Ministry of High Education and Scientific Research
Al-Furat Al-Awsat Technical University
Al-Najaf Technical Institute

Medical Microbiology

Optometry Department
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WEEK	Topics Covered
1	Introduction to Microbiology. Definition: Major Categories: The Role of the Ophthalmic Assistant : Microbes and the Human Body. Pathogenic vs. Non-Pathogenic: Normal Flora: Ocular Defenses: Susceptibility Factors:
2	Classification of Bacteria. 1. Morphology (Shape). Cocci: Bacilli: Spirochetes: ..Gram Character (Staining) Gram-Positive: Gram-Negative:
3	Common Ocular Bacterial Pathogens. Pyogenic (Pus-Producing) Cocci.. Staphylococcus spp .:Streptococcus spp.: Neisseria spp .:Bacilli (Rods) Gram-Negative Rods: Pseudomonas aeruginosa: Gram-Positive Rods:
4	Key Ocular Pathogens. Pyogenic (Pus-producing) Cocci: Staphylococcus: Streptococcus : Neisseria : Significant Bacilli (Rods): Pseudomonas aeruginosa: Bacillus spp.: Comparison: Bacteria vs. Viruses

week	Topics Covered
5	**General classification of bacterial eye infection.** Classification of Bacteria. Common Ocular Bacterial Pathogens.
6	**General classification of viral eye infection.** Fundamental Nature of Viruses. Viral Immunity and Persistence. Major Ocular Viral Pathogens.
7	**General classification of fungal eye infection.** Characteristics of Fungi. Ocular Mycoses (Fungal Infections). Key Fungal Pathogens in Ophthalmology.
8	**General classification of other microbes eye infection.** Chlamydial Organisms. Protozoa (Parasites).
9	**Bacteria (isolation and staining of bacteria)**

week	Topics Covered
10	**General classification of other microbes eye infection. intracellular parasites. Diagnostic Cytology in Eye Scrapings**
11	**Eye hygiene**
12	**Bacteria (isolation and staining of bacteria)**
13	**Bacteria (Gram positive and Gram negative bacteria , their shapes and arrangement)**
14	**Bacteria (culturing media , types and properties)**
15	**Sterilization and disinfection (Physical and chemical methods)**

References

- Stein, H.A., Stein, R.M. and Freeman, M.I., 2017. The ophthalmic assistant: A text for allied and associated ophthalmic personnel. Edinburgh: Elsevier Saunders . TENTH EDITION

- Stein, H.A., Stein, R.M. and Freeman, M.I., 2022. The ophthalmic assistant: A text for allied and associated ophthalmic personnel. Edinburgh: Elsevier Saunders . ELEVENTH EDITION

Unit 1

- This lecture introduces **Medical Microbiology** with a specific focus on its application to optometry and ocular health, covering the nature of microbes, their classification, and their interaction with the human eye.

Pre test :

- 1.Name some common sources of bacteria, viruses, and fungi in your everyday environment.

- 2. Name some common viral conditions affecting the body.

Microbiology

Microbiology is the branch of science that deals with microscopic, unicellular, and cell-cluster organisms. The major microbial categories that may be associated with eye infections are *bacteria*, *viruses*, *fungi*, and *parasites*.

A basic understanding of microbiology is helpful for the ophthalmic assistant, who may be required to take smears, stain the appropriate slide, and assist in taking a culture.

In everyday life, we are constantly in touch with microbes. We wash our hands to lower the number of microbes on our outer skin. We disinfect wounds for the same reason.

Microbiology

- We cover our sneezes and wash our fruit to prevent getting or spreading infectious diseases. We add chlorine to our water supply to inhibit the growth of *pathogenic* (disease-causing) bacteria. We do many things to control the growth of bacteria, but most bacteria are helpful in our daily lives.

- In fact, we could not live without the help of certain bacteria that exist in and on our bodies. For example, bacteria in our gut are necessary for absorption of certain vitamins.
- -Some bacteria actually educate our immune system and help to protect us against pathogenic microbial invaders.

Microbiology

- Certain species of bacteria are normal inhabitants of specific geographic areas of the body and their numbers are controlled by the local environment's moisture, temperature, and available nutrients.
- However, when such bacteria (say from the gut) get into the wrong place (like the eye) they have the potential to cause disease.
- The eye is subject to the same types of infections that may occur in other parts of the body. **Microorganisms** are everywhere in our environment, and fortunately the eye is very resistant to infection.

Microbiology

- Our intact epithelial skin surface resists most microbial invaders. Any break in the skin of the outer eye can act as a portal of entry for microbes, at which point if a significant concentration of microbes, an *inoculum,* is present it may overcome our ocular defenses and cause an infection.
- Ocular trauma, surgery, radiation, severe surface dryness from exposure or inadequate blinking, lid abnormalities, and corneal degenerative changes may create surface disruptions that leave the eye more susceptible to infection.
- Persons with normal ocular surface structures may still be susceptible to diseases if their ability to defend against infectious agents is compromised.

Microbiology

- A compromised immune system can be present in patients with diabetes, acquired immunodeficiency syndrome(AIDS), and those taking immunosuppressive agents, such as oral steroids.
- A variety of organisms can cause ocular disease of the eye**(Box 5.1**). These infectious agents have predilection for certain sites of the eye and usually vary in their severity in causing ocular disease.

Microbiology

Box 5.1 Common organisms causing disease in the anterior segment of the eye

Bacteria

Cocci

Gram positive

Staphylococcus
Streptococcus

Gram negative

Gonococcus
Meningococcus

Bacilli

Gram negative

Pseudomonas
Haemophilus
Moraxella

Gram positive

Corynebacterium
Bacillus
Mycobacterium

Spirochetes

Treponema (syphilis)

Viruses

Herpes simplex
Herpes zoster
Adenovirus

Fungi

Candida
Fusarium
Aspergillus

Chlamydia

Chlamydia trachomatis

Parasites

Acanthamoeba
Microsporidia

Microbiology

- By far the most common infections result from **bacterial** and **viral organisms**.
- **Bacteria** are larger than **viruses** and may easily be seen under magnification by a light microscope.
- **Bacteria** range in **size** from 0.2 to 5um and **viruses** from 0.005 to 0.1 um.
- **Viruses** cannot be seen with e light microscope but can be indirectly viewed with electron microscopes

SUMMARY

1. Fundamentals of Microbiology

•**Definition**: It is the branch of science dealing with microscopic, unicellular, and cell-cluster organisms.

•**Ocular Relevance**: Major categories associated with eye infections include **bacteria**, **viruses**, **fungi**, and **parasites**.

•**Clinical Skills**: Understanding these basics allows ophthalmic assistants to perform essential

•tasks like taking smears, staining slides, and assisting in cultures.

SUMMARY

2. Microbes and the Eye

•**Normal Flora**: Many bacteria are helpful, such as those in the gut that aid vitamin absorption.

• Some inhabit specific body regions as "normal flora," but can cause disease if they enter the wrong location, like the eye.

•**Ocular Defenses**: The eye is naturally resistant to infection thanks to the intact

•epithelial surface.

•**Risk Factors**: Infections often occur when these defenses are breached via:

- •**Trauma or Surgery**: Providing a "portal of entry".
- •**Environmental Factors**: Severe dryness or inadequate blinking.
- •**Systemic Conditions**: Compromised immunity from diabetes, AIDS, or steroids.

Post test

- **1.What is the branch of science that deals with microscopic, unicellular, and cell-cluster organisms?**
- A) Microbiology B) Anatomy
- C) Pathology D) Ophthalmology
- **2.Which of the following is NOT a major microbial category associated with eye infections?**
- A) Bacteria B) Viruses
- C) Parasitic plants D) Fungi

Unit

- his lecture introduces **Medical Microbiology** with a specific focus on its application to optometry and ocular health, covering the nature of microbes, their classification, and their interaction with the human eye.

Bacteria

- Bacteria can be categorized in different ways (Fig. 5.1).
- They are commonly **classified** by **morphology (shape) ,Gram character (dyed color), and ability to live in and use oxygen.**
- **Morphologically**, there are three basic shapes:
- the **round *coci*** , the **rod-shaped *bacilli***, and the **helix-shaped *spirochetes*** (Fig. 5.2).
- Although **the shape** can be used to classify the organism, another important differentiating feature is whether the organism **stains blue** (**gram positive**) or **red** (**gram negative**) with a special stain referred to as **Gram stain**.

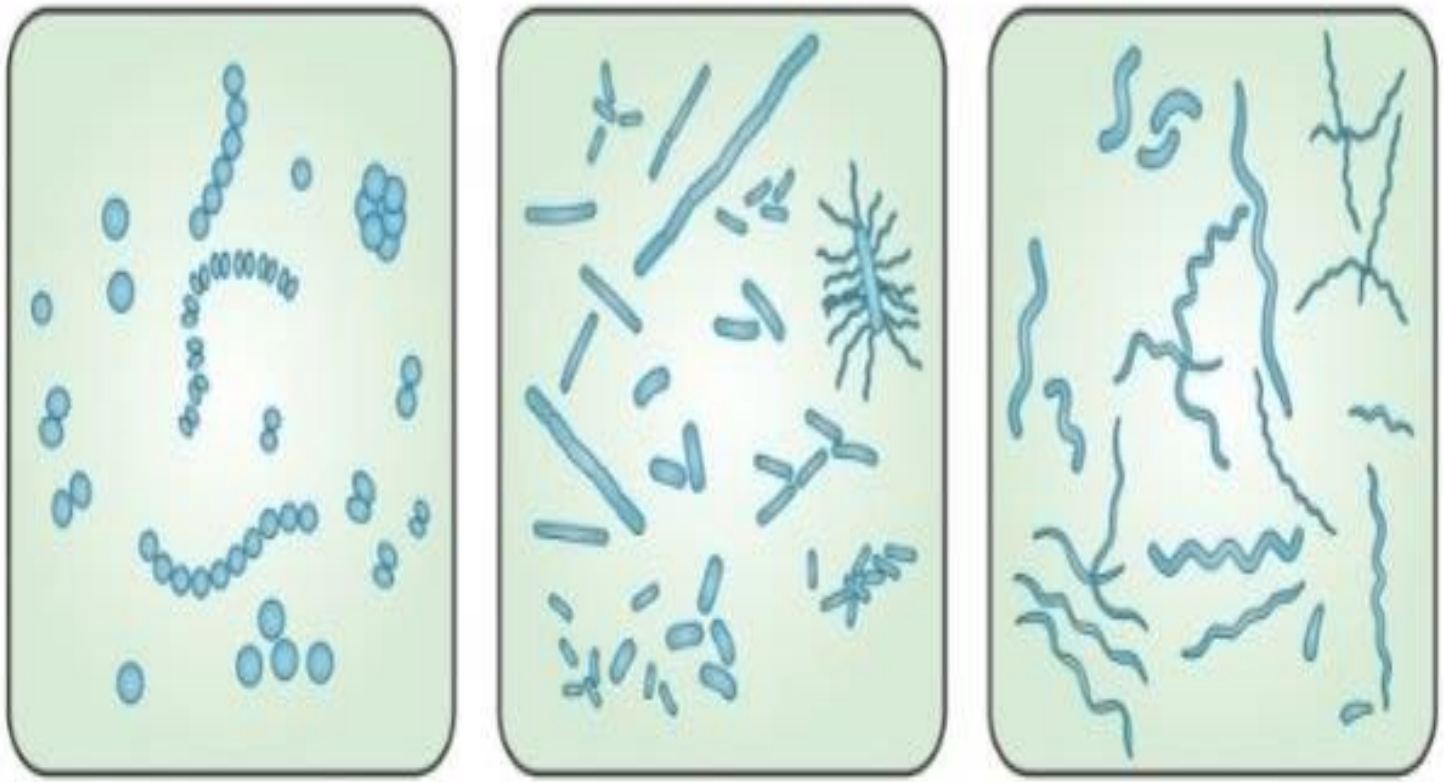


Fig. 5.1 Bacteria.

Bacteria

- The organism's color after Gram staining is referred to as its **Gram character**. A bacterium's Gram character tells us about its cell wall makeup and therefore which antibiotics may be useful
- The ***coccus*** is a round bacterium that arranges itself in a variety of patterns, each with its own characteristics.
- Certain strains of ***Staphylococcus* spp.**, ***Streptococcus* spp.**, and ***Neisseria* spp.** are sometimes referred to as *pyogenic* or *pus-producing bacteria*.
- ***Staphylococcus* spp.** are **gram-positive** organisms that may appear in grape-like clusters or, more commonly, singly or in pairs.

