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EYE INFECTIONS
Outline

- Sites of infection and Specimen Collection
- Bacterial Infections
- Viral Infections
- Fungal Infections
- Parasitic infections
History and Physical Exam

• Ocular pain
  – Uncommon in conjunctivitis
  – Common in keratitis, intraocular involvement

• Age of patient
• Acute (<4 weeks) vs. chronic
• Viral vs. bacterial
• Other factors
# Bacterial Culture?

## TABLE 2

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Serous</th>
<th>Mucoid</th>
<th>Mucopurulent</th>
<th>Purulent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlamydial</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bacterial</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allergic</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Toxic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Present; − = absent.

Adapted with permission from Jackson WB. Differentiating conjunctivitis of diverse origins. Surv Ophthalmol 1993;38(Suppl):91–104.
Conjunctivitis

- Commonly called “Pink Eye”
- Inflammation of the conjunctiva
- Symptoms include: swelling of the conjunctiva and/or eyelids (blepharitis), increased tear production, feeling like a foreign body is in the eye(s) or an urge to rub the eye(s), itching, irritation, and/or burning, discharge (pus or mucus), crusting of eyelids or lashes, especially in the morning, contact lenses that do not stay in place on the eye and/or feel uncomfortable
- Highly contagious
- Most common infectious causes are bacteria and viruses
Specimen Collection:
Conjunctivitis and Blepharitis

• Samples are most commonly collected with soft-tipped applicators (i.e. cotton, Dacron, or calcium alginate swabs).
• Moistened swab prior to collection with sterile medium (i.e. PBS, BSS)
• May apply a topical anesthetic (0.5% proparacaine) prior to obtaining a sample
• Conjunctival cultures are obtained by lowering the bottom eyelid and applying the moistened applicator to the lower bulbar conjunctiva for about 5 seconds without touching the eyelid margin.
• The eyelid margins are cultured similarly by applying the moistened applicator to the eyelashes and margins of both top and bottom eyelids.
• It is good practice to culture the conjunctiva and eyelid of both eyes in cases of conjunctivitis and blepharitis, even if only one eye is symptomatic.
Keratitis

- Infection of the cornea
- Symptoms include: eye pain, eye redness, blurred vision, sensitivity to light, excessive tearing, eye discharge
- Serious condition requiring prompt treatment
- May progress to perforation and blindness if treatment is unsuccessful.
- Most common infectious causes are bacteria and fungi, followed by parasites and viruses
Risk Factors for Keratitis

• Contact lens usage
  – Overnight wear
  – Improper disinfection or cleaning
  – “Topping off” solution
  – Rinsing with tap water
• Immunosuppression
• Underlying disease
• Trauma
Specimen Collection: Keratitis

• Culturing of the cornea should be performed by an ophthalmologist or experienced physician.
• Generally the bacteria are located at the leading edge of an ulcer or infiltrate, and the specimen is obtained with a spatula, blade, or scalpel.
• A corneal specimen could also be obtained by meticulously dabbing the infected area with a soft-tipped applicator (i.e. swab).
• Topical anesthetic should always be applied prior to obtaining a corneal specimen.
• Corneal specimens can be plated on the same agar media with the conjunctiva and eyelid specimens.
• Separate samples must be collected into appropriate transport media for detection of viruses or chlamydiae.
Inoculation of Corneal Scraping

• A general convention is to form Cs on the media designating the cornea.
• Breaking the surface of the agar occurs and is acceptable.
• Inoculation of plates and preparation of slides may need to be done at the patients’ side.
  – Small amounts of material involved
  – Low inoculum
Endophthalmitis

- Inflammation in the intraocular cavity of the eye, including involvement of the vitreous and/or aqueous humors.
- Symptoms include: pain, reduced vision, swelling, and redness in the affected eye which may develop days to weeks after exposure; can also present as an indolent, sub-acute infection with waxing and waning visual acuity and without a large pain component.
- Serious sight threatening disease.
- Most common infectious causes are bacteria, followed by fungi.
- Not caused by viruses or parasites; by convention, infections due to these organisms are included in the term "uveitis" (eg, cytomegalovirus [CMV] retinitis, toxoplasma chorioretinitis).
# Endophthalmitis

