GENERAL PATHOLOGY AND MICROBIOLOGY

PRACTICAL LOGBOOK

DEPARTMENT OF GENERAL PATHOLOGY AND MICROBIOLOGY

This is to certify that Mr./Ms. _____,

College Roll No. _____, Batch No. _____, Year _____, and KMU Roll No. _____, has successfully completed the required practical work in accordance with the prescribed course of study under the supervision of _____ for the academic year _____, as recorded in the practical schedule of this journal.

Professor In-Charge signature with official stamp

_____

Date:_____

LIST OF PRACTICALS PERFOMED

S.NO	Practical	Date
BLOCK D FOUNDATION		
1.	Coagulative Necrosis	
2.	Pathologic Calcification	
3.	Hyperplasia	
4.	Gram Staining	
5.	Culture Media	
BLOCK E INFLAMMATION, INFECTION & AUXILIARY DENTAL MATERIALS MODULE		
6.	Acute Inflammation	
7.	Bacterial Motility	
8.	Biochemical Tests	
9.	Plasmodium	
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11.	Sterilization and Disinfection	
12.	Ziehl Neelsen Staining (Zn Staining)	
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BLOCK F PRE-CLINICAL DENTISTRY I (HEALING, REPAIR & DENTAL RESTORATIONS -I)		
15.	Various Laboratory Instruments and Machine	
16.	Sample collection and Transportation	
17.	Granulation Tissue	
BLOCK G PRE-CLINICAL DENTISTRY II (NEOPLASIA & DENTAL REHABILITATION)		
18.	Ascaris lumbricoides	
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20.	Enterobius vermicularis	
21.	Taenia saginata and Taenia solium	
22.	Hydatid cyst	
23.	Pleomorphic Adenoma	
24.	Squamous Cell Carcinoma	
25.	Basal Cell Carcinoma	
26.	Lipid Profile	

Completed & Checked: _____ Dated: ____/_____/_____

BLOCK – D

Foundation II

Practical # 1

COAGULATIVE NECROSIS

Necrosis: Necrosis is a pathological and irreversible form of cell death resulting from severe cellular injury. It is characterized by denaturation of cellular proteins, loss of cell membrane integrity, leakage of intracellular contents, enzymatic digestion of the dead cells, and an associated inflammatory reaction in the surrounding tissue.

Coagulative necrosis: Coagulative necrosis is a type of irreversible cell death/ necrosis in which injurious stimulus predominantly causes the denaturation of structural and enzymatic proteins, thereby blocking proteolysis of dead cells, resulting in preservation of the basic tissue architecture for a variable period of time despite loss of nuclear staining and cellular viability. It is most commonly caused by ischemia and infarction in solid organs.

Etiology / Causes:

1. Ischemia due to obstruction of blood vessels

   Examples: thrombosis, embolism, atherosclerosis

2. Infarction of solid organs

   Examples: heart, kidney, spleen

3. Chemical and toxic injury

   Examples: strong acids, toxins

4. Physical injury

   Examples: burns, extreme heat or cold

5. Rapidly growing tumors with inadequate blood supply

Note: Ischemic injury to the brain results in liquefactive necrosis due to high lipid content and enzymatic activity.

Pathogenesis:

Severe ischemic or toxic injury leads to denaturation of cellular proteins and inactivation of lysosomal enzymes. Due to inhibition of proteolysis, the dead cells are not immediately digested, and the basic tissue framework remains preserved. Subsequently, inflammatory cells infiltrate the necrotic area, releasing lysosomal enzymes that digest and remove the necrotic tissue.

Morphology: Grossly, the necrotic area appears pale, firm, and well demarcated from surrounding viable tissue. In infarcts, the lesion is often wedge shaped with the apex pointing toward the site of vascular obstruction and the base toward the surface of the organ. Microscopically, the tissue shows preservation of the basic tissue architecture despite loss of cellular viability. The cells exhibit increased eosinophilia of the cytoplasm due to protein denaturation. Nuclear changes are evident in the form of pyknosis, karyorrhexis, and karyolysis, leading to partial or complete loss of nuclear staining. The outlines of the dead cells remain intact, giving rise to characteristic ghost cells. In later stages, inflammatory cell infiltration is seen in the surrounding tissue, which contributes to the removal of necrotic cellular debris.

Microscopic Points of Identification:

1) Preservation of basic tissue architecture
2) Increased eosinophilia of cytoplasm
3) Loss of nuclei showing pyknosis, karyorrhexis, or karyolysis
4) Intact cell outlines producing ghost cells
5) Inflammatory cell infiltration in later stages

Organs Commonly Affected

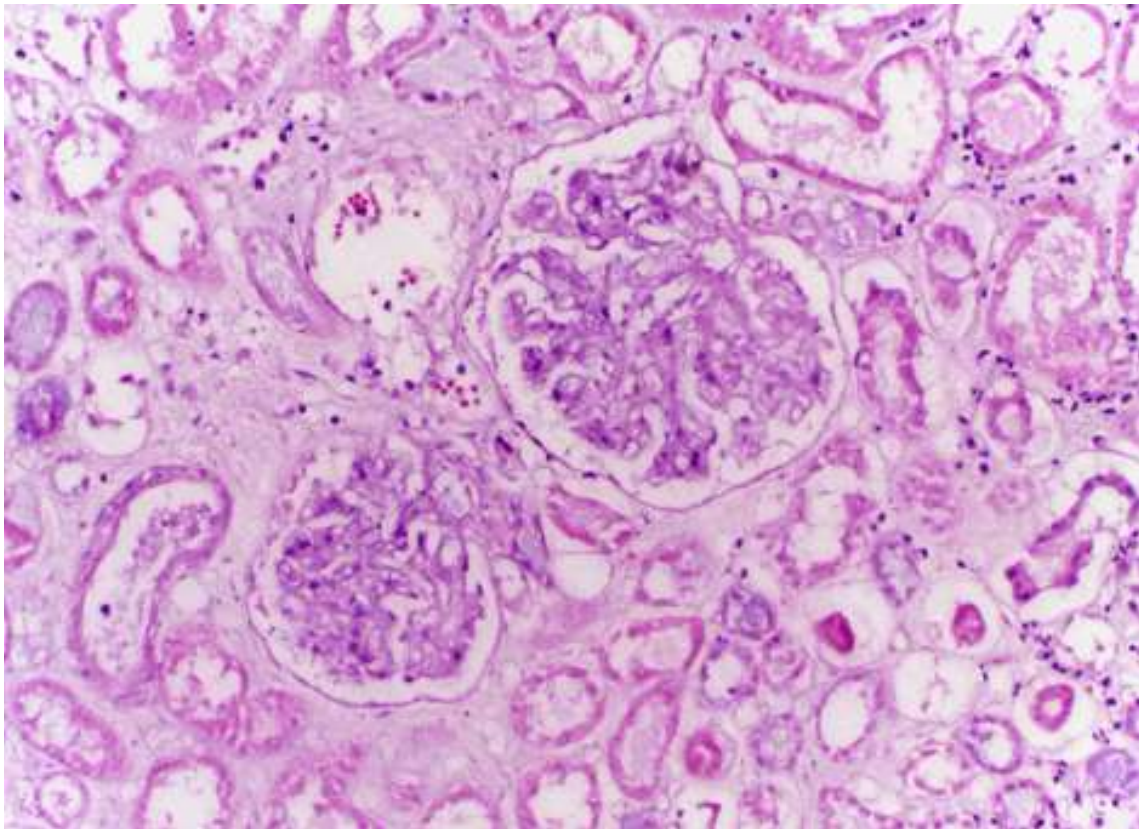
1) Heart
2) Kidney
3) Spleen
4) Adrenal gland
5) Skeletal muscle

Common Clinical Conditions:

1) Myocardial infarction
2) Renal infarction
3) Splenic infarction
4) Ischemic necrosis of solid organs
5) Tumors showing central necrosis

Significance:

1) Indicates irreversible cell injury
2) Leads to loss of function of the affected organ
3) May result in fibrosis and scar formation
4) Important for diagnosis of infarction



The renal parenchyma with coagulative necrosis. The basic tissue architecture is preserved, and the dying cells leave behind characteristic ghost outlines. The cells appear intensely eosinophilic with complete loss of nuclear staining.

References

1. Robbins and Cotran, Basic Pathology
2. Harsh Mohan, Textbook of Pathology
3. Kumar, Abbas, Aster – Robbins Basic Pathology

Student Task:

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Practical No. 2

PATHOLOGICAL CALCIFICATION

Definition

Pathological calcification refers to the abnormal accumulation of calcium salts along with smaller amounts of iron, magnesium, and other minerals in tissues, occurring in association with disease processes.

Types of Pathological Calcification

There are two main types of pathological calcification:

1. Dystrophic calcification
2. Metastatic calcification

1. Dystrophic Calcification

Definition: Dystrophic calcification is the local deposition of calcium salts in dead or dying tissues. It occurs in the absence of any disturbance in calcium metabolism, and serum calcium levels remain normal.

Causes

1) Tissue necrosis of any cause
2) Chronic inflammation
3) Degenerative changes in tissues
4) Old scars and damaged heart valves

Pathogenesis

Dystrophic calcification occurs due to deposition of calcium salts in areas of dead or injured tissue. The calcium binds to phospholipids present in the membranes of necrotic cells. These deposits act as seeding sites for further crystal growth. Progressive deposition of calcium leads to enlargement of these deposits and formation of laminated calcified structures.

Morphology

Gross Features: Calcium salts appear as fine, white granules or clumps. On palpation, the affected area often feels gritty.

Microscopic Features: On routine hematoxylin and eosin staining, calcium salts appear as basophilic, amorphous, granular, and sometimes clumped deposits. They may be present intracellularly, extracellularly, or in both locations. Calcium may be deposited in layers around dead cells forming seeding crystals. Progressive layering can result in lamellated structures known as psammoma bodies, which resemble grains of sand. In some cases, heterotopic bone formation may be seen at the site of calcification.

Common Organs / Sites

1) Areas of necrosis
2) Old scars
3) Atherosclerotic plaques
4) Damaged heart valves
5) Lungs in asbestosis

2. Metastatic Calcification

Definition: Metastatic calcification is the systemic deposition of calcium salts in normal tissues and occurs due to hypercalcemia secondary to disturbances in calcium metabolism.

Causes

1) Hyperparathyroidism
2) Destruction of bone
3) Vitamin D disorders
4) Renal failure

Pathogenesis:

In metastatic calcification, elevated serum calcium levels lead to deposition of calcium salts in otherwise normal tissues. The deposition is favored in tissues that lose acid and therefore become relatively alkaline.

Morphology

The microscopic appearance of calcium salts in metastatic calcification resembles that of dystrophic calcification. Deposits may be non-crystalline, amorphous, or granular and, in some cases, may form hydroxyapatite crystals.

Common Organs Affected

1) Blood vessel walls
2) Kidneys
3) Lungs
4) Gastric mucosa

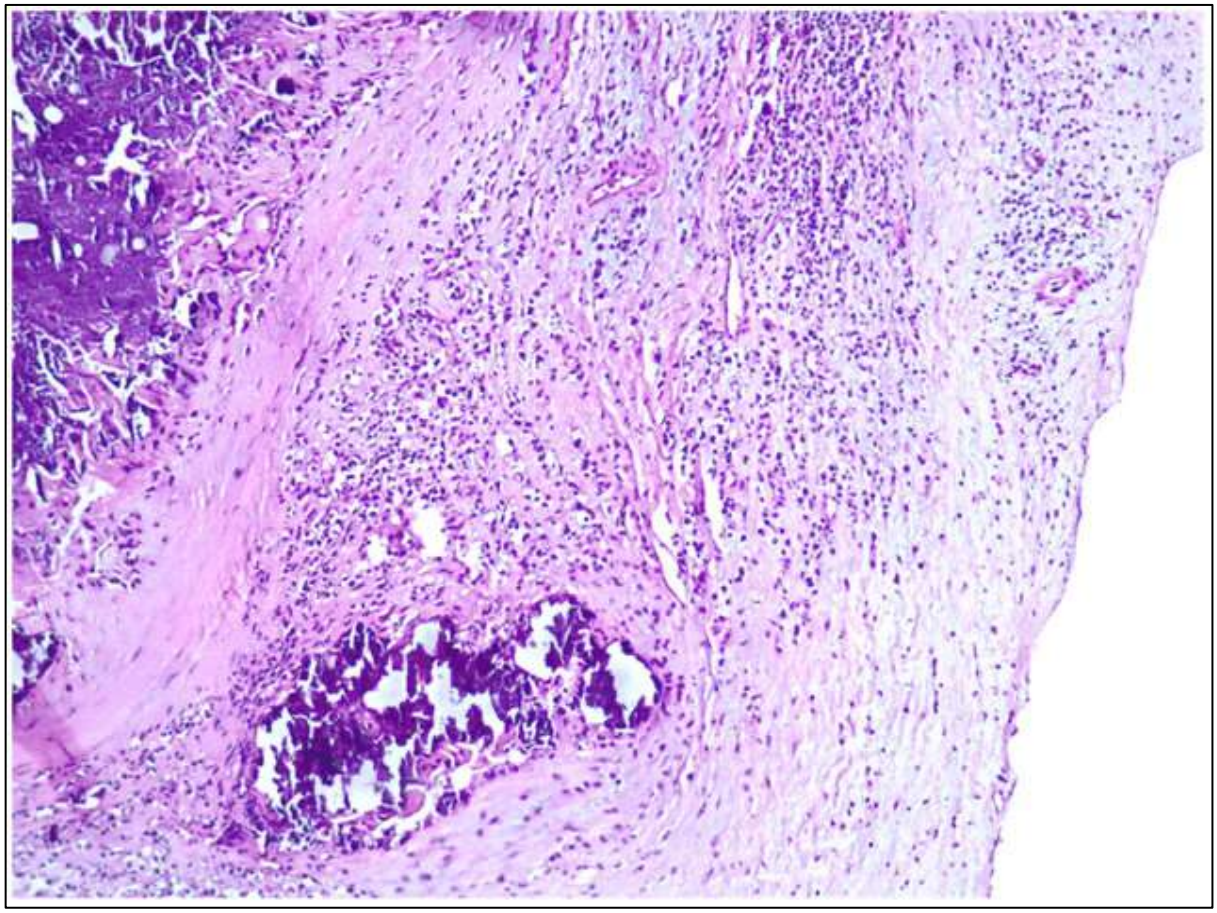
Points of identification: These microscopic features on H&E stain are characteristic of pathological calcification.

1) Presence of basophilic (deep blue-purple) amorphous granular deposits within the tissue.
2) Calcium deposits may be seen intracellularly, extracellularly, or in both locations.
3) Deposits appear as fine granules, coarse clumps, or irregular masses.
4) Lamellated concentric calcified structures (psammoma bodies) may be present.

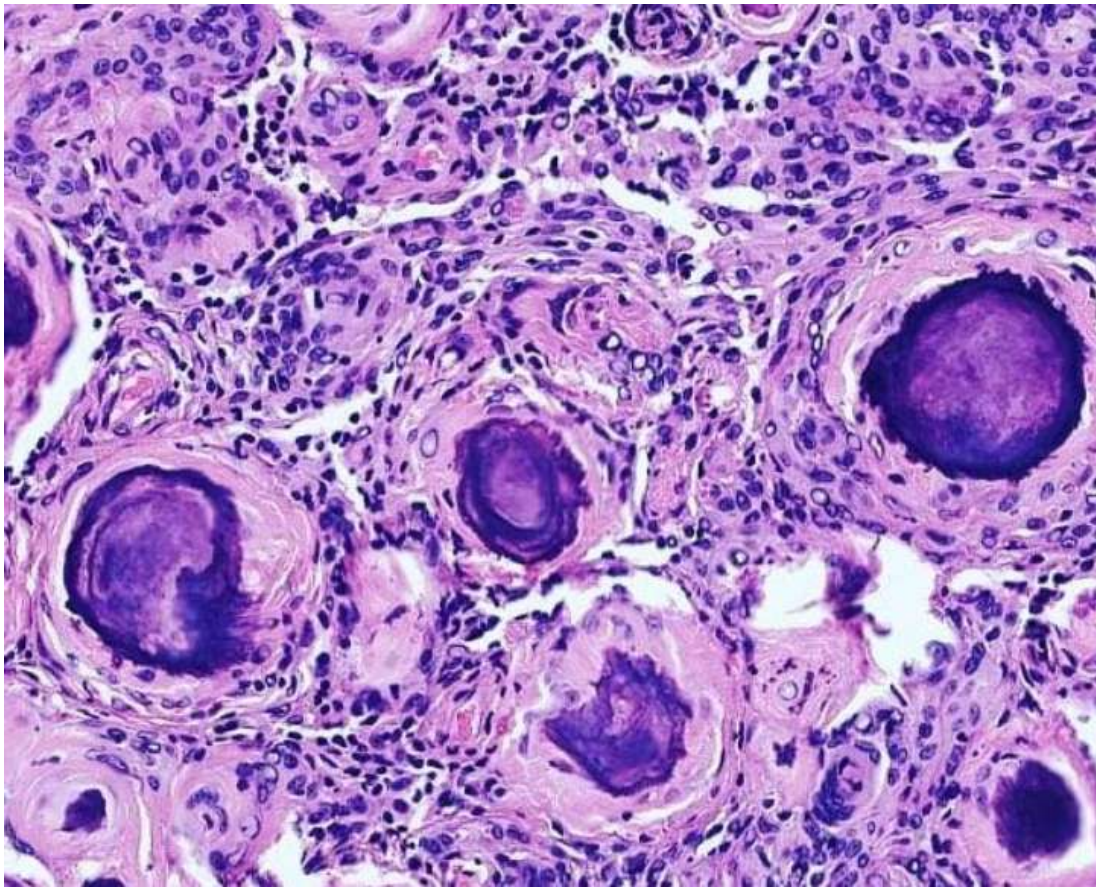
5) Surrounding tissue may show evidence of necrosis, degeneration, or chronic inflammation.
6) In some cases, heterotopic bone formation may be seen at the site of calcification.

Clinical Significance / Complications

1) Interfere with normal tissue function
2) Calcification of heart valves may lead to valvular dysfunction
3) Vascular calcification can cause reduced elasticity of blood vessels
4) Renal calcification may impair kidney function
5) Presence of calcification indicates underlying tissue injury or metabolic disorder



Areas of dystrophic calcifications and abundant inflammatory infiltrate (HE staining)



Psammoma bodies in Psammomatous meningioma

References

1. Robbins and Cotran, Basic Pathology
2. Harsh Mohan, Textbook of Pathology
3. Kumar, Abbas, Aster – Robbins Basic Pathology

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Practical No. 3

Nodular Hyperplasia of Prostate/ Benign Prostatic Hyperplasia (BPH)

Introduction – Hyperplasia:

Hyperplasia is an increase in the number of cells in an organ or tissue in response to a stimulus. It occurs only in tissues with cells capable of dividing and may occur along with hypertrophy. Hyperplasia can be physiologic or pathologic.

1. Physiologic hyperplasia occurs in response to hormones or growth factors, either to increase functional capacity (e.g., proliferation of breast epithelium at puberty or pregnancy) or for compensatory regeneration (e.g., liver after partial hepatectomy, bone marrow after blood loss).

2. Pathologic hyperplasia results from excessive or inappropriate hormonal or growth factor stimulation, as in endometrial hyperplasia or benign prostatic hyperplasia. Although abnormal, pathologic hyperplasia is controlled and can regress if the stimulus is removed.

Definition – Benign Prostatic Hyperplasia (BPH)

BPH is a common disorder in men over 50, characterized by nodular hyperplasia of the prostatic tissue, especially in the transition and periurethral zones. The nodular overgrowth compresses the urethra and leads to lower urinary tract symptoms. Approximately 30% of men in that age group have moderate to severe symptoms of BPH, and histologic evidence of BPH is found in up to 90% of men by age 80. It is not a premalignant lesion

Etiology

Hormonal factors: Interaction of androgens and estrogens, with dihydrotestosterone (DHT) being the main mediator of prostatic growth. DHT is formed from circulating testosterone by the action of 5α-reductase type 2 within the prostate. Risk increases with advancing age and may be influenced by metabolic factors.

Pathogenesis

Hyperplasia occurs predominantly in the transitional zone (periurethral region) of the prostate. Androgenic stimulation (primarily DHT) promotes proliferation of both glandular epithelium and stromal fibromuscular tissue. The enlarging nodules compress the urethra, increasing urethral resistance and causing symptoms. BPH nodules often form a surgical capsule separating hyperplastic tissue from the peripheral zone.

Morphology:

Gross Features: The prostate is enlarged, often weighing 3- to 5- fold greater than normal (60-100 g). The cut surface shows multiple well-demarcated nodules of varying sizes, mainly in the transition/periurethral zone. Nodules are tan-gray to tan-yellow, firm or soft depending on the proportion of stromal versus glandular components. Predominantly glandular nodules may appear more spongy or cystic, whereas stromal nodules are more solid and firm. Differentiation from carcinoma by grossing alone is difficult; carcinoma typically arises in the peripheral zone.

Microscopically there are two types of Hyperplasia seen in BPH: Glandular Hyperplasia which shows proliferation of acini forming papillary infoldings and cystic dilatation. The acini/ glands are lined by tall columnar epithelium with a basal cell layer. The lumina often contain proteinaceous secretions called corpora amylacea.

Stromal Hyperplasia in which there is marked increase in fibromuscular stroma. Nodules may be predominantly stromal or mixed (glandular and stromal). Most cases exhibit a combination of both glandular and stromal hyperplasia.

Common Site

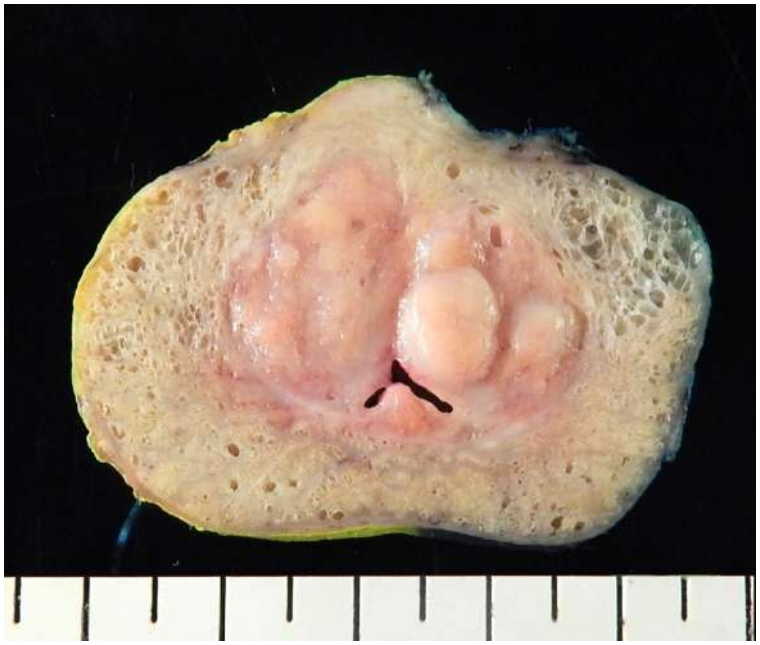
Prostate gland, especially the transitional and periurethral zones around the urethra while most carcinomas (70-80%) arise in peripheral zone.

Clinical Significance / Complications

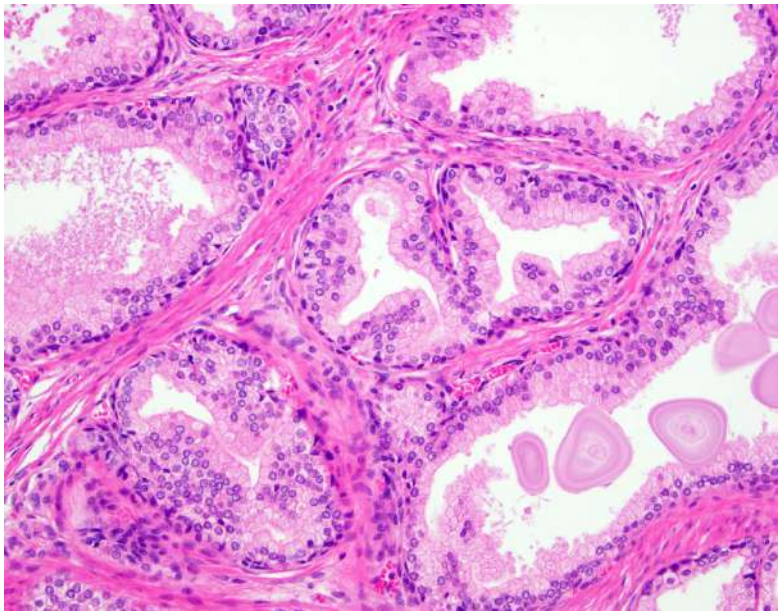
1) Progressive lower urinary tract symptoms, including urinary frequency, urgency, nocturia, weak stream, hesitancy, and incomplete emptying.
2) Acute urinary retention.
3) Bladder stone formation due to chronic urinary stasis.
4) Urinary tract infections.
5) Hydronephrosis and renal impairment from chronic obstruction.

Points of Identification on H&E Slide:

1) Nodular arrangement of hyperplastic glands and stroma.
2) Glandular acini lined by tall columnar epithelial cells with a distinct basal cell layer.
3) Acini lumina containing corpora amylacea.
4) Increased fibromuscular stroma, often around glands.
5) Preservation of basal cells helps differentiate BPH from prostatic carcinoma.



Hyperplastic nodules around the urethra in a cross section of the prostate gland



Glandular hyperplasia in Benign prostatic glands with luminal infoldings, lined by an inner layer of cuboidal to columnar secretory cells by an outer layer of basal cells

References

1. Robbins and Cotran, Basic Pathology
2. Harsh Mohan, Textbook of Pathology
3. Kumar, Abbas, Aster – Robbins Basic Pathology

Student Task:

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Practical # 4

GRAM STAINING

Introduction:

Developed in 1884 by Hans Christian Gram, Gram staining is used to:

a. Identify bacterial morphology (shape and arrangement)
b. Differentiate bacteria into Gram-positive (purple) and Gram-negative (pink/red)

Principle:

Gram-positive bacteria have a thick peptidoglycan cell wall, which retains the crystal violet–iodine complex and appears purple

Gram-negative bacteria have a thin peptidoglycan layer, lose the primary stain during decolorization, and take up the counterstain, appearing pink/red

Requirements

Equipment

a) Glass slides
b) Wire loop
c) Spirit lamp
d) Staining rack

Sample / Specimen: Bacterial culture or clinical specimen

Reagents:

Step	Stain / Reagent	Role
Primary stain	Crystal violet	Stains all bacteria purple
Mordant	Gram's iodine	Forms insoluble complex with crystal violet
Decolorizer	Acetone-alcohol / iodine-acetone	Removes dye from Gram-negative cells
Counterstain	Safranin / diluted carbol fuchsin (1:10)	Stains Gram-negative cells pink

Procedure

Slide preparation

a) Take a clean, grease-free slide and pass over flame

b) Make a thin smear with specimen using a sterile loop
c) Allow to air dry

Fixation

Heat-fix by passing the slide over flame 3–4 times or use methanol fixation

Staining

1. Primary stain: Flood smear with crystal violet for 1 minute, then rinse with water
2. Mordant: Flood with Gram's iodine for 1 minute, then rinse
3. Decolorization: Apply acetone-alcohol for 5-10 seconds, then rinse immediately. It removes the dye mordant complex from the cell. Gram positive stays dark purple due to thick peptidoglycan layer in the wall of gram +ve bacteria and gram negative bacteria becomes colorless. Acetone breaks gentian violet iodine complex.
4. Counterstain: Flood with diluted safranin for 1 minute, then rinse and air dry

Examination

Observe under oil immersion lens (100x)

Bacteria	Color
Gram-positive	Dark purple / blue
Gram-negative	Pink / red

Common Pitfalls

1. Slide not heat-fixed → smear may wash off
2. Over heat-fixing → distorted cell morphology
3. Over-decolorization → Gram-positive appears Gram-negative
4. Under-decolorization → Gram-negative appears Gram-positive
5. Smear too thick → uneven decolorization; Gram-negative may appear Gram-positive
6. Insufficient counterstain time → Gram-negative cells appear faint

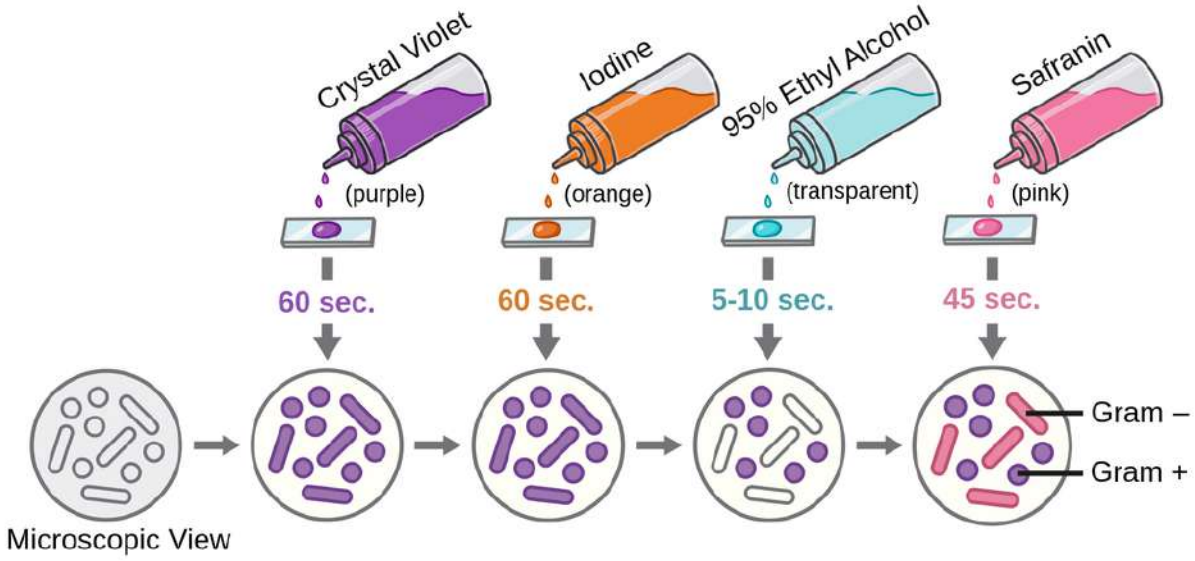
Common examples

Gram-positive bacteria (stain purple)

1. Staphylococcus
2. Streptococcus
3. Bacillus species
4. Clostridium species

Gram-negative bacteria (stain pink/red)

1. Escherichia coli
2. Salmonella species
3. Shigella species
4. Pseudomonas aeruginosa
5. Neisseria gonorrhoeae



References

1. Levinson W. Review of Medical Microbiology and Immunology.
2. Prescott LM, Harley JP, Klein DA. Microbiology.
3. AFIP Manual of Laboratory Medicine

Student Task:

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Practical # 5

CULTURE MEDIA

Introduction

Culture media are artificial nutrient preparations used to grow microorganisms under laboratory conditions. They mimic natural environments to support microbial growth, isolation, and identification.

Culture refers to visible growth of microorganisms on or in a culture medium after inoculation.