Bacteria

- **Staphylococci** frequently are present on the skin and may give rise to boils and styes.
- Not all staphylococci are pathogenic (disease causing); ***Staphylococcus epidermidis*** is a normal floral organism that lives on our skin and seldom causes disease.
- ***Staphylococcus aureus***, however, is the species most commonly associated with skin infections. A particular type known as **methicillin-resistant *Staphylococcus aureus*** (**MRSA**) is a bacterium responsible for several difficult-to-treat infections in humans.

Bacteria

- It is increasingly reported as a pathogen in the skin and other tissue infections.

- **Hospital-acquired MRSA** infections are on the decline, but **community-based MRSA** infections are on the rise.

Bacteria

- **Streptococci** are bullet-shaped gram-positive cocci that are usually arranged in pairs and short chains. Of the streptococcal organisms, the most common agent to affect the eye is ***Streptococcus pneumoniae*** (also known as ***Pneumococcus***, ***Diplococcus***).
- When causing disease, this organism possesses a polysaccharide (slime) capsule. The encapsulated form of this organism protects it from our body's defenses.

Bacteria

- Although ***Pneumococcus*** is a common cause of lobar pneumonia, it can also be the cause of conjunctivitis, a corneal ulcer, or an infection inside the eye referred to as *endophthalmitis*
- The bacterial genus ***Neisseria*** is a gram-negative cocci.
- The species ***Neisseria gonorrhoeae***, also known as the ***gonococcus***, and ***Neisseria meningitidis***, also known as the ***meningococcus***, are diplococci (paired) and have a characteristic kidney-bean shape. They are the causative agents of gonorrhea and meningitis, respectively,. These organisms are very invasive and rapid in their destruction.

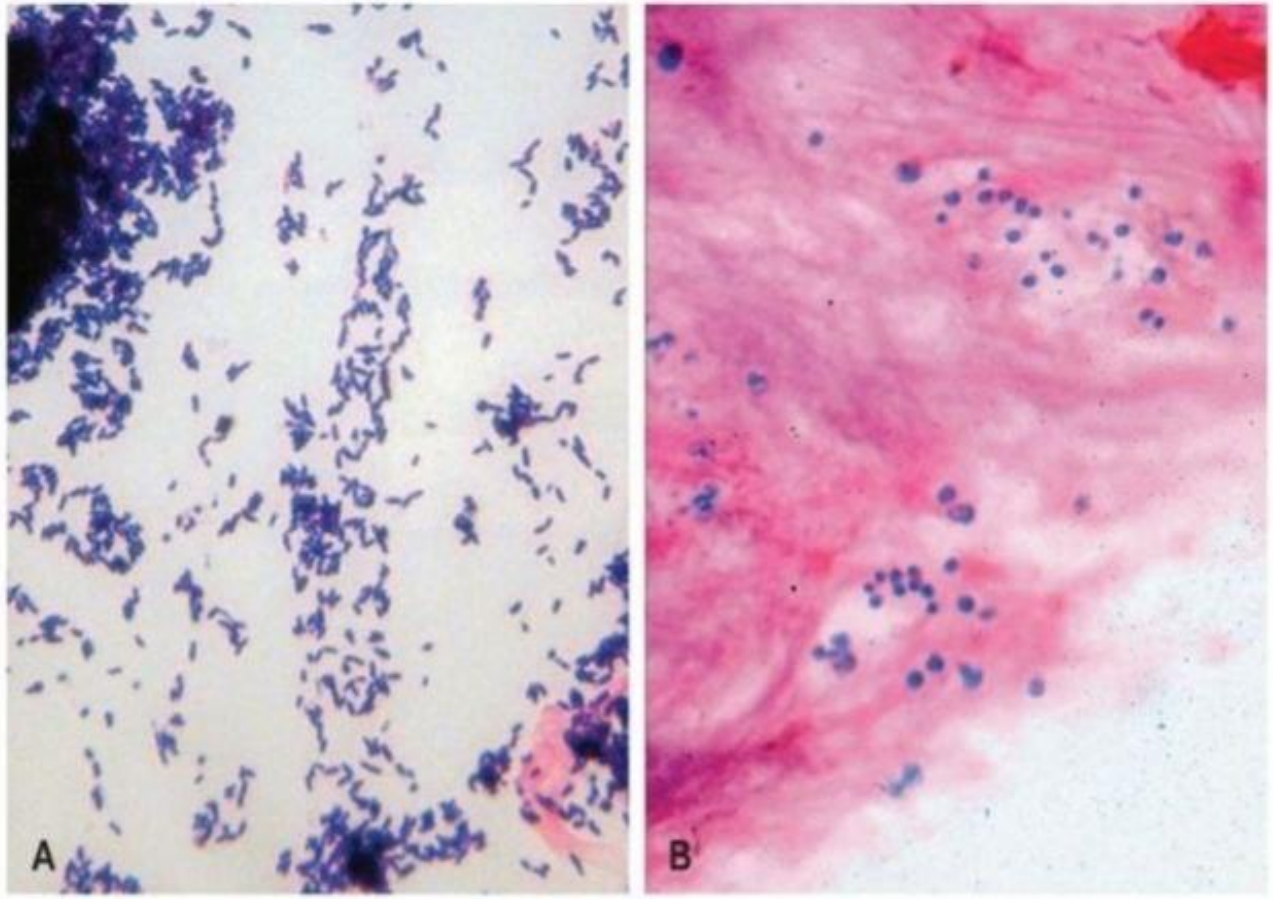


Fig. 5.2 Gram stain showing gram-positive rods on the left (A) and gram-positive cocci on the right (B). (Courtesy of Hans E. Grossniklaus, MD.)

Bacteria

- Another group of bacteria are the **bacilli.**
- All the members of this group are rod shaped, the rods being long or short, plump or slender, curved or straight, smooth or beaded.
- Commonly affecting the eye are the gram-negative rods, **Haemophilus spp.**, **Serratia spp.**, and **Pseudomonas**.
- Historically, the organism **Pseudomonas aeruginosa** has been considered the most devastating gram-negative bacillus because of its very rapid and destructive potential,Pseudomonas is an opportunistic pathogen.

Bacteria

- It cannot usually penetrate our intact skin and it causes disease only when given an opportunity, such as a corneal abrasion. It is the most common cause of corneal ulcers in patients wearing contact lenses. Unless treatment is initiated early, the organism can cause significant visual loss.
- Gram-positive bacilli that affect the eye include **Corynebacterium spp.**, **Bacillus spp.**, and **Mycobacterium spp.**
- **Corynebacteria** are anaerobic rods that are part of our normal flora and live just beneath the outer skin layer.

Bacteria

- **Bacillus spp.** are soil organisms that seldom cause disease but are devastating in their destruction when involved in intraocular infections.
- **Mycobacterial keratitis** is rare but is often associated with previous ocular surgery. It is very difficult to treat and often results in poor visual outcomes.
- The third group, the **spiral-shaped organisms** as found in **Treponema pallidum**, comprises small organisms whose diameter is below the range of resolution of the routine light microscope and which are rarely associated with ocular disease.
- The positive identification of bacterial organisms by microscopic shape and staining reaction alone is not usually possible, and culture characteristics are often necessary.
- The ophthalmologist, however, may frequently make a presumptive diagnosis in association with the clinical picture, but the microscopic picture always remains an important aid.

summary

Bacterial Classification

Bacteria are the most common cause of ocular infection and are categorized by three main features:

- **Morphology (Shape)**:
    1. **Cocci**: Round-shaped.
    2. **Bacilli**: Rod-shaped.
    3. **Spirochetes**: Helix or spiral-shaped.
- **Gram Character**: Determined by a special stain that colors the cell wall.
    - **Gram-positive**: Stains blue.
    - **Gram-negative**: Stains red.
- **Oxygen Use**: The ability of the organism to live in and use oxygen.

summary

Key Ocular Pathogens

- **Pyogenic (Pus-producing) Cocci**:
  - **Staphylococcus**: Gram-positive clusters; includes *S. aureus* (skin infections)
  - and the difficult-to-treat **MRSA**.
  - **Streptococcus**: Gram-positive pairs/chains; *S. pneumoniae* can cause
  - conjunctivitis and internal eye infections (**endophthalmitis**).
  - **Neisseria**: Gram-negative diplococci; highly invasive agents of gonorrhea and meningitis.
- **Significant Bacilli (Rods)**:
  - **Pseudomonas aeruginosa**: A devastating gram-negative rod and the leading
  - cause of corneal ulcers in contact lens wearers.
  - **Bacillus spp.**: Soil organisms that are extremely destructive if they enter the eye.

summary

Comparison: Bacteria vs. Viruses

The lecture notes that while both cause common infections, they differ significantly in size and visibility:

Feature	Bacteria	Viruses
Size	0.2 to 5 μm	0.005 to 0.1 μm
Microscope	Visible under light microscope	Requires electron microscope

Post test

- **What is the most destructive bacterium affecting the outer portion of the eye?**
- **Conjunctivitis caused by this infectious agent often produces enlargement of the preauricular lymph node.**
- a. Staphylococcus
- b. Pneumococcus
- c. Haemophilus
- d. Adenovirus

Unit

- This lecture in **Medical Microbiology** shifts the focus from bacteria to the unique characteristics and clinical manifestations of viruses, particularly those affecting the eye.

Viruses

- Viruses are very different from bacteria. They are made of the genetic material **ribonucleic acid (RNA)** or **deoxyribonucleic acid (DNA)**, never both, plus a bit of **protein**. They are obligate intracellular parasites that cannot live on their own.
- Viruses are very small organisms (**5-300 nm**) that are not visible through a light microscope. Viruses multiply by injecting their genetic material into suitable host cells.
- Once inside, they commandeer the reproductive machinery of the host cell and reprogram it to make more viruses.

Viruses

- Our bodies acquire immunity to most viruses during the course of a viral infection so we can fight off a repeat infection by the same strain of virus in the future.
- However, our bodies are not capable of immunity to all viruses, notably **herpes simplex** and **human immunodeficiency virus (HIV).**
- The herpes simplex virus lives dormant in the nerve ganglia and when activated travels along the nerve root to invade the corneal epithelium and may give rise to a dendritic (branching) or geographic corneal ulcer.

Viruses

- 'The virus can be identified by scraping the advancing edge of the corneal ulcer and inoculating the specimen into a cell culture system. In most cases, however, the clinical diagnosis of herpes simplex virus is readily apparent and a scraping is unnecessary.
- A common disease known as **epidemic keratoconjunctivitis (EKC)** is caused by an **adenovirus.**
- **Adenoviruses** are highly contagious and may affect the upper respiratory tract, the conjunctiva, and the cornea, causing fever, lymph gland enlargement, conjunctivitis, and keratitis.

Viruses

- **Varicella zoster virus** causes **chickenpox** in **children** and is responsible for causing a **vesicular eruption** on the skin referred to as **shingles** in **adults**.

- It resides in the **trigeminal nerve ganglion (CN-V)** and is therefore restricted to only one side of the face, stopping at the midline. Some patients may develop ocular involvement that most commonly manifests as **keratitis** or **iritis**.

Viruses

- **Coronavirus**, **SARS-CoV-2**, causes the infectious disease **COVID-19**, which is a respiratory pathogen.

- The World Health Organization first learned of this new virus from cases in Wuhan, People's Republic of China on 31December 2019, which later resulted in spread of the virus and a worldwide pandemic.

- The virus can cause a **conjunctivitis** in addition to affecting many organs of the body.

summary

1. The Nature of Viruses

Viruses are fundamentally different from bacteria in their structure and life cycle:

•**Composition**: They consist of genetic material—either **DNA or RNA**,

• never both—and a protein coat.

•**Size**: They are extremely small (**5–300 nm**), making them invisible

•under a standard light microscope.

•**Parasitic Life**: As **obligate intracellular parasites**, they cannot live independently.

• They multiply by hijacking a host cell's reproductive machinery to create more viruses.

summary

2. Viral Immunity

While the body develops permanent immunity to many viral strains after an infection,some viruses can evade the immune system and remain in the body indefinitely:

- **Persistent Infections**: Notable exceptions to permanent immunity include
- **Herpes Simplex** and **HIV**.

summary

3. Major Ocular Viral Pathogens

The lecture highlights four key viruses that frequently involve the eye:

Herpes Simplex Virus (HSV)

•**Dormancy**: It lives inactive in the nerve ganglia.