## Chronic
- months to years after intraocular surgery
- *P. acnes*
- Coagulase negative staphylococci
- *Corynebacterium* species
- Yeasts and molds
- *P. aeruginosa*
- *S. aureus*
- *Mycobacterium* species

## Endogenous
- rare; bacteremia or fungemia; immunosuppressive therapy, IVDU or invasive surgical procedures.
- Yeasts
- Molds
- *S. aureus*
- Streptococci
- Enterobacteriaceae
- *Bacillus* species

## Post-traumatic
- penetrating or perforating ocular injuries.
- *Bacillus cereus*
- Fungi
- Streptococci
- *Clostridium* species
- *Microsporidium* species
Risk Factors for Endophthalmitis

• Recent eye surgery or other invasive eye procedure
• Recent eye injury
• Diabetes
• Steroid use
• Immunosuppression
• Fungal bloodstream infection, such as candidemia
Specimen Collection: Endophthalmitis

- Specimens are obtained with a syringe and needle by an experienced ophthalmologist who is aware of all intraocular complications.
- Aqueous and vitreous fluids should be transported to lab as quickly as possible.
- A few drops of aqueous and vitreous should be placed on glass slides for Gram stain. The drops should not be spread over the slide like a blood smear.
- Vitrectomy specimens are often cultured after endophthalmitis. These specimens are vitreous diluted with large volumes of BSSplus (50 to 100 ml) and should be concentrated by centrifuging at 3000rpm for 30 minutes. The pellet can be aliquotted onto slides for staining, and to culture media for microbial isolation.
- Suspended matter in the diluted vitreous sample could be fished-out, placed on a glass slide, and stained for the examination of organisms.
- PCR may be a good alternative.
# Sample Collection Summary

## Table 1. Type of sample and recommended procedure for sample collection in various eye infections

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Type of sample</th>
<th>Recommended device/procedure for sample collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blepharitis</td>
<td>Scales/discharge from lid margin</td>
<td>Forceps/cotton swab</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Fluid/discharge from lower conjunctival sac</td>
<td>Calcium alginate/cotton swab</td>
</tr>
<tr>
<td>Dacryocystitis</td>
<td>Fluid/discharge from lower conjunctival sac</td>
<td>Calcium alginate/cotton swab</td>
</tr>
<tr>
<td>Keratitis</td>
<td>Corneal scraping</td>
<td>Kimura spatula, No. 15 surgical blade, bent needle</td>
</tr>
<tr>
<td>Uveitis</td>
<td>Anterior chamber fluid</td>
<td>Paracentesis (anterior chamber tap) with tuberculin syringe</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>Anterior chamber fluid</td>
<td>Paracentesis (anterior chamber tap) with tuberculin syringe</td>
</tr>
<tr>
<td></td>
<td>Vitreous aspirate</td>
<td>Vitreous syringe</td>
</tr>
<tr>
<td></td>
<td>Vitreous Bioso</td>
<td>Vitrectomy</td>
</tr>
<tr>
<td>Panophthalmitis</td>
<td>Vitreous biopsy</td>
<td>Vitrectomy</td>
</tr>
<tr>
<td></td>
<td>Evisceration contents</td>
<td>Evisceration</td>
</tr>
<tr>
<td>Deep seated stromal infiltrate in keratitis</td>
<td>Corneal biopsy</td>
<td>Lamellar biopsy</td>
</tr>
<tr>
<td>Non-healing keratitis requiring keratoplasty</td>
<td>Corneal buttons</td>
<td>Penetrating keratoplasty</td>
</tr>
<tr>
<td>Contact lens-associated keratitis</td>
<td>Contact lenses, lens cases, and lens solution</td>
<td>Aseptically removed from the eye. Aseptically collected</td>
</tr>
<tr>
<td>Postoperative endophthalmitis following intraocular lens implantation</td>
<td>Intraocular lens</td>
<td>Surgical removal</td>
</tr>
<tr>
<td>Eye injury with iris prolapse/incarceration</td>
<td>Iris tissue</td>
<td>Surgical removal</td>
</tr>
</tbody>
</table>
Normal Flora of the Eye