Uses in practical lab:

a. Isolate bacteria from clinical specimens
b. Identify pathogenic versus non-pathogenic organisms
c. Perform antibiotic sensitivity testing

Classification of Culture Media

A. Based on Physical State

1. Liquid (Broth) Media

   No solidifying agent; cloudy growth indicates bacteria

   Use: Biochemical tests, turbidity observation

   Examples: Peptone water, Nutrient broth

2. Solid Media

   Contains agar (1.5–2%) to support colony formation

   Use: Colony morphology, isolation of pure cultures

   Example: Nutrient agar in Petri plates

3. Semi-solid Media

   Low agar (0.5%) for motility tests and microaerophilic growth

B. Based on Function:

1. Basal / Basic Media

   Simple non-selective media that support growth of non-fastidious organisms

   Use: Routine culture, subculture, general growth

   Examples: Nutrient agar/broth, Peptone water

2. *Enriched Media*

   Basal media plus extra nutrients (blood, serum) for fastidious bacteria

   Use: Grow organisms with special requirements

   Examples: Blood agar (sheep blood) for hemolysis, Chocolate agar for Haemophilus and Neisseria

3. *Selective Media*

   Contains agents that inhibit unwanted microbes and allow specific ones to grow

   Use: Isolate pathogens from mixed specimens

   Examples: MacConkey agar (selects gram-negative), Bismuth Sulphite agar (Salmonella), Alkaline Peptone Water (Vibrio cholerae)

4. *Differential Media*

   Contains indicators to show differences between organisms (color, hemolysis)

   Use: Distinguish species based on biochemical traits

   Examples: MacConkey agar (lactose fermenters pink, non-fermenters colorless), Blood agar (alpha, beta, gamma hemolysis), EMB agar (metallic green for E. coli)

5. *Enrichment Media*

   Liquid media that increase the number of a specific organism before plating

   Use: Boost low-count pathogens to detectable levels

   Examples: Selenite F broth and Tetrathionate broth (Salmonella)

6. *Transport Media*

   Maintain viability of specimens without significant growth

   Use: Preserve clinical samples until culture

   Examples: Stuart's medium, Amie's medium, Kelly-Blair medium

Sterilization of Media

Media are sterilized by steaming or by autoclaving.

Common culture media

1. Blood Agar/ Blood Agar Plate (BAP)

Purpose: To support growth of a wide range of organisms, particularly those exhibiting hemolysis, such as streptococci, staphylococci, and pneumococci.

Preparation:

Blood is obtained from sheep, rabbit, goat, or horse.

Human blood is rarely used due to:

i. Potential antibodies against target microorganisms
ii. Risk of infection
iii. Presence of antibiotics

Ingredients:

i. Nutrient agar
ii. Minerals
iii. Defibrinated blood
iv. Vitamins

Uses:

a. Isolation and identification of hemolytic organisms
b. Observation of colony morphology and hemolysis patterns

Hemolysis Types:

a. Alpha (α) hemolysis: Partial greenish discoloration around colonies (e.g., Streptococcus pneumoniae, Streptococcus viridans). Caused by hydrogen peroxide oxidizing hemoglobin to green methemoglobin.
b. Beta (β) hemolysis: Complete clearing around colonies (e.g., Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas).
c. Gamma (γ) hemolysis: No hemolysis; agar remains unchanged (e.g., Enterococcus faecalis).

2. Chocolate Agar

Purpose: Enriched medium for fastidious organisms such as Haemophilus influenzae and Neisseria species.

Preparation:

Basic nutrient agar is supplemented with lysed blood (heated at 70°C in a water bath). Avoid overheating to prevent granular texture.

Ingredients and Factors:

- Factor V (NAD): Enhances bacterial growth
- Factor X (Hemin): Required for catalase and heme-containing cytochromes

Uses: Diagnosis of H. influenzae and Neisseria species.

3. Enrichment Media

Definition: Liquid media that favor the multiplication of a specific organism by providing selective nutrients or inhibiting competitors.

Examples: Tetrathionate broth, Selenite F broth (used to enrich Salmonella species)

4. Selective Media

Definition: Contain substances that inhibit unwanted organisms while promoting the growth of desired microorganisms.

Examples:

a) Mannitol Salt Agar (MSA)

Purpose: Isolate Staphylococcus aureus from other staphylococci.

Ingredients:

i. Nutrient agar: energy source
ii. Mannitol: fermentable sugar
iii. Phenol red: pH indicator

Principle: S. aureus ferments mannitol, producing acid, which lowers pH and changes medium color from red to yellow.

b) Lowenstein-Jensen (LJ) Medium

Purpose: Isolation of Mycobacterium tuberculosis.

Type: Semisolid slant medium (butt and slope for increased surface area)

Ingredients:

i. Fresh hen's egg: protein source
ii. Salt glycerol: inhibits M. bovis
iii. Malachite green: suppresses unwanted bacteria

Incubation: 37°C for 6–8 weeks; lid loosened every 4th day for oxygen
Observation: Yellow colonies indicate M. tuberculosis growth

Note: M. leprae cannot be cultured artificially; diagnosis uses animal models and Ziehl-Neelsen staining

c) Potassium Tellurite Medium

Purpose: Isolate Corynebacterium diphtheriae (causes pharyngitis, endocarditis in children <5 years)

Ingredients: Chocolate medium + 0.04% potassium tellurite

Principle: Tellurite reduces to metallic tellurium; colonies appear black, often arranged in "Chinese letter" patterns on Gram stain

Other species: C. gravis, C. intermedius, C. mitis

5. Differential Media

Definition: Contain indicators to distinguish closely related microorganisms based on biochemical reactions.

Examples:

1. Blood agar: hemolysis patterns
2. MacConkey agar: lactose fermenters (pink colonies) vs non-fermenters (colorless)
3. EMB agar: metallic green colonies for E. coli

Uses: Differentiation of closely related species or strains based on observable characteristics.

Media	Type	Use
Nutrient Agar	Basic	Isolated colonies for identification
Blood Agar	Enriched & Differential	β-hemolysis seen as clear zones
MacConkey Agar	Selective + Differential	Pink vs colorless colonies
Selenite Broth	Enrichment	Enrich Salmonella before plating
Amie's Medium	Transport	Keeps specimen alive for later culture

Blood Agar

Blood Agar

Nutrient Agar

Macconkey Agar

LJ (Löwenstein-Jensen) medium

References

1. Levinson W. Review of Medical Microbiology and Immunology.
2. Prescott LM, Harley JP, Klein DA. Microbiology.
3. AFIP Manual of Laboratory Medicine

Student Task:

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BLOCK – E

INFLAMMATION, INFECTION & AUXILIARY DENTAL MATERIALS MODULE

Practical # 6

ACUTE INFLAMMATION – ACUTE APPENDICITIS

Aim: To study acute inflammation with reference to acute appendicitis and to identify histopathological features in tissue section.

Introduction:

Inflammation: A protective/ potentially harmful response of vascularized tissue involving host cells, blood vessels, proteins and other mediators that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues resulting from the original insult and to initiate process of repair. May lead to harmful consequences esp. chronic inflammation, - melodramatically referred to it as "the silent killer."

Types of Inflammation:

- Acute inflammation
- Chronic inflammation

Acute inflammation:

It is an immediate and rapid response of vascularized tissue to harmful stimuli. It usually begins within minutes of injury and lasts for a short duration, ranging from a few hours to a few days. The reaction is characterized by increased vascular permeability leading to exudation of fluid and plasma proteins, resulting in edema, along with migration of leukocytes, predominantly neutrophils (polymorphonuclear leukocytes), to the site of injury. The process is generally self-limited, causes minimal tissue damage, and forms an essential part of the innate immune response.

Causes of Acute Inflammation:

1. Mechanical trauma such as cutting and crushing
2. Chemical injury due to acids, alkalis and corrosive substances
3. Physical injury due to heat, cold, radiation and ultraviolet light
4. Infections caused by bacteria, viruses and parasites
5. Immune-mediated injury

Cardinal Signs of Acute Inflammation:

1. Rubor - Redness
2. Calor -Heat
3. Dolor- Pain
4. Tumor- Swelling

5. Functio Laesa - Loss of function

Acute Appendicitis

The vermiform appendix is a narrow, worm-like diverticulum arising from the caecum. It usually measures 2–20 cm in length and contains abundant lymphoid tissue in its wall, making it prone to inflammation.

Layers of Appendix: From outer to inner layer

1. Serosa
2. Muscularis externa- inner circular & outer longitudinal muscles
3. Submucosa - notable for lymphoid follicles
4. Mucosa

Pathogenesis:

Acute appendicitis commonly results from luminal obstruction by lymphoid hyperplasia, fecolith, parasites or foreign bodies. Obstruction leads to increased intraluminal pressure, vascular compromise, bacterial overgrowth and acute inflammation.

Morphology:

Gross Appearance: In early acute appendicitis, the appendix is swollen due to edema and congestion. The serosal surface appears dull, granular and erythematous. Subserosal blood vessels are congested. The normal glistening appearance of the mucosa is lost. In advanced cases, the appendix may show areas of hemorrhage, ulceration or greenish-black discoloration indicating gangrene.

Microscopic Appearance: Variable acute inflammation with predominance of neutrophils; involves some or all layers of the appendiceal wall

Early acute appendicitis:

- Mucosal erosions and scattered crypt abscesses
- Congestion of subserosal blood vessels
- Edema of the wall
- Perivascular infiltration by neutrophils
- Neutrophils present in mucosa, submucosa and muscularis

Acute suppurative appendicitis

- Dense infiltration of neutrophils throughout the wall
- Formation of focal abscesses

- Muscle fiber separation due to edema (muscle splitting)

Gangrenous appendicitis:

- Extensive hemorrhage
- Ulceration of mucosa
- Areas of coagulative necrosis
- Loss of normal tissue architecture

Diagnostic criterion: Presence of neutrophilic infiltration of the muscularis propria is essential for diagnosis of acute appendicitis.

Observe Under Microscope:

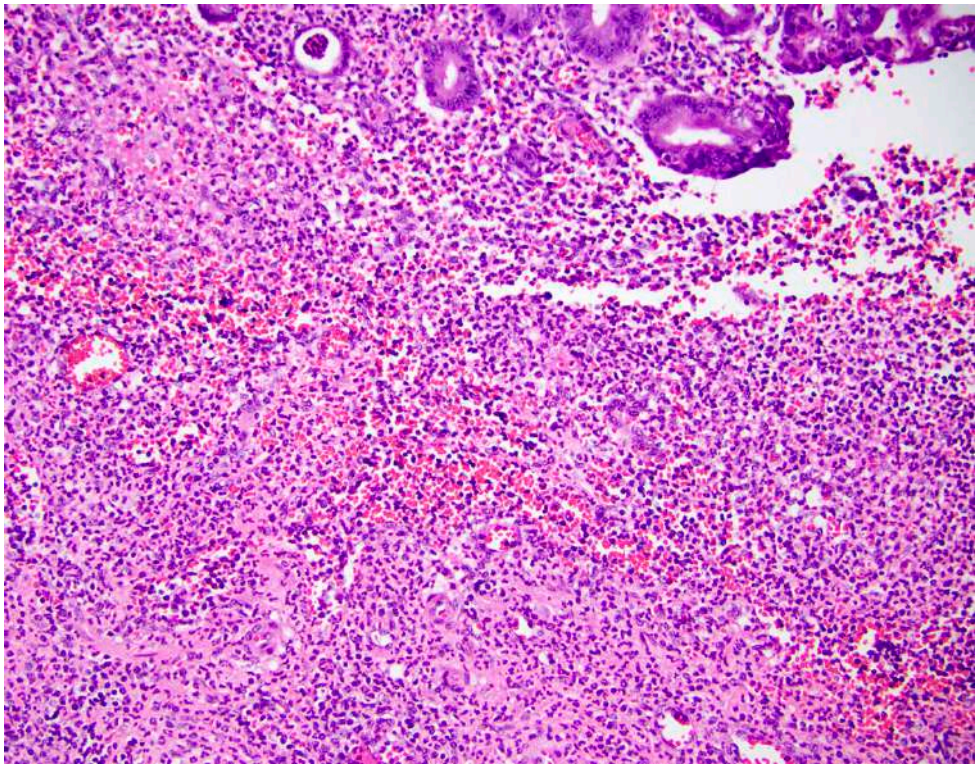
On low power examination, the appendiceal wall appears thickened with congestion and edema. On high power examination, numerous neutrophils are seen infiltrating mucosa, submucosa and muscularis layers. Muscle fibers appear separated due to edema. Mucosal ulceration may be present. In severe cases, areas of necrosis and hemorrhage are seen.

Points of Identification:

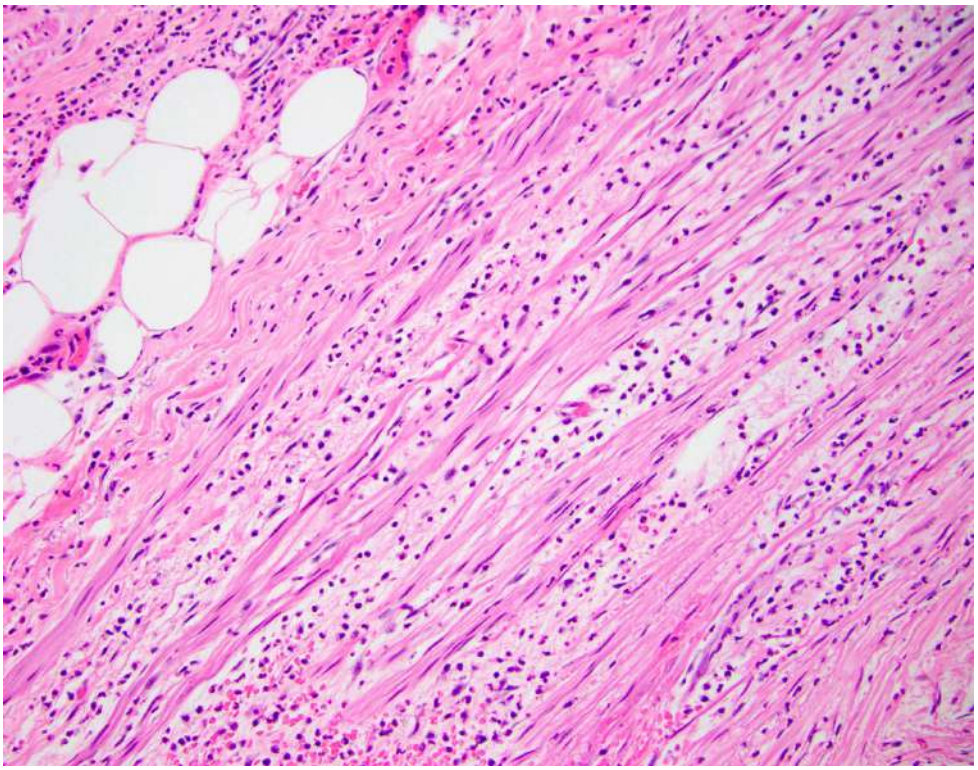
1. Neutrophilic infiltration of muscle layer; most important
2. Muscle splitting due to edema
3. Disruption or ulceration of mucosa
4. Congested blood vessels
5. Occasionally an obstructive element in lumen

References:

1. Robbins and Cotran, Basic Pathology
2. Harsh Mohan, Textbook of Pathology
3. Kumar, Abbas, Aster – Robbins Basic Pathology



Marked neutrophilic infiltration of appendiceal wall



Marked neutrophilic infiltration of appendiceal muscle layer

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 7

BACTERIAL MOTILITY

Aim:

To determine the motility of bacteria and differentiate between motile and non-motile organisms

Introduction:

Bacterial motility is an important phenotypic characteristic that contributes to colonization, invasion, biofilm formation, and pathogenicity. Motility is usually mediated by flagella, though other mechanisms such as axial filaments and gliding motility may also occur. Demonstration of motility is a valuable preliminary test in microbiological identification and aids in differentiating morphologically similar organisms.

Principle:

When live bacteria are suspended in a liquid medium and observed under a microscope, motile organisms exhibit true directional movement and change their position within the field. Non-motile organisms do not show active movement and may only demonstrate Brownian motion, which is a random, oscillatory movement caused by collision with water molecules and does not result in a change of position.

Common motile organisms:

1. Salmonella species
2. Escherichia coli
3. Vibrio cholerae
4. Proteus species
5. Listeria monocytogenes
6. Campylobacter species
7. Treponema pallidum
8. Leptospira
9. Borrelia species

Methods of demonstrating motility:

a. Hanging drop method
b. Semi-solid motility media such as SIM (Sulfide Indole Motility) medium and motility agar with TTC (Triphenyl Tetrazolium Chloride) indicator

This practical describes the hanging drop method.

Hanging Drop Method:

Requirements

1. Clean glass slide/ hollow-ground (concavity) slide
2. Cover slip
3. Normal saline or broth culture
4. Inoculating wire loop
5. Plasticine or petroleum jelly
6. Compound microscope
7. Fresh bacterial culture

Procedure:

1. Clean a glass slide thoroughly and place a circular ring of plasticine or petroleum jelly on it or use hollow-ground (concavity) slide
2. Using a sterile inoculating loop, prepare a light suspension of the bacterial culture in normal saline.
3. Place a small drop of this suspension in the center of a clean cover slip.
4. Invert the glass slide over the cover slip so that the plasticine ring surrounds the drop.
5. Gently press the slide to seal the edges.
6. Carefully invert the entire assembly so that the drop hangs freely from the cover slip.
7. Place the preparation on the microscope stage.
8. Focus initially using the 10× objective to locate the hanging drop.
9. Examine under the 40× objective, partially closing the condenser iris diaphragm to improve contrast.
10. Observe the movement of organisms, especially near the margins of the drop.

Results and interpretation:

a. Motile organisms show active, directional movement across the field with a definite change in position.
b. Non-motile organisms show no true movement and may exhibit only Brownian motion, characterized by a to-and-fro oscillation around a fixed point.

Precautions

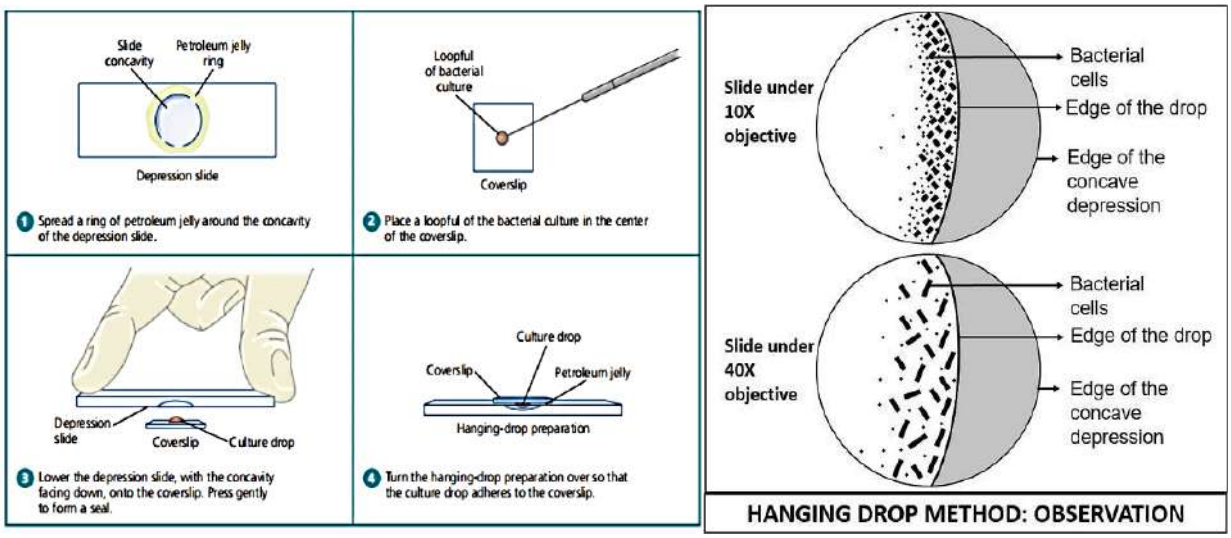
1. Use fresh bacterial cultures.
2. Ensure the drop does not touch the slide.
3. Avoid air bubbles in the preparation.
4. Adjust illumination properly for better contrast.

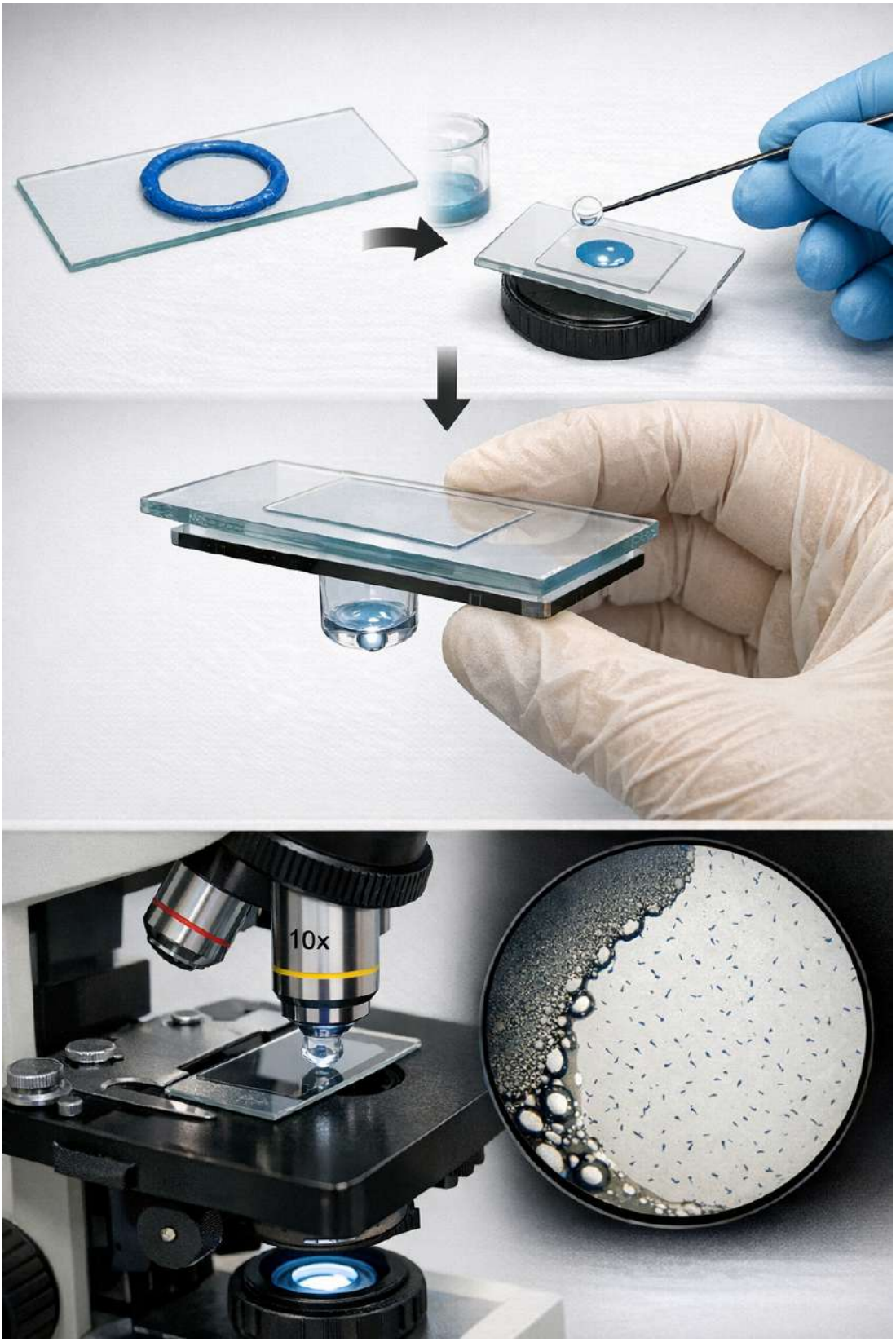
Limitations

1. Brownian motion may be confused with true motility.
2. Old or damaged cultures may show reduced or absent motility.
3. The hanging drop method carries a higher risk of aerosol exposure.
4. The method does not indicate the type or arrangement of flagella

Utility and clinical significance:

1. The motility test is useful in the preliminary identification of bacteria in clinical microbiology.
2. It helps differentiate closely related organisms such as Salmonella and Shigella.
3. Motility is often associated with increased virulence and invasiveness of pathogens.
4. Culture-based motility methods are preferred in routine diagnostic laboratories for safety and reliability, while microscopic methods are useful for rapid screening.





Step By Step Hanging Drop Method

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 8

BIOCHEMICAL TESTS

Introduction:

Biochemical tests are laboratory procedures used to identify and differentiate bacteria based on their metabolic activities and enzyme production. They help in diagnosis, treatment planning, and epidemiological studies. In routine diagnostic laboratories, biochemical tests form a bridge between culture characteristics and definitive bacterial identification.

Significance of Biochemical Tests:

- Help in identification and differentiation of bacteria
- Assist in confirmation of pathogenic organisms
- Useful in guiding appropriate antimicrobial therapy
- Important for academic learning and practical examinations

CATALASE TEST

Principle:

Some bacteria produce the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen. The release of oxygen in the form of bubbles indicates a positive reaction. This test is mainly used to differentiate staphylococci from streptococci.

Chemical reaction:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Hydrogen Peroxide — Water — Oxygen

Requirements:

- 3% hydrogen peroxide
- Clean glass slide or test tube
- Sterile wooden stick or glass rod
- Bacterial culture

Methods:

The test can be performed by slide method or tube method.

a. **Slide Method**
    - Place one drop of 3% hydrogen peroxide on a clean glass slide.
    - Using a sterile wooden stick or glass rod, pick a small amount of the test organism.
    - Mix the organism with hydrogen peroxide.
    - Observe for immediate effervescence.
b. **Tube Method**
    - Take a clean test tube and add 1 ml of 3 % hydrogen peroxide.
    - Using a sterile loop, add the test organism to the tube.
    - Observe for formation of oxygen bubbles.

Interpretation

- Immediate bubbling indicates catalase positive reaction.
- No bubble formation indicates catalase negative reaction.

Positive organisms

- Staphylococcus aureus
- Staphylococcus epidermidis
- Staphylococcus saprophyticus

Negative organisms

- Streptococcus species
- Enterococcus species

Controls:

- Positive control: Staphylococcus aureus
- Negative control: Streptococcus pyogenes

Catalase Test

1. Place a drop of 3% H_2O_2

2. Add bacterial colony using a sterile loop

3. H_2O_2 → H_2O, O_2

Interpretation

Staphyllococcus spp

POSITIVE

Coagulum forms

NEGATIVE

Staphylococcus spp

Staphyllococcus spp

NEGATIVE

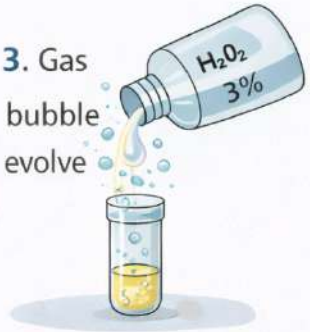
No bubbles ceem

Catalase Test (Tube Method)

1. Sterilize wire loop

2. Transfer the bacterial colony into the test tube containing 3% H_2O_2

3. Gas bubble evolve



Interpretation

TEST

Clotted plasma

Staphyllococcus spp

NEGATIVE

Unclotted plasma

Streptococcus spp

COAGULASE TEST

Principle:

Coagulase is an enzyme that converts fibrinogen in plasma into fibrin, resulting in clot formation. This test is used to differentiate Staphylococcus aureus from other staphylococci.

Types of Coagulase:

- Bound coagulase (clumping factor) detected by slide test
- Free coagulase detected by tube test

Requirements:

- Fresh human or rabbit plasma
- Clean glass slide
- Test tubes
- Bacterial culture

Methods:

- The test can be performed by slide method or tube method.

a. Slide Method

- Place a drop of normal saline on two ends of a clean slide.
- Emulsify the test organism in both drops.
- Add a drop of plasma to one suspension.
- Mix gently and observe for clumping within 5–10 seconds.
- The second drop serves as control for autoagglutination.

b. Tube Method

- Take 0.5 ml of diluted plasma (1:6) in a test tube.
- Add 4–5 drops of broth culture or bacterial colony suspension.
- Incubate at 35–37°C.
- Examine for clot formation at 1, 3, and 6 hours.

Interpretation

- Clot formation indicates coagulase positive.
- No clot formation indicates coagulase negative.

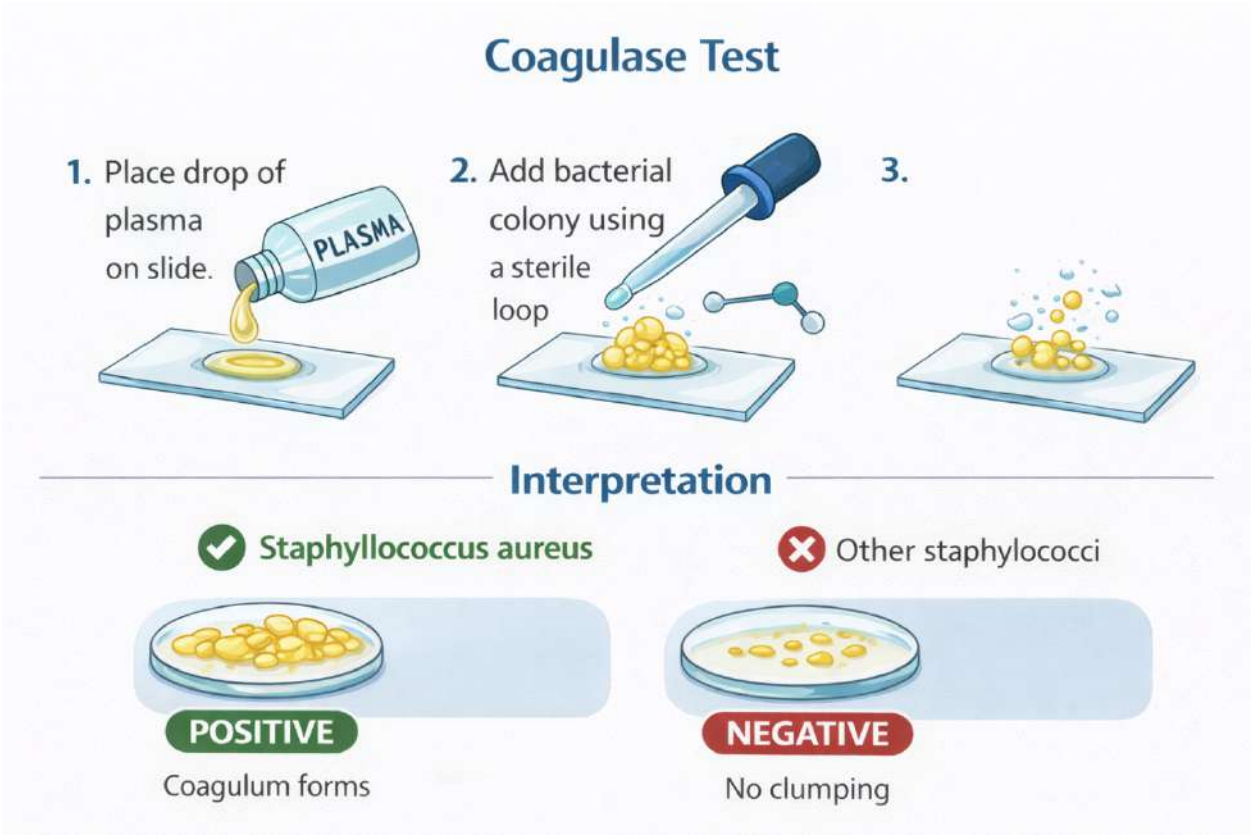
Positive organism

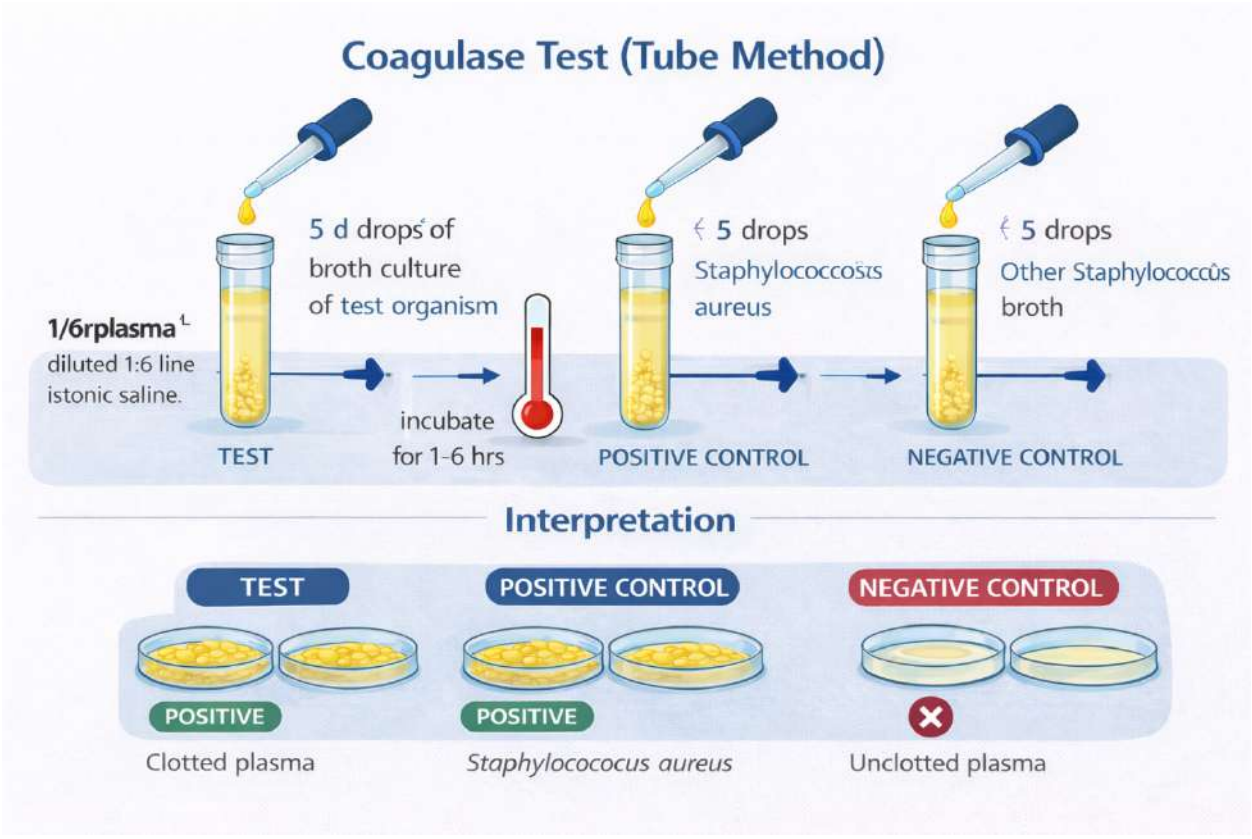
- Staphylococcus aureus

Negative organisms

- Staphylococcus epidermidis
- Staphylococcus saprophyticus

Note: *If slide test is negative, tube test must be performed before reporting the organism as coagulase negative.*





OXIDASE TEST

Principle:

The oxidase test detects the presence of cytochrome c oxidase enzyme in bacteria. Organisms producing this enzyme oxidize the reagent to produce a purple colored compound. This test helps in identification of organisms such as Pseudomonas, Neisseria, and Vibrio species.