•**Ocular Signs**: When activated, it invades the corneal epithelium, often causing

•a **dendritic** (branching) or geographic ulcer.

•**Diagnosis**: While it can be identified via cell culture, it is usually diagnosed

•based on its distinct clinical appearance.

summary

Adenovirus

•**Condition**: It is the cause of **Epidemic Keratoconjunctivitis (EKC)**, which is highly contagious.
•**Symptoms**: It can lead to fever, lymph gland enlargement, conjunctivitis, and keratitis.

Varicella Zoster Virus (VZV)

•**Manifestations**: Responsible for chickenpox in children and **shingles** in adults.
•**The Midline Rule**: Because it resides in the **trigeminal nerve ganglion (CN-V)**,
•the resulting skin eruption is restricted to one side of the face and stops at the midline.
•**Eye Involvement**: Common ocular manifestations include keratitis or iritis.

Coronavirus (SARS-CoV-2)

•**Pathogenesis**: The cause of **COVID-19**, primarily a respiratory pathogen first identified in Wuhan in late 2019.
•**Ocular Sign**: In addition to systemic organ involvement, it can cause **conjunctivitis**.

summary

Virus	Nerve Involvement	Common Ocular Finding
Herpes Simplex	Nerve ganglia (dormant)	Dendritic corneal ulcer
Adenovirus	Upper respiratory tract	EKC (highly contagious)
Varicella Zoster	Trigeminal Nerve (CN-V)	Shingles / Keratitis
SARS-CoV-2	Respiratory system	Conjunctivitis

Post test

- **Which microbe has a size of (5-300 nm) and is invisible under a routine light microscope?**
- A) Fungi B) Bacilli C) Viruses D) Parasites
- **How do viruses take control of a host cell?**
- A) By covering the cell with a thick layer of protein.
- B) By reprogramming the host cell's reproductive machinery to make more viruses.
- C) By absorbing all the oxygen from the host cell.
- D) By performing binary fission outside the cell.

Unit

- This lecture from outlines the role of fungi in ocular health, emphasizing that while these infections are less frequent than bacterial or viral ones, they are often linked to specific environmental factors and injuries.

Fungi

- Fungal ocular infections are much less common than bacterial and viral infections.
- Molds and mildew_are fungi, Athlete's foot and ringworm are two common skin diseases caused by fungi.
- Fungi are larger than bacteria and grow either as a mass of branching interlacing filaments (Fig. 5.3) or as rounded yeast forms.
- They are typically,found in soil and moist environments

Fungi

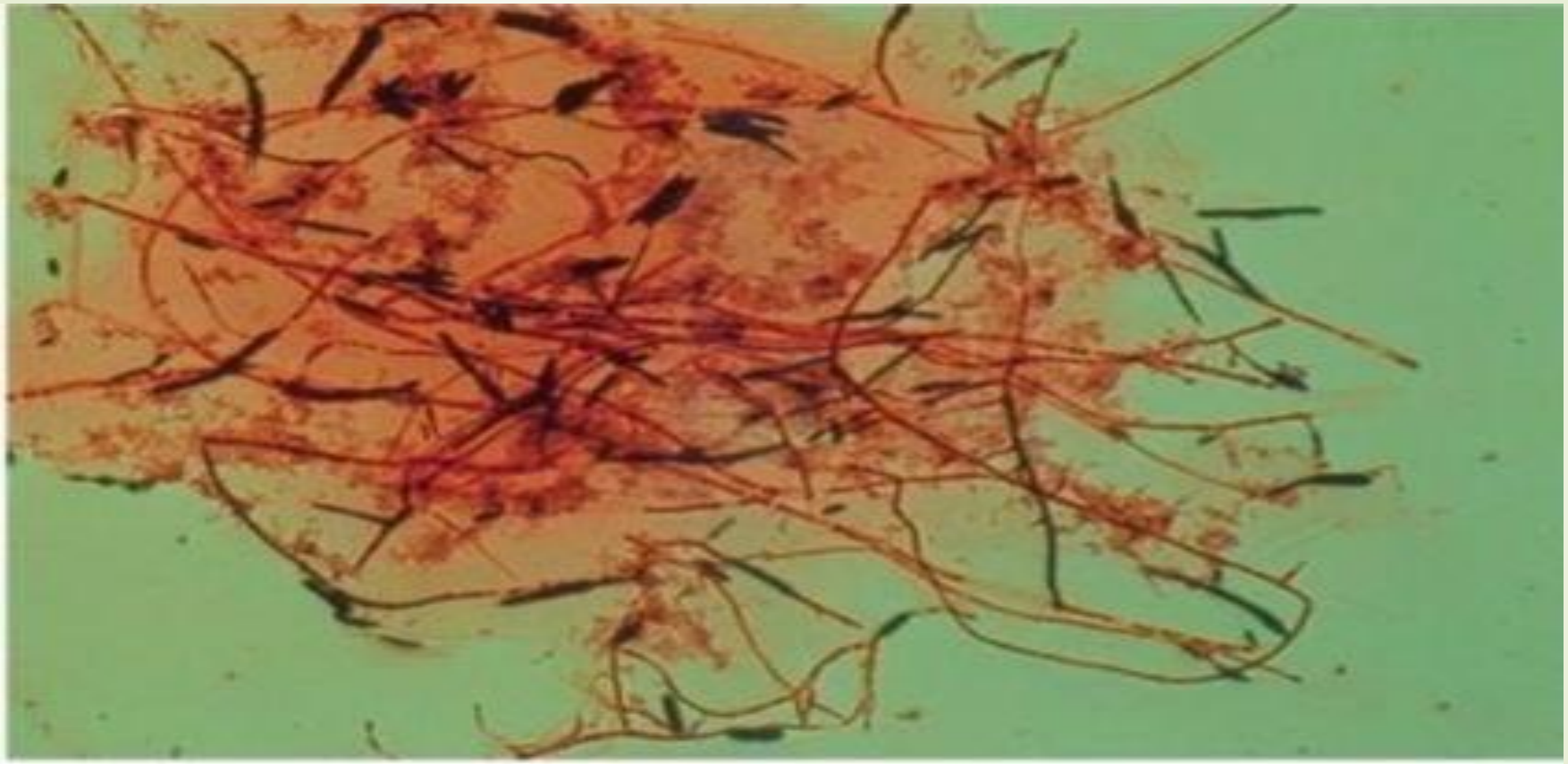


Fig. 5.3 Gram stain of *Fusarium* sp. fungi.

Fungi

- Ocular fungal infections are more likely to occur in the outer eye, cornea, and occasionally in the lacrimal sac.
- Ocular mycoses (fungal infections) are typically associated with trauma involving plant matter.
- A typical history IS one in which a Patient's cornea is scratched by a twig or leaf, and several days later the eye becomes red and inflamed.
- The most common fungal infection of the eye is caused by **Candida albicans** (Fig, 5.4), which is a common yeast that also grows on moist skin and on mucous membranes as normal flora, but may Overgrow and cause disease

Fungi

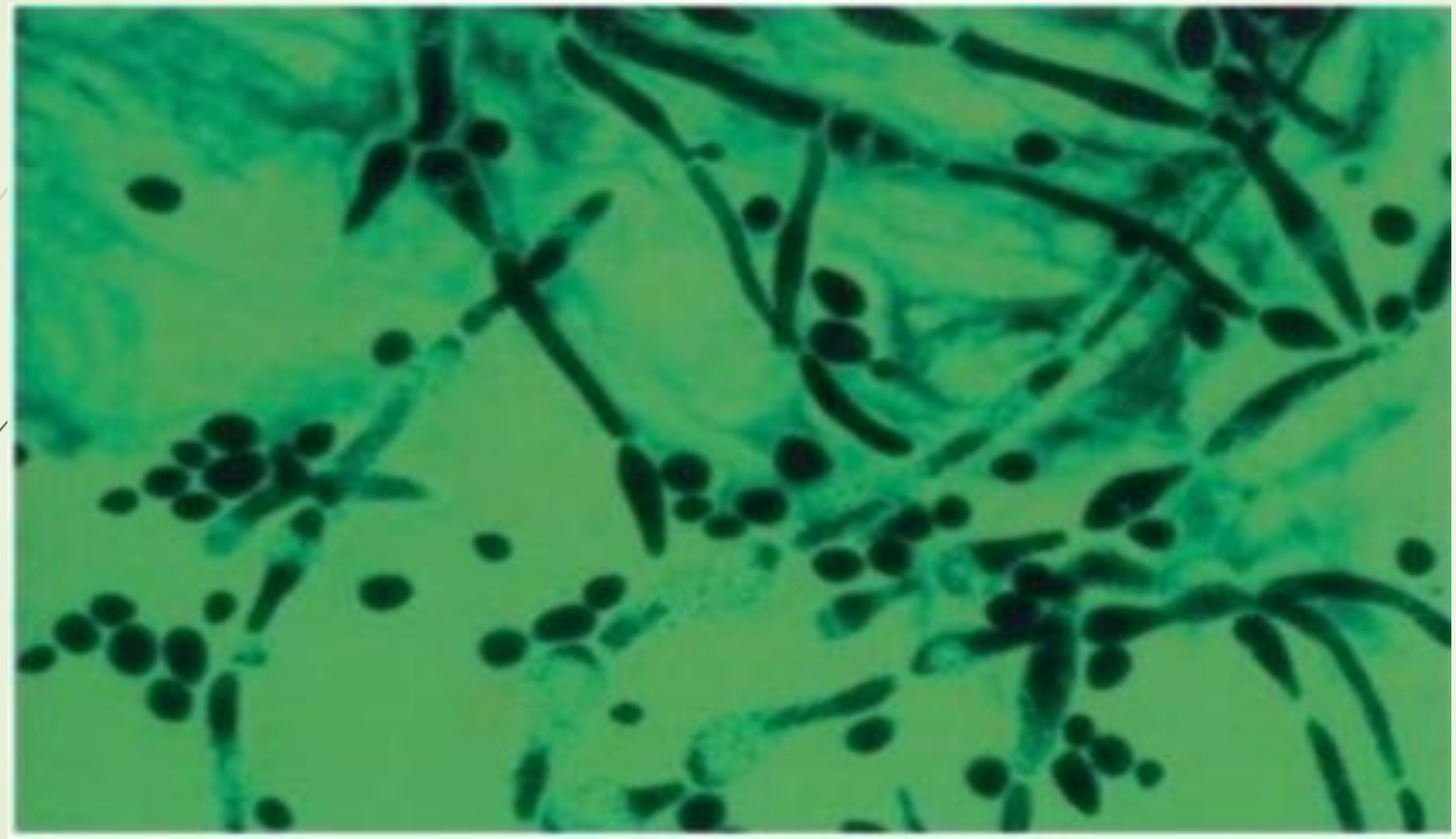


Fig. 5.4 *Candida albicans*. Note both yeast and hyphae.

Fungi

- Other fungi that may cause eye infections are the branching fungi **Aspergillus spp.** and **Fusarium spp.** both being more common in warm and moist climates.

- The largest outbreak of **fungal keratitis** ever recorded occurred in 2006; It was caused by a **Fusarium sp.** and was associated with a newly introduced contact lens multipurpose lens care product.

Summary

1. Overview of Ocular Fungi

•**Prevalence**: Fungal infections of the eye are much less common than those caused
• by bacteria or viruses.
•**Structure**: Fungi are larger than bacteria and typically appear as rounded **yeast**
• forms or as a mass of branching **filaments** (hyphae).
•**Habitat**: These organisms thrive in soil and moist environments.

Summary

2. Clinical Presentation and Transmission

•**Location**: Infections typically affect the cornea, the outer eye, and occasionally the lacrimal sac.

•**The "Plant Trauma" Link**: Most ocular mycoses are associated with trauma involving plant matter, such as a scratch from a leaf or twig.

•**Progression**: A patient typically presents with a red, inflamed eye several

•days after the initial injury.

Summary

3. Key Ocular Fungal Pathogens

•***Candida albicans***: This is the most common cause of ocular fungal infections.

• It is a yeast that exists as normal flora on the skin and mucous membranes but can become pathogenic if it overgrows.

•***Fusarium spp.***: A branching fungus found in warm, moist climates.

• It was famously linked to a massive 2006 outbreak of fungal keratitis caused by a specific contact lens multipurpose solution.