- *Corynebacterium sp.* (“diphtheroids”)
- *Propionibacterium sp.*
- Coagulase negative staphylococci
- *Neisseria sp.*
- *Moraxella sp.*
- *Streptococci* (non-hemolytic)
- Gram-negative rods (rare)

- Presence of bacteriostatic substances (lysozyme, IgA, and IgG), decreased temperature of conjunctiva due to evaporation of tears, exposure and moderate blood supply also inhibits the bacterial growth.
Common Bacterial Pathogens

• Typically requires a compromised epithelial surface
• Most common cause of microbial keratitis
• Common organisms
  – *Staphylococcus* sp.
  – *Streptococcus pneumoniae* and viridans sp.
  – *Haemophilus* sp.
  – *Moraxella* sp.
  – *Neisseria* sp
  – Enteric Gram negative rods (*Serratia*)
Invasive Bacteria

• Only a few bacteria are capable of invading an intact epithelial surface:
  – Corynebacterium diphtheriae
  – Listeria monocytogenes
  – Shigella sp.
  – Neisseria gonorrhoeae
  – Haemophilus aegyptius
  – Invasive strains of Pseudomonas aeruginosa
Case 1

- 54 yo F
- Bilateral conjunctivitis
- No history of eye injury or prior eye complaints
- Swabs from each eye were sent laboratory for bacterial culture
Culture Work Up

• After ~48 h incubation
• Small, round, and convex colonies on SBA and CHOC
• Small Gram positive coryneform rods
• Catalase positive
• Oxidase negative
Corynebacterium macginleyi

- Lipophilic
- Detected, almost exclusively from ocular surfaces of symptomatic patients
- Only very rarely recovered from a study of healthy eyes
- Conjunctivitis, suture-related keratitis, corneal ulcer, post operative endophthalmitis
- Extraocular involvements have also been reported
- Susceptibility to commonly used topical antibiotics, with some resistance to erythromycin and clindamycin being observed; emerging resistance in fluoroquinolones
- Clinicians also empirically prescribing topical antibiotics without performing culture may encounter resistant strains
Sequence Based ID

Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship of NML 080212 to its closest *Corynebacterium* species.

Neighbor-joining phylogenetic tree based on partial *rpoB* gene sequences, showing the relationship of NML 080212 to its closest *Corynebacterium* species.

**Moraxella sp.**

- Colonizing the nasopharynx and on other mucous membranes
- *M. catarrhalis* = Gram negative diplococci

*M. lacunata*

*M. nonliquefaciens*

- Gram negative rods
- Catalase and oxidase positive and do not produce acid from carbohydrates
- Butyrate disk positive
- Produce destructive proteases and endotoxins
- Cause of conjunctivitis and bacterial keratitis; painless ulcer
- Ulcer may take several weeks to heal
- Predisposing factors thought to include alcoholism, diabetes; however, disease occurs in healthy patients with persistent corneal epithelial defect or corneal pathology that enhances their susceptibility
**Haemophilus spp.**

- *H. influenzae* biogroup *aegyptius*
- Koch-Weeks bacillus
  - Firstly described by Koch in 1883, who observed the organism in eye secretions from Egyptian patients with conjunctivitis
  - Weeks made a similar observation 3 years later in the United States
- Highly contagious epidemic purulent conjunctivitis
- Transmitted by flies
- Brazilian purpuric fever
  - Outbreak in the 1980s in Brazil
  - High mortality, children between 1-4
  - Purulent meningitis, bacteremia, high fever, vomiting, purpura, vascular collapse
  - Rapid mortality
  - Pathogenesis not established
Case 2