Reagent:
Tetramethyl-p-phenylenediamine dihydrochloride [Freshly prepared 10 g/L solution of tetramethyl-p-phenylenediaminedihydrochloride (Sigma).]

Requirements:

- Oxidase reagent
- Filter paper or blotting paper
- Glass slide or petri dish
- Wooden stick or glass rod
- Bacterial culture

Procedure:

- Place a piece of filter paper on a clean slide or petri dish.
- Add 2–3 drops of oxidase reagent to the paper.
- Using a sterile wooden stick, pick a colony from culture plate.
- Smear the organism onto the reagent-soaked paper.
- Observe for color change within 5–10 seconds.

Interpretation:

- Development of blue or purple color indicates oxidase positive.
- No color change indicates oxidase negative.

Positive organisms:

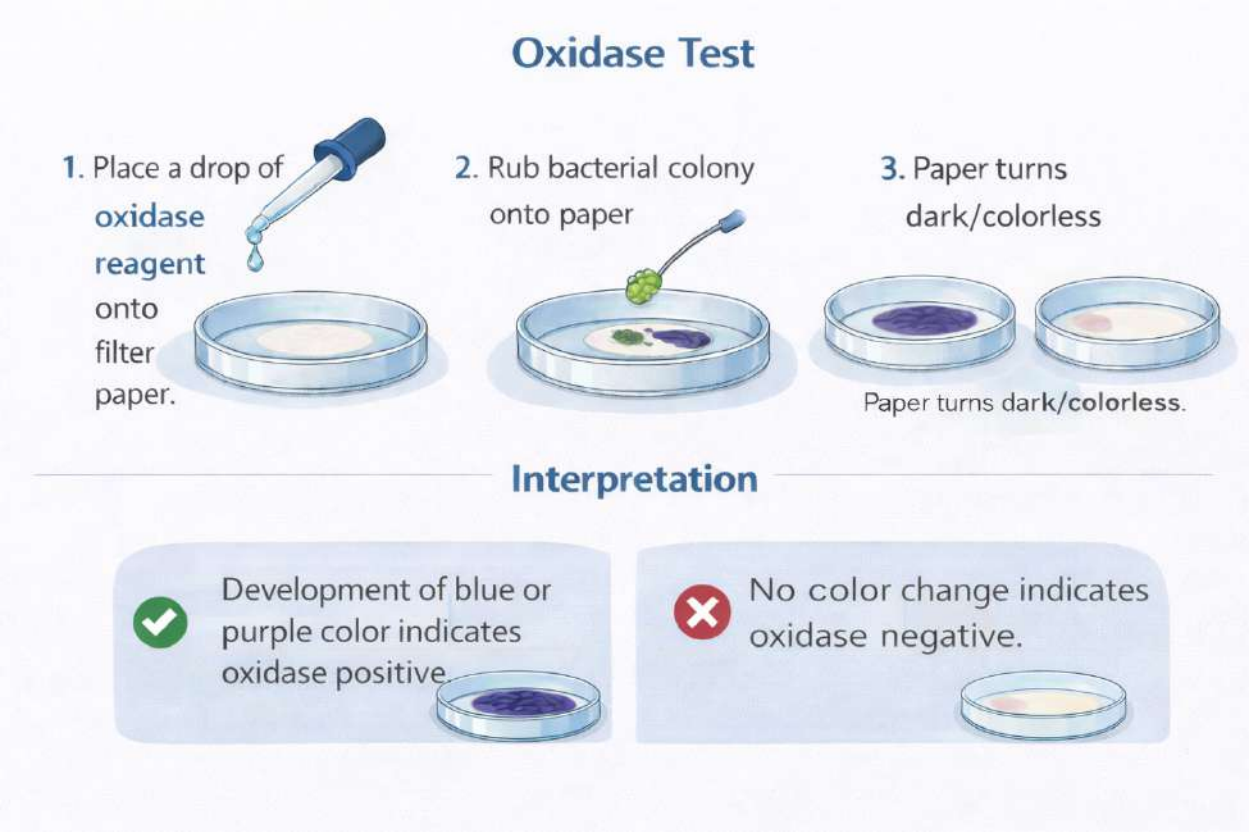
- Pseudomonas aeruginosa
- Neisseria gonorrhoeae
- Vibrio cholera
- Campylobacter jejuni

Negative organisms:

- Escherichia coli
- Klebsiella species
- Salmonella species

Controls:

- Positive control: Pseudomonas aeruginosa
- Negative control: Escherichia coli



Note:

- Use wooden stick instead of metal loop for oxidase test to avoid false positive results.
- Blood agar containing glucose may give false oxidase reactions.
- Tube catalase method is preferred when testing organisms from blood agar to avoid false positives due to red blood cells.

Biochemical tests such as catalase, coagulase, and oxidase are simple, rapid, and reliable methods for bacterial identification. Proper technique, correct interpretation, and use of controls are essential for accurate results. These tests are routinely asked in BDS practical examinations.

References

- Ananthanarayan and Paniker's Textbook of Microbiology, latest edition
- Levinson, Review of Medical Microbiology and Immunology, latest edition
- AFIP Manual of Laboratory Medicine
- CDC Laboratory Identification of Bacteria Guidelines

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 9

PLASMODIUM – LIFE CYCLE AND IDENTIFICATION IN BLOOD SMEAR

Aim: To study the life cycle of Plasmodium and to identify malaria parasite in peripheral blood smear.

Organism: Plasmodium species causing malaria in humans.

- Plasmodium vivax,
- Plasmodium falciparum,
- Plasmodium malariae,
- Plasmodium ovale,
- Plasmodium knowlesi

Hosts:

- Definitive host: Female Anopheles mosquito (sexual cycle occurs)
- Intermediate host: Human (asexual cycle occurs)

Life cycle

The life cycle of Plasmodium is completed in two hosts and consists of three main phases:

1. Pre-erythrocytic (hepatic) schizogony,
2. Erythrocytic schizogony and
3. Sexual cycle (sporogony)

1. Human phase (asexual cycle)

A. Infective stage:

The infective stage for humans is the sporozoite. When an infected female Anopheles mosquito bites a human, sporozoites present in its saliva are injected into the bloodstream.

B. Pre-erythrocytic (hepatic) schizogony:

The sporozoites rapidly enter liver cells (hepatocytes). Inside the liver cells, they grow and multiply asexually to form schizonts over a period of 5–16 days depending on the species. Each schizont contains thousands of merozoites.

In Plasmodium vivax and Plasmodium ovale, some parasites remain dormant in the liver as hypnozoites, which may reactivate weeks or months later causing relapse.

C. Erythrocytic schizogony:

Merozoites are released into the bloodstream and invade red blood cells. Inside red blood cells, the parasite passes through ring form, trophozoite and schizont stages. The infected red blood cells rupture, releasing new merozoites which infect fresh red blood cells. This cyclic rupture causes fever and clinical manifestations of malaria.

The duration of erythrocytic cycle varies from 24 to 72 hours depending on species.

D. Gametocyte formation:

Some merozoites do not continue asexual multiplication. Instead, they differentiate into sexual forms called gametocytes, which circulate in the peripheral blood.

2. Mosquito phase (sexual cycle)

A. Ingestion of gametocytes:

When a female Anopheles mosquito bites an infected human, it ingests red blood cells containing gametocytes.

B. Gametogenesis and fertilization:

In the mosquito stomach, red blood cells rupture releasing gametocytes. Male gametocytes form microgametes and female gametocytes form macrogametes. Fusion of male and female gametes produces a diploid zygote.

C. Ookinete and oocyst formation:

The zygote elongates to form a motile ookinete, which penetrates the mosquito midgut wall and develops into an oocyst beneath the outer lining.

D. Sporogony:

Within the oocyst, repeated division occurs producing thousands of sporozoites. After 8–15 days, the oocyst ruptures releasing sporozoites into the mosquito body cavity. These sporozoites migrate to the salivary glands of the mosquito.

Infective Stage:

- Humans: Sporozoite
- Mosquito: Gametocyte

Pathogenesis: Destruction of red blood cells during erythrocytic schizogony leads to anemia, fever, chills and splenomegaly. Plasmodium falciparum may cause severe complications such as cerebral malaria.

Laboratory Diagnosis:

1. Peripheral blood smear examination
2. Rapid diagnostic tests detecting malarial antigens

Prevention and Control: Early diagnosis and complete treatment Use of insecticide-treated bed nets Indoor residual spraying Elimination of mosquito breeding sites

Malaria

Malaria is a protozoal disease caused by parasites of the genus Plasmodium and transmitted to humans by the bite of an infected female Anopheles mosquito. The disease is characterized by fever with chills, anemia and splenomegaly. Severity depends on the Plasmodium species and host immunity.

Important Species of Plasmodium

1. *Plasmodium vivax:* Most common species in India. Causes benign tertian malaria. Infects young red blood cells. Schüffner's dots present. Can cause relapse due to hypnozoites.
2. *Plasmodium falciparum:* Most dangerous species. Causes malignant tertian malaria. Infects red blood cells of all ages. No relapse but causes severe complications such as cerebral malaria. Appliqué forms and banana-shaped gametocytes seen.
3. *Plasmodium malariae:* Causes quartan malaria. Infects older red blood cells. Band-shaped trophozoites seen.
4. *Plasmodium ovale:* Rare. Causes mild tertian malaria. Oval red blood cells with fimbriated edges. Schüffner's dots present.
5. *Plasmodium knowlesi:* Zoonotic malaria. Causes daily fever (quotidian malaria). Morphology resembles Plasmodium malariae.

Identification in Peripheral Blood Smear

Peripheral blood smear examination is the gold standard for laboratory diagnosis of malaria.

Two types of blood films are prepared: Thick and thin

Thin blood film:

Used for species identification. Red blood cells remain intact. Parasite morphology is clearly seen.

Observe under microscope: Under oil immersion objective, red blood cells containing intracellular parasites are seen.

Ring-shaped trophozoites are observed inside RBCs.

In Plasmodium vivax and Plasmodium ovale, RBCs appear enlarged and show fine reddish granules known as Schüffner's dots.

In Plasmodium falciparum, multiple delicate ring forms may be seen within a single RBC and characteristic banana-shaped gametocytes may be observed.

In Plasmodium malariae, band-shaped trophozoites are seen stretching across the RBC.

Thick blood film:

Used for detection of malaria parasites when parasitemia is low. Red blood cells are lysed during staining. Parasites appear concentrated in the smear. Species identification is difficult.

Observe under microscope:

On examination, red blood cells are not visible.

Darkly stained malaria parasites are seen scattered in the field.

Ring forms, trophozoites or gametocytes may be observed as dense structures against a pale background.

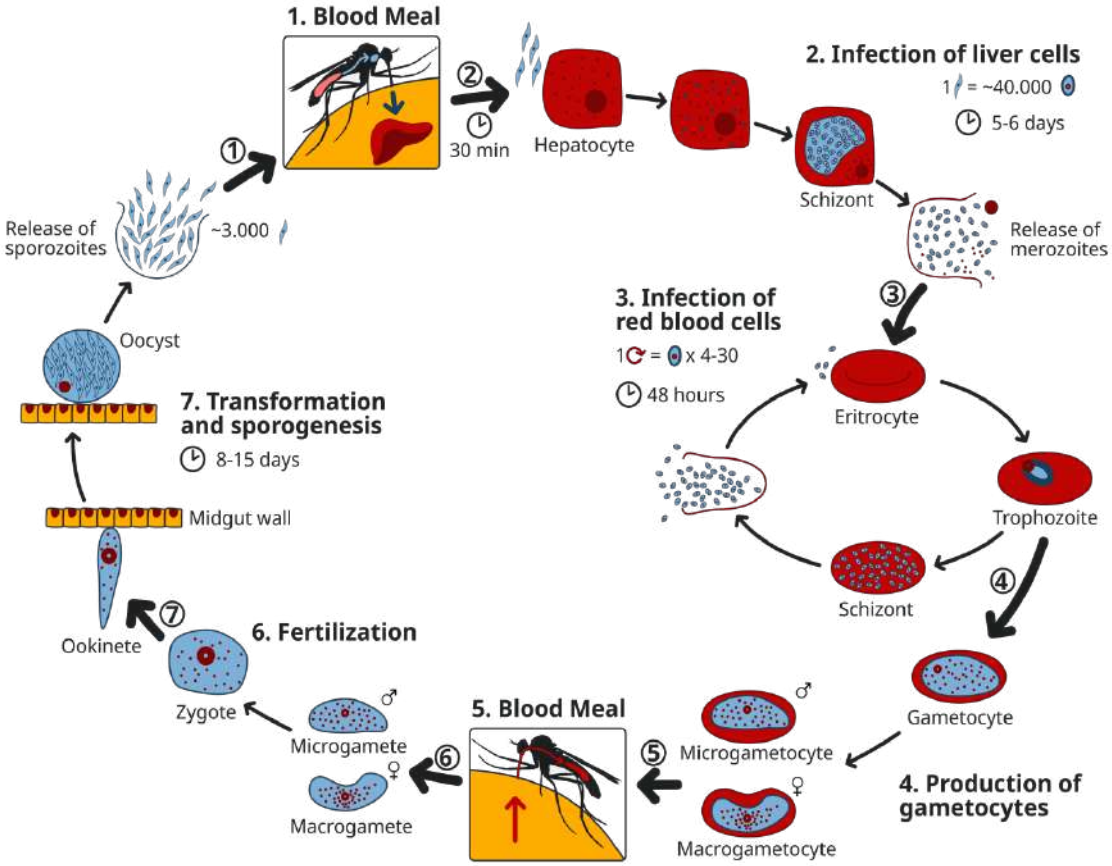
Thick film is mainly used to confirm presence of malaria parasite.

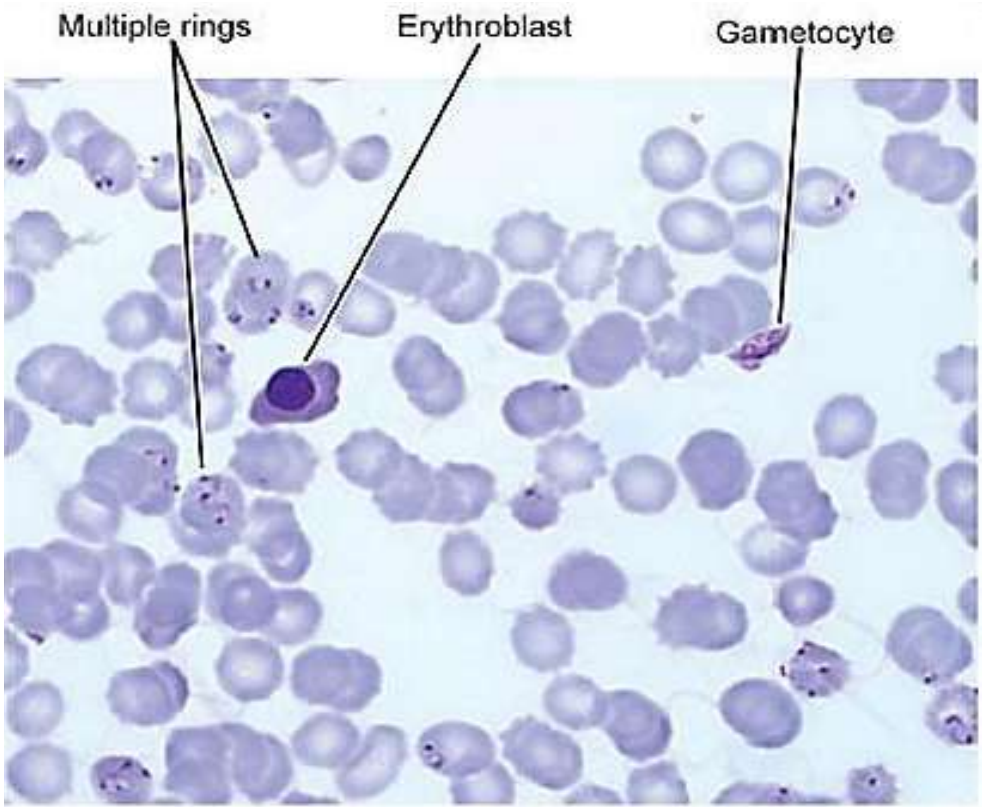
Practical Identification Points:

1. If ring forms are seen inside rbcs – malaria parasite present.
2. If multiple ring forms per RBC – suggest Plasmodium falciparum.
3. If enlarged rbcs with Schüffner's dots – Plasmodium vivax or Plasmodium ovale.
4. If banana-shaped gametocytes – Plasmodium falciparum.
5. If band-shaped trophozoites – Plasmodium malariae.

References

1. Levison W. Review of Medical Microbiology and Immunology. Mcgraw-Hill Education.
2. Ananthanarayan R, Paniker CKJ. Textbook of Microbiology. Universities Press.
3. World Health Organization. Malaria Fact Sheets and Guidelines.
4. Centers for Disease Control and Prevention. Malaria Biology and Life Cycle.
5. Text & Practical Manual of Pathology, Part 1


1. Blood Meal
2. Infection of liver cells
1 = ~40.000
5-6 days
30 min
Hepatocyte
Schizont
Release of merozoites
Release of sporozoites
~3.000
3. Infection of red blood cells
1 = x 4-30
48 hours
Eritrocyte
Trophozoite
Schizont
4. Production of gametocytes
Gametocyte
Microgametocyte
Macrogametocyte
5. Blood Meal
6. Fertilization
Microgamete
Macrogamete
Zygote
Ookinete
7. Transformation and sporogenesis
8-15 days
Midgut wall
Oocyst


Multiple rings
Erythroblast
Gametocyte

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 10

LEISHMANIA

1. Introduction

Leishmaniasis is a vector-borne parasitic disease caused by obligate intracellular protozoa of the genus Leishmania. The disease is transmitted to humans by the bite of an infected female sandfly (genus Phlebotomus in the Old World and Lutzomyia in the New World).

Approximately 21 species pathogenic to humans are known. Important species include:

- Leishmania donovani complex – L. donovani, L. infantum
- Leishmania tropica
- Leishmania mexicana
- Leishmania braziliensis

Reservoir: Domestic animals, especially dogs, act as important reservoir hosts in many regions. In India, humans are the main reservoir for L. donovani.

2. Morphological Forms

A. Amastigote (Leishman–Donovan body)

- Seen in human tissues
- Shape: Round or oval, 2–4 µm
- Non-flagellated
- Found inside macrophages
- Contains nucleus and kinetoplast
- This is the diagnostic stage

B. Promastigote

- Seen in sandfly and in culture (NNN medium)
- Elongated, spindle-shaped body
- Free anterior flagellum present
- This is the infective stage for humans

3. Life Cycle of Leishmania

A. Human Host

1. Infected sandfly injects promastigotes during blood meal (infective stage)
2. Promastigotes are phagocytosed by neutrophils at the bite site

3. Parasites are released and taken up by macrophages
4. Inside macrophages, promastigotes transform into amastigotes (diagnostic stage)
5. Amastigotes multiply by binary fission and infect other reticuloendothelial cells of liver, spleen, and bone marrow

B. Sandfly Host

1. Sandfly ingests infected macrophages during blood meal
2. Amastigotes are released in the midgut of sandfly
3. They transform into promastigotes
4. Promastigotes multiply and migrate to foregut and proboscis
5. Sandfly becomes infective and transmits parasite during next bite

4. Types of Leishmaniasis

A. Visceral Leishmaniasis (Kala-azar)

- Caused by Leishmania donovani
- Incubation period: 2–8 months
- Affected organs: spleen, liver, bone marrow

Clinical features:

- Prolonged fever
- Massive splenomegaly
- Hepatomegaly
- Anemia and weight loss
- Darkening of skin in Indian kala-azar

Prognosis:

- Fatal if untreated

B. Cutaneous Leishmaniasis

- Caused by Leishmania tropica and Leishmania mexicana
- Produces skin ulcers at the site of bite

Clinical features:

- Painless ulcer with raised margins
- Heals slowly leaving scar

C. Mucocutaneous Leishmaniasis

- Caused by Leishmania braziliensis
- Occurs months to years after healing of skin lesion
- Involves mucous membranes of nose, palate, and pharynx

Clinical importance:

- Destructive lesions
- Does not heal spontaneously
- Requires treatment

5. Epidemiology and Transmission

- Vector: Female sandfly (Phlebotomus and Lutzomyia)
- Reservoirs:
    - Dogs, rodents, small carnivores in Africa, Middle East, China
    - Humans in India

6. Pathogenesis

- Amastigotes multiply within macrophages
- Destruction of reticuloendothelial cells
- Involvement of liver, spleen, and bone marrow
- Leads to immunosuppression

7. Laboratory Diagnosis

A. Direct Microscopy

- Demonstration of amastigotes inside macrophages
- Specimens used:
    - Bone marrow aspirate
    - Splenic aspirate
    - Skin lesion smear

B. Culture

- NNN (Novy–MacNeal–Nicolle) medium

C. Serological Tests

- rK39 dipstick test
- ELISA

D. Skin Test

- Montenegro test indicating past exposure

8. Points of Identification of Leishmania under

- Numerous intracellular amastigotes seen within macrophages
- Each amastigote is small, round to oval in shape
- Presence of nucleus and rod-shaped kinetoplast
- Organisms are non-flagellated
- Background shows macrophages of bone marrow or tissue smear

9. Treatment

- Sodium stibogluconate
- Liposomal Amphotericin B is drug of choice for visceral leishmaniasis
- Miltefosine is an effective oral drug

10. Prevention and Control

- Protection from sandfly bites
- Use of insecticides and vector control
- Early detection and treatment of cases
- No effective vaccine available

11. Important Points

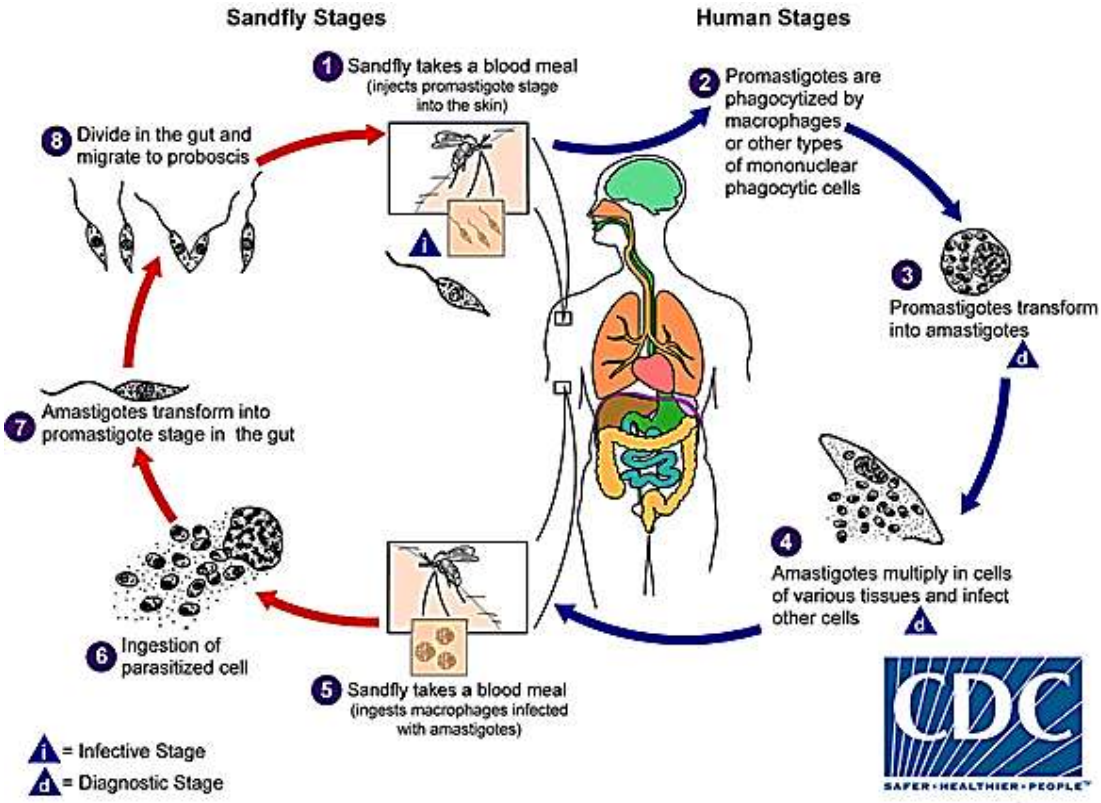
- Infective stage: Promastigote
- Diagnostic stage: Amastigote
- Vector: Female sandfly
- Disease caused by Leishmania donovani: Kala-azar
- Common practical slide: Bone marrow smear showing Leishman–Donovan bodies

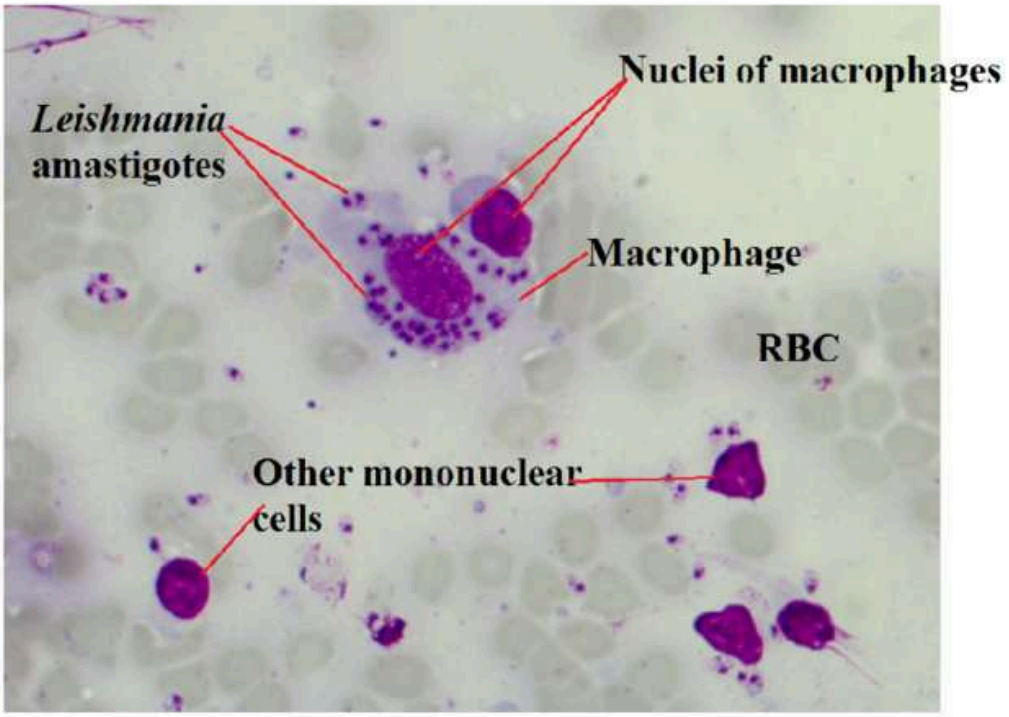
12. The **gold standard test** for diagnosis of leishmaniasis is:

- Demonstration of amastigotes (Leishman–Donovan bodies) in tissue aspirates by microscopy.

References

1. Ananthanarayan and Paniker's Textbook of Microbiology, latest edition
2. Levinson, Review of Medical Microbiology and Immunology, latest edition
3. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases
4. CDC – Leishmaniasis, Parasites and Health section
5. WHO Technical Report Series on Leishmaniasis


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal
(injects promastigote stage
into the skin)
2 Promastigotes are
phagocytized by
macrophages
or other types
of mononuclear
phagocytic cells
3 Promastigotes transform
into amastigotes
4 Amastigotes multiply in cells
of various tissues and infect
other cells
5 Sandfly takes a blood meal
(ingests macrophages infected
with amastigotes)
6 Ingestion of
parasitized cell
7 Amastigotes transform into
promastigote stage in the gut
8 Divide in the gut and
migrate to proboscis
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE


Nuclei of macrophages
Leishmania
amastigotes
Macrophage
RBC
Other mononuclear
cells

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 11

STERILIZATION AND DISINFECTION

Definitions

Sterilization: Complete killing of all microorganisms including bacterial spores and viruses on an object.

Disinfection: Killing most vegetative pathogenic microbes (does not reliably kill spores).

Antiseptics: Chemical agents safe for use on skin or mucous membranes (e.g., 70% alcohol, iodophors).

Classification of Methods

A. Physical Methods

1. Heat

- Dry heat: Flaming, red heat, hot air oven
- Moist heat: Boiling, pasteurization, autoclaving

2. Filtration

- Membrane filters (0.22 µm) remove microbes from fluids and air.
- Principle: Mechanical removal of microbes by porous filters (e.g., nitrocellulose 0.22 µm).
- Uses: Sterilizing heat-labile fluids (sera, IV solutions), air purification.
- Note: Does not remove toxins or very small viruses reliably.

3. Radiation

- Non-ionizing (UV): Low penetration; Surface sterilization, air cabinets; forms thymine dimers in DNA.
- Ionizing (X, gamma): Deep penetration; High-energy (gamma, X-rays) for bulk sterilization of disposables

Heat Sterilization (Most common in labs & clinics)

a) **<u>Dry Heat</u>**
   - Flaming / Red heat: Direct flame for needles, loops.
   - Hot Air Oven: 160 °C for 1–2 hours (glassware, oils, powders).
   - Mechanism: Oxidation & protein denaturation.

b) <u>Moist Heat</u>

- *Boiling (100 °C):* Kills vegetative forms but not spores.
- *Pasteurization:* Preserves food quality; used for milk/beverages
    - Batch: 62 °C for 30 min
    - Flash: 72 °C for 15 s
    - Ultra-pasteurization: 82 °C for 3 s
- *Autoclave (Standard):*
    - 121 °C, 15 psi, 15–20 min – kills spores reliably.
    - Higher cycles (e.g., 132–134 °C) shorten time.
    - Monitoring: mechanical, chemical, biological (spore) indicators recommended weekly.
- *Tyndallization/ fractional sterilization:* Intermittent steaming over 3 days for heat-sensitive media.