•***Aspergillus spp.***: Another filamentous fungus common in tropical or moist environments that can lead to serious eye infections

Post test

- **Which of the following is a physical characteristic that distinguishes fungi from bacteria?**
- a..Fungi are larger than bacteria and grow in filaments or yeast forms.
- B.Fungi are always unicellular and never form clusters..
- C.Fungi consist only of RNA and protein..
- D.Fungi are significantly smaller than bacteria.

- **the largest recorded outbreak of fungal keratitis in 2006 was caused by which organism?** .أFusarium spp..ب Adenovirus.ج Pseudomonasد .Candida

- **What is the most common fungal infection of the eye?**
- A.Aspergillus spp b..Candida albicans c.Fusarium spp. d.ringworm

Unit

- This lecture covers specialized microorganisms and the diagnostic techniques used to identify various types of ocular inflammation.

Other microbes

- **Chlamydial** organisms are technically classified as bacteria but deserve a classification of their own.

- They are **intracellular parasites** that are larger than viruses but smaller than most bacteria.

- **Chlamydia** is the most widespread sexually transmitted bacterial disease in the United States.

Other microbes

- In North America the most common chlamydial eye disease is adult inclusion **keratoconjunctivitis**, which is usually spread from an infected sexual partner.

- **Chlamydia** may be transferred to infants while passing through the birth canal and result in an eye infection called **ophthalmia neonatorum** (eye disease of the newborn).

Other microbes

- Outside of North America, (in North Africa, the Middle East, and South Asia), another chlamydial disease, **trachoma,** remains epidemic and a serious cause of ocular- morbidity.
- Diagnosis of inclusion **keratoconjunctivitis** can be made by obtaining a scraping of the conjunctiva and looking for inclusion bodies (microscopic foreign particles) in the cytoplasm of the epithelial cells.
- In infant chlamydial disease, the probability of finding inclusion bodies is higher than in adults, culture or specialized immunofluorescent test can also be used to confirm the diagnosis.

Other microbes

- **Protozoa** are small single-celled parasites that eat organic matter including bacteria and infrequently may cause ocular infections.
- ***Acanthamoeba*** and ***Microsporidia*** are two protozoa capable of affecting the eye.
- ***Acanthamoeba*** can cause significant ocular morbidity. The organism is ubiquitous; it can be found in freshwater, soil, swimming pools,and hot tubs.
- It is capable of causing **keratitis**, which can progress despite the best available medication.

Other microbes

- Unhygienic contact lens use is the major risk factor for acquiring this infection; it feeds on bacteria in the contact lens case.
- This risk is increased when wearers use homemade saline, rinse lenses with tap water, or swim while wearing contact lenses.
- **Corneal transplantation** may be required to restore vision but unfortunately there is approximately a 30% recurrence rate of the parasite in the graft.

Other microbes

- **Microsporidia** are small simple single-celled parasites that may infect compromised hosts.
- They are most commonly found in **HIV-positive individuals** and occasionally in other compromised hosts , such as those on prolonged steroid use.
- They are too small to be seen by simple light microscopy but may be detected by immunoassay or electron microscopy.

Other microbes

- Bacteria, viruses, fungi, chlamydia, and parasites are among the causes of infectious inflammations of various parts of the human body, including the eye.
- Various terms are used to denote the specific site of inflammation, as noted in Table 5.1. Each part of the eye is susceptible to attack by a large variety of organisms that have in common a predisposition to attack these specific areas.

Other microbes

Table 5.1 Cytology in eye scrapings

Finding	Possible diagnosis
Polymorphonuclear cells	Bacterial
Mononuclear cells	Viral
Eosinophils	Allergy
Inclusion bodies	Chlamydia
Giant cells	Herpesvirus

summary

1. Chlamydial Organisms

Chlamydia occupies a unique space in microbiology, sitting between viruses and bacteria.

•**Biological Traits**: They are technically classified as bacteria but act as **intracellular parasites**. They are larger than viruses but smaller than most bacteria.

•**Adult Inclusion Keratoconjunctivitis**: This is the most common chlamydial eye disease
in North America, often spread through sexual contact.

•**Ophthalmia Neonatorum**: An infection passed to infants during birth as they travel
through the birth canal.

•**Trachoma**: An epidemic disease in North Africa, the Middle East, and South Asia that
remains a serious cause of vision loss (morbidity).

•**Diagnosis**: Inclusion bodies (microscopic foreign particles) are found in the cytoplasm of epithelial cells during conjunctival scrapings

summary

2. Protozoa (Parasites)

Protozoa are single-celled organisms that can lead to severe ocular damage if they infect the eye.

Acanthamoeba

- **Environment**: Found in freshwater, soil, swimming pools, and hot tubs.
- **Risk Factors**: Primarily associated with **unhygienic contact lens use**, such as using tap water, homemade saline, or swimming with lenses.
- **Clinical Impact**: It causes a severe keratitis that often resists standard medication.
- **Treatment**: May require a corneal transplant, although the parasite has a **30% recurrence rate** in the new graft.

Microsporidia

- **Targets**: Infects compromised hosts, most notably HIV-positive patients or those on long-term steroid therapy.
- **Detection**: Because they are too small for standard light microscopy, they require electron microscopy or immunoassays.

summary

- **3. Diagnostic Cytology in Eye Scrapings**
- A critical part of this lecture is the use of **cytology** to diagnose the cause of an infection based on the types of cells found in a scraping.

- **4. Key Terminology**

•Keratitis: Inflammation of the cornea.
•Conjunctivitis: Inflammation of the conjunctiva.
•Endophthalmitis: Infection inside the eye.
•Ophthalmia Neonatorum: Eye disease specifically affecting newborns.

Post test

- **How are Chlamydial organisms technically classified in microbiology?.**Î
- A.As a type of bacteria b..As a multi-celled parasite
- c.As a fungal mold d.As a type of virus.
- **contact lens wearer who rinses their lenses with tap water or swims in their lenses is at high risk for which infection?**
- a.Microsporidia b.Herpes Simplex
- c.Candida albicans d.Acanthamoeba
- **When examining a conjunctival scraping, the presence of 'Eosinophils' most likely indicates which condition?.**Î
- A.Viral infection. b.Chlamydia
- c.Allergic condition d.Bacterial infection

Unit

- The lecture focuses on the laboratory methods used to identify ocular pathogens and the clinical indications for performing these tests.

Clinical indications for smears and cultures

- Smears are obtained and cultures are grown to identify causative organisms of an ocular infection. A smear is a sample of discharge or tissue cells obtained by scraping.
- **Gram** and **Giemsa stains** are commonly used; Gram stain is used to examine for bacteria and Giemsa stain is used for cytology. Although many ocular infections occur in which a clinical diagnosis can be made and smears and cultures are unnecessary, in a number of specific conditions, laboratory studies are desirable:
- • Acute purulent conjunctivitis or chronic conjunctivitis (Fig. 5.5)
- • Conjunctivitis in the newborn infant
- •Corneal ulcers that possibly have a bacterial, fungal, or parasitic cause (Figs. 5.6and5.7)

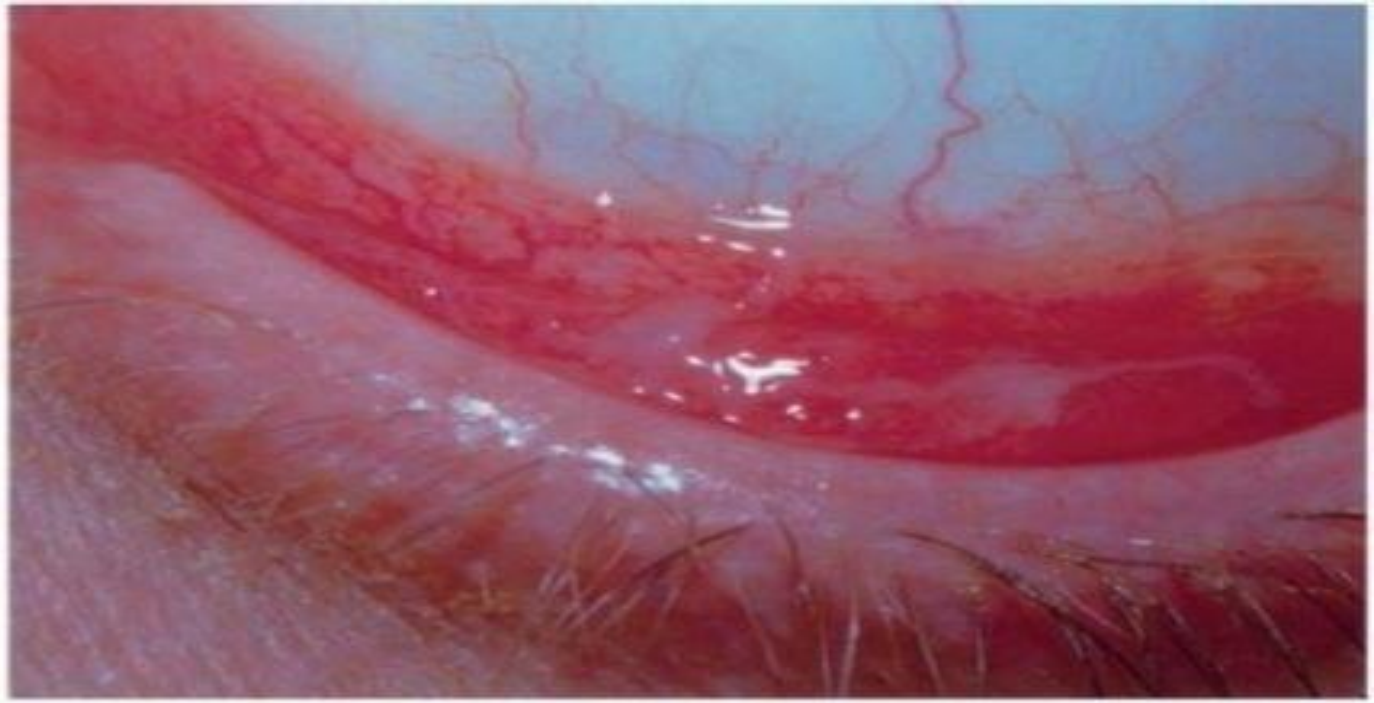


Fig. 5.5 Adult *Chlamydia* inclusion conjunctivitis. Note conjunctival follicles and discharge.

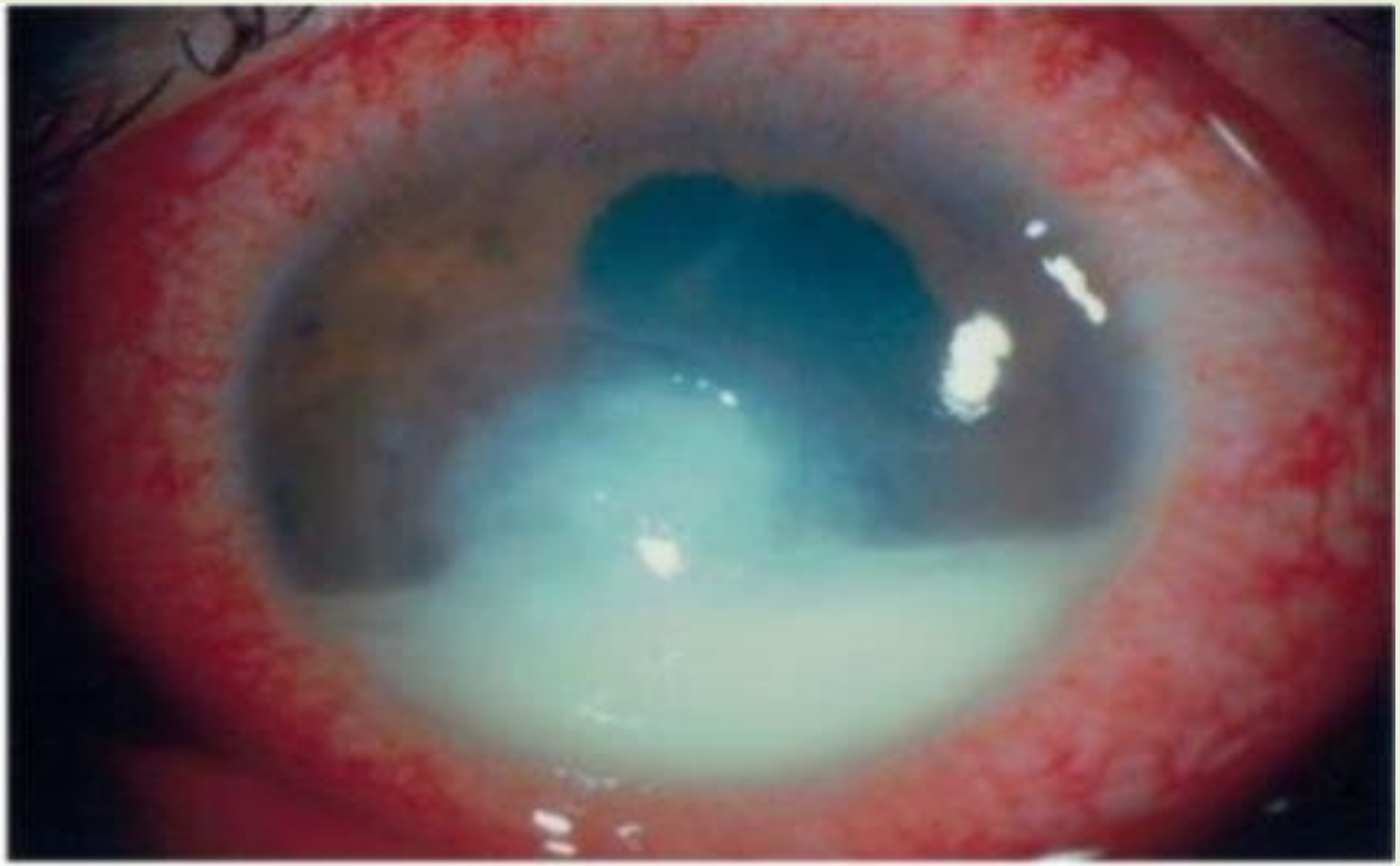


Fig. 5.6 *Streptococcus pneumoniae* corneal ulcer with hypopyon.

