:
- Because of difficulty in viral isolation, diagnosis of EBV infection depends on the detection of antibodies to various viral components.
- During acute infection, first IgM and then IgG antibodies to viral capsid antigens (VCA) appear. Anti-VCA IgG may persist for the life of the patient.
- Antibodies to early antigens (EA) also rise during the acute phases of the disease and subsequently decrease to low or undetectable levels in most individuals.
- Antibodies to EBV nuclear antigens (EBNA) appear weeks to months later, providing serologic evidence of past infection.

PCR
- Nested PCR
- Real-time PCR
Picornaviridae

- **Enterovirus** (enteroviruses)
  a) Polioviruses types 1, 2 and 3
  b) **Coxsackieviruses** A1-A24, B1-B6
  c) Echoviruses 1–34
  d) **Enteroviruses** 68-71
- **Rhinovirus** (rhinoviruses)
- **Hepatovirus** (hepatitis A virus)
- **Parechovirus** (parechoviruses)
- **Aphthovirus** (foot-and-mouth disease viruses)
- **Cardiovirus** (cardioviruses)
Picornaviruses – coxsackieviruses and Enteroviruses

- **Aseptic Meningitis**

- **acute febrile illness**
  etiological agent is *Coxsackie viruses A, B and Echoviruses*
  - may also lead to polio-like paralysis

- **Respiratory Tract Disease**
  - Coxsackie viruses A21/A24; Echoviruses 11/20

- **Acute Hemorrhagic Conjunctivitis**
  - *Enterovirus 70 and CoxsackieVirus A24*

- **Diabetes** insulin-dependent
  - *Coxsackie B virus destruction of the Islets of Langerhans*
Enterovirus type 70 & coxsackievirus A24

**Disease association:**
Acute hemorrhagic conjunctivitis (AHC)

**Laboratory diagnosis:**
- Virus isolation
  - monkey kidney tissue culture
  - human embryo kidney tissue culture
- PCR
  - Nested PCR
  - Real-time PCR

**Specimens:**
Conjunctival Swab
Orthomyxoviridae

- Influenza virus

on the basis of antigenicity of virus proteins (NP and MP) Classified into three main groups:

- Influenza A
- Influenza B
- Influenza C
Influenza A

• Further classified by Hemagglutinin (H) and Neuraminidase (N) sub-types

• Infects a wide variety of warm blooded animals, including domestic and wild birds and mammals

• Human subtypes include H1N1, H3N2, H1N2, and H2N2

• Current circulating strains are H1N1, H3N2

• Avian subtypes include H1 to H16 and N1 to N9

• Bird ➔ human H5N1, H9N2, H7N7, H7N2, H7N3
Avian subtypes of Influenza

A/H5N1 outbreak, an influenza-like illness typically appeared early in the course of the disease, and conjunctivitis was seen in some patients.

A/H7N7 infections mainly result in conjunctivitis and/or an influenza-like illness.

Severe congestion of the conjunctivae
Laboratory Diagnosis of Influenza

Specimens:
• Conjunctival Swab

➢ Virus Isolation - virus may be readily isolated from Conjunctival Swab.

➢ Antigen Detection – can be done by IFT or EIA

➢ Serology - a retrospective diagnosis may be made by serology. CFT most widely used. HAI and EIA may be used to give a type-specific diagnosis

➢ RNA Detection – RT-PCR assays give the best sensitivity and specificity.
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<td>Orthopoxvirus</td>
<td>Variola Major (Smallpox virus) man</td>
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<tr>
<td></td>
<td>Variola Minor (Alastrim virus)</td>
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<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>
</tr>
<tr>
<td></td>
<td>Orf virus (milker’s nodules)</td>
</tr>
<tr>
<td>Lporipoxvirus</td>
<td>Not important to man</td>
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<td>Avipoxvirus</td>
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<td>Yatapoxvirus</td>
<td>Yaba monkey tumor virus</td>
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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

Disease association:
- Infection produces 1 or more umbilicated nodules on the skin and eyelid margin and, less commonly, on the conjunctiva.
Laboratory diagnosis of Molluscum contagiosum

- Molluscum contagiosum virus cannot be cultured using standard techniques such as PCR.

- Diagnosis is based on the detection of the characteristic eyelid lesions in the presence of a follicular conjunctivitis.

- No testing available in routine clinical laboratories.
• Papillomaviridae
  – Similar to polyomaviruses
  – Diameter: 55nm
  – Genome size: 6.8 - 8.4kbs
    (larger than polyomaviruses)
  – In humans: May cause warts and genital cancers.
  – Human Papillomavirus (HPV)
Human Papilloma Virus

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

Appearance
A wart is a rough, raised part of the skin. Warts are often a different shade from the surrounding skin and are usually painless. The strain of HPV that affects the eyes usually causes flat warts, which are small and smooth and erupt in large numbers, and filiform warts, which are thread-like in shape. Flat warts tend to be more common in children than in adults. Eyes infected with HPV are not usually at risk of more severe symptoms than warts.
Laboratory Diagnosis of HPV Infections

- 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
Sampling for eye infections

- SPECIMEN COLLECTION
- SPECIMEN TRANSPORT AND STORAGE
- SPECIMEN PROCESSING
Sampling

external

internal
CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

- External specimens should be placed into Virus Transport Medium (VTM) immediately after collection.
- Samples collected after the application of fluorescent dye to the patient’s eye do not appear to affect the isolation of virus by cell culture.
TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

- Specimens that may be delayed should be refrigerated prior to transportation to the laboratory.

- Serum, aqueous and vitreous samples that may be delayed for a long time for examination or nucleic acid extraction should be stored at -20°C.
SAFETY CONSIDERATIONS

• Viruses associated with infections of the eye are in **Hazard Group 2**

• Laboratory procedures that may give rise to infectious aerosols, eg vortexing swabs, must be conducted in a **microbiological safety cabinet** and the **operator should wear gloves**.

• Chance **contact of infected gloved hand** with the operator’s eye **must be avoided** as laboratory acquired infection would be a likely outcome.
Thank you for your attention