B. Chemical Methods

Ideal disinfectants/storylands should be: Broad spectrum, non-toxic, stable, not inactivated by organic matter.

A) Cell Membrane Damage
    - Alcohols: 70% ethanol/isopropyl – skin antiseptic.
    - Detergents: Benzalkonium chloride – surface antiseptic.
    - Phenolics: Lysol, chloroxylenol, chlorhexidine.

B) Protein & Enzyme Inactivation
    - Halogens: Iodine, povidone-iodine – skin prep.
    - Chlorine compounds: Hypochlorite (bleach).
    - Heavy metals: Silver nitrate/silver sulphadiazine.
    - Oxidizers: Hydrogen peroxide, potassium permanganate.
    - Aldehydes: Formaldehyde, glutaraldehyde (sporicidal).

C) Gaseous Sterilants
    - Ethylene oxide: For heat-sensitive equipment (plastics).
    - Formaldehyde gas: Irritant; occasional use for environments.

Object	Preferred Method
Surgical instruments	Autoclave
Rubber/ plastic catheters	Boiling / chemical sterilant
Heat-sensitive scopes	High-level disinfection / chemicals
Plastic syringes (disposable)	Gamma / ethylene oxide
Glassware, oils	Hot air oven
Skin prior to surgery	Alcohol + iodophor
Culture media	Autoclave / Tyndallization
Air	Filtration

Monitoring of Sterilization

Best practice (Centers for Disease Control and Prevention/ American Dental Association). (CDC/ADA):

- Use biological indicators (spore tests) regularly to confirm sterilization efficacy.
- Use chemical indicators inside every package to ensure exposure to sterilant conditions.
- Record mechanical parameters (temperature, time, pressure)

Cleaning & Disinfection Principles (CDC)

- Cleaning always precedes disinfection/sterilization.
- Remove visible soil to improve effectiveness.
- Critical items (penetrate sterile tissue) → sterilize.
- Semicritical (contact mucosa) → sterilize or high-level disinfect.
- Non-critical (contact intact skin) → surface disinfection

Equipment: AUTOCLAVE

Aim: To sterilize instruments, dressings, culture media and other materials using steam under pressure**.**

Principle:

Autoclave works on the principle of moist heat sterilization using saturated steam under pressure. When pressure is increased, the boiling point of water rises above 100°C. Steam at high temperature penetrates materials and coagulates and denatures proteins of microorganisms, including bacterial spores, leading to their death.

Temperature and Pressure Settings:

- Standard autoclave cycle: Temperature: 121°C Pressure: 15 lb/in² (15 psi) Time: 15–20 minutes
- Other cycles: 126°C for 10 minutes 132–134°C for short-duration sterilization of certain items

Parts of Autoclave:

1. Strong metallic chamber with lid
2. Pressure gauge
3. Temperature gauge
4. Safety valve
5. Steam release valve
6. Heating element
7. Water reservoir
8. Perforated tray or basket

Procedure:

1. Water is added to the autoclave chamber up to the required level.
2. Articles to be sterilized are wrapped or placed in perforated trays.
3. Lid is closed tightly to make the chamber air-tight.
4. Heating is started and steam is generated.
5. Air inside the chamber is expelled through the steam outlet.
6. Pressure and temperature rise to desired levels.
7. Articles are exposed to steam at 121°C under 15 psi pressure for 15–20 minutes.
8. After completion, heating is stopped and pressure is allowed to return to normal.
9. Lid is opened only after complete pressure release.

Characteristics of Effective Steam:

a. Steam should be saturated and free from air
b. Temperature should be near condensation point
c. No suspended water droplets

Items Sterilized by Autoclave:

- Culture media
- Surgical instruments
- Dressings, gauze and linen
- Rubber goods and gloves (like silicone/neoprene)
- Glassware (except heat-sensitive)

- Intravenous fluids

Items Not Suitable for Autoclaving:

- Oils and fats
- Powders
- Heat-sensitive plastics
- Sharp instruments prone to corrosion

Indicators Used in Autoclave:

1. Chemical indicators: Brown's tube: Colour change from red to green indicates proper temperature exposure Bowie–Dick test: Indicates adequate steam penetration and air removal
2. Biological indicators: Spores of Bacillus stearothermophilus Absence of growth after incubation indicates effective sterilization

Advantages:

a. Reliable and effective method of sterilization
b. Kills spores and all microorganisms
c. Non-toxic
d. Economical

Disadvantages:

a. Not suitable for heat-sensitive materials
b. Can corrode metal instruments if not dried properly
c. Requires proper monitoring

Precautions:

1. Do not overload the autoclave
2. Ensure complete removal of air
3. Follow recommended temperature and time
4. Allow pressure to return to zero before opening

Utility in Hospital and Laboratory

1. Routine sterilization in microbiology laboratories
2. Sterilization of surgical supplies
3. Preparation of sterile culture media
4. Infection control in hospitals

References:

1. Levison W. Review of Medical Microbiology and Immunology. McGraw-Hill Education.
2. Ananthanarayan R, Paniker CKJ. Textbook of Microbiology.
3. CDC Guidelines for Disinfection and Sterilization in Healthcare Facilities.
4. Text & Practical Manual of Pathology, Part 1.

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 12

ZIEHL NEELSEN STAINING (ZN STAINING)

Introduction:

Ziehl Neelsen staining is a differential staining technique used for demonstrating acid fast bacilli, mainly Mycobacterium species. It was developed by Ziehl and Neelsen between 1881 and 1883. This stain is routinely used for the diagnosis of tuberculosis, leprosy, and other infections caused by acid fast organisms.

Mycobacteria are aerobic, non-motile, rod-shaped bacteria measuring about 0.5–10 micrometers in length. They contain large amounts of complex lipids, waxes, and mycolic acid in their cell wall, which makes them resistant to decolorization by acid-alcohol. In stained smears, the bacilli are often seen lying parallel to each other.

ZN staining is a standard method for demonstrating acid fast bacilli in clinical specimens such as sputum, pus, tissue sections, and body fluids.

Organisms Demonstrated by ZN Stain

1. Mycobacterium tuberculosis
2. Mycobacterium leprae
3. Mycobacterium ulcerans
4. Nocardia species (weakly acid fast)
5. Legionella species (weakly acid fast)

Principle:

When mycobacteria are treated with a strong basic dye such as carbol fuchsin containing phenol, the dye penetrates the lipid-rich cell wall. Once stained, these organisms resist decolorization by acid or acid-alcohol. Hence, they are called acid fast bacilli. Non–acid fast organisms lose the primary stain during decolorization and take up the counterstain, providing a contrasting background.

Acid Fast Bacilli Acid fast bacilli are organisms that retain carbol fuchsin even after treatment with acid decolorizing agents. The red color of the bacilli is due to the primary stain, while the background color is provided by the counterstain.

Reagents:

1. Primary stain: Carbol fuchsin
    a. Basic fuchsin (dye)

    b. Phenol or carbolic acid (mordant)
2. Decolorizer: Acid alcohol
    a. Sulphuric acid 20 percent
    b. Ethyl alcohol 95 percent
3. Counterstain:
    a. Methylene blue or malachite green

Requirements

1. Microscope
2. Clean glass slides
3. Spirit lamp
4. Slide rack
5. Clinical specimen
6. Staining reagents

Specimens

1. Sputum
2. Pus
3. Tissue sections
4. Body fluids
5. Skin smears (for leprosy)

Procedure

1. Prepare a thin smear on a clean glass slide, air dry, and fix by gently passing through flame.
2. Flood the smear with carbol fuchsin and heat until steam rises. Do not boil or allow the stain to dry. Keep the stain on the slide for about 5 minutes, reheating intermittently.
3. Rinse the slide gently with water.
4. Decolorize with acid alcohol for 2–5 minutes or until the smear appears pale pink.
5. Wash with water.
6. Apply counterstain methylene blue for 1–2 minutes.
7. Wash with water, air dry, and examine under microscope.

Microscopic Examination

- First examine the smear under low power to assess distribution.
- Then examine under oil immersion objective for acid fast bacilli.

Result:

- Acid fast bacilli appear bright red or pink
- Background appears blue or green depending on counterstain used

Grading of AFB in Smear:

- More than 100 bacilli per field: +++
- 10–100 bacilli per field: ++
- 1–10 bacilli per field: +
- 1–9 bacilli per entire smear: scanty (numbering done)
- No bacilli seen: negative

Identification under Microscope:

- Slender, red-colored rod-shaped bacilli
- Seen against blue or green background
- Bacilli may appear singly or in parallel groups

Modifications of Ziehl Neelsen Staining:

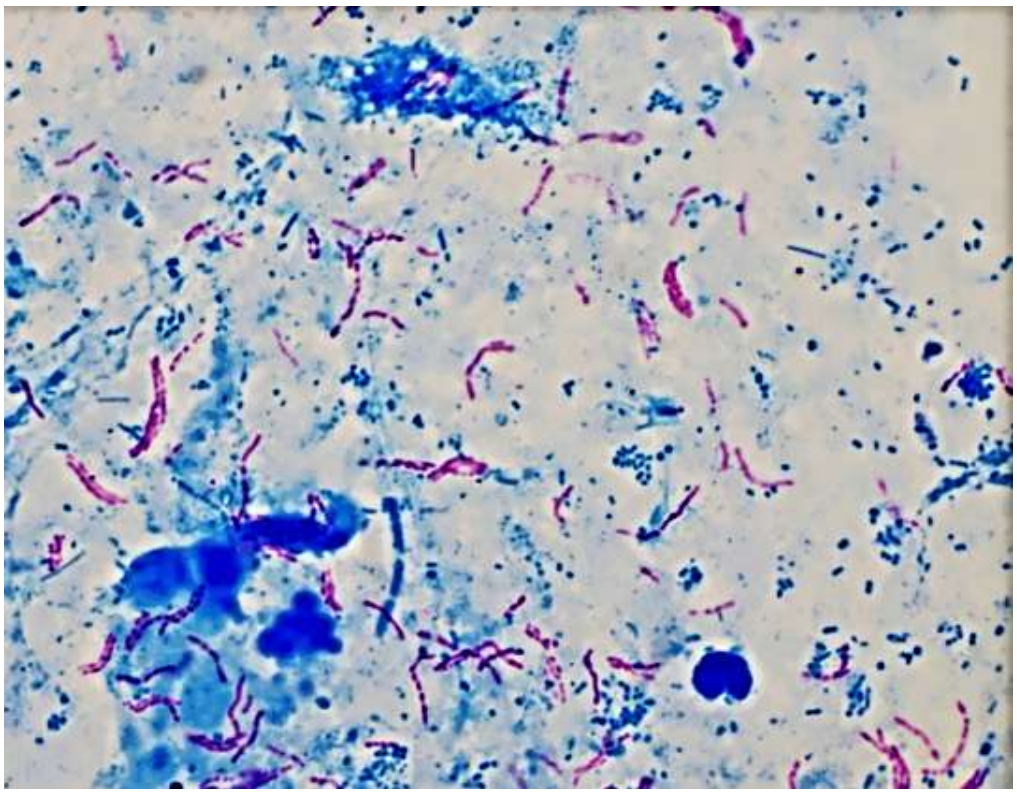
- For Mycobacterium leprae: decolorization with 5 percent sulphuric acid
- For Nocardia and Legionella in tissue sections: decolorization with 1 percent sulphuric acid

Precautions:

- Do not allow carbol fuchsin to boil or dry on slide
- Adequate heating is necessary for proper staining
- Proper decolorization is essential to avoid false results

References

4. Levinson W. Review of Medical Microbiology and Immunology.
5. Prescott LM, Harley JP, Klein DA. Microbiology.
6. AFIP Manual of Laboratory Medicine



Beaded Acid Fast Bacilli

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 13

CHRONIC CHOLECYSTITIS

Introduction

Chronic inflammation:

Chronic inflammation is inflammation of prolonged duration, lasting for weeks to months, in which active inflammation, tissue injury, and healing occur simultaneously. It may follow acute inflammation or arise insidiously without a preceding acute phase.

Causes of chronic inflammation:

- Persistent infections by microorganisms
- Immune-mediated diseases such as autoimmune disorders
- Prolonged exposure to toxic agents
- Recurrent episodes of acute inflammation

General features of chronic inflammation:

- Infiltration with mononuclear cells such as lymphocytes, plasma cells, and macrophages
- Tissue destruction caused by persistent inflammatory cells
- Attempts at healing by fibrosis and angiogenesis

Chronic inflammatory cells

- *Macrophages:* Derived from blood monocytes. They perform phagocytosis, antigen presentation, cytokine secretion, and play a major role in tissue destruction and repair.
- *Lymphocytes:* Include B lymphocytes and T lymphocytes. B cells produce antibodies via plasma cells, while T cells mediate cellular immunity and regulate immune responses.
- *Plasma cells:* Derived from B lymphocytes and responsible for antibody production.
- *Eosinophils:* Seen in allergic conditions, parasitic infections, and some malignancies.
- *Fibroblasts:* Responsible for collagen production and fibrosis during healing.
- *Giant cells:* Formed by fusion of macrophages when phagocytosis is ineffective. Types include Langhans giant cells, foreign body giant cells, and Touton giant cells.

Effects of chronic inflammation:

- Fibrosis leading to scarring and loss of normal organ architecture
- Organ dysfunction due to replacement of parenchyma by fibrous tissue
- Persistent immune activation as seen in autoimmune diseases

CHRONIC CHOLECYSTITIS:

It is a classic example of chronic inflammation where persistent injury leads to fibrosis, architectural distortion, and loss of normal function of gall bladder. Histopathological examination using H&E stain is essential for diagnosis and identification of characteristic features such as chronic inflammatory infiltrate and Rokitansky–Aschoff sinuses.

Etiology:

- Persistence or recurrence of acute cholecystitis
- Chronic irritation by gallstones
- Recurrent mechanical trauma to gallbladder mucosa
- Bacterial infection, commonly by intestinal organisms such as Escherichia coli

Morphology:

Gross pathology:

- The gallbladder is usually contracted.
- The serosa is smooth and glistening or may appear dull due to fibrosis.
- The wall is thickened and firm with a grey-white appearance.
- The lumen often contains clear or greenish mucoid bile with gallstones.

Microscopic features (H&E stain)

- Gallbladder wall shows chronic inflammatory cell infiltration predominantly lymphocytes, plasma cells, and macrophages
- Mucosa may be flattened or focally ulcerated
- Marked fibrosis of the wall
- Presence of Rokitansky–Aschoff sinuses, which are outpouchings of mucosal epithelium extending into the muscular layer
- Muscular hypertrophy may be present

Point of identification of H&E slide:

- Thickened gallbladder wall
- Infiltration by lymphocytes and plasma cells
- Fibrosis of the muscular layer
- Rokitansky–Aschoff sinuses lined by columnar epithelium
- Absence of neutrophil-rich acute inflammatory infiltrate

Complications of chronic Cholecystitis:

- Porcelain gallbladder due to extensive calcification
- Gallbladder empyema
- Perforation of gallbladder
- Cholecystoenteric fistula
- Increased risk of gallbladder carcinoma
- Obstructive jaundice due to stone migration

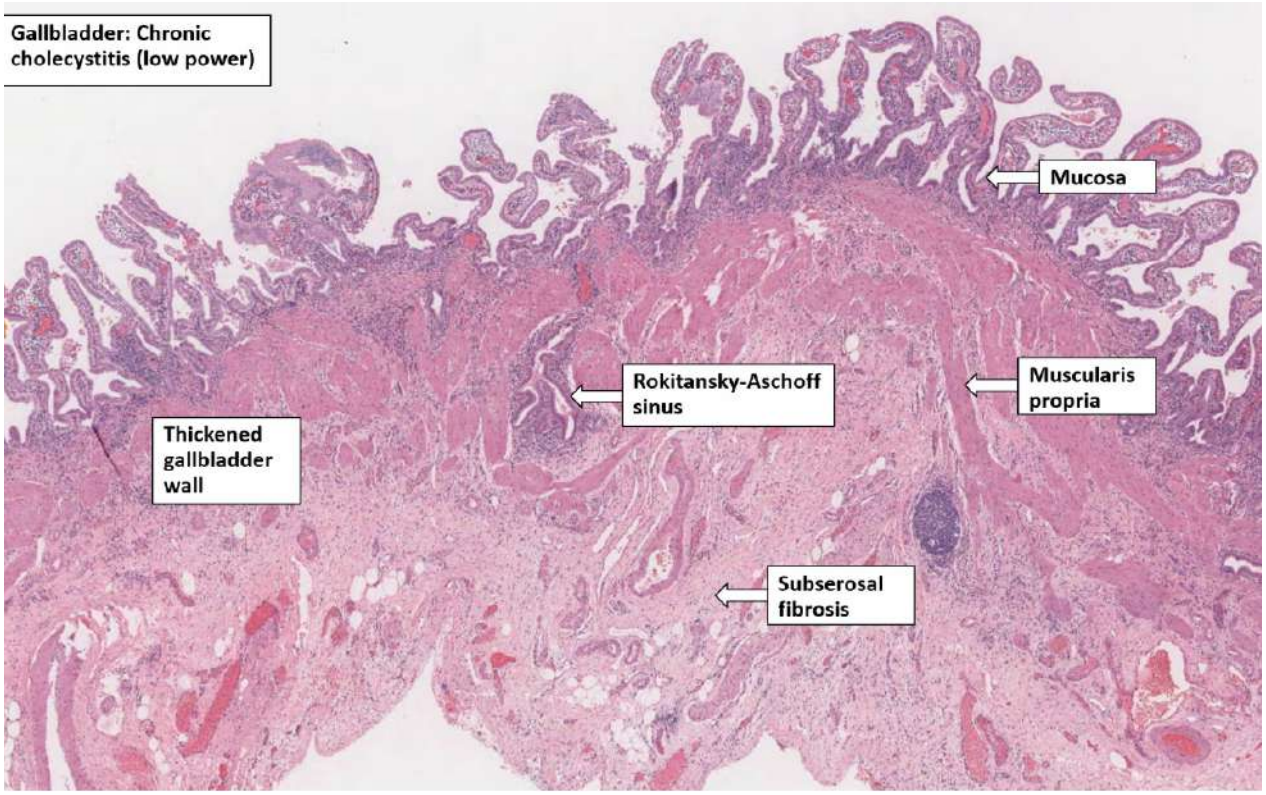
Clinical significance:

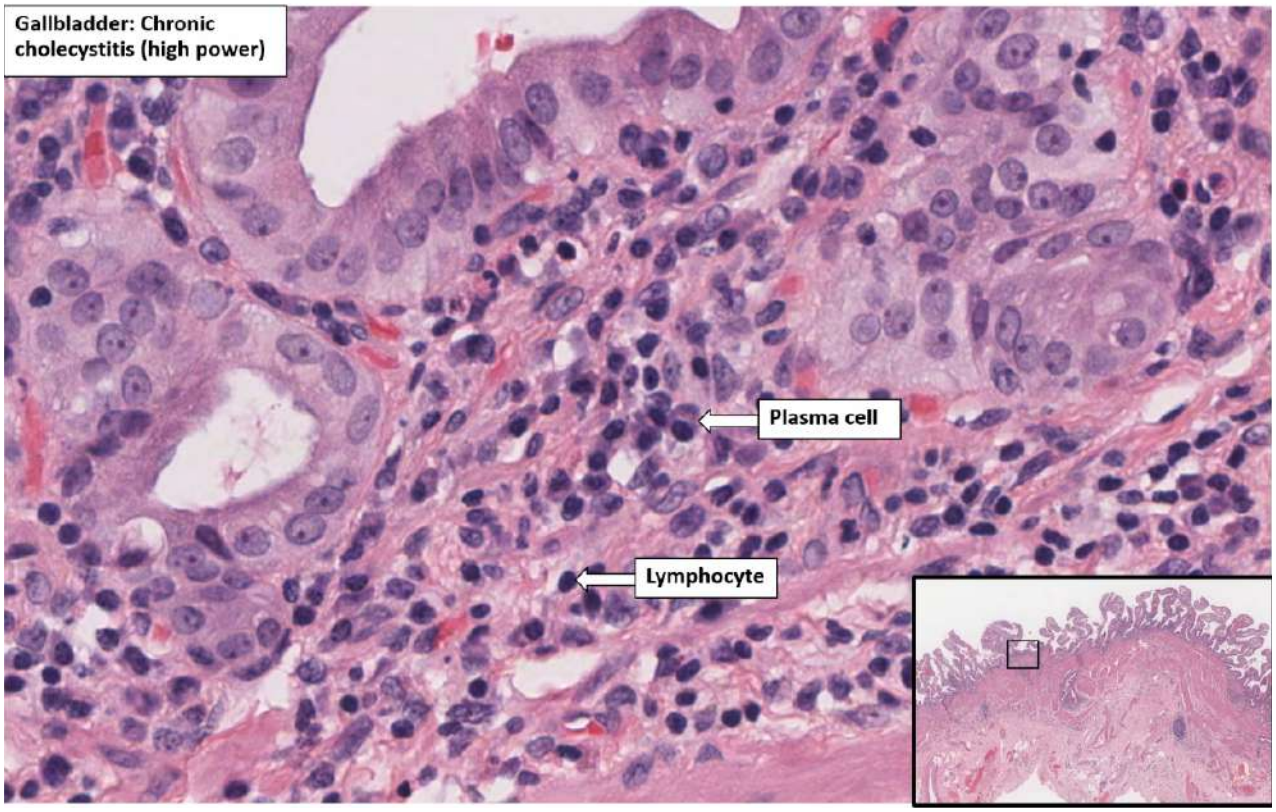
Chronic cholecystitis is a common cause of recurrent right upper quadrant abdominal pain. Long-standing disease may lead to serious complications and is an important risk factor for gallbladder carcinoma.

Chronic cholecystitis represents a classic example of chronic inflammation where persistent injury leads to fibrosis, architectural distortion, and loss of normal function. Histopathological examination using H&E stain is essential for diagnosis and identification of characteristic features such as chronic inflammatory infiltrate and Rokitansky–Aschoff sinuses.

References:

1. Robbins and Cotran, Basic Pathology
2. Harsh Mohan, Textbook of Pathology
3. Kumar, Abbas, Aster – Robbins Basic Pathology


Gallbladder: Chronic cholecystitis (low power)
Mucosa
Rokitansky-Aschoff sinus
Muscularis propria
Thickened gallbladder wall
Subserosal fibrosis


Gallbladder: Chronic cholecystitis (high power)
Plasma cell
Lymphocyte

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 14

GRANULOMA

Introduction

Granuloma is a special form of chronic inflammation characterized by the formation of a localized collection of activated macrophages called epithelioid cells, usually surrounded by lymphocytes and fibroblasts. Granuloma formation occurs when the body attempts to contain and isolate a persistent agent that is difficult to eradicate. Tuberculosis is the most common cause of granulomatous inflammation encountered in routine pathology practice.

Definition of granuloma:

A granuloma is a circumscribed microscopic collection of epithelioid macrophages, often associated with multinucleated giant cells and surrounded by a rim of lymphocytes and fibroblasts.

Types of granuloma

1. Immune granuloma
    - Formed in response to persistent antigenic stimuli
    - Example: tuberculosis, leprosy, fungal infections
    - Often shows central caseous necrosis
2. Foreign body granuloma
    - Formed in response to non-antigenic material
    - Example: sutures, silica, talc
    - Giant cells are prominent and necrosis is usually absent

Tuberculosis as the most common cause:

Tuberculosis is a chronic specific granulomatous inflammation caused by Mycobacterium tuberculosis. The granuloma formed in tuberculosis is called a tubercle. It typically shows central caseous necrosis surrounded by epithelioid cells, Langhans giant cells, lymphocytes, and fibrosis depending on the stage.

Etiology Granulomatous Inflammation:

Category	Examples
Bacterial	Mycobacterium tuberculosis, Mycobacterium leprae, Treponema pallidum
Fungal	Histoplasma capsulatum, Cryptococcus neoformans, Coccidioides immitis
Parasitic	Schistosoma species
Foreign body	Silica, asbestos, sutures, talc
Immune mediated	Sarcoidosis, Crohn's disease
Others	Wegener granulomatosis, berylliosis

Pathogenesis:

Persistent injurious agent
(e.g. Mycobacterium tuberculosis, foreign body)
↓
Agent resists destruction by host immune response
↓
Chronic inflammation with recruitment of macrophages and T lymphocytes
↓
Activation of CD4+ Th1 cells by IL-12 from macrophages and dendritic cells
↓
Secretion of IFN-γ and TNF-α by Th1 cells and NK cells
↓
IFN-γ mediated activation of macrophages (M1 type)
↓
Activated macrophages show increased microbicidal activity and antigen presentation
↓
Macrophages transform into epithelioid cells
↓
Fusion of epithelioid cells to form multinucleated giant cells
↓
Formation of granuloma with central epithelioid cells and giant cells surrounded by lymphocytes.

Cell Types in Granuloma:

1) Epithelioid cells: Modified macrophages with elongated pale nuclei and eosinophilic cytoplasm
2) Giant cells: Formed by fusion of macrophages

    Types of giant cells

    1. Langhans giant cells
        - Nuclei arranged peripherally in a horseshoe or ring pattern
        - Seen commonly in tuberculosis
    2. Foreign body giant cells
        - Nuclei scattered irregularly throughout cytoplasm
        - Seen in foreign body granulomas
3) Lymphocytes: Form a rim around the granuloma
4) Fibroblasts: Seen in healing stages causing fibrosis

Microscopic features of granuloma (H&E stain)

- Central area may show caseous necrosis, especially in tuberculosis
- Surrounding zone of epithelioid cells
- Presence of multinucleated giant cells
- Peripheral rim of lymphocytes
- Fibrosis may be present in older lesions

Point of identification of granuloma on H&E slide

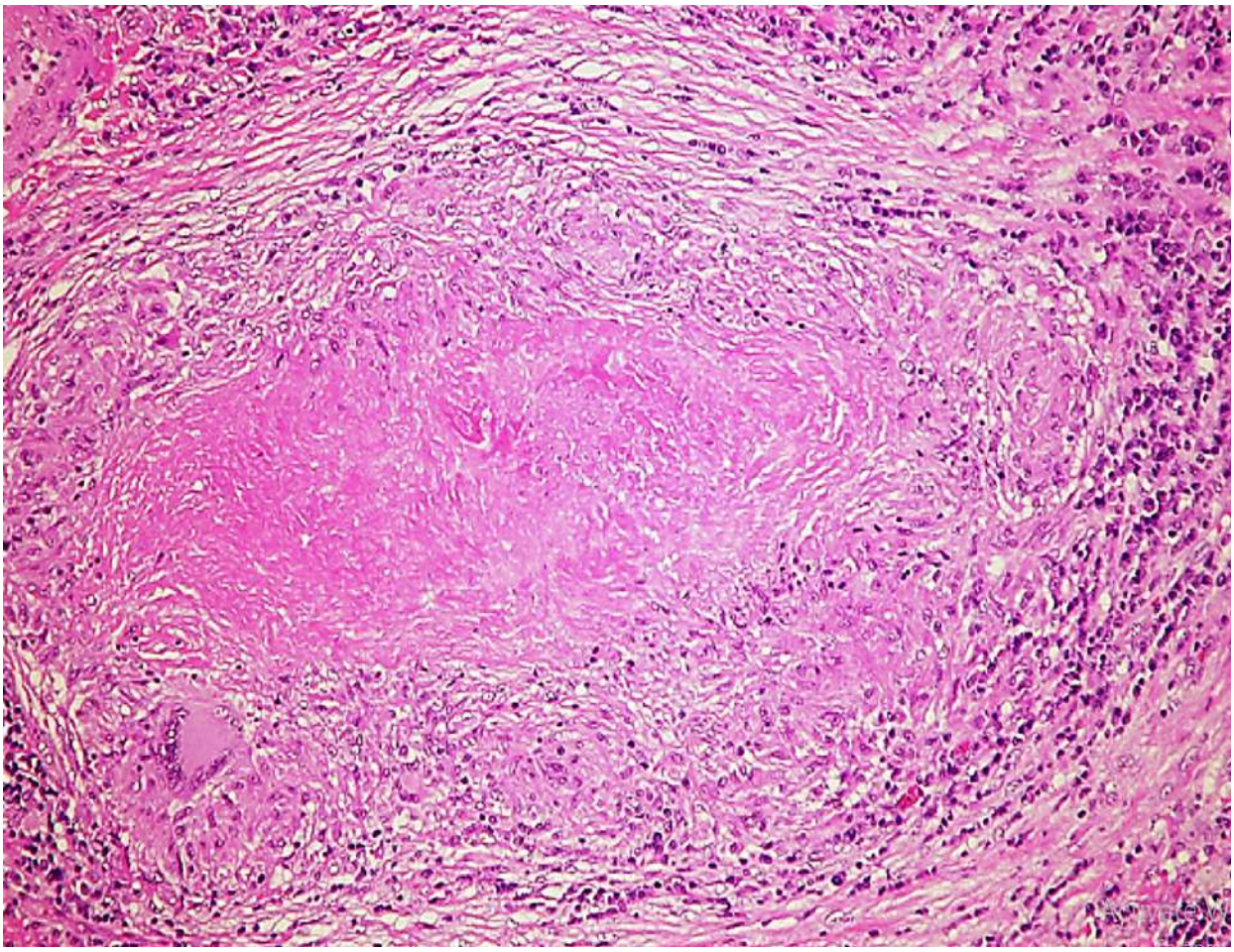
- Well-defined circumscribed lesion
- Aggregates of epithelioid macrophages
- Presence of multinucleated giant cells
- Peripheral rim of lymphocytes
- Central caseous necrosis may or may not be present
- Features consistent with chronic granulomatous inflammation

Note: Histopathological features are suggestive of granulomatous inflammation, most commonly due to tuberculosis in endemic areas. Acid fast bacilli are not seen on routine H&E stain and require Ziehl–Neelsen staining for confirmation in tuberculous granuloma.

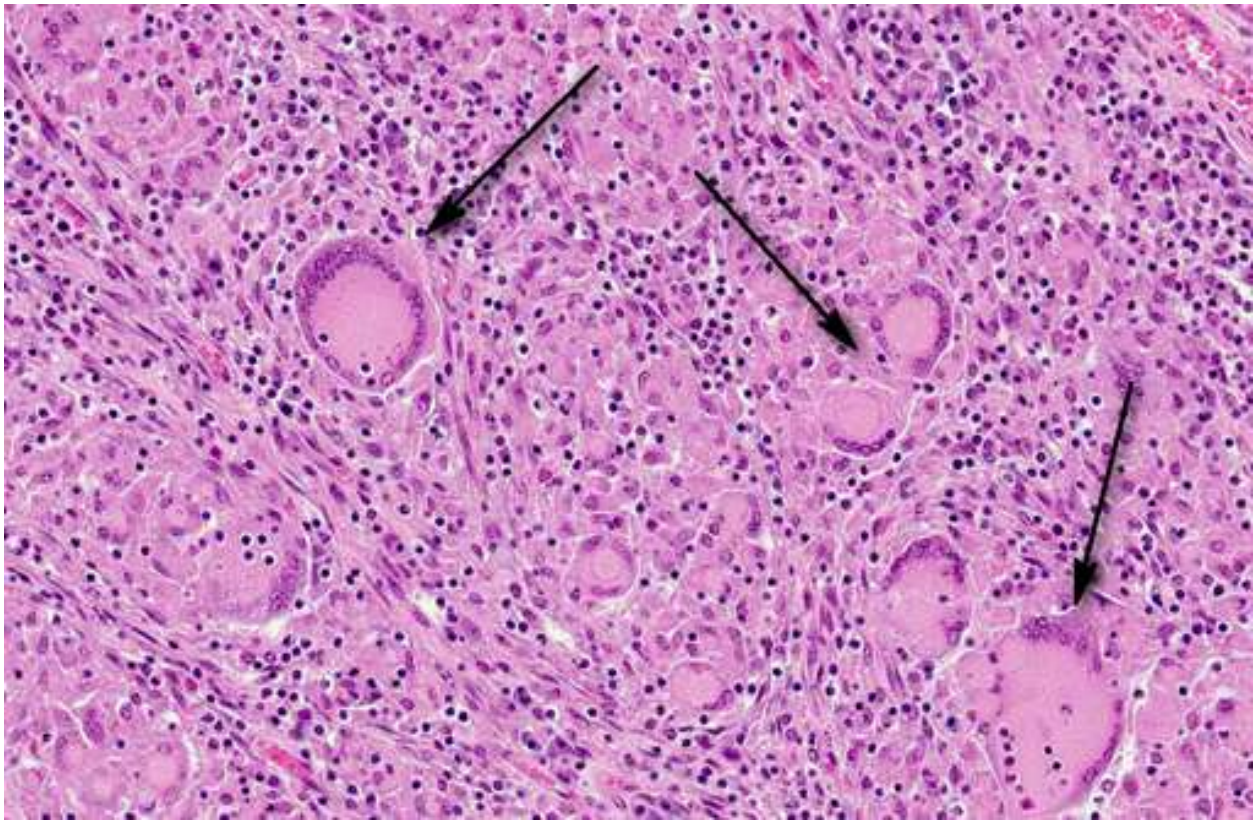
References:

1) Robbins and Cotran Pathologic Basis of Disease, latest edition
2) Levinson W. Review of Medical Microbiology and Immunology, latest edition

3) Harsh Mohan, Textbook of Pathology, latest edition



Typical Tuberculous granuloma with central caseous necrosis and Langhans type giant cells



Multinucleated giant cells of the Langhans type

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

BLOCK – F

PRE-CLINICAL DENTISTRY I

(Healing, Repair & Dental Restorations -I)

Practical # 15

VARIOUS LABORATORY INSTRUMENTS AND MACHINE

A. Microscope:

A microscope is a precision optical and imaging instrument that uses a system of lenses and illumination to produce a magnified, high-resolution image of minute structures such as cells, microorganisms, tissues, and crystals, which are not visible to the unaided human eye.