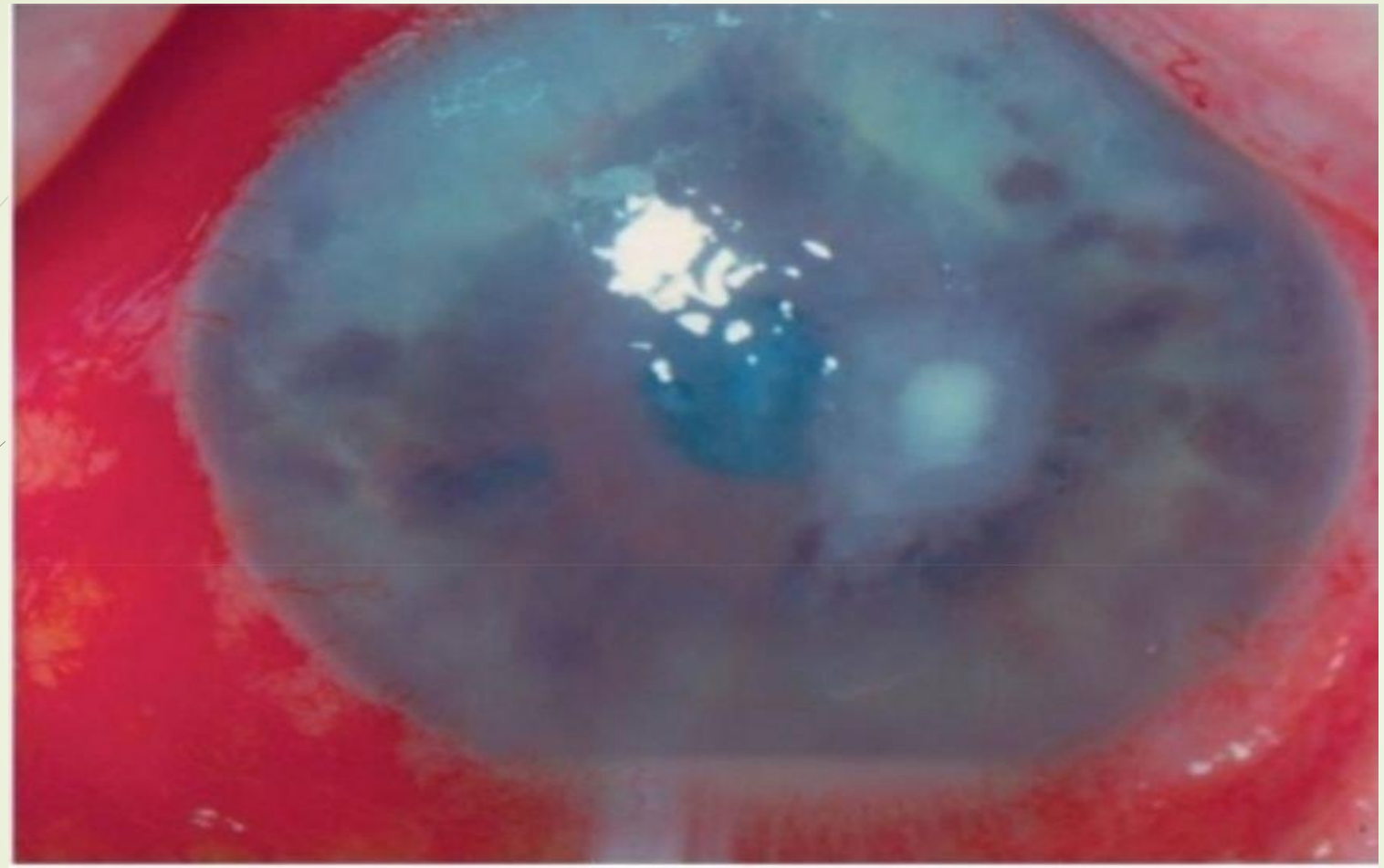


Fig. 5.7 *Aspergillus* sp. fungal corneal ulcer.

Clinical indications for smears and cultures

- Less commonly, infections of the lids, tear passages (Fig. 5.8), intraocular structures (endophthalmitis), or wounds (e.g, after surgery or trauma) may result, which require smears and cultures by a variety of specialized techniques.

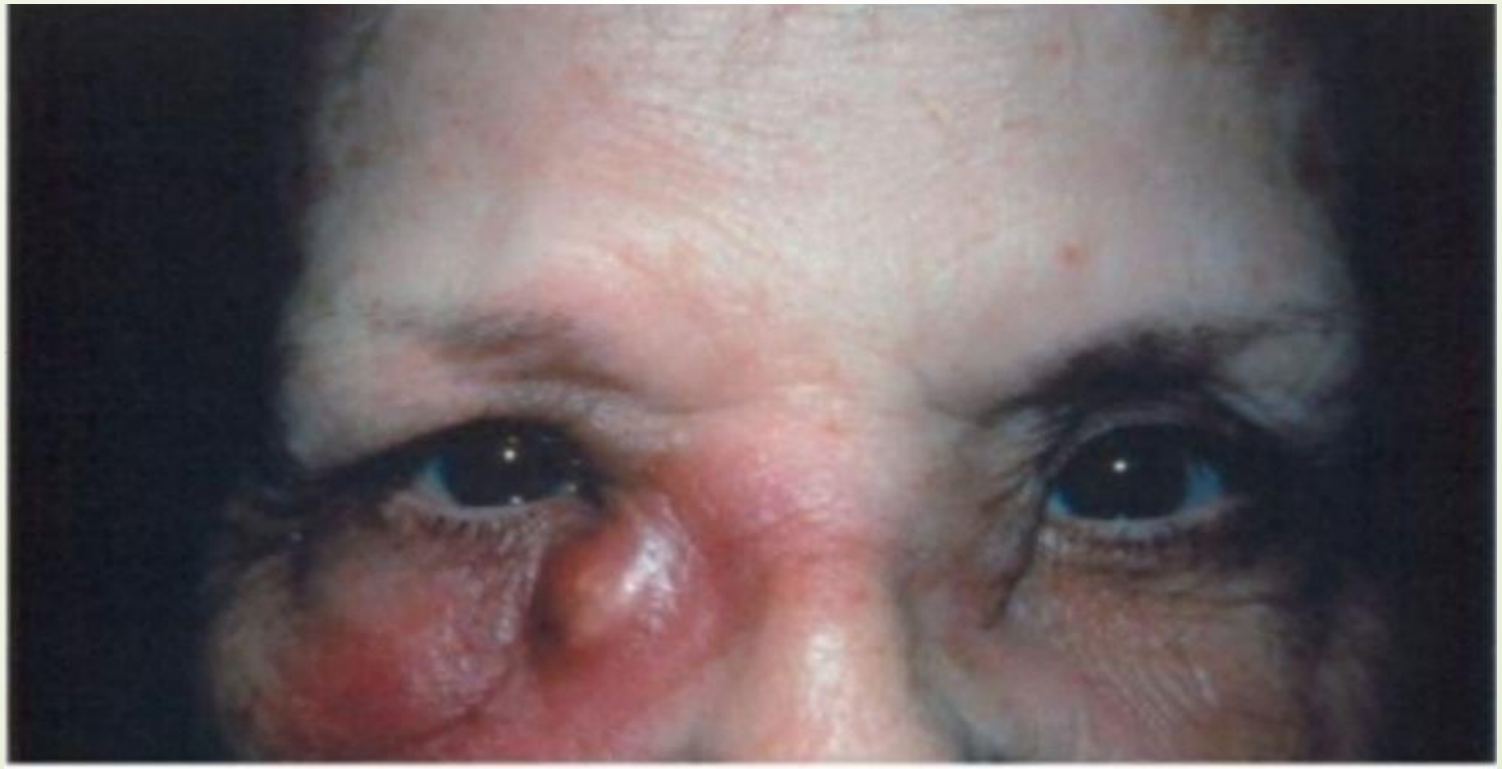


Fig. 5.8 Dacryocystitis.

Clinical indications for smears and cultures

- ***Cytology*** is the study of cell types; conjunctival cytology is useful in differentiating types of ocular inflammations.

- The *conjunctiva* is a clear, loose, vascular tissue that reacts to infection by becoming irritated with inflammatory cells and edematous fluid.

- Microbial organisms may be difficult to find in smears but the type of inflammatory cells that predominate may give a clue as to the type of infection (Table 5.2).

Table 5.2 Inflammation of the eye

Part affected	Condition
Lids	Blepharitis
Cornea	Keratitis
Conjunctiva	Conjunctivitis
Tear sac	Dacryocystitis
Uveal tract	Uveitis
Iris	Iritis
Ciliary body	Cyclitis
Iris and ciliary body	Iridocyclitis
Choroid	Choroiditis
Retina	Retinitis
Optic nerve	Optic neuritis
Inner ocular coats	Endophthalmitis
All ocular coats	Panophthalmitis
Orbital tissue surrounding eye	Orbital cellulitis

Clinical indications for smears and cultures

- The presence of polymorphonuclear leukocytes (PMNs) is most characteristic of bacterial infections, lymphocytes of viral infections, and eosinophils of allergic conditions.

- Smears are of particular importance in conjunctivitis of the newborn infant. Gram- and Giemsa -stained smears can help to differentiate among the likely causes of chlamydia, gonorrhea, or herpesvirus.

Clinical indications for smears and cultures

- The cornea is one of the few tissues in the body without blood vessels; it commonly reacts to infection by ulcerative necrosis.
- Smears are an important aid to early diagnosis, particularly in bacterial and fungal corneal ulcers.
- Results of the smears are usually available within minutes to hours and allow the ophthalmologist to choose antimicrobial agents that: are generally effective against bacterial or fungal organisms.

Clinical indications for smears and cultures

- Culture results are usually available within 1 to 3 days, which allows modification of the initial antibiotic therapy so as to achieve the best possible clinical response.
- The etiologic agents of bacterial ulcers vary somewhat with regard to geographic location and patient population. ***Pseudomonas*** tends to be the most common cause in the southern United States and ***Staphylococcus*** the most common in Canada and the northern United States.

Summary

1. Diagnostic Tools: Smears vs. Cultures

Laboratory studies are essential for determining the exact cause of an infection and selecting the most effective treatment.

- **The Smear:** A sample of tissue cells or discharge obtained by scraping.
  - **Gram Stain:** Used to identify **bacteria**.
  - **Giemsa Stain:** Used for **cytology** (the study of cell types).
  - **Timing:** Results are available in minutes to hours, allowing for immediate
  - "presumptive" treatment.
- **The Culture:** Involves growing the organism in a controlled environment.
  - **Timing:** Results typically take 1 to 3 days.
  - **Purpose:** Allows the doctor to modify and refine antibiotic therapy for
  - the best clinical outcome.