- A 29-year old man presented redness and ocular discharge from both eyes for 13 days
- Conjunctiva was markedly inflamed and there was
- Intense dilatation of the conjunctival vessels without small petechial hemorrhages with purulent discharge.
- New sexual partner about 3 weeks prior, but there was no evidence of genitourinary symptoms.
Culture Work Up

- Conjunctival swab sent to the lab
- Gram negative diplococci seen on Gram stain and recovered on chocolate agar
Neisseria gonorrhoeae

- Hyperacute bacterial conjunctivitis
- Sexually active adults
- Characterized by copious, purulent discharge; pain; and diminished vision loss within 12 hours of inoculation
- Can progress in an extremely rapid and fulminant fashion, leading to corneal perforation
- Risk of serious sequelae and visual loss is greatly reduced if promptly managed.
- The incubation period ranges from 3–19 days
- The urethral symptoms precede the ocular symptoms from one to several weeks
- Treat with systemic abx
- Antimicrobial resistance—combination therapy using two antimicrobials with different mechanisms of action (e.g., a cephalosporin plus azithromycin)
Chlamydia Conjunctivitis

• Inclusion conjunctivitis is a common, primarily sexually transmitted disease that occurs in both newborns (ophthalmia neonatorum) and adults (adult inclusion conjunctivitis).

• Inclusion conjunctivitis is caused by the bacterium called *Chlamydia trachomatis*, (associated with serotypes D through K)

• Symptoms include redness of the eye(s), swelling of the eyelids, and discharge of pus

• Likely to appear 5 to 12 days after birth in neonates

• Prenatal screening and treatment of pregnant women is the best method for preventing chlamydia conjunctivitis among neonates
Diagnostic Testing

• Culture
  – The culture must contain epithelial cells; exudates are not sufficient.
  – Collected samples are placed in 2.0 ml of Chlamydial transport medium. (sometimes called VTM-viral transport media or UTM-universal transport media)
  – Fastidious and will not survive unless refrigerated (short-term) or frozen (long-term) (-75 degrees C).

• DFA
  – FDA approved for ocular specimens

• NAAT
  – Not FDA approved but likely the most sensitive method
  – Can be testing using same collection methods as other sites (swab based collection kit)
Trachoma

• Almost 8 million people are visually impaired by trachoma; 500 million are at risk of blindness from the disease throughout 57 endemic countries
• Caused by Chlamydia trachomatis, (associated with serotypes A through C)
• Spread through direct personal contact, shared towels and cloths, and flies that have come in contact with the eyes or nose of an infected person, areas that lack adequate access to water and sanitation
• Repeated trachoma infections can cause severe scarring of the inside of the eyelid and can cause the eyelashes to scratch the cornea (trichiasis).
• Permanently damages the cornea and can lead to irreversible blindness.
• Physicians treating immigrant and refugee populations, or those practicing internationally, may encounter chronic trachoma cases and should be familiar with its presentation and management.
• The World Health Organization has targeted trachoma for elimination by 2020 through an innovative, multi-faceted public health strategy known as S.A.F.E.:
  – Surgery to correct the advanced, blinding stage of the disease (trichiasis),
  – Antibiotics to treat active infection,
  – Facial cleanliness and,
  – Environmental improvements in the areas of water and sanitation to reduce disease transmission
Case 3

• 45 yo male presented with redness and worsening pain in his left eye 3 days post-cataract surgery

• History of insulin-dependent diabetes mellitus.

• A rapid diagnosis of endophthalmitis was made by Gram staining of vitreous fluid

• Despite the administration of intravitreal and systemic treatment the infection progressed, requiring the enucleation of the eye on the same day.
**Bacillus cereus**

- Large Gram positive, spore forming rods with square ends
- Flat, spready, rough matted colonies on SBA
- β hemolytic
- Catalase positive
- Motile
- Lecithinase positive on EYA
- Ubiquitous in the environment
- Difficult to differentiate from *B. thuringiensis*
Bacillus cereus Pathogenesis

• Endophthalmitis can be exogenous or endogenous
• Ocular entrance of the bacterium results in a massive destruction within 12 to 18 h
  – Tissue-destructive exotoxins (hemolysin, collagenase, phospholipase)
  – Bacterial swarming—morphological differentiation
  – Permeability of the blood-retinal barrier

Propionibacterium acnes

- Normal skin flora
- Pleomorphic Gram positive rod
- Grows best anaerobically but can be recovered aerobically—slow growing
- Endophthalmitis is a relatively new clinical entity, rare
- Notify the lab if this organism is on the differential
Nocardia spp.