Types of microscopes and uses:

- *Compound light microscope:* Most commonly used type. Routine examination of stained and unstained specimens.
- *Oil immersion microscope:* Detailed study of bacteria and fine cellular structures.
- *Dark field microscope:* Visualization of thin, unstained organisms such as spirochetes.
- *Phase contrast microscope:* Observation of living, unstained cells with good contrast.
- *Fluorescent microscope:* Detection of fluorochrome-stained organisms and antigens.
- *Immunofluorescence microscope:* Identification of specific antigens or antibodies.
- *Electron microscope:* Study of viruses and ultrastructure of cells.
- *Transmission electron microscope:* Examination of intracellular details.
- *Scanning electron microscope:* Study of surface morphology in three dimensions.
- *Confocal microscope:* Optical sectioning and three-dimensional imaging in research.

Parts of a Compound light microscope

1. *Foot piece (base):* Provides stability to the microscope and houses the light source.
    a. Light source / illuminator: Provides illumination for the specimen; usually an inbuilt LED or halogen lamp in modern microscopes.
    b. Power switch and brightness control knob: Used to switch the microscope on or off and adjust light intensity.
2. *Body:* Supports the optical and mechanical components and includes the following:
    a. Sub-stage condenser: Concentrates and focuses light on the specimen; diaphragm controls intensity and field of illumination.
        i. Iris diaphragm lever: Controls the amount of light entering the condenser to improve contrast.
        ii. Field diaphragm: Controls the diameter of the light beam and helps in proper illumination alignment.
    b. Stage: Platform for holding the specimen slide; may be fixed or mechanical to allow movement in horizontal planes.
        i. Mechanical stage controls: Allow precise movement of the slide in horizontal (x and y) directions.
        ii. Stage clips: Hold the slide in position on a fixed stage.
    c. Nosepiece: Revolving part that holds objectives of different magnifications.

d. Objectives: Lens systems that provide primary magnification: Scanner x4, low power x10, high power x40, oil immersion x100.
e. Focusing knobs:
    i. Coarse adjustment knob: It is used for rapid focusing by moving the stage or body tube up and down. It is mainly used with scanner and low-power objectives and should not be used with high-power or oil immersion objectives to avoid damage to the slide or lens.
    ii. Fine adjustment knob: It is used for precise and accurate focusing by producing small vertical movements of the stage. It is essential for high-power and oil immersion objectives to obtain a sharp and clear image.

3. *Eyepiece:* Used to view the image formed by the objective; commonly x 10 magnifications. May be monocular or binocular.
4. *Body tube / head:* Maintains proper distance between objective and eyepiece and aligns the optical path.

Uses of compound microscope:

1. Examination of stained blood smears for red blood cells, white blood cells, and platelets.
2. Identification of bacteria and fungi in stained preparations.
3. Observation of parasites, ova, cysts, and larvae in clinical specimens.
4. Study of histological tissue sections in pathology.
5. Examination of urine sediments and crystals.
6. Study of cells and cellular morphology in cytology.
7. Observation of microorganisms in microbiology practical work.
8. Routine teaching and diagnostic purposes in medical and dental laboratories.

Oil immersion microscope:

- Oil immersion microscopy is used for the examination of very small structures such as bacteria and fine cellular details that cannot be resolved clearly under dry objectives. In this method, a special oil immersion objective of x100 magnification is used along with immersion oil.
- The immersion oil has a refractive index similar to that of glass. When oil is placed between the cover slip and the objective, it replaces air in the light path and reduces light refraction and scattering. This allows more light to enter the objective, thereby improving resolution and image clarity.
- Oil immersion microscopy is commonly used in bacteriology for examination of gram stained smears, acid-fast bacilli, and blood parasites.
- After observation, the oil should be cleaned immediately from the slide and the objective to prevent damage to the lens.

Care of the microscope:

1. Protect the microscope from heat, dust, moisture, and direct sunlight.
2. Clean lenses daily using lens paper or soft tissue; wipe gently, do not rub.

3. Clean oil immersion objective immediately after use.
4. Cover the microscope when not in use.
5. Switch off power and unplug after use.

Troubleshooting:

- No light: Loose power connection, fused bulb, closed diaphragm, or improper objective position.
- Insufficient light: Low illumination setting, condenser too low, dirty lenses.
- Too bright light: Excessive illumination for the objective in use.
- Flickering light: Loose electrical connections or defective bulb.
- No focus under high power: Slide placed upside down.
- Bubbles in oil immersion: Insufficient oil contact; clean and re-apply oil.

References:

1. AFIP Manual of Laboratory Medicine
2. Ananthanarayan and Paniker's Textbook of Microbiology, latest edition
3. Bancroft and Gamble, Theory and Practice of Histological Techniques
4. Davidson's Principles and Practice of Medicine (laboratory techniques section)

Compound Microscope

B. Hematology Analyzer

A hematology analyzer is an automated laboratory instrument used for quantitative and qualitative analysis of blood cells to perform complete blood count (CBC), including red blood cells, white blood cells, platelets, hemoglobin, and related indices. It plays a crucial role in diagnosis, monitoring, and screening of hematological and systemic diseases.

Types of hematology analyzers:

Based on automation

- Fully automated analyzers: Blood sample is directly aspirated and processed by the instrument with minimal operator intervention. Used in medium to large laboratories.
- Semi-automated analyzers: Require manual steps such as dilution. Limited parameters. Largely obsolete.

Based on WBC differentiation

1. *3-part differential analyzers:* Classify WBCs into lymphocytes, monocytes, and granulocytes. Used for routine screening in small laboratories and clinics.
2. *5-part differential analyzers:* Differentiate neutrophils, lymphocytes, monocytes, eosinophils, and basophils using flow cytometry and light scatter. Used in hospitals and diagnostic centers for detailed evaluation.
3. *Advanced / 6-part analyzers:* Provide additional parameters such as reticulocyte count, nucleated RBCs (NRBC), immature granulocytes, and abnormal cell flags. Used in tertiary care centers and hematology reference labs.

Basic principles of operation:

- *Electrical impedance (Coulter principle):* Cells suspended in an electrolyte pass through a small aperture. Each cell causes a change in electrical resistance, generating a pulse. Pulse number indicates cell count and pulse height reflects cell size. Used for RBC, WBC, and platelet counting.
- *Optical / light scattering method*: Cells pass single-file through a laser beam. Light scattered at different angles reflects size, granularity, and nuclear complexity. Used for WBC differential counts.
- *Flow cytometry:* Combines hydrodynamic focusing with light scatter and fluorescence to precisely identify and differentiate cells.
- *Fluorescence technology:* Fluorescent dyes bind to DNA or RNA, enabling detection of reticulocytes, NRBCs, and abnormal cells.
- *Photometry (spectrophotometry):* Hemoglobin is measured after RBC lysis by converting hemoglobin into a stable compound and measuring light absorbance.

Common parts of a hematology analyzer:

- Sample aspiration system / autoloader
- Reagent system (diluent, lysing reagent, stains)
- Hydraulic system for fluid movement and waste disposal
- Detection chambers (aperture, flow cell)
- Data processing unit and software
- Display and user interface
- Printer and LIS connectivity

Tests performed by hematology analyzer

1. *Complete blood count (CBC)*
    a. Hemoglobin concentration
    b. Red blood cell count (RBC)
    c. White blood cell count (WBC)
    d. Platelet count
2. *Red cell indices*
    a. Mean corpuscular volume (MCV)
    b. Mean corpuscular hemoglobin (MCH)
    c. Mean corpuscular hemoglobin concentration (MCHC)
    d. Red cell distribution width (RDW)
3. *White cell analysis*
    a. Total leukocyte count
    b. Differential leukocyte count (3-part or 5-part)
4. *Platelet parameters*
    a. Mean platelet volume (MPV)
    b. Platelet distribution width (PDW)
    c. Plateletcrit (PCT)
5. *Advanced parameters (in advanced analyzers)*
    a. Reticulocyte count
    b. Nucleated red blood cells (NRBC)
    c. Immature granulocytes
    d. Abnormal cell flags and histograms

Uses with clinical relevance

1. Diagnosis
    - Anemia (iron deficiency, megaloblastic, hemolytic)
    - Infections (bacterial, viral, parasitic)
    - Leukemia and other hematological malignancies
    - Bleeding and platelet disorders
2. Monitoring:

- Chemotherapy and radiotherapy response
- Chronic diseases such as renal failure and inflammatory disorders

3. Screening
    - Routine health check-ups
    - Pre-operative evaluation
    - Antenatal screening
4. Blood banking
    - Donor screening and component preparation

Identification points:

- Automated CBC report with histograms and scattergrams
- RBC indices (MCV, MCH, MCHC, RDW)
- WBC differential count
- Platelet count and platelet indices
- Abnormal cell flags prompting peripheral smear review

Care and maintenance:

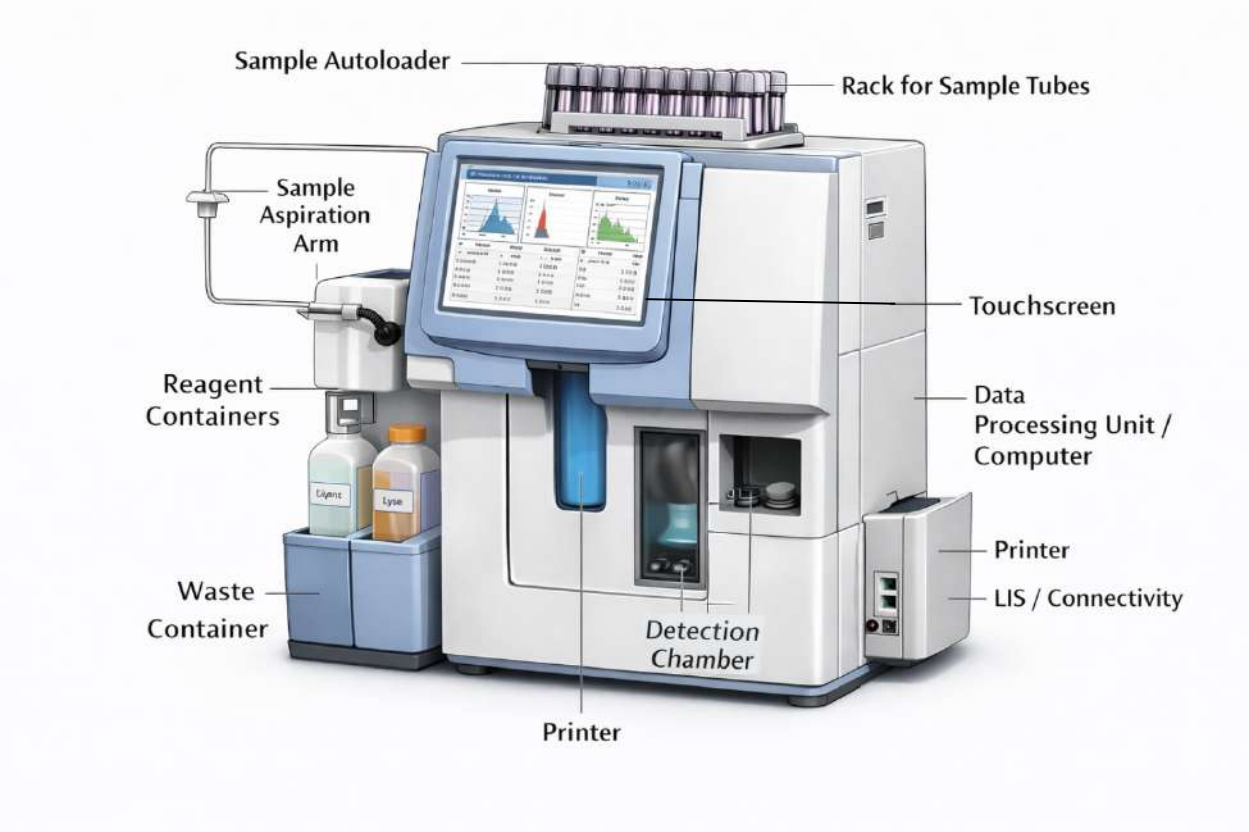
- Use EDTA anticoagulated blood samples only
- Run daily internal quality control samples
- Perform regular cleaning cycles and reagent replacement
- Calibrate as per manufacturer's guidelines
- Ensure proper temperature control and power supply
- Regular preventive maintenance and service support

Examples of hematology analyzers:

- Beckman Coulter series
- Sysmex analyzers
- Abbott Cell-Dyn series
- Roche Cobas systems

References

- Dacie and Lewis Practical Haematology
- Bain BJ. Blood Cells: A Practical Guide
- CLSI guidelines on hematology analyzers
- AFIP Manual of Laboratory Medicine



Hematology Analyzer

C. Clinical Chemistry Analyzer

A clinical chemistry analyzer is an automated laboratory instrument used to quantitatively measure biochemical constituents in biological fluids such as serum, plasma, urine, and cerebrospinal fluid. It plays a vital role in diagnosis, monitoring, and prognosis of metabolic, endocrine, hepatic, renal, and cardiovascular disorders.

Types of chemistry analyzers

1. Based on automation

    a. Semi-automated analyzers: Require manual pipetting of samples and reagents. Used in small laboratories with low workload.
    b. Fully automated analyzers: Perform sample aspiration, reagent dispensing, incubation, measurement, calculation, and reporting automatically. Used in hospitals and reference laboratories.
2. Based on throughput

    a. Low-throughput analyzers – small clinics
    b. Medium-throughput analyzers – diagnostic centers

c. High-throughput analyzers – tertiary care hospitals

3. Based on analytical technology

    a. Colorimetric / spectrophotometric analyzers
    b. Ion-selective electrode (ISE) based analyzers
    c. Immunochemistry analyzers
    d. Advanced systems using tandem mass spectrometry (LC-MS/MS)

4. Based on sample and reagent handling systems
    a. Continuous flow analyzers – obsolete
    b. Discrete (random access) analyzers – most commonly used
    c. Centrifugal analyzers – limited use

Common basic laboratory chemistry analyzers include:

- Semi-auto chemistry analyzer
- Fully automated chemistry analyzer
- Photometric (colorimetric) analyzer
- Spectrophotometer
- Electrolyte analyzer (ISE based)
- Blood gas analyzer
- Random access chemistry analyzer
- Batch chemistry analyzer
- Dry chemistry analyzer (slide-based systems, e.g., Vitros)

Principle of operation

Most clinical chemistry analyzers are based on colorimetry and spectrophotometry, governed by the Beer–Lambert law.

Beer–Lambert law
The absorbance of light by a solution is directly proportional to the concentration of the absorbing substance and the path length of light through the solution.
Absorbance = $\varepsilon \times c \times l$
where ε is molar absorptivity, c is concentration, and l is path length.

Colorimetry: Colorimetry measures the intensity of color produced when an analyte reacts with a reagent to form a colored compound. A filter is used to select a broad wavelength band in the visible spectrum corresponding to maximum absorption of the colored product.

Uses:

- Glucose estimation

- Urea
- Creatinine
- Total protein
- Bilirubin

Limitations

- Lower specificity
- Interference from hemolysis, lipemia, and turbidity

Spectrophotometry

Spectrophotometry is an advanced and more precise form of colorimetry. A monochromator (prism or diffraction grating) isolates a very narrow and specific wavelength of light. Measurements can be performed in ultraviolet, visible, and infrared ranges.

Advantages over colorimetry

- Higher accuracy and sensitivity
- Better wavelength specificity
- Reduced analytical interference

Advanced spectrometric techniques

1. UV spectrophotometry: Used for enzyme assays such as ALT, AST, LDH by measuring change in absorbance of NADH/NAD^+.
2. Reflectance spectrophotometry: Used in dry chemistry analyzers.
3. Mass spectrometry (LC-MS/MS): Highly specific and sensitive method used for therapeutic drug monitoring, hormones, toxicology, and newborn screening.

Basic tests commonly performed by chemistry analyzer

1. *Carbohydrate metabolism*
    - Blood glucose
2. *Renal function tests*
    - Urea
    - Creatinine
    - Uric acid
3. *Liver function tests*
    - Total bilirubin
    - Direct bilirubin
    - AST (SGOT): Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase)

- ALT (SGPT): Alanine aminotransferase (Serum glutamic pyruvic transaminase)
- Alkaline phosphatase (ALP)

4. *Total protein*
    - Albumin
5. *Lipid profile*
    - Total cholesterol
    - Triglycerides
    - HDL cholesterol
    - LDL cholesterol (calculated or direct)
6. *Electrolytes and minerals*
    - Sodium
    - Potassium
    - Calcium
    - Chloride
    - Phosphorus
7. *Enzymes*
    - Amylase
    - Lipase
    - Creatine kinase (CK)

Uses with clinical relevance

1. Liver function tests:
    - ALT, AST, ALP, bilirubin – hepatitis, cirrhosis, obstructive jaundice
2. Renal function tests:
    - Urea, creatinine – acute and chronic kidney disease
3. Metabolic disorders:
    - Glucose – diabetes mellitus
    - Uric acid – gout
4. Lipid profile
    - Cholesterol, triglycerides – cardiovascular risk assessment
5. Electrolytes and minerals
    - Sodium, potassium, calcium – fluid and electrolyte imbalance
6. Therapeutic drug monitoring
    - Antiepileptics, antibiotics, immunosuppressants

Care and maintenance

- Use clean, non-hemolyzed samples
- Perform daily calibration and quality control
- Maintain reagent storage temperature
- Clean probes and cuvettes regularly
- Follow manufacturer’s preventive maintenance schedule

Quality control significance:

- Ensures accuracy and reproducibility
- Detects systematic and random errors
- Prevents clinical misinterpretation
- Modern analyzers use automated *Levey–Jennings charts* and *Westgard rules*

Examples of chemistry analyzers:

- Roche Cobas series
- Beckman Coulter AU series
- Abbott Architect
- Siemens Dimension systems

References

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics
2. Bishop ML. Clinical Chemistry: Principles, Techniques, and Correlations
3. AFIP Manual of Laboratory Medicine

Chemistry Analyzer

D. Incubator

An incubator is a laboratory instrument designed to maintain controlled environmental conditions such as temperature, humidity, and specific gas concentrations within an enclosed chamber to allow optimal growth and maintenance of microorganisms, cells, and biological materials.

Principle and working

Incubators work by maintaining a stable internal environment using an electric heating system regulated by thermostats and microprocessor-controlled sensors. Heat is distributed uniformly by air circulation fans or liquid circulation systems. Humidity is maintained by water reservoirs, and gas concentration (CO_2, N_2) is regulated through external gas cylinders and flow regulators. Modern incubators use digital displays and alarm systems to monitor deviations from preset conditions.

Types of incubators with uses

1. Simple (hot air) incubator
    a. Controls temperature only, usually between 25–60°C.
    b. Uses: routine bacterial culture, incubation of biochemical tests such as Widal and Coombs test.
2. Anaerobic incubator
    a. Provides oxygen-free environment by replacing air with nitrogen or gas mixtures.
    b. Uses: cultivation of anaerobic bacteria such as Clostridium species.
3. CO_2 incubator
    a. Maintains 5–10% CO_2 with controlled humidity and temperature.
    b. Uses: culture of fastidious organisms and tissue/cell cultures.
4. Cell culture incubator
    a. Precisely controls temperature, humidity, and CO_2 concentration.
    b. Uses: mammalian cell culture, virology, research applications.
5. Shaking incubator (advanced)
    a. Provides controlled temperature with continuous agitation.
    b. Uses: aerobic microbial growth, enzyme and fermentation studies.

Common temperature settings:

- 37°C – human pathogenic bacteria
- 25–28°C – fungi
- 55°C – thermophilic organisms

Clinical and laboratory uses:

- Growth and isolation of bacteria, fungi, and yeast
- Maintenance of microbial and cell cultures
- Incubation of serological and biochemical reactions
- Research in microbiology, pathology, and biotechnology
- Quality control testing in pharmaceutical and diagnostic labs

Care and maintenance

- Clean and disinfect chamber periodically with detergent, disinfectant, and alcohol
- Avoid unnecessary opening of door
- Monitor temperature, humidity, and gas levels regularly
- Check gas cylinders daily for adequate supply
- Ensure proper calibration of sensors

Troubleshooting (common)

- No temperature rise – faulty heating element or thermostat
- Uneven temperature – fan malfunction or overcrowding
- Contamination – improper cleaning or frequent door opening
- CO_2 fluctuation – empty gas cylinder or faulty regulator

Identification points

- Insulated chamber with shelves
- Thermostat and digital display
- Temperature and gas sensors
- Used for microbial culture at controlled temperature

References:

1. AFIP Manual of Laboratory Medicine
2. Mackie & McCartney Practical Medical Microbiology
3. WHO Laboratory Biosafety Manual

Incubator

E. BACTEC Blood Culture System (BD)

The BACTEC system (Becton, Dickinson and Company) is a widely used automated blood culture system designed for the rapid detection of bacteria and fungi in blood and other normally sterile clinical specimens. It uses specialized culture bottles with enriched media and continuously monitors microbial growth by detecting carbon dioxide (CO_2) production, enabling early diagnosis and prompt initiation of appropriate antimicrobial therapy.

Principle of Detection

- Microorganisms growing in the culture bottle metabolize nutrients and produce CO_2.
- The CO_2 concentration increases, leading to a chemical or fluorescent change in a sensor embedded at the bottom of the bottle.
- This change is detected by the instrument using fluorescence-based detection technology.
- When a predefined threshold is reached, the bottle is flagged as positive.

Operational Mechanism

Media Bottles

- Uses proprietary blood culture bottles such as:
    - BACTEC Plus Aerobic/F
    - BACTEC Plus Anaerobic/F
- Bottles contain enriched broth, resins to neutralize antibiotics, and a CO_2-sensitive fluorescent sensor.

Continuous Monitoring

- Inoculated bottles are loaded into automated instruments (e.g., BD BACTEC FX, FX40).
- Bottles are incubated and monitored automatically.
- Each bottle is scanned at frequent intervals (approximately every 10 minutes) for changes indicating microbial growth.

Detection and Alert

- Once growth is detected, the instrument:
    - Flags the bottle as positive
    - Generates an audible and visual alert
- Positive bottles are removed for:
    - Gram staining
    - Subculture
    - Identification and antimicrobial susceptibility testing

Benefits

- **Speed**: Significantly reduces time to detection (TTD) compared to conventional manual blood culture methods, which is critical for early targeted antibiotic therapy.
- **High Sensitivity**: Capable of detecting low-level bacteremia and fungemia.
- **Automation**: Continuous incubation and monitoring reduce manual workload and human error.
- **Scalability**: Available in different configurations (e.g., FX, FX40) to suit small to large laboratory workloads.

Applications

- **Blood cultures**: Primary application for detection of:
    - Bacteremia
    - Septicemia
    - Fungemia

- **Sterility testing**:
    - Screening platelet concentrates and other sterile biological products
- **Detection of fastidious organisms**:
    - Effective for organisms such as *Brucella spp.*, *HACEK group*, and certain slow-growing bacteria

Note:

- Mycobacterial detection is performed using **separate BACTEC systems** with different media and detection principles.
- *BACTEC systems for tuberculosis*
    1. BACTEC 460 TB (radiometric system) (Older, largely discontinued)
        - Principle: detects release of radioactive CO_2 produced from metabolism of ^{14}C-labelled palmitic acid by Mycobacterium tuberculosis.
    2. BACTEC MGIT 960 (non-radiometric system) - (Most commonly used)
        - Principle: detects mycobacterial growth by fluorescence due to oxygen consumption in MGIT tubes.
- **GeneXpert MTB/RIF is the gold-standard rapid molecular test for MTB.**

GeneXpert MTB/RIF is a cartridge-based, automated real-time PCR assay used for rapid detection of Mycobacterium tuberculosis complex directly from clinical specimens. It simultaneously detects MTB DNA and rifampicin resistance by targeting the rpoB gene. The test provides results within about 2 hours, has high sensitivity and specificity, and is recommended by WHO as the initial diagnostic test for pulmonary tuberculosis, especially in HIV-infected patients and suspected drug-resistant TB.

References

1. AFIP Manual of Laboratory Medicine
2. Mackie & McCartney Practical Medical Microbiology
3. CDC Laboratory Guidelines for Mycobacterial Diagnostics

F. BIOSAFETY CABINET

In microbiology, a biosafety cabinet (BSC) is a ventilated laboratory enclosure that uses controlled airflow and HEPA filtration to provide a contained work area, protecting laboratory personnel, the environment, and the specimen from exposure to infectious aerosols generated while handling microorganisms such as bacteria, viruses, and fungi. It ensures operator safety and maintains aseptic conditions during microbiological procedures.

Functions:

- Personnel protection: prevents inhalation of infectious aerosols during handling of pathogens such as Mycobacterium tuberculosis, Brucella, and viral cultures
- Product/sample protection: maintains sterility of cultures, media, and clinical specimens
- Environmental protection: filters exhaust air to prevent laboratory contamination

Working principle – General

- Room air is drawn through the front opening, forming an inward airflow barrier
- Air flows across the work surface, capturing aerosols generated during procedures
- Contaminated air passes through HEPA filters that remove 99.97–99.99% of particles ≥0.3 microns
- Filtered air is either exhausted outside or recirculated depending on cabinet class

Types of biosafety cabinets with uses:

Class I

- Provides personnel and environmental protection only
- No protection for the product
- HEPA-filtered exhaust air
- Used for procedures involving low-to-moderate risk agents where sterility is not critical

Class II

- Provides personnel, product, and environmental protection
- Most commonly used in microbiology laboratories
- Uses HEPA-filtered vertical laminar airflow
- Subtypes A1, A2, B1, B2 differ in exhaust pattern and chemical handling capacity
- Used for routine bacteriology, virology, mycology, cell culture, and diagnostic specimen processing

Class III

- Maximum containment cabinet
- Totally enclosed, gas-tight system with glove ports
- Exhaust air is double HEPA-filtered
- Used for high-risk pathogens (BSL-4) such as Ebola virus (reference standard)

Clinical and laboratory uses with examples

- Processing sputum for tuberculosis culture and smear preparation
- Handling blood and body fluid specimens suspected of HIV or hepatitis viruses
- Subculturing bacterial isolates like Salmonella and Shigella
- Viral culture work and cell line maintenance
- Aerosol-generating procedures such as vortexing, pipetting, and centrifuge loading

Care and maintenance

- Cabinet should be placed away from doors, windows, and high-traffic areas
- HEPA filters must be certified and tested periodically
- Work surface should be disinfected before and after use
- Avoid overcrowding and rapid hand movements
- UV light (if present) used only when cabinet is not in operation

Troubleshooting (practical points)

- Alarm sounding: check airflow obstruction or sash position
- Contamination of cultures: ensure proper aseptic technique and airflow integrity
- Reduced airflow: check filter status and cabinet certification

References

4. AFIP Manual of Laboratory Medicine
5. Mackie & McCartney Practical Medical Microbiology
6. CDC Laboratory Guidelines for Mycobacterial Diagnostics

G. Tissue processor

A tissue processor is an automated laboratory instrument that carries fixed tissue specimens through dehydration, clearing, and wax impregnation to prepare them for embedding and sectioning.

Types and uses

1. Manual tissue processor
    a. Tissues are transferred manually between reagents.
    b. Use: Small laboratories, low workload.
2. Automatic tissue processor
    a. Reagent changes, timing, agitation, and temperature are computer controlled.
    b. Use: Routine histopathology laboratories for biopsy and surgical specimens.

Clinical relevance:

- Uniform processing of tissues
- Reduced processing time
- Improved tissue morphology
- Essential for accurate histopathological diagnosis

Automated Tissue Processor

H. Embedding station

- Used to embed processed tissues in molten paraffin wax to form blocks for sectioning.

I. Microtome

- Used to cut thin, uniform tissue sections (3–5 µm) for microscopic examination.

J. Water bath

- Used to float and flatten paraffin tissue sections before mounting on glass slides.

K. Centrifuge

- Used to separate components of blood, urine, and other body fluids based on density.

L. Biosafety cabinet / laminar airflow

- Used to provide a sterile and safe working environment while handling infectious or sensitive biological materials.

Rapid Diagnostic Tests in Clinical Laboratory

Introduction

Rapid tests are simple diagnostic assays that provide results within minutes, require minimal equipment, and are useful in emergency situations and resource-limited settings. They are mainly used as screening tests. Because of limitations such as false negatives, inability to quantify pathogen load, or species differentiation, positive or doubtful results should be confirmed by appropriate gold standard tests like microscopy, ELISA, or PCR.

1. Immunochromatographic test (ICT) for malaria

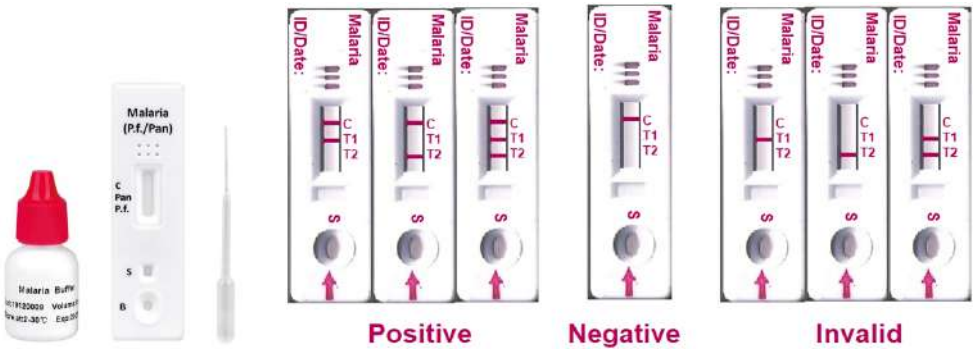
Principle: Lateral flow immunoassay that detects malaria parasite antigens in whole blood. The immuno-chromatographic (ICT) test device is coated with monoclonal antibodies to histidine-rich protein-2 (specific to P. falciparum) and pan-specific or species-specific plasmodium lactate dehydrogenase (specific to all four plasmodium species- pLDH or aldolase). Antigen–antibody reaction produces a visible colored test line, with a control line indicating test validity.

Interpretation:

- Negative: only control line present
- Positive: control line with one or more test lines (species dependent)
- Invalid: control line absent

Uses: Rapid screening and diagnosis of malaria in endemic areas and emergencies, especially where microscopy is not immediately available.

Gold standard: Microscopic examination of thick and thin blood smears. PCR is used in low parasitemia or for species confirmation.



2. Hepatitis B and C Immunochromatographic Test (ICT based)

Principle: Lateral flow immunoassays detecting HBsAg (hepatitis B) or anti-HCV antibodies (hepatitis C) use antigen–antibody reactions on a membrane. ICT tests (also known as lateral

flow immunoassays) are qualitative tests that typically use a blood sample (serum, plasma, or whole blood) and provide results within 10-20 minutes without the need for sophisticated laboratory equipment.