Summary

2. When are Laboratory Studies Needed?

While many infections are diagnosed through clinical observation, specific conditions require laboratory confirmation:

- **Newborns:** Any case of conjunctivitis in an infant.
- **Severe Inflammation:** Acute purulent or chronic conjunctivitis.
- **Ulcers:** Corneal ulcers that may be bacterial, fungal, or parasitic in nature.
- **Surgical/Traumatic Wounds:** Infections following surgery or eye injuries.
- **Deep Infections:** Inflammation of the tear passages (Dacryocystitis) or the interior of the eye (Endophthalmitis).

Summary

3. Cytology: Identifying the Cause by Cell Type

The specific type of inflammatory cells present in a conjunctival smear provides a major clue to the type of infection:

Dominant Cell Type	Likely Diagnosis
Polymorphonuclear leukocytes (PMNs)	Bacterial Infection
Lymphocytes	Viral Infection
Eosinophils	Allergic Condition

Summary

4. Ocular Tissue Reactions

Different parts of the eye react differently to microbial invasion:

•**Conjunctiva:** As a vascular tissue, it reacts with **edema** (swelling) and inflammatory cell infiltration.

•**Cornea:** Because it lacks blood vessels, it reacts via **ulcerative necrosis**

Summary

5. Key Clinical Terminology

The lecture concludes with the standardized medical terms used to describe inflammation in specific ocular structures:

- **Blepharitis:** Inflammation of the lids.
- **Keratitis:** Inflammation of the cornea.
- **Uveitis:** Inflammation of the uveal tract.
- **Dacryocystitis:** Inflammation of the tear sac.
- **Orbital Cellulitis:** Inflammation of the tissue surrounding the eye.
- **Panophthalmitis:** Inflammation involving all coats of the eye.

Post test

- **What is the primary difference between a smear and a culture?**
- A.A smear is taken from the blood, while a culture is taken from the eye.
- B.A smear is a sample for direct microscopic examination, while a culture involves growing organisms on media.
- C.A smear identifies viruses, while a culture identifies bacteria.
- D.There is no difference; the terms are interchangeable

- **In a newborn infant with conjunctivitis, why are Gram and Giemsa stains specifically indicated?.**
- A.To differentiate between Chlamydia, Gonorrhea, or Herpesvirus.
- B.To see if the baby's eyes will change color.
- C.To determine the baby's blood type.
- D.To check for allergic reactions to breast milk

Unit

- The lecture provides practical instructions on the collection and staining of ocular samples for diagnostic purposes.

Taking smears

- For rapid diagnosis, smears obtained from infective material are most valuable.
- A smear is a sample of discharge or infected tissue that is placed on a glass slide.
- To obtain a good sample of a superficial tissue, a gentle scraping with a platinum spatula may be done.
- The actual sampling of infected tissues should be performed by the ophthalmologist or under his or her direct supervision.

Taking smears

- All corneal lesions must be sampled by the ophthalmologist because this type of operative procedure has certain hazards. 'The material for culture and smear is taken from the advancing edge of the corneal lesion with a platinum spatula, scalpel blade, or swab.
- The ophthalmic assistant, however, may easily obtain the sample of conjunctival discharge found in the **lower fornix** in cases of conjunctivitis.
- The exudate is collected with a small sterile swab or platinum spatula , either from the lower cul-de-sac or from the inner canthus (**Fig. 5.9**).

Taking smears

- It is spread as thinly and evenly as possible on the surface of a clean glass slide. The slide then can be stained for bacterial or cytologic differentiation and identification. When a culture is taken, it is important to avoid contamination of the applicator by the lashes and lid margin.

Fig. 5.9 Swabbing of the lower lid margin for culture.

Making a stain

- The routine stains of smears that may be performed by the ophthalmic assistant in the office are Gram stain for bacteria and fungi and the Giemsa and Diff-Quik (Baxter Diagnostics) stains for determining tissue cell type or the presence of inclusion bodies.

- Inclusion bodies often are characteristic of certain types of infection.

Making a stain

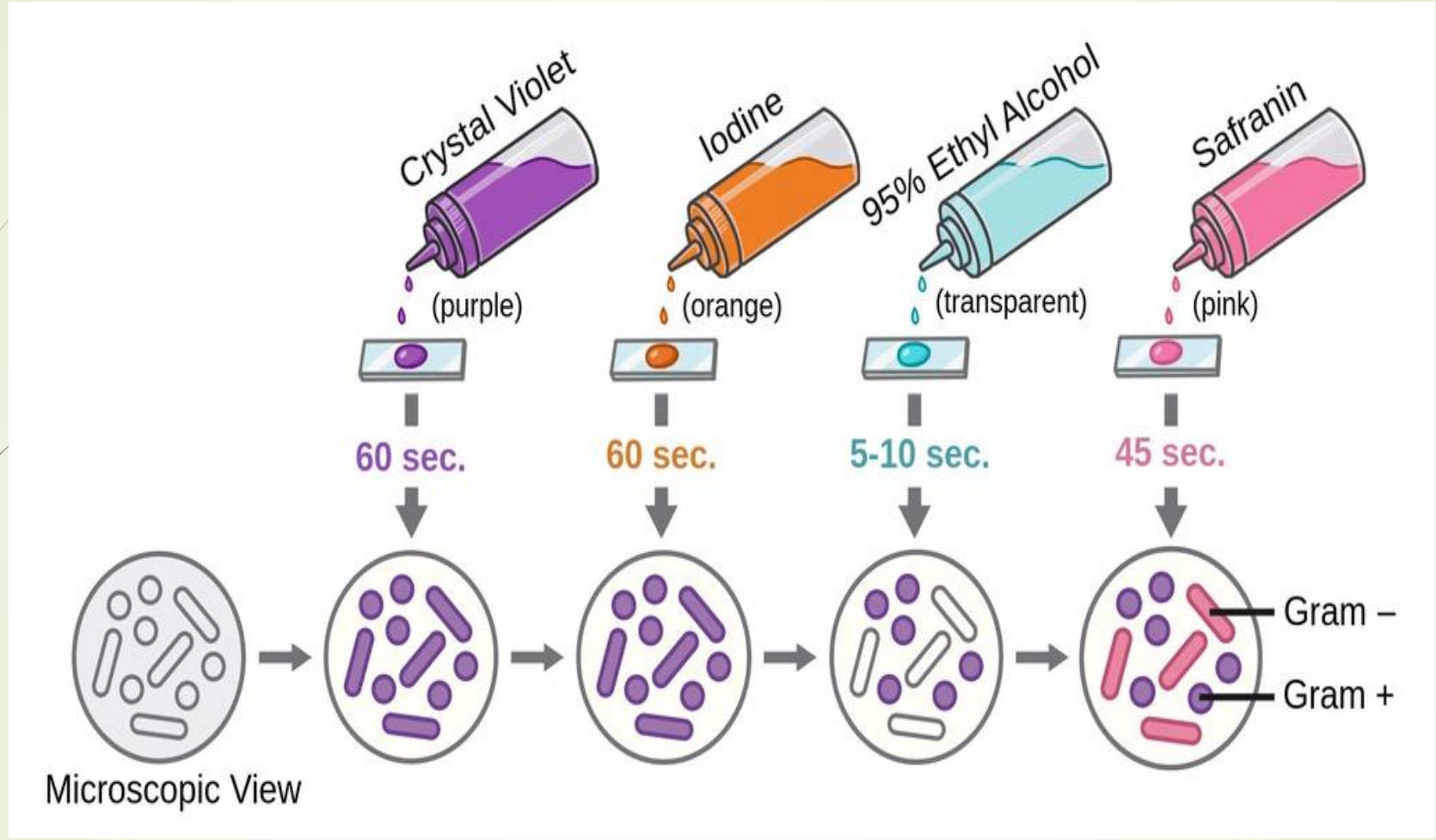
- Gram stain is useful in determining the type of bacteria or fungi responsible for the infection, whereas the Giemsa and Diff-Quik stains are useful in determining the inflammatory cellular response to a particular agent.
- The ophthalmic assistant should learn about Gram, Giemsa, and Diff-Quik stains. Diff-Quik is used in place of Giemsa as a rapid stain used for cytology. Giemsa stains are time consuming and are seldom used today.

Gram stain for bacterial identification

- 1. Fix smear by gentle heating.
- 2. Cover with crystal or gentian violet solution for 40 to 60 seconds.
- 3. Rinse with water.
- 4.Cover with Gram's iodine for 1 minute; pour off solution.
- 5. Decolorize with 95% ethyl alcohol or acetone for 5 to10seconds.

Gram stain for bacterial identification

- 6.Rinse with water.
- 7.Cover with Safranin counterstain for 40 to 60 seconds.
- 8. Wash with water, blot, and dry.
- Gram-positive organisms stain dark blue/purple and gram-negative organisms stain red/pink.


Crystal Violet
Iodine
95% Ethyl Alcohol
Safranin
(purple)
(orange)
(transparent)
(pink)
60 sec.
60 sec.
5-10 sec.
45 sec.
Gram –
Gram +
Microscopic View

Diff-Quik stain for cytologic identification

- 1.Dip slides in Diff-Quik fixative for 10seconds (10 dips).
- 2. Dip slides in Diff-Quik solution 1 for 10seconds (10 dips).
- 3. Dipslides in Diff-Quik solution 2 for 12seconds (12 dips).
- 4. Rinse slides with distilled or deionized water and let them dry.
- 5. Dip in xylene.
- 6. Mount.

Diff-Quick Staining Protocol

Commercial Diff-Quick Stains

Methanol

Eosinophilic Stain

Basophilic stain

Diff-Quick is a Romanowsky Stain. Romanowsky stains are a group of stains that include a combination of acidic and basic dyes. These stains are commonly used for the differential staining of blood smears and other cellular preparations.

summary

1. Techniques for Taking Smears

A smear is a sample of discharge or infected tissue used for rapid identification of pathogens.

- **Clinical Roles**:
  - **Ophthalmologist**: Must perform all samplings of **corneal lesions** due to the risks involved in the procedure.
  - **Ophthalmic Assistant**: Can safely collect **conjunctival discharge** from the lower fornix or inner canthus.
- **Collection Tools**: Samples are typically taken using a platinum spatula, a scalpel blade, or a sterile swab.
- **Best Practices**: Material from corneal lesions should be taken from the **advancing edge** of the lesion. It is vital to avoid touching the eyelashes or lid margins to prevent contamination of the sample.

summary

2. Laboratory Staining Methods

The lecture outlines the two primary types of stains used in an office or clinical setting to differentiate between various infections.

Gram Stain (Bacterial/Fungal Identification)

- **Purpose**: Used to determine the type of bacteria or fungi causing the infection.
- **Gram-Positive**: Organisms will appear **dark blue or purple**.
- **Gram-Negative**: Organisms will appear **red or pink**.

Cytological Stains (Giemsa and Diff-Quik)

- **Purpose**: Used to determine the inflammatory cell response or the presence of **inclusion bodies**.

- **Modern Practice**: **Diff-Quik** is preferred today because it is a much faster alternative to the time-consuming Giemsa stain

summary

3. Staining Protocols

The Gram Stain Procedure

1.Fix the smear with gentle heat.

2.Cover with **crystal/gentian violet** (40–60 seconds), then rinse.

3.Apply **Gram’s iodine** (1 minute), then pour off.

4.Decolorize with **ethyl alcohol or acetone** (5–10 seconds), then rinse.

5.Apply **Safranin counterstain** (40–60 seconds), wash, and dry.

summary

The Diff-Quik Procedure

1.Dip in **fixative** (10 times).

2.Dip in **Solution 1** (10 times).

3.Dip in **Solution 2** (12 times).

4.Rinse with distilled water, dry, and mount using xylene.

Post test

- **Which rapid staining method is currently used in place of the time-consuming Giemsa stain for cytology?**
- A.Diff-Quik b.Gentian Violet c.Acetone d.Safranin

- **. From which specific part of a corneal lesion should the sample for culture and smear be taken?**
- A.The advancing edge of the lesion.
- B.The lacrimal punctum.
- C.The center of the ulcer.
- D.The upper eyelid

Unit

- This lecture, outlines the essential protocols for collecting and cultivating microorganisms in clinical settings, with a specific focus on eye infections.