- Large group of environmental gram-positive branching, filamentous rods
- “Aerobic actinomycetes”
  - Mycobacteria, Corynebacteria, Nocardia, Rhodococcus, Gordonia, Tsukamurella
- Corneal infection is by far the most common ocular infection
- Reported after accidental and surgical trauma including refractive surgery
- Molecular methods have expanded the spectrum of pathogenic Nocardia species
  - >80 Nocardia species have been described and >30 have been implicated in human disease
Nocardia in the Lab

- Direct smears show gram-positive, beaded, fine, right-angled branching filaments (<1 µm diameter)
- Prolonged incubation weeks may be required
- Grow on nonselective media used for bacteria, fungi, mycobacteria
- Buff/pigmented, waxy colonies → Develop a dry, chalky appearance
- Earthy “musty basement” odor
Nocardia spp. Modified Acid Fast Stain

• Varying degrees of acid-fastness depending on the mycolic acid composition of the cell wall and the types of media used
  – Modified Kinyoun stain uses 1% H₂SO₄ as the decolorizing agent
Identification of *Nocardia* Species

- DNA gene sequencing is required
- Molecular techniques have enabled rapid, accurate species identification recognition and characterization of numerous new species
- Initial analyses used genetic variation within 16S rRNA gene region
- Other targets provide greater discrimination
  - *secA1*
  - SecA1 protein is an essential component of the preprotein translocase ATPase that provides the driving force for the export of proteins across the bacterial cytoplasmic membrane
  - Good separation of all of the clinically relevant type and reference strains
  - Finer species distinctions among closely related species than 16S rRNA gene sequence analysis

Nocardia Species

**Pseudomonas aeruginosa**

- Keratitis usually starts with a small ulcer that rapidly spreads
- Can lead to corneal perforation
- Less commonly, the infection is more indolent
- Thin elongated GNR
- Oxidase positive
- β-hemolytic, metallic sheen, fruity odor
- Non-lactose fermenter
- Produces pyocyanin
Case 4

• A 42-year-old male veterinarian was injured when a dog's abscessed tooth fractured during manual extraction and struck the veterinarian in his right eye.
• Patient presented with pain, light sensitivity, and blurred vision.
• Corneal edema traumatic corneal laceration associated with contusive endothelial dysfunction and iritis.
• A corneal scraping for was sent to the lab.
• Heavy growth was noted in all “C” streaks on both blood and chocolate agar plates on the next day. Anaerobic cultures were negative.
• The Gram stain showed Gram-negative rods that were thin and fusiform with tapered ends.
Capnocytophaga canimorsus

- Eye infection is rare
- Sepsis, meningitis
- Splenectomy/hyposplenism, alcoholism
- Normal flora of dogs and cats
- Dog bite, scratch or lick
- Case fatality rate 25-30%
- Long thin Gram-negative rod with tapered ends
- Requires CO₂
- Unable to grow on MacConkey agar,
- Indole, urease, nitrate reduction negative
- Oxidase and catalase positive
- Gliding motility
Antimicrobial Susceptibility Testing

- The antibiotic discs contain obtainable serum level of the drug and not the level obtainable in the tear film or cornea or intraocular space by usual topical or intraocular therapy.
- Often 1000 times greater achievable in the eye.
- Organisms reported as resistant may be susceptible in ophthalmic situation.
- Broth dilution procedures may be useful to determine minimum inhibitory concentration (MIC).
- Especially in endophthalmitis, as the effective peak concentration should be 2–4 times higher than the MIC.
- Clinical response is best indicator.
Viral Infections