Hepatitis B (HBV): The tests typically detect the Hepatitis B surface antigen (HBsAg), a protein present on the virus's surface, indicating a current infection.

Hepatitis C (HCV): The tests detect antibodies to the Hepatitis C virus (anti-HCV). A positive antibody test indicates exposure to the virus but does not distinguish between a past resolved infection and a current active one; a separate molecular (PCR or RNA) test is needed to confirm active infection.

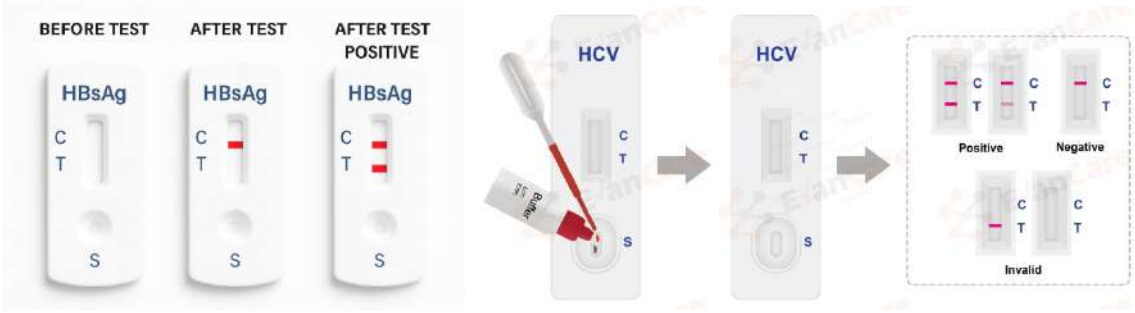
Interpretation

- Positive: both control and test lines visible
- Negative: only control line visible

Uses: Initial screening of hepatitis B and C in clinical and field settings.

Limitations: Rapid tests less effective at detecting low viral loads, distinguishing infection stages, or identifying new infections during the "window period" (the time between infection and the appearance of detectable antibodies or antigens) compared to molecular techniques

Gold standard: ELISA for serological confirmation and PCR for detection of viral DNA/RNA and assessment of active infection.



3. ELISA for Hepatitis B and C

Principle: Enzyme-linked immunosorbent assay (ELISA) is an immunological method used to detect specific viral antigens such as HBsAg or antibodies such as anti-HCV in serum or plasma. It is based on a specific antigen–antibody reaction on a solid phase, usually a microtiter plate. The patient sample binds to the immobilized antigen or antibody, after which an enzyme-labeled conjugate is added to form an immune complex. Following washing steps, a

chromogenic substrate is added, and the enzyme–substrate reaction produces a color change. The intensity of the color is proportional to the amount of antigen or antibody present and is measured spectrophotometrically for qualitative or quantitative interpretation.

Interpretation: Optical density (absorbance) is measured by an ELISA reader and compared with a cutoff value. Values above cutoff are reactive.

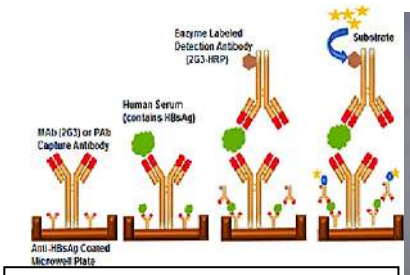
- Absorbance > cutoff → Positive
- Absorbance < cutoff → Negative

Uses: Laboratory-based screening of blood donors and patients with higher sensitivity than rapid tests.

Gold standard: PCR or nucleic acid testing to confirm active infection. For HBV, additional serological markers are assessed.

Uses of ELISA other than virology:

- Detection of bacterial and parasitic infections (e.g., typhoid, toxoplasmosis, amoebiasis)
- Diagnosis of autoimmune disorders by detecting autoantibodies (e.g., ANA, anti-dsDNA, rheumatoid factor)
- Detection of hormones such as hCG, insulin, and thyroid hormones
- Allergy testing by measurement of specific IgE antibodies
- Tumor marker estimation (e.g., PSA, AFP, CA-125)
- Screening for blood transfusion–related infections and compatibility testing
- Therapeutic drug monitoring and measurement of cytokines and other biomarkers



ELISA Basic principle

ELISA Microplate

ELISA Microplate

4. Polymerase Chain Reaction (PCR) for HBV, HCV, and HIV

Principle: A molecular technique that detects viral infections by amplifying specific target sequences of viral DNA or RNA. The viral nucleic acid is first extracted from the sample and then amplified through repeated thermal cycles using sequence-specific primers and a thermostable polymerase. This amplification increases even minute quantities of viral genetic material to detectable levels, allowing highly sensitive and specific identification of the pathogen, with real-time PCR also enabling quantification of viral load.

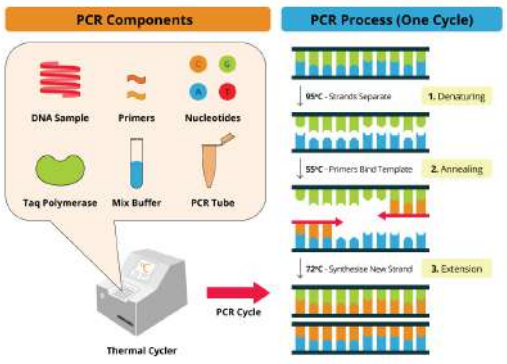
Interpretation: Presence or absence of viral genome; real-time PCR also provides viral load and cycle threshold values.

Uses: Confirmation of active infection, early diagnosis, monitoring treatment response, and assessing virological cure or suppression.

Gold standard: PCR itself is considered the gold standard for confirming active viral replication.

Uses of PCR other than virology

- Detection and identification of bacterial, fungal, and parasitic infections (e.g., Mycobacterium tuberculosis, Chlamydia, Plasmodium)
- Diagnosis of genetic disorders by detecting gene mutations or deletions
- Prenatal and neonatal screening for inherited diseases
- Oncology: detection of oncogenes, tumor suppressor gene mutations, and minimal residual disease
- Forensic medicine: DNA fingerprinting and individual identification
- Transplant medicine: HLA typing and graft monitoring
- Detection of antimicrobial resistance genes
- Research applications including gene cloning and expression analysis



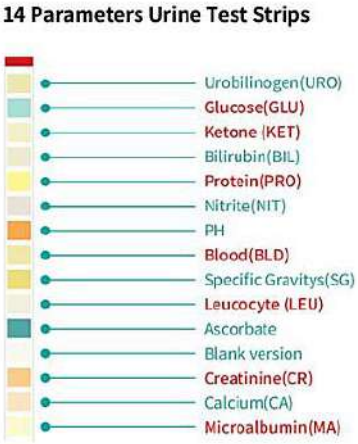
5. Urine Dipstick Test

Principle: A urine dipstick is a plastic reagent strip containing multiple chemically impregnated pads, each specific for a particular urinary constituent such as glucose, protein, blood, ketones, nitrites, leukocyte esterase, and pH. When the strip is immersed in urine, these pads undergo specific enzymatic or chemical reactions with the corresponding analytes, resulting in characteristic color changes. The color intensity correlates with the concentration of the substance present and is interpreted by visual comparison with a standardized color chart or by an automated reader, allowing rapid screening for metabolic, renal, and urinary tract disorders.

Interpretation: Color changes are compared visually with a standard chart at specified times.

Uses: Screening for urinary tract infection, diabetes mellitus, renal disorders, and liver disease.

Gold standard: Microscopic urine examination, urine culture for UTI, and specific biochemical tests such as blood glucose or 24-hour urine protein.



6. Pregnancy test

Principle: Lateral flow immunoassay detecting human chorionic gonadotropin (hCG) in urine or blood.

Interpretation:

- Positive: Test line along with control line
- Negative: Control line only
- Invalid: Control line absent

Uses: Rapid detection of pregnancy.

Gold standard: Quantitative serum hCG estimation and ultrasonography.

Test	Principle	Interpretation	Use	Gold Standard
ICT Malaria	Detects specific antigens (HRP-2 for *P. falciparum* or pLDH/Aldolase for all species) using gold-labeled antibodies.	Presence of a Test (T) line and Control (C) line is positive; C line only is negative.	Emergency screening and field diagnosis.	**Microscopy** (Thick/Thin blood films).
Rapid Hep B (HBsAg)	Lateral flow immunoassay detecting the Hepatitis B surface antigen.	Two lines (C and T) = Positive; One line (C) = Negative.	Initial screening for HBV infection.	**ELISA** or **PCR** (for viral load).
Rapid Hep C (Anti-HCV)	Detects antibodies against the Hepatitis C virus using immobilized antigens.	Visible pink dots or lines at test sites indicate reactivity.	Screening for exposure to HCV.	**PCR** (HCV RNA) to confirm active infection.
Pregnancy Test (uHCG)	Detects human chorionic gonadotropin (hCG) hormone in urine.	Two lines = Pregnant; One line = Not pregnant.	Early detection of pregnancy.	**Serum HCG** (quantitative) or Ultrasound.
Urine Dipstick	Chemical reagents on pads change color (e.g., glucose oxidase for glucose, diazonium salt for nitrites).	Color change matched against a manufacturer's chart.	Screening for UTIs, diabetes, and kidney disorders.	**Urine Culture** (for UTI) or **24-hour urine protein**.

Student Task

S. No.	Equipment / Instrument Observed	Clinical Use Explained (Yes/No)	Basic Principle Understood (Yes/No)	Demonstration Attended (Yes/No) Lecturer Signature
1	Compound microscope (binocular)			
2	Microbiology incubator			
3	CO_2 / anaerobic incubator			
4	Automated hematology analyzer			
5	Clinical chemistry analyzer			
6	BACTEC Blood Culture System (BD)			
7	Tissue processor			
8	Embedding station			
9	Microtome			
10	Water bath			
11	Centrifuge			
12	Biosafety cabinet / laminar airflow			

Dated:_____

S. No.	Tests Observed	Lecturer signature	Remarks
1	ICT for Malaria		
2	Rapid Hepatitis B test (HBsAg)		
3	Rapid Hepatitis C test (Anti-HCV)		
4	Urine pregnancy test (hCG)		
5	Urine dipstick test		
6	ELISA (HBsAg / Anti-HCV)		
7	PCR (HBV / HCV / HIV)		

Dated:_____/_____/_____

Practical # 16

Sample collection and Transportation

Introduction

Proper sample collection and transport are crucial for accurate laboratory diagnosis. Errors at this stage can lead to false results irrespective of test quality. The basic steps include correct patient identification and documentation, proper specimen collection using aseptic techniques, appropriate labeling, and timely transport to the concerned laboratory section under recommended conditions.

General steps in specimen handling

Patient documentation and registration

The patient is registered at the reception with details including hospital number, name, age, sex, unit/address, and investigations requested. Identity and entitlement are verified. A receipt mentioning tests and report timing is issued before specimen collection.

Specimen collection

Specimens must be collected from the correct site, in adequate quantity, using sterile containers, preferably before starting antimicrobial therapy. Proper labeling with patient details, date, time, and type of specimen is mandatory. Relevant clinical information should be mentioned on the request form.

1. Blood specimen collection (venepuncture):

1) Verify patient identity using name, hospital number, and request form.
2) Explain the procedure to the patient and obtain cooperation.
3) Wash hands thoroughly and wear disposable gloves.
4) Arrange all required materials (syringe/vacutainer, needle, tourniquet, antiseptic swabs, and labeled tubes).
5) Seat the patient comfortably with the arm supported.
6) Apply tourniquet and select a suitable vein, preferably the antecubital vein.
7) Clean the puncture site with 70% alcohol using circular motion from center outward and allow to dry.
8) For blood culture, disinfect the site with iodine or povidone-iodine, allow to act, then remove with alcohol.
9) Do not touch the cleaned site again to avoid contamination.
10) Insert the sterile needle into the vein and withdraw required volume of blood gently to prevent hemolysis.
11) Release tourniquet once blood flow is established.
12) Withdraw the needle and apply firm pressure with sterile swab until bleeding stops.
13) Transfer blood into pre-labeled containers without touching the rim or inside of the tube.

14) Dispose of needle and sharps immediately in puncture-proof container.
15) Remove gloves and perform hand hygiene.

Avoiding contamination in blood collection

- Use sterile, single-use needles and syringes
- Proper skin antisepsis and adequate drying time
- Avoid touching needle, puncture site, or container openings
- Use aseptic technique especially for blood culture samples

Transport: Whole blood is stored at 4°C, serum at 2–8°C, and frozen at –20°C or below for reference laboratories when required.

Blood for culture

Blood for culture is collected under strict aseptic conditions and preferably before initiation of antibiotic therapy to improve pathogen recovery. Two or more blood culture samples are recommended, collected from different venepuncture sites (preferably from different veins) and at different times, to increase diagnostic yield and to help differentiate true bacteremia from skin contamination.

Tube Cap Color	Additive / Preservative	Primary Use
Purple / Lavender	**EDTA (Ethylenediaminetetraacetic acid)**	Hematology (e.g., CBC, ESR, blood films)
Light Blue	**Sodium Citrate (3.2% or 3.8%)**	Coagulation studies (PT, APTT, D-dimer)
Red	**No additive / Clot activator**	Serum tests (e.g., electrolytes, LFTs, RFTs)
Green	**Heparin (Lithium or Sodium Heparin)**	Plasma chemistry (e.g., ammonia, some hormones)
Gray	**Sodium fluoride + Potassium oxalate**	Glucose estimation, lactate, ethanol levels

2. Sputum specimen

1) Explain the purpose and procedure of sputum collection to the patient.
2) Collect the specimen preferably in the early morning before eating, drinking, or brushing teeth.
3) Collect it near an open window or balcony or in an open bathroom with good ventilation or Outdoors, if possible, away from other people. The patient should avoid closed rooms and crowded areas to reduce the risk of spreading infection, especially in suspected tuberculosis or respiratory infections.
4) Ask the patient to rinse the mouth thoroughly with clean water only; antiseptic mouthwash should not be used.
5) Provide a clean, sterile, wide-mouthed, leak-proof container and instruct the patient not to touch the inside of the container or lid.
6) Ask the patient to take 2–3 deep breaths to expand the lungs.
7) Instruct the patient to cough deeply from the chest and bring up sputum (phlegm), not saliva.
8) Expectorate the sputum directly into the container without contaminating the rim or inner surface.
9) Ensure the sample is thick, purulent sputum rather than clear saliva.
10) Close the container immediately and tightly after collection.
11) Label the container with patient name, date, time, and type of specimen.
12) Send the specimen to the laboratory promptly; if delay is unavoidable, store at 2–8°C.
13) If the patient is unable to cough up sputum, chest physiotherapy, steam inhalation, or nebulization may be used to induce sputum. In infants or uncooperative patients, gastric aspirate may be collected under medical supervision.
14) For diagnosing conditions like tuberculosis (TB), the standard protocol is to collect three sputum specimens over two to three days.

Prevention of contamination

- Rinse mouth with water before collection to remove food debris
- Avoid saliva, nasal secretions, and postnasal discharge
- Use only sterile containers
- Do not touch the inner surface of container or lid
- Transport specimen without delay

3. Urine specimen

Purpose of Urine Collection

- To diagnose infections (e.g., UTI), kidney diseases, metabolic disorders.

- To perform tests like urine culture, routine examination, proteinuria screening, etc.

Types of Urine Specimens

1. Midstream clean-catch: Culture for UTI, routine tests
2. First morning urine: Pregnancy test, concentrated sample
3. Catheterized sample: If patient is catheterized
4. Suprapubic aspiration: Pediatric patients, when contamination must be avoided
5. 24-hour urine collection: Protein, creatinine clearance, etc.
6. Random urine sample: Often used for routine screening

Instructions to the Patient (Midstream Clean-Catch)

1. Wash hands thoroughly.
2. Clean genital area with clean water or antiseptic wipes
    a. Females: Front to back.
    b. Males: Retract foreskin if applicable.
3. Begin urinating, discard the first few milliliters, and collect the midstream urine in a sterile, screw-cap container.
4. Close the container tightly without touching the inside of the lid or cup.

Transport Guidelines

- Label the container clearly: name, date, time of collection.
- Transport to the lab within 1 hour of collection.
- If delay exceeds 1–2 hours, refrigerate the sample at 2–8°C to prevent bacterial overgrowth.
- For culture, do not freeze and avoid using preservatives unless specified.
- Use urine transport tubes (with boric acid) if refrigeration isn't possible and transport will take longer.

Common Errors to Avoid

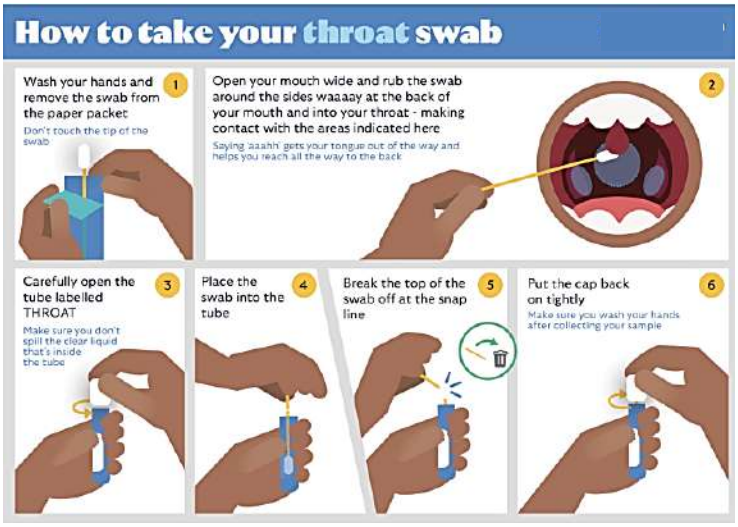
- Using non-sterile containers
- Collecting only the first voided urine (may lead to contamination)
- Delayed transport without refrigeration
- Labeling errors or missing patient info

4. Throat and Nasal swab

- Throat swabs are collected under good illumination and direct vision using a sterile swab, preferably before starting antibiotics. The swab is gently rubbed over areas of

inflammation, exudate, ulcers, or tonsillar crypts while avoiding contact with the tongue, cheeks, or teeth to prevent contamination with oral flora. The procedure should be quick and gentle to minimize discomfort and gag reflex.

- Nasopharyngeal swabs are collected by trained personnel using flexible, sterile swabs. The swab is inserted gently along the floor of the nasal cavity until resistance is felt at the nasopharynx, rotated gently to absorb secretions, and then withdrawn slowly. This method provides a higher yield for respiratory pathogens, including viruses.
- After collection, swabs are immediately placed into *appropriate transport media* (such as Stuart, Amies, or viral transport medium) to preserve organism viability. Specimens are labeled correctly and transported promptly to the laboratory, preferably at recommended temperatures, to ensure accurate culture or molecular testing.



5. Biopsy and Surgical specimens

Specimens are placed in wide-mouthed containers with sufficient fixative. Routine specimens are fixed in **10% neutral buffered formalin (NBF),** and employing a fixative-to-specimen volume ratio of at least **10:1**. Frozen section samples are sent in normal saline without fixative. Containers are properly labeled, and request forms include site of biopsy and brief clinical history. Bone specimens are accompanied by radiographs when indicated.

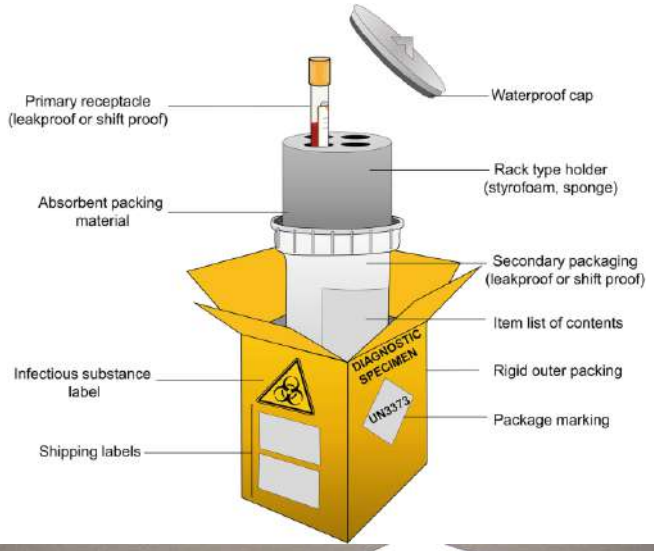
Transport of Specimens

- All laboratory specimens should be considered potentially infectious and handled with standard precautions to ensure safety, specimen integrity, and accurate results.

- *Safety:* Standard precautions must be followed during transport, including hand hygiene and use of appropriate personal protective equipment. Needles and sharps should never be transported with specimens and must be disposed of immediately after use.
- *Specimen integrity:* Correct specimen type, appropriate container, and proper handling are essential. Blood samples must be collected in the correct tubes and transported without undue delay to prevent hemolysis, clotting, or degradation of analytes.
- *Temperature control:* Specimens must be transported at recommended temperatures. Some samples are transported at room temperature, others require refrigeration at 2–8°C, and certain specimens need freezing at –20°C or below. Insulated containers with ice packs or gel packs are used when required.
- *Triple packaging system:* Specimen transport follows the triple packaging system.
    - The primary container is a leak-proof, securely closed specimen container.
    - The secondary container is a leak-proof protective covering with absorbent material to contain spills.
    - The outer container is a rigid box that protects against physical damage during transport.
- *Labeling and documentation:* Each specimen container must be labeled immediately after collection with patient name, identification number, date, and time. The request form with clinical details should be placed separately from the specimen to avoid contamination.
- *Regulatory compliance:* Most routine diagnostic specimens are classified as Category B (biological substances that are not in a form generally capable of causing life-threatening disease in otherwise healthy individuals) and transported according to standard guidelines. Proper labeling of the outer container and availability of responsible contact details are essential.
- *Coordination:* Urgent or special specimens should be communicated to the laboratory in advance to ensure timely and appropriate processing.
- *Adherence to these principles* ensures reliable results, protects healthcare personnel, and maintains laboratory quality standards.
- *Example:* Sputum for tuberculosis should be transported promptly or refrigerated at 2–8°C to prevent overgrowth of commensals, while blood culture bottles must be kept at room temperature and not refrigerated. Viral swabs require transport in viral transport medium under cold conditions to maintain infectivity for accurate detection.

Specimen	Transport medium	Temperature	Urgency
Blood (routine	Plain/anticoagulant tube	Room	Immediate

tests)		temperature	
Blood culture	Culture bottle	Room temperature	Immediate
Sputum	Sterile container	2–8°C if delayed	Prompt
Urine	Sterile container	2–8°C if delayed	Within 1–2 hours
Throat/nasal swab	Transport medium	2–8°C	Immediate
Biopsy specimen	**10% neutral buffered formalin (NBF)**	Room temperature	Prompt



Triple Packaging

Student task

S.No.	Type of Specimen	Sample Collection and Transport Method	Lecturer signature	Remarks
1	Blood (venepuncture)	Peripheral venous blood collection		
2	Blood for culture	Aseptic collection from two sites		
3	Sputum	Early morning deep cough method		
4	Urine	Midstream clean-catch method		
5	Throat swab	Swab from tonsillar area		
6	Nasopharyngeal swab	Deep nasal swab technique		
7	Biopsy specimen	Surgical tissue in fixative		
8	Sample transport	Triple Packing System		

Dated:_____/_____/_____

Practical # 17

GRANULATION TISSUE

Introduction

Wound healing is a complex, well-coordinated biological process that restores tissue integrity following injury. It involves a sequence of overlapping phases: inflammation, proliferation, and remodeling. Healing occurs by two main mechanisms: regeneration, where lost cells are replaced by the same type of cells, and repair, where damaged tissue is replaced by fibrous tissue (scar formation). The choice of mechanism depends on the type of tissue injured and the extent of damage. In most clinical wounds, healing occurs predominantly by repair, with granulation tissue playing a central role.

Types of wound healing

- *Healing by primary intention:* occurs in clean, incised wounds with minimal tissue loss; characterized by minimal granulation tissue and small scar.
- *Healing by secondary intention:* occurs in large wounds with extensive tissue loss; characterized by abundant granulation tissue, wound contraction, and larger scar.
- *Healing by tertiary intention (delayed primary closure):* wound is left open initially and closed later after infection is controlled.

Granulation tissue is newly formed, young mesenchymal tissue that develops during wound healing. It is composed of proliferating capillaries, fibroblasts, and inflammatory cells, and fills the gap between wound edges. It appears pink and granular grossly due to abundant neovascularization.

Granulation tissue is essential for wound repair as it:

- Fills the tissue defect and provides a scaffold for further healing
- Supplies oxygen and nutrients through newly formed capillaries
- Supports re-epithelialization over the wound surface
- Serves as the precursor to mature fibrous scar tissue

Components of granulation tissue:

- Newly formed capillaries (angiogenesis)
- Proliferating fibroblasts
- Inflammatory cells, mainly macrophages, with lymphocytes and occasional neutrophils
- Loose extracellular matrix with early collagen deposition

Formation:

- 0–3 days: Inflammation with neutrophils and macrophages
- 3–7 days: Proliferation of fibroblasts and endothelial cells, formation of granulation tissue
- 1–2 weeks onwards: collagen deposition, reduction in vascularity
- Weeks to months: remodeling and maturation into fibrous scar

Morphology:

Gross features

- Pink to red, soft, moist tissue
- Granular appearance due to abundant capillaries
- Bleeds easily on touch
- Seen at the base of healing wounds

Microscopic features (H&E stain) [Points of identification]

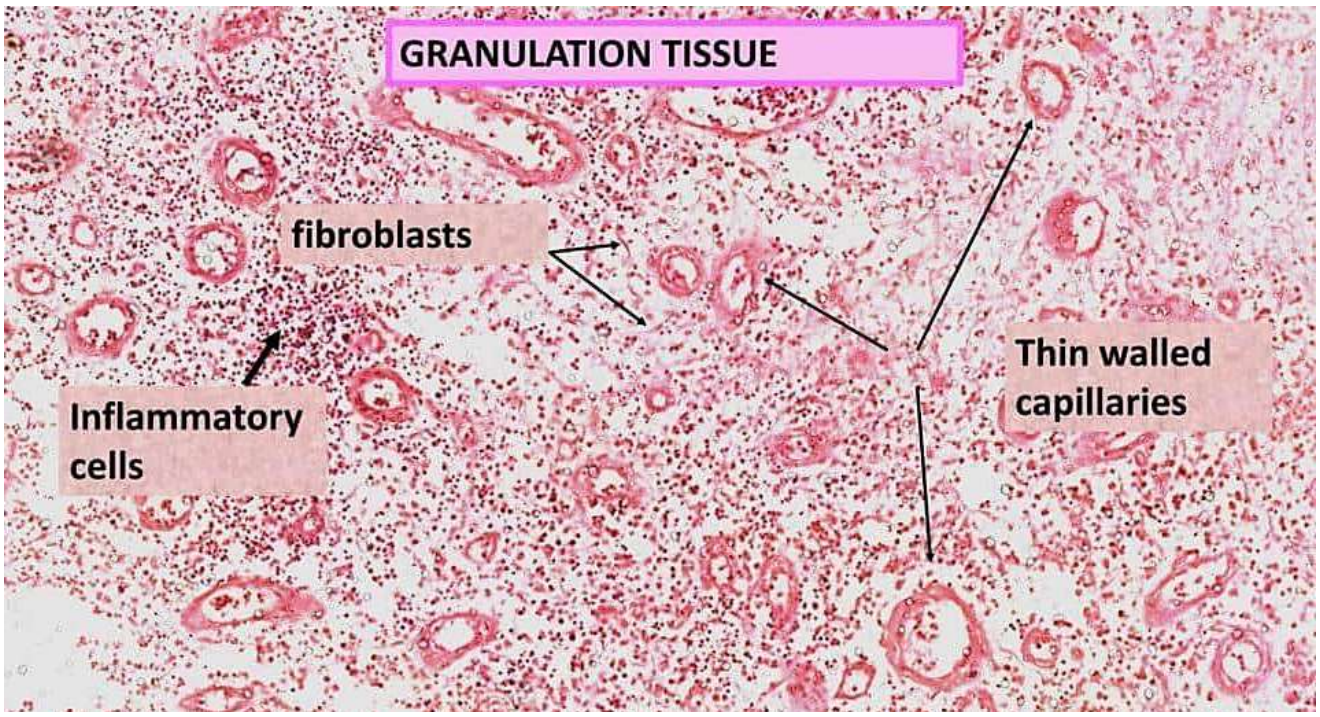
- Numerous newly formed, thin-walled capillaries lined by plump endothelial cells (angiogenesis)
- Proliferating plump fibroblasts arranged loosely in the stroma
- Loose, edematous extracellular matrix with early collagen (mainly type III)
- Mixed inflammatory infiltrate, predominantly macrophages, with lymphocytes and plasma cells
- Absence of dense, organized collagen bundles seen in mature scars.

Abnormalities and Complications of Wound Healing

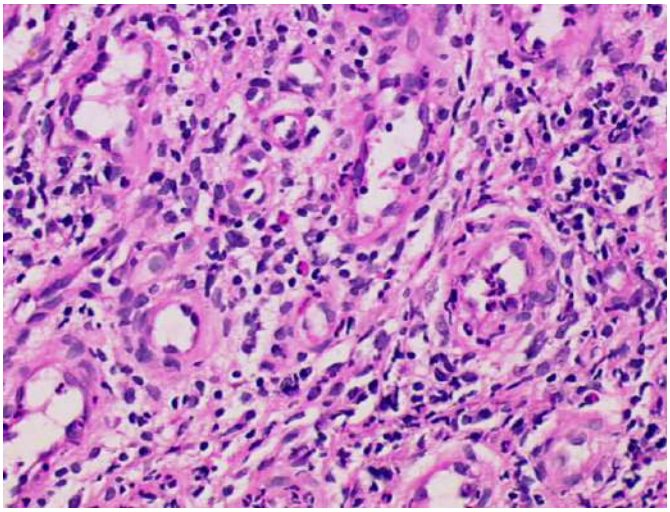
a. Defective healing (chronic wounds):
    - Venous ulcers due to chronic venous insufficiency
    - Arterial and diabetic ulcers due to ischemia and neuropathy
    - Pressure sores due to prolonged pressure and ischemia
b. Excessive repair:
    - Hypertrophic scar: raised, collagen-rich scar confined to wound margins
    - Keloid: excessive collagen extending beyond wound boundaries
    - Exuberant granulation tissue (proud flesh): excess granulation tissue preventing re-epithelialization
c. Other complications
    - Wound dehiscence: reopening of wound due to infection, malnutrition, obesity, or increased intra-abdominal pressure
    - Contractures: excessive wound contraction causing deformity, commonly after burns

References

4. Robbins and Cotran, Basic Pathology
5. Harsh Mohan, Textbook of Pathology
6. Kumar, Abbas, Aster – Robbins Basic Pathology



H&E Slide Granulation Tissue (Low Power)



H&E Slide Granulation Tissue (High Power)

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

BLOCK – G

PRE-CLINICAL DENTISTRY II

(Neoplasia & Dental Rehabilitation)

Practical # 18

ASCARIS LUMBRICOIDES

Introduction

Stool examination is a simple, inexpensive, and essential laboratory procedure used for the detection of intestinal parasites. Ascaris is the largest intestinal nematode infecting humans and is highly prevalent in areas with poor sanitation. Microscopic examination of stool provides direct evidence of infection. As per CDC DPDx guidelines, stool microscopy remains the standard and most reliable diagnostic method for intestinal Ascaris infection. The presence of eggs indicates active intestinal infection and ongoing transmission potential.

Aim: To identify the eggs of Ascaris lumbricoides in stool by microscopic examination.

Specimen: Fresh stool sample collected in a clean, dry, urine-free container.