Specimen collection for culture

- Cultures are an important aid in diagnosis of infections.
- Cotton, synthetic, or calcium alginate tipped swabs are used to collect specimens from the eyelids, conjunctiva, or cornea for culture.
- The physician may want to inoculate the specimen directly onto culture media in the office or send the specimen swab to the laboratory for plating on the appropriate growth media.

Specimen collection for culture

- Specimen collection swabs with transport media (culturettes) are commercially available to keep organisms viable while in transit.
- The swab may be moistened in the transport media or other sterile fluid before specimen collection and then immediately placed into the transport liquid and taken to the microbiology laboratory.
- Once a specimen is collected, it is important that it be immediately inoculated onto the proper culture medium and incubated at the proper temperature for best results. In the bacteriology laboratory, the swabs are routinely platedon nutrient-rich culture media (**Fig. 5.10**).

Fig. 5.10 Bacterial colonies of *Serratia marcescens* on a chocolate agar plate.

Culture media

- Specific culture media are used to grow particular organisms.
- The most common types of nutrient-rich culture media include **blood agar, chocolate agar, thioglycolate broth, and Sabouraud-dextrose agar.**

- **Blood agar** is a medium enriched with 5% sheep's blood. It will grow most aerobic bacteria and can differentiate bacterial colonies that produce hemolysin , an exotoxin that lyses red blood cells.
- Some pathologic species of streptococci (e.g., the responsible organism for strep throat) produce hemolysin on blood agar, lysing the red blood cells, thereby creating a clear zone around the colony.

Culture media

- This allows the physician to confirm or rule out the presence of pathologic streptococci by looking at the culture plate.
- **Chocolate agar** is a very rich growth medium and is used to grow fastidious organisms, such as Haemophilus and Neisseria spp.
- There is no chocolate in chocolate agar:
- it is nutrient agar with 10% sheep or horse blood that has been heated to release nutrients from the blood cells which gives it a chocolate-brown appearance.
- Any organism that can grow on blood agar can grow on chocolate agar.

Culture media

- **Thioglycolate broth** is a liquid growth medium that is used to grow anaerobic bacteria.
- The broth chemically removes oxygen to maintain an anaerobic environment. It is important that the specimen be completely submerged into the thioglycolate broth to protect it from oxygen,
- **Sabouraud-dextrose agar** is used to grow yeast and fungi while suppressing bacterial growth.
- Bacteria can usually be identified within 24 hours. Viruses and chlamydia are not grown on these culture media.
- Chlamydia and fungi require specific cell cultures for growth and identification, which may take from 1 day to 3weeks for results.
- These specimens are collected and placed in viral/chlamydial transport media, placed on ice (4°C),and transported to the microbiology laboratory for culture.

Culture media

- When the physician orders a specimen to be sent for 'culture and sensitivity," the request is to isolate and grow the bacteria and then test the organisms for sensitivity to a standard battery of antibiotics.
- Discs soaked in various antibiotic solutions are placed on the culture medium along with the bacteria to test which antibiotic will inhibit the growth of susceptible bacteria.
- If the bacterium is sensitive to a particular drug, an area of "no growth" will be visible around the disc.

Other aids to identify organisms

- Biopsies have some value in the diagnosis of ocular infections. A biopsy is the removal of a piece of tissue, which is then either fixed in preservative (eg., formalin) and sent to the pathology laboratory or placed unfixed in a sterile jar and sent to the bacteriology laboratory for culture.

- An example of a condition that is readily diagnosed by a biopsy is the molluscum nodule on the lid margins, which is caused by a large virus (**Fig. 5.11**).

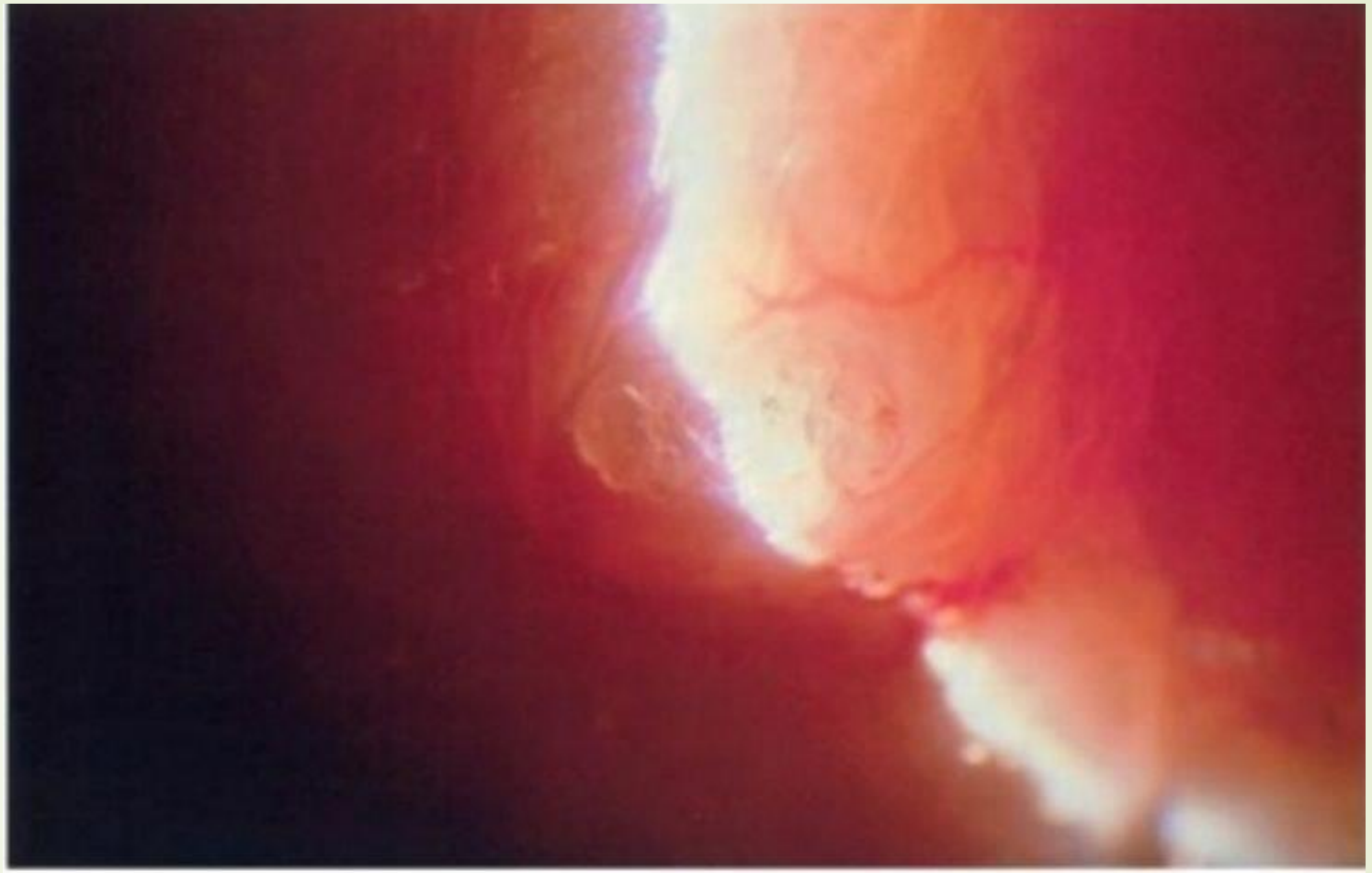


Fig. 5.11 Eyelid lesion (molluscum contagiosum) caused by a virus.

Other aids to identify organisms

- Skin tests are another diagnostic aid that may be performed in the office or hospital clinic. Various infections,such as tuberculosis, toxoplasmosis, and some fungi produce a skin reaction if the patient has been infected.

- Many known causes of eye disease may be attributed to infectious agents, such as bacteria, viruses, chlamydia , fungi, and parasites. Other unknown causes of diseases may also someday be ascribed to these organisms as improved techniques in isolating and identifying microbes develop.

- Unfortunately, only the exterior portions of the eye are readily available for routine sampling for identification of organisms. However, the ophthalmologist may sample the aqueous and/or vitreous fluids for culture by aspirating through a syringe and needle.

summary

- **1.Specimen Collection for Culture**
- Cultures are vital for diagnosing infections accurately.
- **Collection Tools:** Swabs made of cotton, synthetic materials, or calcium alginate are used to sample the eyelids, conjunctiva, or cornea.
- **Transport Media:** "Culturettes" are used to keep organisms viable during transit. Swabs may be moistened with sterile fluid or transport media before collection.
- **Immediate Processing:** Specimens must be immediately inoculated onto culture media and incubated at the correct temperature to ensure the best result

summary

- **II. Common Culture Media**
- Different media are used to grow and identify specific types of pathogens
- Medium Type -Description and Function
- **Blood Agar** .Enriched with 5% sheep's blood. It grows aerobic bacteria and differentiates species that produce hemolysin, which lyses red blood cells to create a clear zone around the colony.
- **Chocolate Agar** .Nutrient agar with 10% blood heated to release nutrients, giving it a brown appearance. It is used for fastidious organisms like Haemophilus and Neisseria.
- **Thioglycolate Broth** .A liquid medium that chemically removes oxygen to grow anaerobic bacteria. The specimen must be completely submerged to be protected from oxygen.
- **Sabouraud-dextrose Agar** .Used specifically to grow yeast and fungi while suppressing the growth of bacteria

summary

- **III. Diagnostic Aids and Testing**
- **Culture and Sensitivity:** This process involves isolating bacteria and testing them against various antibiotic-soaked discs. If the bacteria are sensitive, a "no growth" zone appears around the disc.
- **Viral and Chlamydia Identification:** These cannot grow on standard agar and require specific cell cultures. Specimens must be transported on ice (4°C).
- **Biopsies:** Tissue removal for pathology (fixed in formalin) or bacteriology (unfixed) helps diagnose conditions like molluscum contagiosum.
- **Skin Tests:** Used to identify reactions to infections such as tuberculosis or toxoplasmosis

summary

- **IV. Ocular Sampling Sites**
- While external portions of the eye are the easiest to sample, internal samples of aqueous or vitreous fluids can be obtained by an ophthalmologist using a syringe and needle for deeper culture analysis.

Post test

- **Which culture medium is the 'gold standard' for growing fungi?.**Î
- A.Thioglycolate Broth B.Blood Agar
- C.Sabouraud-dextrose Agar D.Chocolate Agar

- **In an antibiotic sensitivity test, what does a 'zone of no growth' around a paper disc indicate?.**Î
- A.The bacteria are sensitive to that antibiotic.
- B.The culture medium is contaminated.
- C.The antibiotic has expired.
- D.The bacteria are resistant to that antibiotic

- **which viral condition on the lid margin is frequently diagnosed using a biopsy?.**Î
- A.Adenovirus B.Molluscum nodule C.Herpes Simplex D. trachoma

Unit

- This lecture focuses on the essential techniques and agents used for sterilization and disinfection in a laboratory setting.

Sterilization and Disinfection

- **Sterilization**: is the killing or removal of all microorganisms , including bacterial spores which are highly resistant.

- **Disinfection:** is the killing of many, but not all microorganisms (or reduction of number of contaminating organisms to a level that cannot cause infection, some organisms and bacterial spores may survive).

- **Disinfectants**: are chemicals that are used for disinfection. Disinfectants should be used only on inanimate objects.

Sterilization and Disinfection

- **Antiseptics**: are mild forms of disinfectants that are used externally on living tissues to kill microorganisms, e.g. on the surface of skin and mucous membranes.

- **Bacteriostatic:** is a condition where the multiplication of the bacteria is inhibited without killing them.

- ■ **Bactericidal:** is that chemical that can kill or inactivate bacteria. Such chemicals may be called variously depending on the spectrum of activity, such as bactericidal , veridical , fungicidal, microbicidal , sporicidal, tuberculocidal or germicidal.