• Most common cause of conjunctivitis
• Collection of cell-rich specimens usually results in the highest sensitivity.
• Flocked nylon swabs have shown excellent yield compared to those from cotton swabs
Adenoviral Conjunctivitis

- Incubation 5-10 days
- Clinical symptoms 5-15 days
- Transmission
  - Direct contact with conjunctival secretions
  - Respiratory fomites
- Most common in children
- Associated with many different serotypes (1-4, 7)
- Can be recovered in viral culture, but molecular testing more sensitive
Ocular Adenoviral Disease

- Pharyngoconjunctival fever
  - Serotypes 3,4,7

- Epidemic keratoconjunctivitis
  - Serotypes 8 and 19
  - Corneal sequelae (subepithelial corneal infiltrates)
  - Viral particles can be infectious ~ 1 month

- Acute hemorrhagic conjunctivitis
  - More commonly caused by coxsackievirus A24 and enterovirus 70
# Adenovirus

Adapted from MCM, 10th Ed.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Common Serotype</th>
<th>Target Population</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>URI</td>
<td>1-3, 5, 7</td>
<td>Infants, children</td>
<td>NP or throat swab</td>
</tr>
<tr>
<td>LRI</td>
<td>3, 4, 7, 21</td>
<td>Infants, children, immunocompromised (IC)</td>
<td>NP or throat swab</td>
</tr>
<tr>
<td>ARD</td>
<td>4, 7</td>
<td>Military recruits</td>
<td>NP or throat swab Lung tissue, BAL</td>
</tr>
<tr>
<td>Acute (hemorrhagic) conjunctivitis or keratoconjunctivitis</td>
<td>1-4, 7, (11)</td>
<td>Children</td>
<td>Conjunctival swab or scraping</td>
</tr>
<tr>
<td></td>
<td>8, 9, 37</td>
<td>Any age</td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic cystitis</td>
<td>11</td>
<td>Children, IC</td>
<td>urine</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>1-3, 5, 7</td>
<td>Infants, children, IC</td>
<td>Liver tissue, blood</td>
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<tr>
<td>Disseminated/ Organ Specific Disease</td>
<td>1, 2, 5, 7, 11, 21, 34, 35</td>
<td>Newborns, children, IC</td>
<td>Blood, organ tissue, CSF</td>
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<tr>
<td>STI</td>
<td>2, 37</td>
<td>Teens, adults</td>
<td>Lesion swab</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>40,41</td>
<td>Children &lt;2</td>
<td>stool</td>
</tr>
</tbody>
</table>
Viral Conjunctivitis and Keratitis

• Herpes Simplex
  – Manifests in the form of follicular conjunctivitis or keratoconjunctivitis with preauricular adenopathy and often with periocular skin involvement

• Varicella-Zoster Virus
  – Approximately 4% of patients with chicken pox have involvement of the conjunctiva or cornea in the form of papules or vesicles
Cytomegalovirus

- Herpes virus
- Seroprevalence rates ranging between 40 to >90% percent of the adult population
- Establishes latent infection after the resolution of acute, typically asymptomatic, infection
- Cultured from multiple sites, including urine, blood, throat, cervix, saliva, semen, stool, tears, and breast milk
- Symptomatic disease usually manifests as a mononucleosis syndrome in immunocompetent patients
- Symptomatic CMV disease in immunocompromised individuals can affect almost every organ of the body, resulting in fever of unknown origin, pneumonia, hepatitis, encephalitis, myelitis, colitis, uveitis, retinitis, and neuropathy.
  - Re-infection vs. reactivation
CMV Retinitis

- Symptoms include blurring or loss of central vision, scotomata ("blind spots"), floaters, or photopsia ("flashing lights"), depending upon the anatomic site of retinal destruction and whether or not retinal detachment has occurred
- Reactivation of latent infection
- Likely results from hematogenous spread
- Uncommon among immunocompetent individuals
- Most common serious ocular complication of AIDS
- ART dramatically reduced incidence by 80% or more
  - Improved morbidity and mortality related to ocular involvement
  - Slowed rates of retinal progression and ocular complications
- Clinical symptoms may occur with higher CD4 counts during immune reconstitution
- CMV viremia detected by polymerase chain reaction (PCR), antigen assays, or blood culture are **NOT** used to make a diagnosis of CMV retinitis
- PCR has been useful for detection of CMV in aqueous fluid from immunocompetent patients presenting with anterior uveitis
Case 5

A pediatric ophthalmologist inquires as to how to make the diagnosis of congenital rubella.