Important Precautions for Stool Examination

- Stool should be fresh and examined as early as possible
- Avoid contamination with urine or water
- Antiseptics should not be used for cleaning the container
- Warm stools are preferable for parasite detection
- Do not refrigerate stool meant for ova and parasite examination
- If blood or mucus is present, that portion should be examined
- Ideally, three consecutive stool samples should be examined to rule out infestation

Method of Stool Examination

- Naked eye (gross) examination
- Microscopic examination

Naked eye examination significance

1. *Presence of adult worms:* Direct evidence of heavy Ascaris infection.
2. *Mucus without blood:* Suggests intestinal irritation seen in helminthic infestation rather than bacterial dysentery.
3. *Greasy or oily appearance:* Indicates malabsorption due to chronic parasitic infection.
4. *Undigested food particles:* Suggests impaired digestion or rapid intestinal transit.
5. *Altered consistency (intermittent loose stools):* Seen in chronic worm infestation affecting gut motility (helminthic)

Microscopic examination:

- Direct saline smear
- Iodine (Lugol's iodine) preparation

In saline preparation, motility of parasites can be observed. Iodine preparation enhances visualization of ova and cysts by staining internal structures.

Stool Examination significance:

Stool examination helps in detecting:

- Ova, larvae, cysts, trophozoites of parasites
- Presence of RBCs and WBCs
- Fat globules in malabsorption
- Occult blood in gastrointestinal pathology

ASCARIS LUMBRICOIDES

Classification

Phylum: Nematoda

Common name: Roundworm

Habitat: Adult worms are found in the lumen of the small intestine (jejunum) of humans.

Geographical distribution: Worldwide, more common in tropical and subtropical regions with poor sanitation.

Hosts

- Definitive host: Human
- Intermediate host: None

Mode of transmission: Ingestion of embryonated (infective) eggs present in contaminated soil, water, or raw vegetables.

Disease caused: Ascariasis

Life cycle:

- Adult female worms lay eggs in the intestine
- Eggs are passed in feces
- In soil, fertilized eggs embryonate and become infective
- Humans ingest infective eggs
- Larvae hatch in intestine, migrate via bloodstream to lungs
- Larvae ascend trachea, are swallowed, and return to intestine
- They mature into adult worms and continue the cycle

Morphology of eggs of Ascaris lumbricoides

Types of eggs seen in stool

1. *Fertilized eggs (with double shell)*
    - Shape: Oval or round
    - Size: Approximately 50–70 µm
    - Color: Yellow-brown
    - Shell: Thick, rough, mammillated albuminous outer coat and smooth inner shell
    - Contents: Single unsegmented ovum with granular cytoplasm
2. *Unfertilized eggs*
    - Shape: Elongated
    - Size: Approximately 50–90 µm
    - Shell: Thin and irregular
    - Contents: Coarse granular material, no organized ovum
3. *Decorticated fertilized eggs*
    - Albuminous outer coat absent
    - Shell appears smooth and colorless
    - Internal ovum clearly visible

Points of Identification of Ascaris Egg

- Oval or round egg with thick shell
- Yellow-brown color (if albuminous coat present)
- Mammillated outer layer in fertilized eggs
- Large size compared to other helminthic eggs
- Presence of unsegmented ovum with granular cytoplasm

Clinical Features:

- Often asymptomatic in mild infection
- Abdominal pain, malnutrition, anemia
- Protein malnutrition in children
- Löffler's syndrome during larval lung migration (cough, fever, eosinophilia)
- Intestinal obstruction in heavy infestation
- Allergic manifestations such as rashes

Complications:

- Intestinal obstruction
- Volvulus or intussusception
- Biliary or pancreatic duct obstruction
- Growth retardation and malnutrition in children
- Pulmonary eosinophilia

Laboratory Diagnosis:

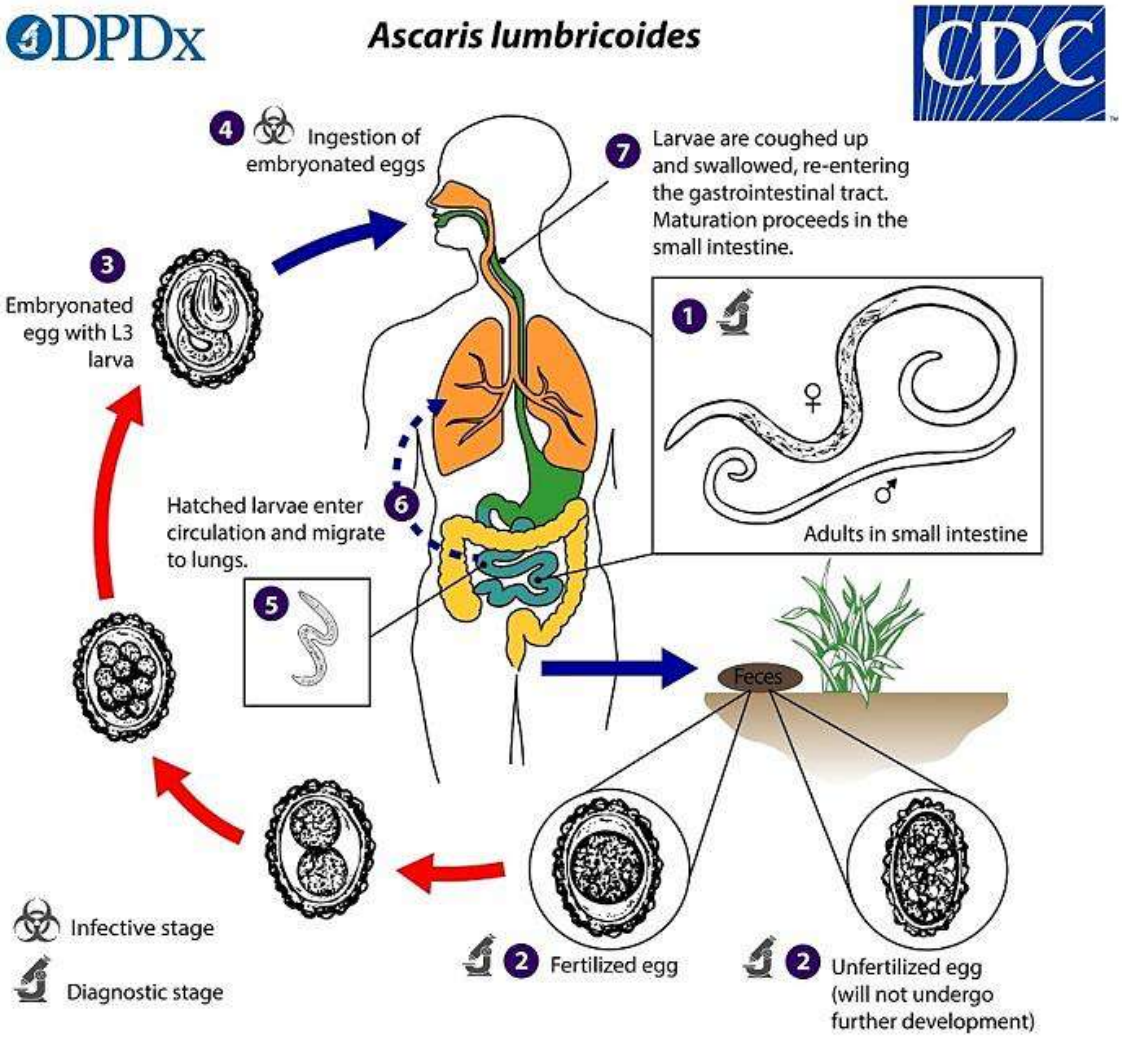
- Microscopic demonstration of eggs in stool (saline or iodine mount)
- Adult worms may be passed in stool or vomitus
- Peripheral blood eosinophilia

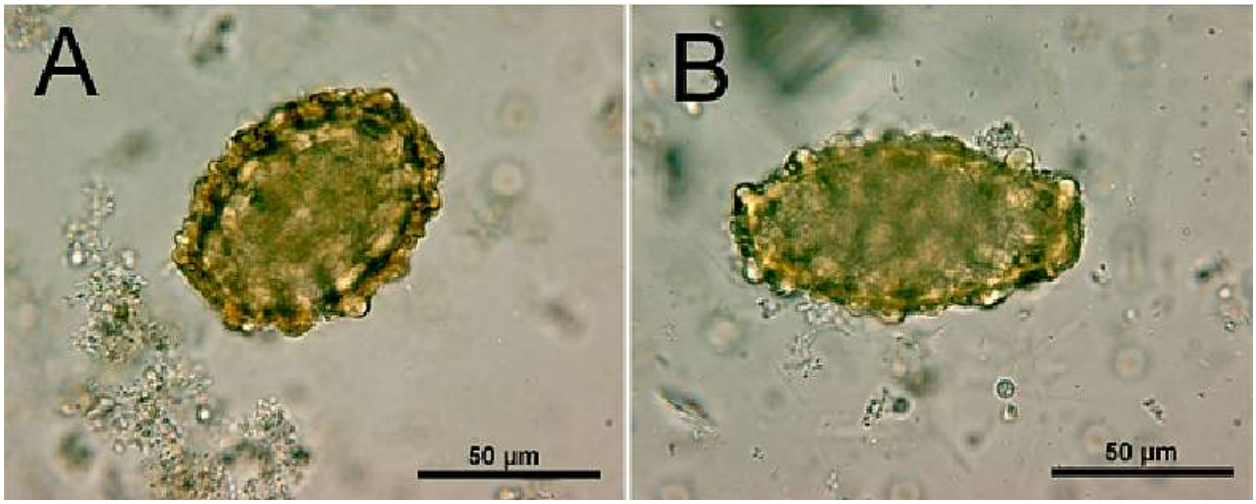
Prophylaxis and Prevention:

- Proper disposal of human feces
- Improved sanitation and safe drinking water
- Washing of vegetables thoroughly
- Personal hygiene and hand washing
- Health education

References:

1. Robbins and Cotran. Pathologic Basis of Disease. Elsevier
2. Levinson W. Review of Medical Microbiology and Immunology. McGraw-Hill
3. AFIP Laboratory Manual


DPDx
Ascaris lumbricoides
CDC
4 Ingestion of embryonated eggs
7 Larvae are coughed up and swallowed, re-entering the gastrointestinal tract. Maturation proceeds in the small intestine.
3 Embryonated egg with L3 larva
1
♀
♂
Adults in small intestine
Hatched larvae enter circulation and migrate to lungs.
6
5
Feces
Infective stage
Diagnostic stage
2 Fertilized egg
2 Unfertilized egg (will not undergo further development)



Microscopic images of A.lumbricoides eggs: fertilized (A) and non-fertilized (B)

Student Task:

Instructor's signature:_____ Dated: _____/_____/_____

Practical # 19

ANKYLOSTOMA DUODENALE

Introduction

Stool examination is an essential diagnostic procedure for detecting intestinal helminths. In hookworm infection, diagnosis is made by identifying characteristic eggs of Ancylostoma duodenale in stool under the microscope. Hookworm infection is a major cause of iron deficiency anemia, especially in tropical and subtropical regions with poor sanitation. Microscopic identification of eggs in stool remains the standard method for laboratory diagnosis.

Aim: To identify hookworm eggs (Ancylostoma duodenale / Necator americanus) in stool by microscopic examination.

Specimen: Fresh stool sample collected in a clean, dry, urine-free container.

Classification

Phylum: Nematoda

Ancylostoma duodenale: Common name: Old World hookworm

Necator americanus: Common name: New World hookworm

Disease: Hookworm disease / Hookworm infection

Habitat: Adult worms live attached to the mucosa of the small intestine of humans.

Geographical distribution: Widely distributed in tropical and subtropical regions where warm, moist soil favors larval development.

Hosts

- Definitive host: Human
- Intermediate host: None

Mode of transmission

- Infection occurs by skin penetration of infective filariform larvae present in fecally contaminated soil, commonly through bare feet.

Life cycle:

- Eggs are passed in feces
- Eggs hatch in soil to release rhabditiform larvae
- Larvae develop into infective filariform stage
- Filariform larvae penetrate skin
- Larvae migrate via bloodstream to lungs
- Ascend trachea, swallowed, and reach small intestine
- Mature into adult worms and attach to intestinal wall
- Eggs are produced and passed in feces

Morphology of hookworm egg

- Shape: Oval or elliptical
- Size: Approximately 60–65 µm × 40 µm
- Color: Colorless
- Shell: Thin, transparent hyaline shell
- Contents: Segmented ovum with 4–16 blastomeres
- Clear space present between shell and embryo

Points of Identification

- Oval, colorless egg
- Thin transparent shell
- Segmented ovum (4–16 blastomeres)
- Clear space between embryo and shell
- Eggs of Ancylostoma and Necator are morphologically indistinguishable

Pathogenesis:

- Adult worms attach to intestinal mucosa and suck blood
- Continuous blood loss leads to iron deficiency

Clinical Features:

- Ground itch at site of skin penetration
- Cough and bronchitis during larval lung migration
- Abdominal pain and diarrhea
- Weight loss
- Eosinophilia
- Microcytic hypochromic anemia

Complications:

- Severe iron deficiency anemia
- Protein-energy malnutrition
- Growth retardation in children
- Cardiac failure in severe anemia

Laboratory Diagnosis:

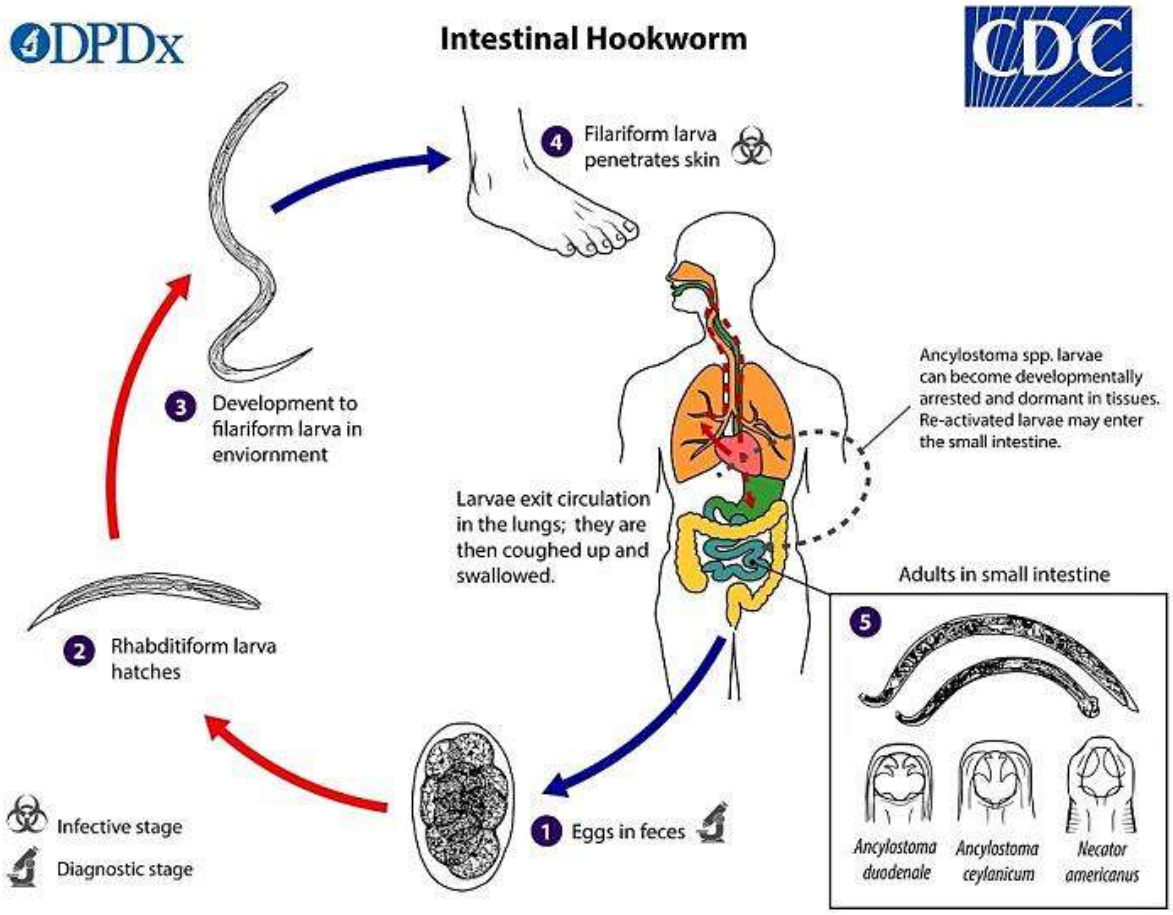
- Microscopic detection of eggs in stool (saline or iodine mount)
- Peripheral eosinophilia
- Reduced hemoglobin levels

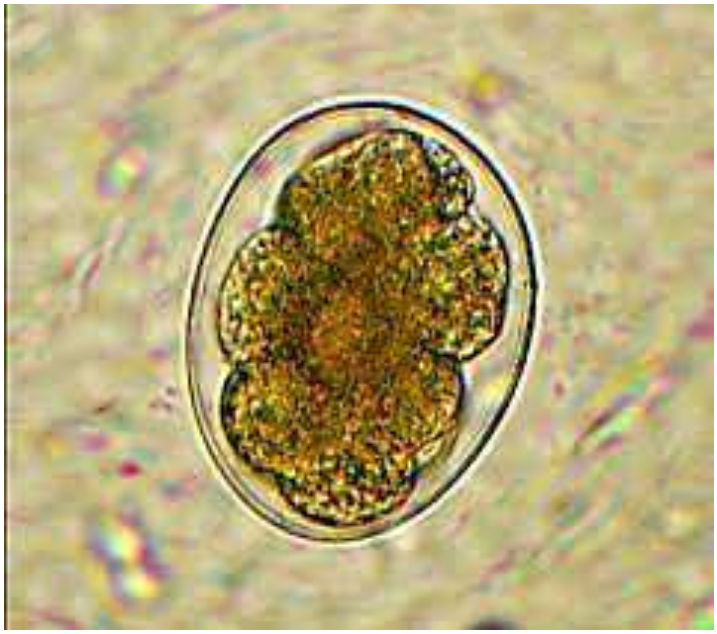
Prevention and Control:

- Wearing footwear
- Proper disposal of human feces
- Improved sanitation
- Health education

References:

1. Robbins and Cotran. Pathologic Basis of Disease. Elsevier
2. Levinson W. Review of Medical Microbiology and Immunology. McGraw-Hill
3. AFIP Laboratory Manual





Microscopic images of Hookworm

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 20

ENTROBIUS VERMICULARIS

Introduction:

Enterobius vermicularis is a common intestinal nematode causing pinworm infection, especially in children. Diagnosis is usually made by demonstrating characteristic eggs collected from the perianal region rather than routine stool examination. Rapid maturation of eggs and frequent reinfection make enterobiasis a common community infection worldwide.

Aim: To identify eggs of Enterobius vermicularis under the microscope.

Common Name:

Pinworm / Threadworm

Specimen: Perianal sample collected using transparent adhesive tape (Scotch tape method) or stool sample in selected cases.

Classification:

Phylum: Nematoda

Geographical distribution: Worldwide (cosmopolitan), more common in children, overcrowded settings, and institutionalized populations.

Habitat: Adult worms reside in the caecum, appendix, colon, and rectum of humans.

Hosts:

- Definitive host: Human
- Intermediate host: None

Mode of transmission:

- Ingestion of embryonated eggs from contaminated hands, food, or fomites
- Autoinfection by scratching perianal area and transferring eggs to mouth
- Person-to-person transmission through contaminated clothes, bedding, and surfaces
- Retroinfection may occur when larvae hatch on perianal skin and migrate back into rectum

Disease: Enterobiasis (pinworm infection)

Life cycle:

- Eggs are ingested by humans
- Larvae hatch in the small intestine
- Adult worms develop in the colon
- Gravid females migrate at night to perianal region
- Eggs are deposited on perianal skin
- Eggs become infective within 4–6 hours
- Reinfection is common

Morphology of Enterobius egg:

- Shape: Asymmetrical, ovoid
- Size: Approximately 50 µm × 20 µm
- Color: Transparent and colorless
- Shell: Thin, double-layered shell
- One side flattened (planoconvex)
- Contents: Coiled larva or granular mass

Points of Identification of Enterobius Egg:

- Asymmetrical ovoid shape
- One side flattened and the other convex
- Transparent, colorless egg
- Thin double-layered shell
- Presence of developing larva

Adult worm: Adult worms are small, white, thread-like. Females measure 8–13 mm and males 2–5 mm in length.

Clinical Features:

- Intense perianal itching, especially at night
- Disturbed sleep and insomnia
- Teeth grinding in children
- Abdominal pain
- Irritability and restlessness

Complications:

- Secondary bacterial infection due to scratching
- Vulvovaginitis in females

- Appendicitis
- Recurrent infection due to autoinfection

Laboratory Diagnosis:

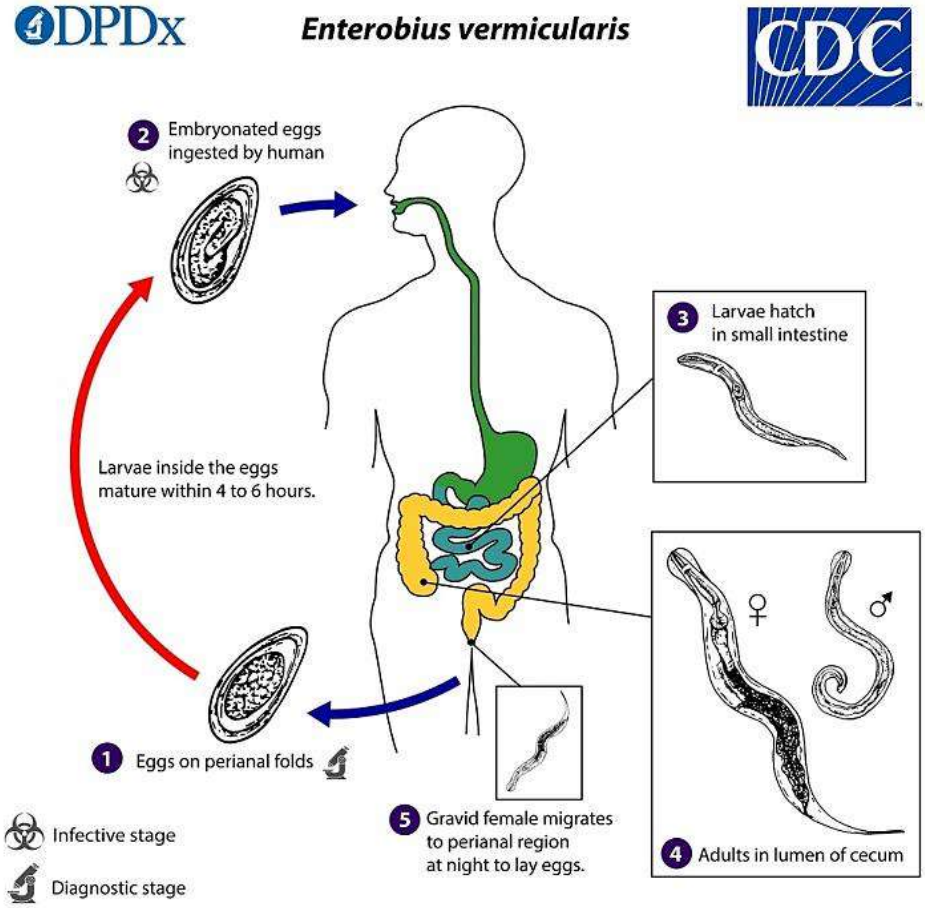
- Scotch tape method showing characteristic eggs
- Adult worms may be seen in diapers or perianal region
- Stool examination usually negative

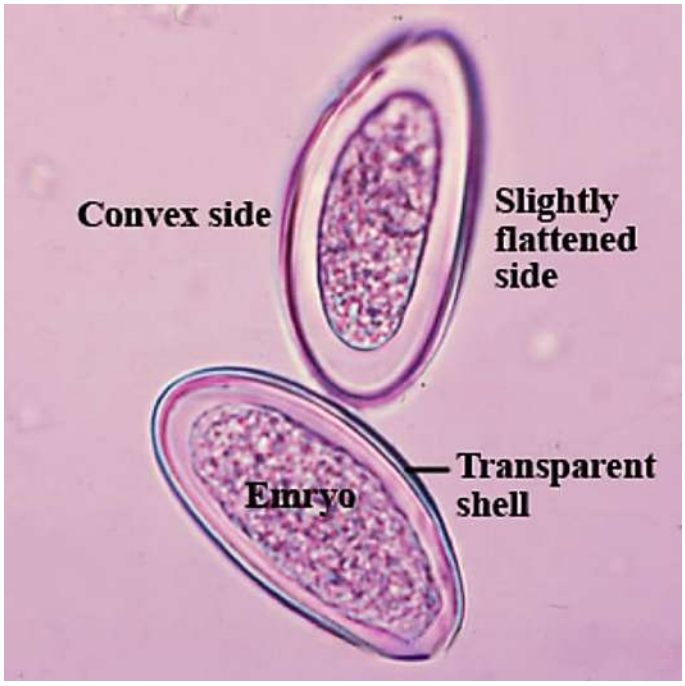
Prevention and Control

- Proper hand hygiene
- Regular washing of clothes and bed linen
- Trimming of fingernails
- Simultaneous treatment of family members

References:

1. Robbins and Cotran. Pathologic Basis of Disease. Elsevier
2. Levinson W. Review of Medical Microbiology and Immunology. McGraw-Hill
3. AFIP Laboratory Manual





Enterobius Egg

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 21

TAENIA SOLIUM AND TAENIA SAGINATA

Introduction:

Stool examination is an important laboratory investigation for the diagnosis of intestinal cestode infections. Taenia saginata and Taenia solium are common tapeworms infecting humans. In routine parasitology, Taenia infection is identified by demonstrating characteristic eggs or gravid proglottids in stool under the microscope. Although the eggs of both species are morphologically identical, correct identification is important because Taenia solium can cause cysticercosis, a serious tissue infection.

Aim: To identify eggs of Taenia saginata / Taenia solium in stool by microscopic examination.

Specimen: Fresh stool sample collected in a clean, dry, urine-free container.

Common names:

- Taenia saginata – Beef tapeworm
- Taenia solium – Pork tapeworm

Classification:

- Phylum: Platyhelminthes
- Class: Cestoda

Habitat: Adult worms reside in the small intestine of humans.

Geographical distribution: Worldwide; endemic in parts of Asia, Latin America, and Europe.

Hosts:

- Definitive host: Human
- Intermediate host:
    - Cattle for Taenia saginata
    - Pig for Taenia solium
- Humans may also act as intermediate hosts in Taenia solium infection.

Mode of transmission:

- Taeniasis is acquired by ingestion of raw or undercooked beef (T. saginata) or pork (T. solium) containing cysticerci
- Cysticercosis occurs by ingestion of Taenia solium eggs through fecally contaminated food or water

Diseases:

- Taeniasis
- Cysticercosis (only with Taenia solium)

Morphology of Taenia egg:

- Shape: Spherical
- Size: 30–40 µm in diameter
- Color: Yellow to brown
- Shell: Thick, dark, radially striated embryophore
- Contents: Hexacanth embryo with three pairs of hooklets
- Eggs of Taenia saginata and Taenia solium are morphologically identical

Points of Identification of Taenia Egg:

- Spherical yellow-brown egg
- Thick radially striated shell
- Presence of hexacanth embryo
- Three pairs of hooklets
- Species differentiation not possible by egg morphology

Adult worm morphology:

Taenia saginata

- Scolex with four suckers and no hooks (unarmed)
- Gravid proglottids have 15–20 uterine branches
- Adult worm length usually 5–8 m
- Causes only intestinal taeniasis

Taenia solium

- Scolex with four suckers and a double row of hooks (armed)
- Gravid proglottids have 5–10 uterine branches
- Adult worm length 2–4 m

- Causes taeniasis and cysticercosis

Life cycle:

- Eggs or gravid proglottids are passed in human feces
- Cattle or pigs ingest eggs from contaminated vegetation
- Oncospheres hatch, penetrate intestinal wall, and migrate to muscles
- Develop into cysticerci
- Humans ingest cysticerci in undercooked meat
- Cysticerci develop into adult worms in the intestine
- In Taenia solium, ingestion of eggs by humans leads to cysticercosis

Clinical Features:

- Often asymptomatic
- Abdominal discomfort
- Diarrhea or constipation
- Flatulence
- Weight loss
- Passage of proglottids

Complications:

Taenia saginata

- Intestinal obstruction (rare)
- Appendicitis
- Nutritional deficiency

Taenia solium

- Cysticercosis
- Neurocysticercosis causing seizures
- Raised intracranial pressure
- Visual impairment

Laboratory Diagnosis:

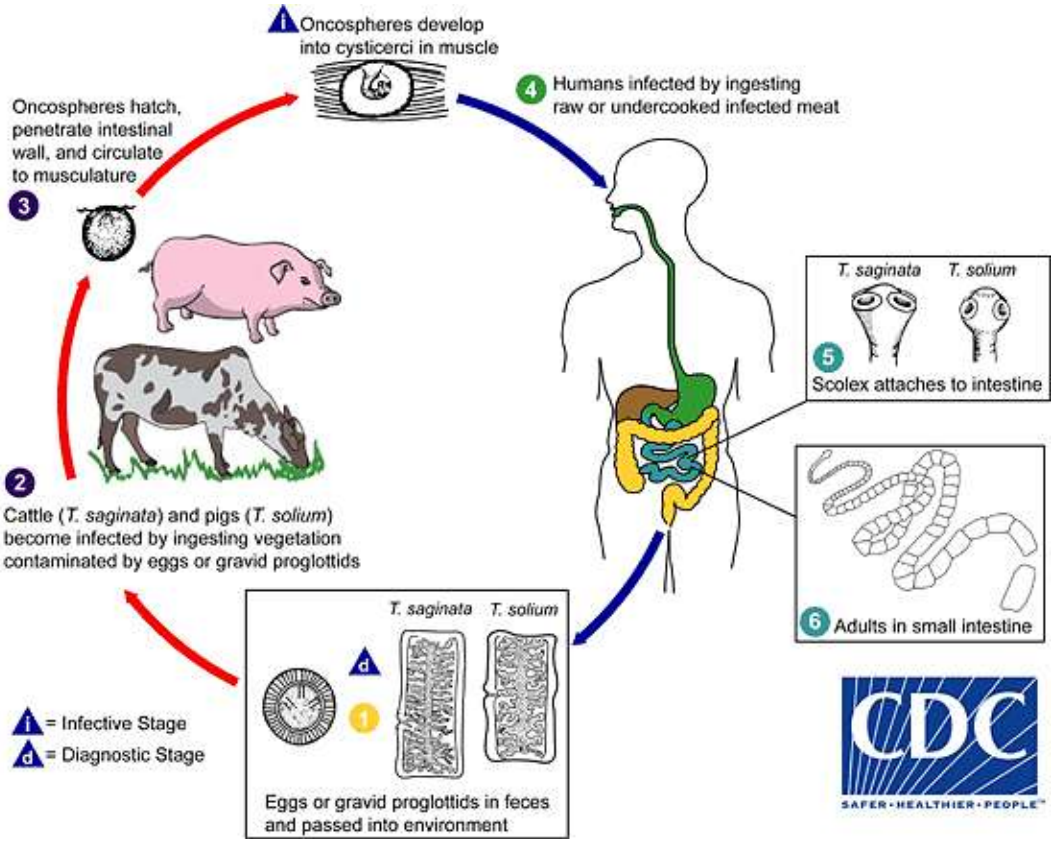
- Microscopic demonstration of Taenia eggs in stool
- Detection of gravid proglottids
- History of passage of segments

Prevention and Control:

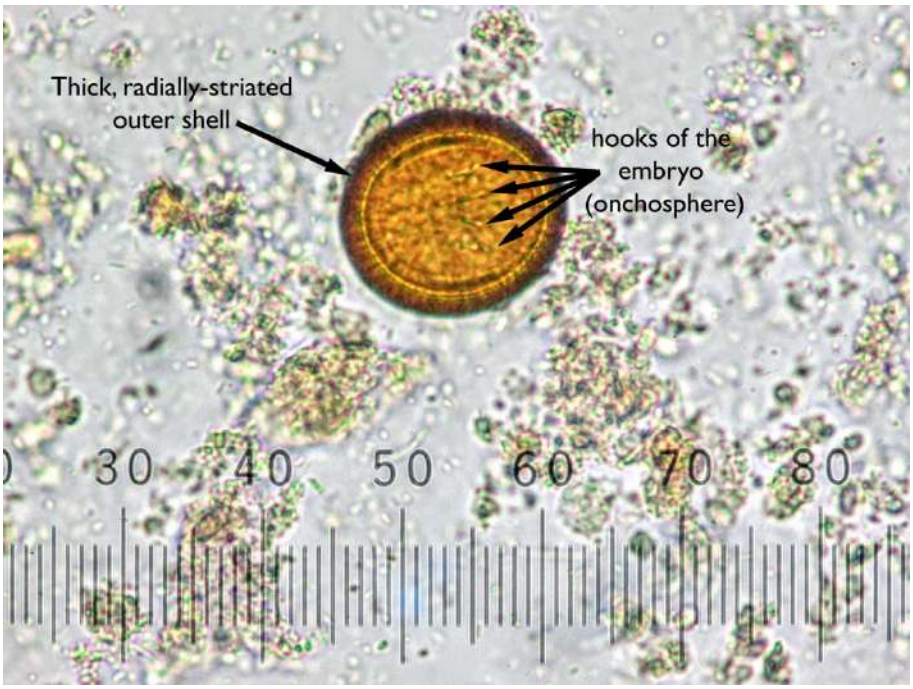
- Adequate cooking of beef and pork
- Meat inspection
- Proper disposal of human feces
- Personal hygiene and sanitation

References:

1. Robbins and Cotran. Pathologic Basis of Disease. Elsevier
2. Levinson W. Review of Medical Microbiology and Immunology. McGraw-Hill
3. AFIP Laboratory Manual



Taenia life cycle



Taenia egg under microscope

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 22

HYDATID CYST

Introduction:

Hydatid disease is a zoonotic parasitic infection caused by the larval stage of Echinococcus granulosus. The cyst is a pathological structure formed in human tissues as a result of accidental ingestion of parasite eggs. Humans act as dead-end intermediate hosts, and the disease commonly affects liver and lungs. Identification is based on the gross appearance of the cyst and its contents.