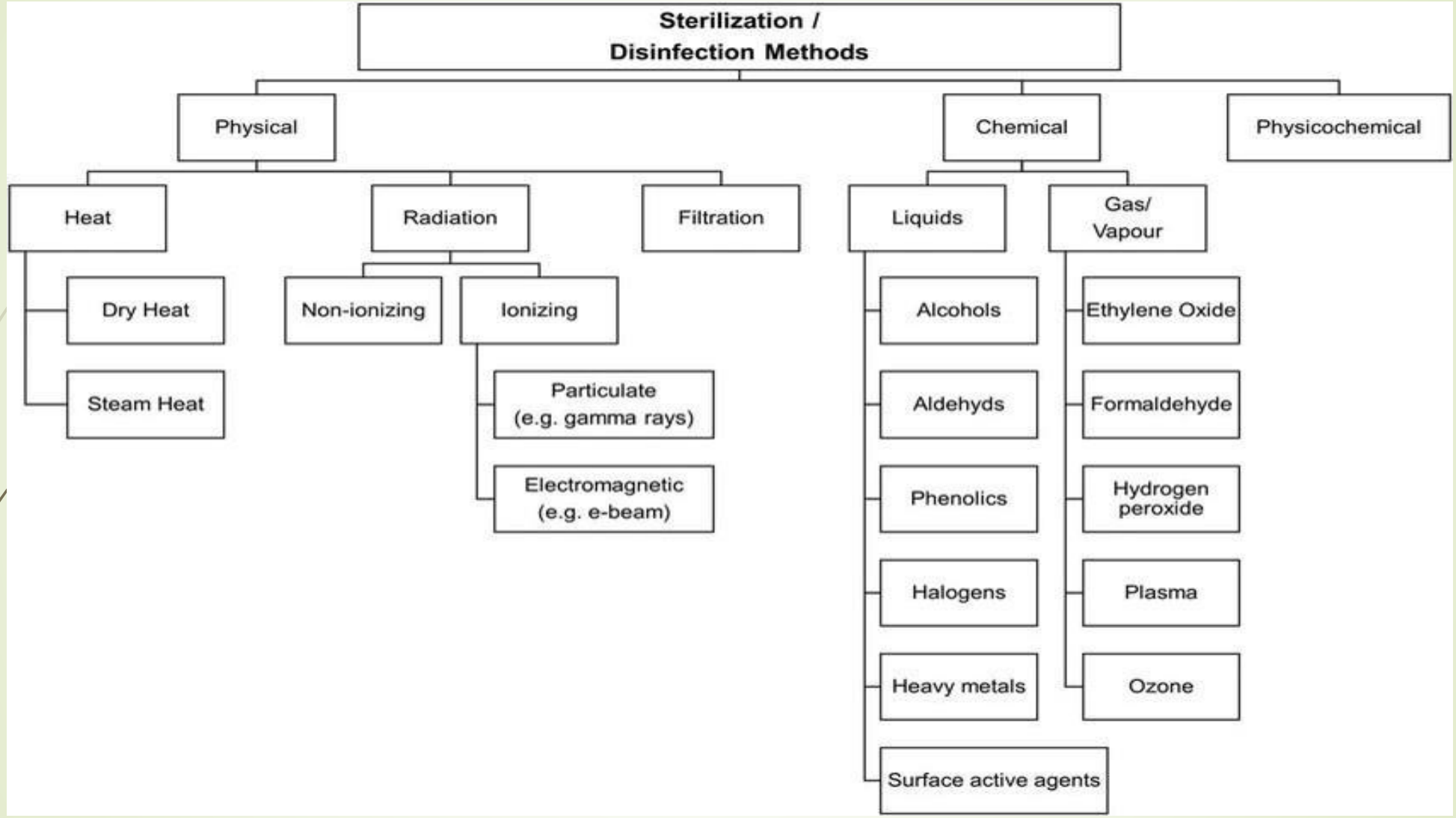
USES OF STERILIZATION

- 1. Sterilization for Surgical Procedures: Gloves, aprons, surgical instruments, syringes etc. are to be sterilized.

  2. Sterilization in Microbiological works like preparation of culture media, reagents and equipment where a sterile condition is to be maintained.

STERILIZATION METHODS

- **1. Physical Method.**
  - (a) Thermal (Heat) methods
    - I- Dry heat (red heat, flaming & hot air oven)
    - II- Moist heat (below 100 °C, at 100 °C & above 100 °C)
  - (b) Radiation methods
    - I- Non-ionizing radiation (Ultraviolet & Infrared radiation)
    - II-Ionizing radiation (X-ray & gamma ray)
  - (c) Filtration methods
- **2. Chemical Method (liquid & gaseous)**


Sterilization /
Disinfection Methods
Physical
Chemical
Physicochemical
Heat
Radiation
Filtration
Liquids
Gas/
Vapour
Dry Heat
Steam Heat
Non-ionizing
Ionizing
Particulate
(e.g. gamma rays)
Electromagnetic
(e.g. e-beam)
Alcohols
Aldehyds
Phenolics
Halogens
Heavy metals
Surface active agents
Ethylene Oxide
Formaldehyde
Hydrogen
peroxide
Plasma
Ozone

HEAT STERILIZATION : DRY HEAT

- 1. **Red heat:** bacteriological loops and tips of forceps are sterilized by holding them in Bunsen flame till they become red hot.
- 2. **Flaming:** scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed over a Bunsen flame, but not heating it to redness. Even though most vegetative cells are killed.
- 3. **Hot air oven:** This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160 °C) for duration of one hour in an electrically heated oven.

HEAT STERILIZATION: MOIST HEAT

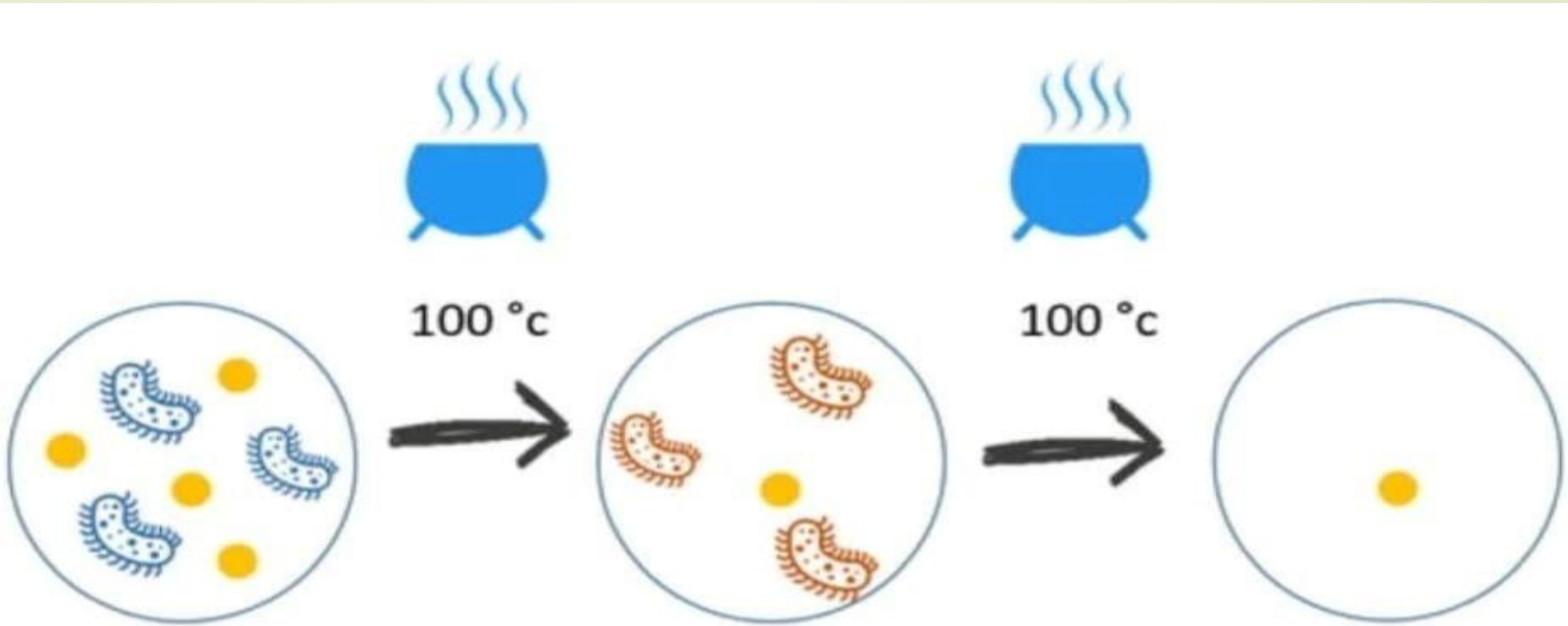
- Moist heat acts by coagulation and denaturation of proteins.
- **1. At temperature below 100 °C**

- **>Pasteurization:** employed by Louis Pasteur. Currently this procedure is employed in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63 °C for 30 minutes) and flash method (heated at 72 °C for 15 seconds) followed by quickly cooling to 13 °C. This method is suitable to destroy most milk borne pathogens like *Salmonella, Mycobacteria, Streptococci, Staphylococci and Brucella*, however *Coxiella* may survive pasteurization.

- >**Clothes sterilization** at 72 °C -80 °C by washing them for 5-10 minutes.

HEAT STERILIZATION: MOIST HEAT

- **2. At temperature of 100 °C:**
- **Boiling:** Boiling water (100 °C) kills most vegetative bacteria and viruses immediately.
- **Steam at 100 °C**: Media such as TCBS, DCA and selenite broth are sterilized by steaming. Sugar and gelatin in medium may get decomposed on autoclaving, hence they are exposed to free steaming for 20 minutes for three successive days. This process is known as tyndallisation (after John Tyndall) or fractional sterilization or intermittent sterilization. The vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days. **The success of process depends on the germination of spores**



TYNDALLIZATION

HEAT STERILIZATION: MOIST HEAT

- **3. At temperature above 100 °C:** (Autoclaving).
- In an autoclave the water is boiled in a closed chamber.
- As the pressure rises, the boiling point of water also raises.
- At a pressure of 15 lbs inside the autoclave, the temperature is said to be 121 °C.
- Exposure of articles to this temperature for 15 minutes sterilizes them.
- To destroy the infective agents associated with spongiform encephalopathies (prions), higher temperatures or longer times are used; 135 °C or 121 °C for at least one hour are recommended

RADIATION

- Two types of radiation are used, ionizing and non-ionizing.
- Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. **Since radiation does not generate heat, it is termed "cold sterilization".**
- **Non-ionizing radiation:**

  >Ultraviolet & infrared radiation

  > Used in sterilizing large number of disposables in short time.

- **Ionizing radiation:**

  >Such as X-ray & gamma ray

Filtration

- Filtration does not kill microbes, it separates them out.
- Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved.
- **It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution**.
- Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydatid fluid.

CHEMICALS METHODS OF STERILIZATION

- **Disinfectants**: are those chemicals that destroy pathogenic bacteria from inanimate surfaces.
- Some chemicals have very narrow spectrum of activity and some have very wide.
- Those chemicals that can sterilize are called **chemisterilants.**
- Those chemicals that can be safely applied over skin and mucus membranes are called **antiseptics.**
- An ideal antiseptic or disinfectant should have following properties:

PROPERTIES OF IDEAL ANTISEPTICS

- Should have wide spectrum of activity.
- Should be able to destroy microbes within practical period of time.
- Should be non-toxic, non- allergenic , non-irritative or non-corrosive.
- Should be active in the presence of organic matter .
- Should not have bad odour.
- Should not leave non-volatile residue or stain.
- Should make effective contact and be wettable.
- Should be active in any pH.
- Should be speedy.
- Should have high penetrating power.
- Should be stable.
- Should have long shelf life.
- Efficacy should not be lost on reasonable dilution.
- Should not be expensive and must be available easily.

Aseptic technique

- Aseptic technique is a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by pathogens.

- Aseptic technique in medical microbiology lab may involves (wearing gloves, work at sterile area, flaming of top side of flasks and tubes before and after opening, etc.).

Post test

- What is the difference between:

  1. sterilization and disinfection?

  2. bacteriostatic and bactericidal?

  3. tantalization and pasteurization?

- What is "aseptic technique" refer to?
- Enumerate the methods of moist heat sterilization with examples.
- What is the principle of moist heat sterilization?
- When you should be use filtration? Give examples.
- What are the physical conditions in:

  1. Autoclave? 2. Hot-air oven?

summary

- Many known causes of eye disease may be attributed to infectious agents, such as bacteria, viruses, chlamydia, fungi, and parasites. Other unknown causes of diseases may also someday be ascribed to these organisms as improved techniques in isolating and identifying microbes develop.
- Unfortunately, only the exterior portions of the eye are readily available for routine sampling for identification of organisms. However, the ophthalmologist may sample the aqueous and/or vitreous fluids for culture by aspirating through a syringe and needle.