- 15 months old
- Ocular findings consistent with rubella (cataract and microcornea) and additional congenital heart defects.
- The mother was reported to have rubella during pregnancy
- Baby was tested for rubella at birth but was negative

The ophthalmologist is planning to remove the cataract from the one eye in a couple of weeks, and she wants to know how to send the specimen and what testing should be ordered.
What Information is Essential to the Case?

• Foreign travel?
• Was the baby immunized with the MMR?
• How was the mother diagnosed? Any lab data?
• What is the best test to order?
• Who performs testing for Rubella?
• What specimens are appropriate to test?
Congenital Rubella Syndrome

• A constellation of congenital abnormalities
  – Ophthalmologic, cardiac, auditory, or neurologic
• 85% of cases if maternal infection in first 12 weeks of gestation, 50% during 13-16 weeks, 25% during 2nd trimester
• Transmitted through direct or droplet contact from nasopharyngeal secretions
• Most cases are IgM positive at birth to 3 months of age
• Can be confirmed by increasing concentrations of IgG over 7-11 months of life
• False positive and false negatives do occur
• Considered contagious until at least 1 year of age
• 1 dose MMR at 12-15 months, 2nd dose at 4-6 years
Rubella in the US

Reported cases of rubella and congenital rubella syndrome (CRS) — National Notifiable Diseases Surveillance System, United States, 2004–2012

During 2004-2012, 79 cases of rubella and six cases of CRS were reported in the United States.
Diagnostic Testing

• Immunization history helpful in determining utility of serology testing
• Testing of specimens is coordinated through public health
• Virus is shed close to a year after infection
  – Serum, NP swab, urine and eye tissue all acceptable specimens
  – Ocular tissue (cataract) likely to be positive the longest
Case Resolution

- Born and resided in Albania for her 1st year
- Baby was immunized = serology is of little value
- No diagnostic information from the mother
- Testing sent to CDC
- Coordinated through public health and state lab
- NP swab and urine were negative = patient not actively shedding virus
- PCR from tissue = positive
Fungal Infections

- Only about 5-10% of cases are caused by fungi
- More common in warmer climates
- Yeast typically from patient’s own flora
- Mold infection typically acquired through trauma
- No clinical feature can be considered absolutely pathognomonic of fungal etiology
Case 6

• 23 yo female presented to her local ophthalmologist with pain, photophobia, and decreased vision in the right eye
• Examination showed a geographic corneal ulcer
• Corneal scraping was sent for bacterial, viral, and fungal culture
Microbiology Work Up

- Viral culture negative
- Bacterial culture—Coagulase negative Staph
- Fungal culture +
“Scotch Tape Prep”

• Morphological examination of fungal structures

• Lactophenol Cotton Blue
  – Lactic Acid acts as a clearing agent and helps preserve the fungal structures
  – Phenol kills the fungus
  – Glycerol is slightly viscous and prevents drying of the prepared slide specimen.
  – Cotton Blue is an aniline dye which adds color to the fungal structures.
Fusarium sp.