Aim: To study and identify a hydatid cyst specimen with naked eye.

Common name

- Dog tapeworm
- Hydatid tapeworm

Specimen: Hydatid cyst obtained from an affected organ, commonly liver or lung.

Classification:

- Phylum: Platyhelminthes
- Class: Cestoda

Causative organism: Echinococcus granulosus

Habitat:

- Adult worm lives in the small intestine of dogs and other canines
- Larval stage (hydatid cyst) occurs in tissues of sheep and humans

Hosts:

- Definitive host: dog
- Intermediate host: sheep
- Accidental intermediate (dead-end) host: human

Mode of transmission:

- Ingestion of eggs through food, water, or hands contaminated with dog feces
- Hand-to-mouth transmission after contact with infected dogs

Disease:

- Hydatid cyst disease (echinococcosis)

Morphology of Hydatid cyst (gross)

- *Size:* Small (under 5 cm) to "giant" (>10 cm or even several liters)
- *Shape:* Typically spherical or ovoid
- *Color:* Usually pearly white, grayish, or yellowish
- *Layers*:
    - Outer Layer: A firm, fibrous, and opaque outer wall called the pericyst
    - Middle Layer: A white, elastic, laminated, and avascular middle layer (ectocyst/laminated membrane)
    - Inner Layer: A thin, delicate, transparent, germinal inner layer (endocyst)
- *Contents:* Clear, colorless, watery fluid (hydatid fluid) inside the cyst
- *Daughter Cysts:* Smaller, spherical secondary cysts sometimes found floating freely within the main cyst fluid or attached to the germinal layer
- *Hydatid Sand:* A fine, gritty sediment visible at the bottom of a jar (if the specimen is in fluid), consisting of free protoscolices and hooklets

Points of identification of hydatid cyst specimen

- Large fluid-filled cyst
- Thick laminated outer wall
- Inner germinal layer
- Daughter cysts present inside the parent cyst
- Hydatid sand seen as fine granular sediment
- Usually obtained from liver or lung

Life cycle:

- Dogs ingest organs of sheep containing hydatid cysts
- Adult worms develop in dog intestine and pass eggs in feces
- Sheep and humans ingest eggs from contaminated environment
- Eggs release larvae that migrate to organs and form hydatid cysts
- Life cycle is completed only in dogs

Pathogenesis:

- Hydatid cyst acts as a space-occupying lesion
- Pressure effects depend on organ involved
- Rupture of cyst may release antigens causing severe allergic reactions

Clinical features:

- Often asymptomatic for years
- Liver involvement: abdominal pain, hepatomegaly
- Lung involvement: cough, chest pain, breathlessness

Complications:

- Rupture of cyst leading to anaphylactic shock
- Secondary infection of cyst
- Compression of vital organs
- Spread of daughter cysts

Laboratory diagnosis:

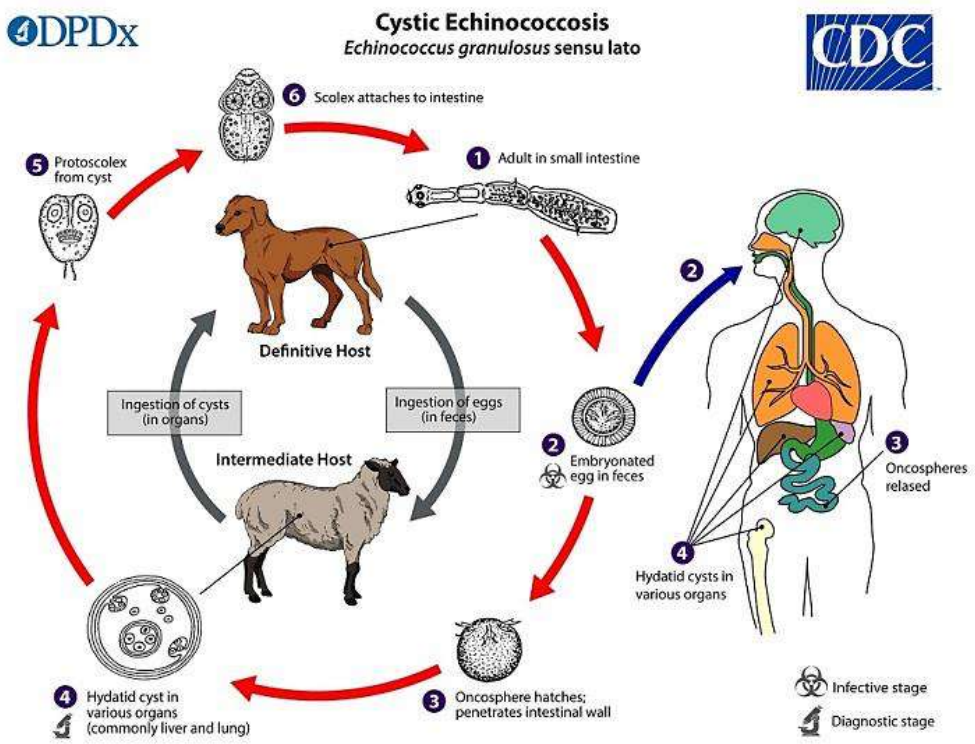
- Eggs are not found in human stool
- Imaging techniques such as ultrasound, CT scan
- Serological tests including ELISA and indirect hemagglutination
- Demonstration of hydatid sand in aspirated fluid

Prevention and Control:

- Proper disposal of sheep viscera
- Prevent dogs from eating raw offal
- Regular deworming of dogs
- Personal hygiene and hand washing

References:

1. Robbins and Cotran. Pathologic Basis of Disease. Elsevier
2. Levinson W. Review of Medical Microbiology and Immunology. McGraw-Hill
3. AFIP Laboratory Manual



Hydatid Cyst

Student Task:

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Practical # 23

PLEOMORPHIC ADENOMA

Introduction to Neoplasia:

Neoplasia is defined as an abnormal mass of tissue formed due to uncontrolled, excessive, and uncoordinated cell proliferation that persists even after removal of the stimulus.

Benign tumors

- Slow growing
- Well circumscribed or encapsulated
- Do not invade surrounding tissues
- Do not metastasize
- Cells resemble normal tissue

Malignant tumors

- Rapid growth
- Poorly circumscribed and infiltrative
- Invade surrounding tissues
- Can metastasize
- Cellular atypia and mitoses present

Pleomorphic Adenoma:

Pleomorphic adenoma is the most common benign salivary gland tumor. It is classically called a mixed tumor because it shows epithelial and mesenchymal-like components, although it arises from a single germ layer (ectoderm). More than one neoplastic cell type—mixed tumors, usually derived from one germ cell layer.

Common site

- Parotid gland – most common
- Submandibular gland
- Minor salivary glands, especially palate

Nature of tumor

- Benign salivary gland neoplasm
- Mixed tumor with epithelial and myoepithelial differentiation
- Single cell origin from ductal reserve cells or myoepithelial cells

Morphology:

Gross features

- Well-circumscribed, rounded mass
- Usually less than 6 cm
- Cut surface is gray-white
- Myxoid, cartilaginous, or translucent areas present

Histological features

- Marked morphological heterogeneity
- Mixture of epithelial and stromal components
- Duct-like structures lined by cuboidal or columnar cells
- Myoepithelial cells arranged in sheets or strands
- Abundant myxoid, chondroid, or hyaline stroma
- Islands of cartilage-like tissue
- No cellular atypia or significant mitotic activity

Point of identification

1. Mixed appearance with epithelial ducts and myxoid/chondroid stroma
2. Presence of ductal and myoepithelial cells
3. Cartilage-like areas within tumor
4. Well circumscribed benign pattern

Clinical features

- Painless, slow-growing swelling
- Firm, mobile mass
- Usually asymptomatic

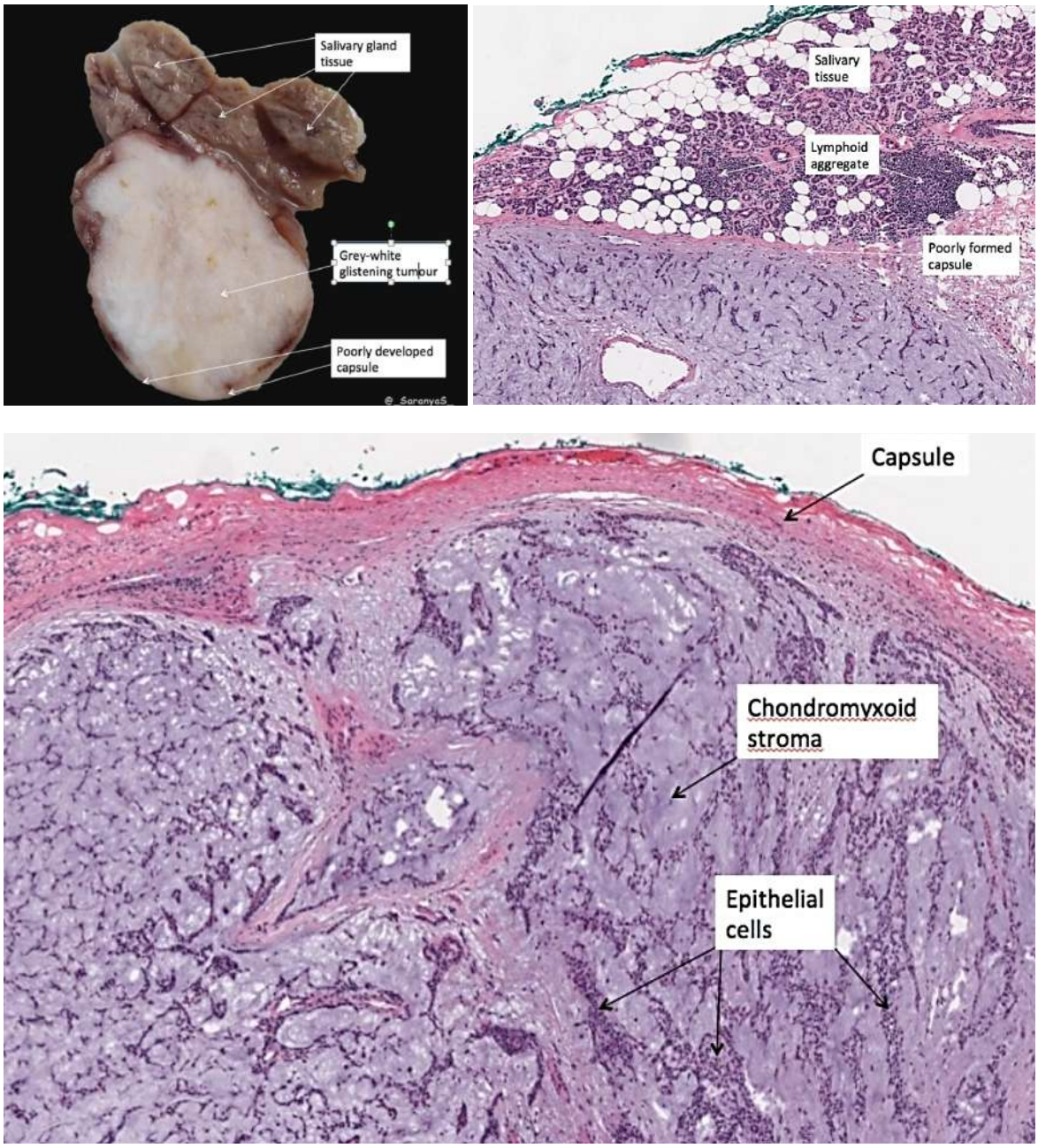
Behavior and complications

- Recurrence if inadequately excised
- Higher recurrence after enucleation
- Malignant transformation risk increases with long-standing lesions
- Can transform into carcinoma ex pleomorphic adenoma

Treatment: Complete surgical excision with margin

References:

- Robbins and Cotran pathologic basis of disease
- Shafer's textbook of oral pathology
- Neville et al. oral and maxillofacial pathology



Pleomorphic Adenoma Macroscopic and Microscopic images (H&E stain)

Student Task:

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Practical # 24

SQUAMOUS CELL CARCINOMA

Introduction:

Squamous cell carcinoma is a malignant epithelial tumor arising from stratified squamous epithelium. It is the most common malignancy of the oral cavity and head and neck region.

General characteristics:

- Malignant tumor of squamous epithelial origin
- Locally invasive with potential for metastasis
- Commonly associated with tobacco, alcohol, betel quid use, and HPV infection
- Shows variable differentiation with keratin production

Common sites in oral cavity:

- Tongue (ventral surface)
- Floor of mouth
- Buccal mucosa
- Gingiva
- Lower lip

Morphology:

Gross features:

- Ulcerative or exophytic growth
- Irregular, indurated margins
- Non-healing ulcer
- May show leukoplakia or erythroplakia in surrounding mucosa

Microscopic features:

- Malignant squamous epithelial cells invading underlying connective tissue
- Loss of normal epithelial architecture
- Islands, nests, and cords of tumor cells in stroma
- Cellular pleomorphism and hyperchromatism
- Increased nuclear-cytoplasmic ratio
- Abnormal and frequent mitotic figures
- Individual cell keratinization
- Keratin pearl formation (concentric layers of keratinized cells) in well-differentiated scc

Histological Grade:

- Well-differentiated – prominent keratin pearls
- Moderately differentiated – reduced keratinization
- Poorly differentiated – minimal or absent keratinization
- Undifferentiated

Point of identification:

- Invasion of malignant squamous cells into connective tissue
- Presence of keratin pearls
- Cellular pleomorphism and hyperchromatic nuclei
- Abnormal mitoses
- Loss of basement membrane continuity

Biological behavior:

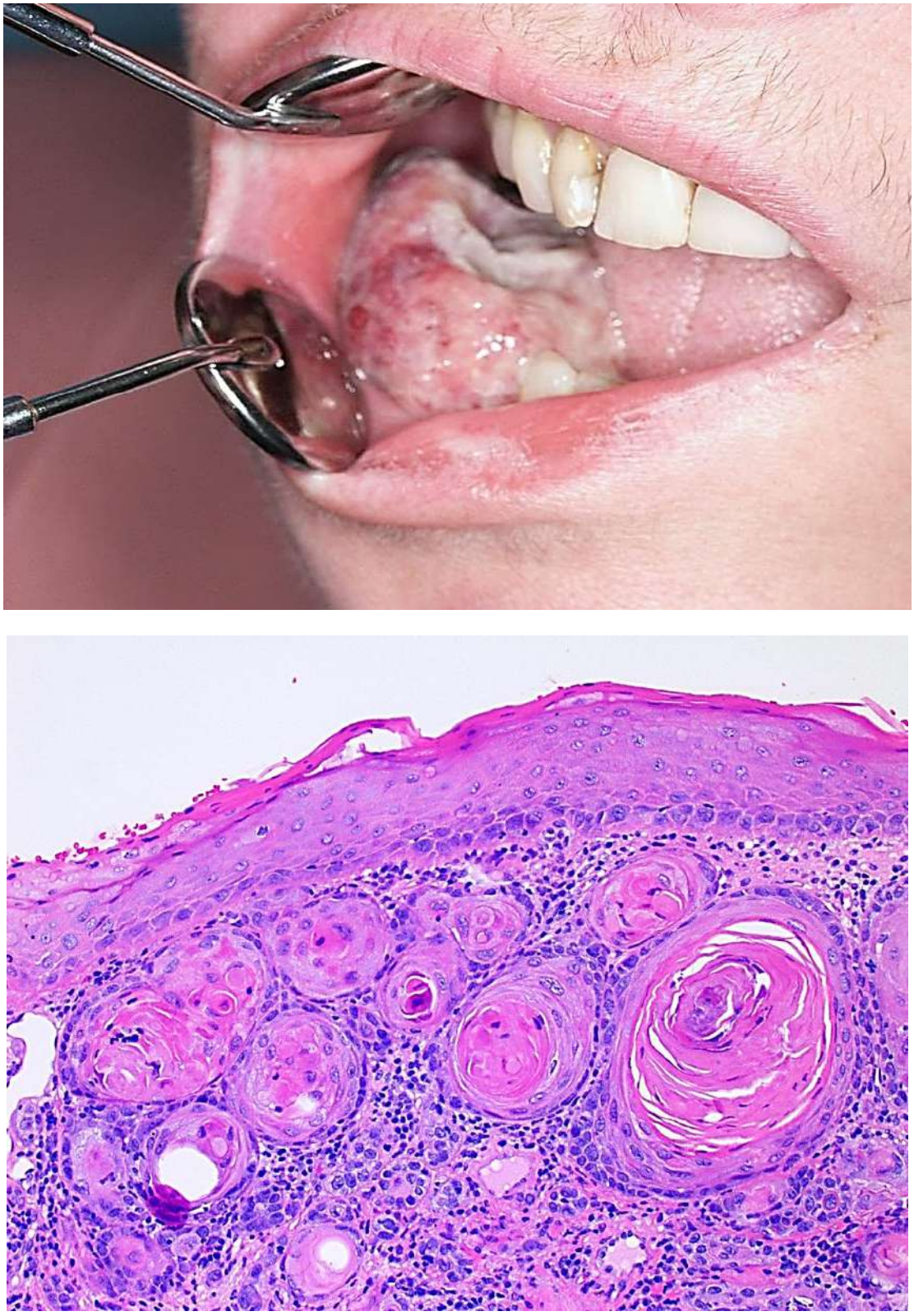
- Locally aggressive
- Spreads to cervical lymph nodes
- Distant metastasis to lungs, liver, bone in advanced cases

Prognostic factors:

- Stage at diagnosis
- Lymph node involvement
- Hpv-positive tumors have better prognosis

References:

1. Robbins and cotran pathologic basis of disease
2. Shafer's textbook of oral pathology
3. Neville et al. Oral and maxillofacial pathology



Gross and H&E Photomicrograph of Oral SCC

Student Task:

Instructor's signature:_____ **Dated: _____/_____/_____**

Practical # 25

BASAL CELL CARCINOMA

Introduction:

Basal cell carcinoma is a malignant epithelial tumor arising from the basal cells of the epidermis or follicular epithelium. It is the most common malignant tumor in humans. Although malignant, it shows slow growth, marked local invasiveness, and very rare metastasis.

General characteristics:

- Malignant tumor of basal epithelial origin
- Locally aggressive with tissue destruction
- Metastasis is exceptionally rare
- Strongly related to chronic ultraviolet radiation exposure
- Commonly occurs on sun-exposed skin of head and neck
- May be associated with genetic syndromes such as gorlin syndrome

Common sites:

- Face (nose, eyelids, nasolabial folds)
- Scalp
- Neck
- Other sun-exposed areas

Morphology:

Gross features

- Small nodular, pearly lesion
- May show central ulceration with rolled borders (rodent ulcer)
- Surface may appear smooth, shiny, or pigmented
- Neglected lesions may cause extensive local tissue destruction

Microscopic features (H&E slide)

- Tumor composed of nests, islands, or cords of basaloid cells
- Basaloid cells have hyperchromatic nuclei and scant cytoplasm
- Peripheral palisading of nuclei at the edges of tumor nests
- Mitotic figures may be present but pleomorphism is usually mild
- Tumor cells resemble basal layer of epidermis
- Absence of keratin pearl formation (helps differentiate from squamous cell carcinoma)

Point of identification:

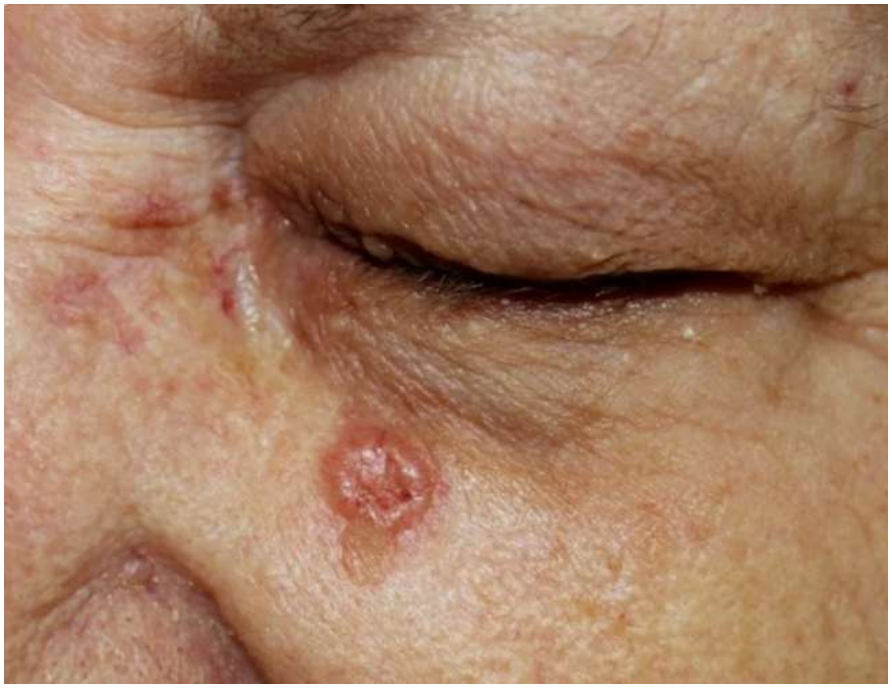
- Nests of basaloid cells
- Peripheral nuclear palisading
- Retraction clefts between tumor and stroma
- Fibromyxoid stromal background
- Absence of keratin pearls

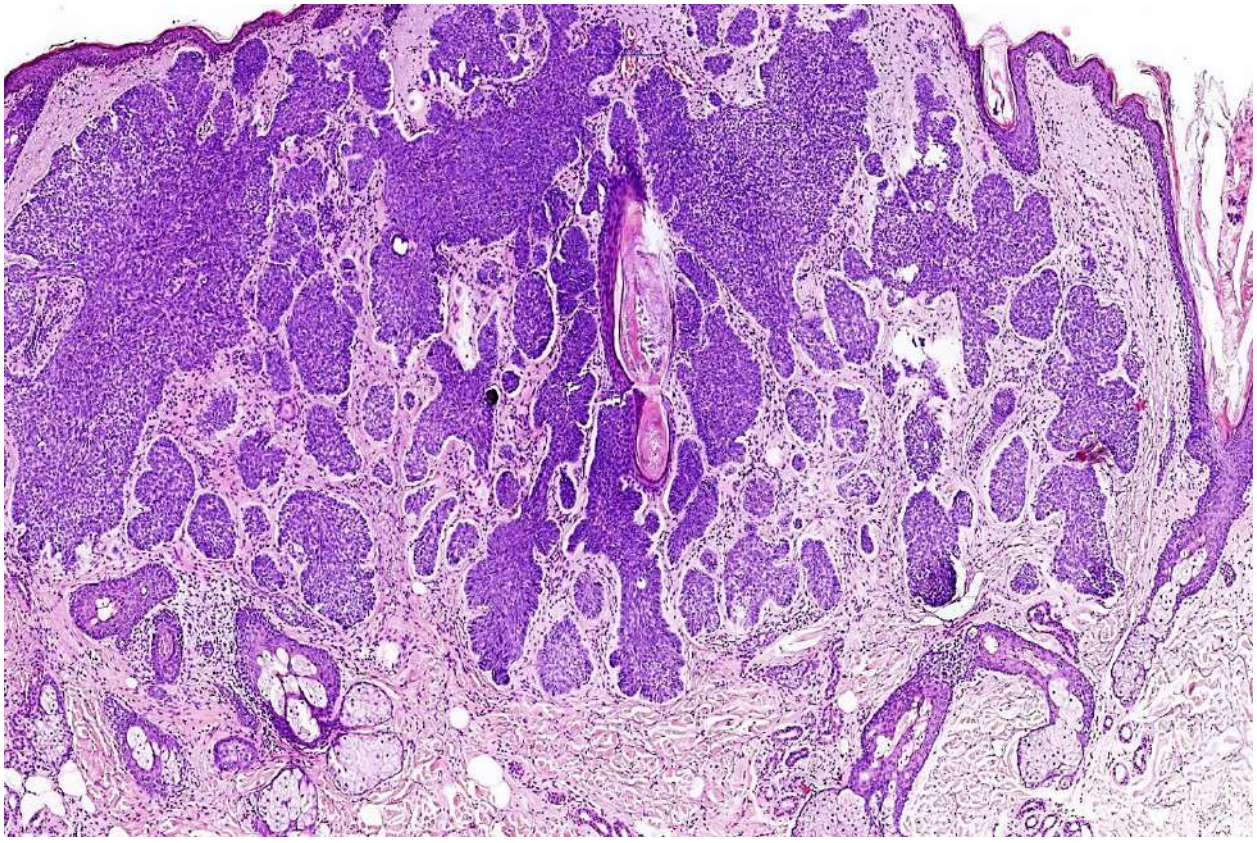
Behavior and Prognosis:

- Slow growing but locally destructive
- Recurrence possible if incompletely excised
- Excellent prognosis with proper treatment

References

1. Robbins and cotran pathologic basis of disease
2. Shafer's textbook of oral pathology
3. Neville et al. Oral and maxillofacial pathology





Clinical and H&E photomicrograph of BCC

Student Task:

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Practical # 26

LIPID PROFILE

Introduction:

Lipids are hydrophobic organic compounds essential for energy storage, cell membrane structure, and hormone synthesis. In blood, lipids circulate as lipoproteins. Abnormal lipid levels play a major role in atherosclerosis and cardiovascular disease.

Main lipids assessed in blood:

- Total cholesterol (TC)
- Triglycerides (TG)
- High-density lipoprotein cholesterol (HDL-C)
- Low-density lipoprotein cholesterol (LDL-C)

Sample collection:

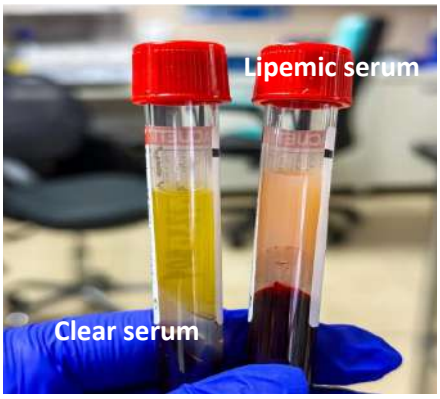
- Sample type: Venous blood
- Option 1: Serum (Most Common for Lipid Panels)
    - Vacutainer: Plain Red-Top (no additive) or SST (Gold/Tiger Top with gel and clot activator).
    - Process: Allow blood to clot (around 30 mins), then centrifuge to separate serum.
- Option 2: Plasma (For Faster Turnaround)
    - Vacutainer: Light Green Top (Lithium Heparin with gel separator).
    - Process: Centrifuge immediately to obtain plasma (no clotting needed).

Patient Preparation and Precautions

- Fasting required: 8–12 hours
- Patient should avoid fatty meals and alcohol before test
- Only water allowed during fasting
- Avoid vigorous exercise before sample collection

Why fasting is required

- Post-meal chylomicrons increase triglycerides
- Non-fasting sample may appear lipemic
- Fasting ensures true baseline lipid levels



Normal serum appearance

- Clear and transparent
- Pale yellow
- No turbidity

Lipemic serum

- Appears milky, cloudy, or creamy after centrifugation
- Due to high triglyceride levels (chylomicrons/vldl)
- Commonly seen after fatty meals or in hypertriglyceridemia

Common causes of lipemic serum

- Non-fasting sample
- Uncontrolled diabetes mellitus
- Alcohol abuse
- Familial hyperlipidemia
- Pancreatitis

Machine: Chemistry Analyzer

Parameters Assessed in Lipid Profile and Reference Values (Fasting)

- Total cholesterol: 125–200 mg/dl
- Triglycerides: 40–150 mg/dl
- HDL-C: 40–59 mg/dl
- LDL-C: 50–129 mg/dl

Interpretation:

- **Total Cholesterol:** Desirable <200 mg/dL Target
- **Triglycerides:** Desirable <150 mg/dL Target
- **HDL-C (Good Cholesterol):** Higher HDL (>=60 mg/dL) is considered protective
    - <40 mg/dL (men) or <50 mg/dL (women) are risk factors
- **LDL-C (Bad Cholesterol):** <100 mg/dL is optimal
    - <70 mg/dL for very high-risk individuals (like those with diabetes or heart disease)

Uses of lipid profile

- Assessment of cardiovascular risk
- Diagnosis of hyperlipidemia
- Monitoring therapy (statins, lifestyle changes)
- Evaluation of metabolic disorders

Risk

- High total cholesterol / LDL-C: Increased risk of atherosclerosis and coronary artery disease
- Low HDL-C: Increased cardiovascular risk
- High Triglycerides: Risk of Pancreatitis and Metabolic syndrome

References

- Robbins and cotran pathologic basis of disease
- Harper's illustrated biochemistry
- Teitz fundamentals of clinical chemistry

Parameter	Value Range mg/dl	Risk Category	Interpretation
Total Cholesterol	<200	Desirable	Total cholesterol is within normal limits; low risk of atherosclerosis.
	200–239	Borderline High	Mildly increased cholesterol; moderate cardiovascular risk.
	≥240	High Risk	Markedly increased cholesterol indicating high risk of coronary artery disease.
Triglycerides	<150	Normal	Triglyceride level is normal; no immediate metabolic risk.
	150–199	Borderline High	Mild elevation suggesting early dyslipidemia.
	200–499	High	Significantly elevated; increased risk of cardiovascular disease.
	≥500	Very High	Very high triglycerides with risk of acute pancreatitis.
HDL-C	≥60	Protective	High HDL provides protective effect against heart disease.
	<40 (men)	Risk	Low HDL increases risk of atherosclerosis.
	<50 (women)	Risk	Low HDL is a cardiovascular risk factor.
LDL-C	<100	Optimal	LDL is optimal; low risk of plaque formation.
	100–129	Near Optimal	Acceptable LDL level; mild risk.
	130–159	Borderline High	Moderately raised LDL; increased cardiovascular risk.
	160–189	High	High LDL indicating high risk of coronary artery disease.
	≥190	Very High Risk	Very high LDL with severe risk of atherosclerosis.
LDL-C (Very High-Risk Patients)	<70	Target	Target LDL level in diabetes and established heart disease.

Student Task:

Parameter	Reference Range (mg/dl)	Desirable / Target Value	Patient Value	Risk Category	Interpretation
Total Cholesterol	125–200	<200			
Triglycerides	40–150	<150			
HDL-C	40–59	≥60 protective			
LDL-C	50–129	<100 optimal			

Instructor's signature:_____ **Dated: _____/_____/_____**

END OF PRACTICAL LOGBOOK

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