- Environmental fungus
- Outbreaks have been directly associated with the use of specific brand of contact lens solution

Ovoid microconidia on slender phialides

Canoe-shaped macroconidia
**Aspergillus fumigatus**

- Colony appearance: Green to blue-green with dark center and white periphery, white to tan reverse
- Microscopic features: Compact uniserate phialides producing a columnar head
Differentiating Aspergillus Species

Conidial head shape: Columnar vs. Radial

Phialides: Uniseriate vs. Biseriate

Conidiophore: Smooth vs. Rough

*A. terreus

*Additional features: length and density of conidial chains, length of conidiophore, presence of cleistothecia or Hülle cells, and pigment
Purpureocillium lilacinum

- Ubiquitously isolated from soil and vegetation
- Is an infrequent cause of human disease
- Most reported cases involve patients with compromised immune systems, indwelling foreign devices or intraocular lens implants
- Shows a special tropism for ocular structures (about 50% of reported cases)
- Previously called Paecilomyces lilacinus
Microscopic appearance

- Conidiophores often branched
- Phialides thin and elongate at the tips, grouped in brush-like clusters at the ends of the conidiophores
- Conidia oval to fusoid in long chains
- Microscopic morphology: Conidiophores attach to metulae (secondary branches) that carry flask-shaped phialides.
- The overall organization resembles a brush-broom.
How to differentiate *Penicillium* from *Purpureocillium*

• *Penicillium* has phialides with thicker apices and these apices tend to have a nearly parallel orientation in tight clusters and round (vs. oval) conidia
Alternaria, Bipolaris, Curvularia

• Thermally monomorphich, dematiaceous molds
• Macroscopic morphology:
  – Surface: Greenish, dark gray, black (when mature)
  – Reverse: Dark
• Microscopic morphology:
  – Closely related and demonstrate conidia that resemble one another
  – Conidia of Alternaria spp. can be differentiated from others based upon the presence of both transverse and longitudinal septations
Case 7

- 65 yo female who wears contact lenses
- Foreign body sensation in the left eye
- After 10 days the patient had severe pain and photophobia
- With diagnosis of corneal ulcer the antibacterial medication was started
- After 5 days no improvement
- Corneal scraping and contact lens sent to microbiology laboratory for culture
Acanthamoeba keratitis

- Microscopic, free-living ameba, or amoeba (single-celled living organism)
- Found worldwide in the environment in water and soil.

Risk Factors
- Storing and handling lenses improperly
- Disinfecting lenses improperly
- Swimming, using a hot tub, or showering while wearing lenses
- Coming into contact with contaminated water
- Having a history of trauma to the cornea
Acanthamoeba Diagnosis

Cysts
- 10-25 µm in diameter
- Two walls:
  - wrinkled fibrous outer wall (exocyst);
  - inner wall (endocyst) that may be hexagonal, spherical, star-shaped or polygonal

Trophs
- 15-45 µm
- Pleiomorphic with spine-like processes called acanthapodia
Onchocerciasis

• Aka “River Blindness”
• Considered a neglected tropical disease
• Caused by *Onchocerca volvulus*
• Vector is *Simulium* blackfly
  – Repeated bites required
  – Larvae enter bite wound, adults mature and reside in subcutaneous tissue
• Primarily in Africa and Yemen
• Symbiotic relationship with *Wolbachia* bacteria
• Usually diagnosed in a skin snip or skin nodule biopsy
Loiasis

- *Loa loa*
- African eye worm
- Infection is often asymptomatic and becomes evident when the adult worm crosses the conjunctiva of the eye
- Vector for is biting mango flies, a member of the genus *Chysops*.
  - Larvae enter bite wound, adults mature and reside in subcutaneous tissue
- *Loa loa* is endemic to parts of Western Africa, especially in the rainforests of the Congo and Sudan.
- Does not carry *Wolbachia*
Differentiation of Microfilariae

Wuchereria bancrofti
Loa loa
Brugia malayi
Mansonella perstans
Mansonella ozzardi
Onchocerca volvulus
Mansonella streptocerca

PINK SHEATH
Diagnosis of *L. loa* in Blood Smear

- Sheathed
- 230-250 µm long in stained blood smears and 270-300 µm in 2% formalin. The
- Tail is tapered and nuclei extend to the tip of the tail.
Diagnosis of *O. volvulus* in Skin Snip

- Unsheathed
- 300-315 µm in length
- Tail tapers to a point and is often sharply bent.
- The nuclei do not extend to the tip of the tail.
- Typically reside in skin
- May be found in blood or urine during heavy infections, or invade the eye and cause a condition known as river blindness.