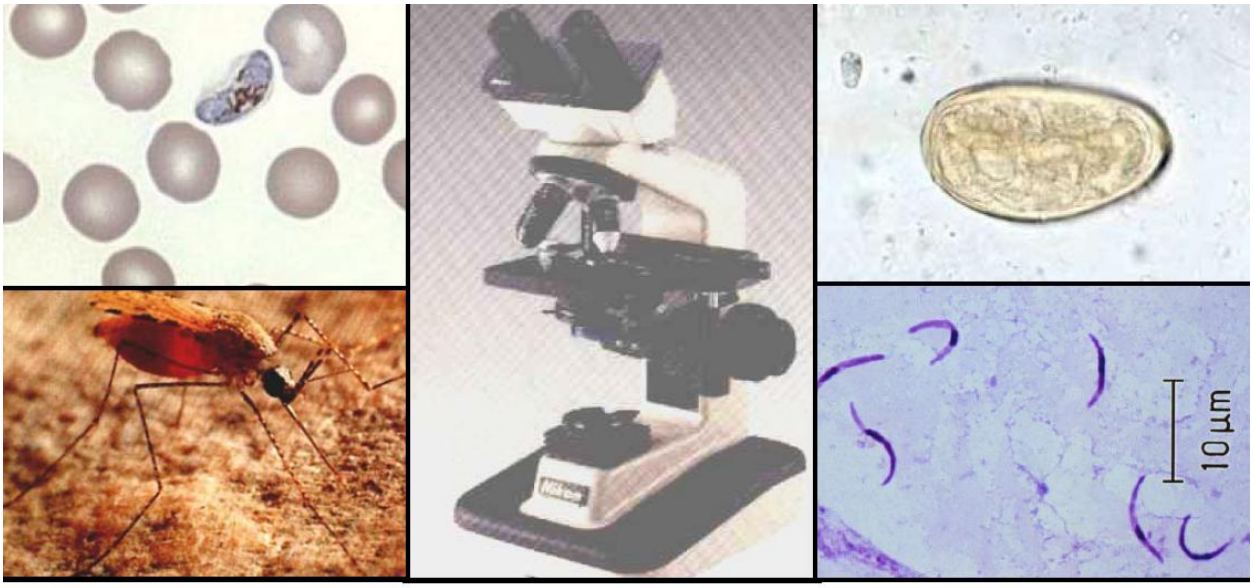
Diagnostic parasitology2



Lecture 1

Introduction to Diagnostic Medical Parasitology

Laboratory Safety – Care of the Microscope – Specimens for Parasitological Diagnosis

---

1. Introduction to Diagnostic Medical Parasitology

Diagnostic Medical Parasitology is the branch of laboratory medicine concerned with the **identification, detection, and differentiation of parasites** that infect humans. These parasites include **protozoa, helminths, and arthropods**.

The main goal of diagnostic parasitology is to:

- Identify the causative parasite
- Determine the stage of infection
- Assist clinicians in selecting appropriate treatment

- Help in epidemiological studies and disease control

Diagnosis depends on:

- Proper specimen collection
- Correct laboratory techniques
- Skilled microscopic examination
- Use of immunological and molecular methods when required

Parasitic infections are especially common in developing countries due to:

- Poor sanitation
- Contaminated water and food
- Close contact with animals
- Inadequate health education

---

2. Laboratory Safety (Handling Specimens)

All clinical specimens must be considered **potentially infectious** and handled according to biosafety guidelines.

General Laboratory Safety Rules:

- Wear personal protective equipment (PPE):
  lab coat, gloves, face mask
- Wash hands before and after handling specimens
- Never eat, drink, or smoke in the laboratory
- Avoid mouth pipetting
- Cover cuts or wounds before work
- Disinfect work surfaces before and after procedures

Handling of Parasitological Specimens:

- Specimens must be properly labeled
- Containers should be leak-proof
- Avoid aerosol formation during processing
- Dispose of contaminated materials in biohazard containers

Figure 1: Laboratory safety measures and personal protective equipment

---

3. Care of the Microscope

The microscope is the most important tool in parasitology laboratories. Proper care ensures accurate diagnosis and prolongs instrument life.

Rules for Microscope Care:

- Carry the microscope with both hands
- Clean lenses using lens paper only
- Do not touch lenses with fingers
- Do not use excessive immersion oil
- Remove oil immediately after use
- Switch off light source after finishing work
- Cover the microscope when not in use

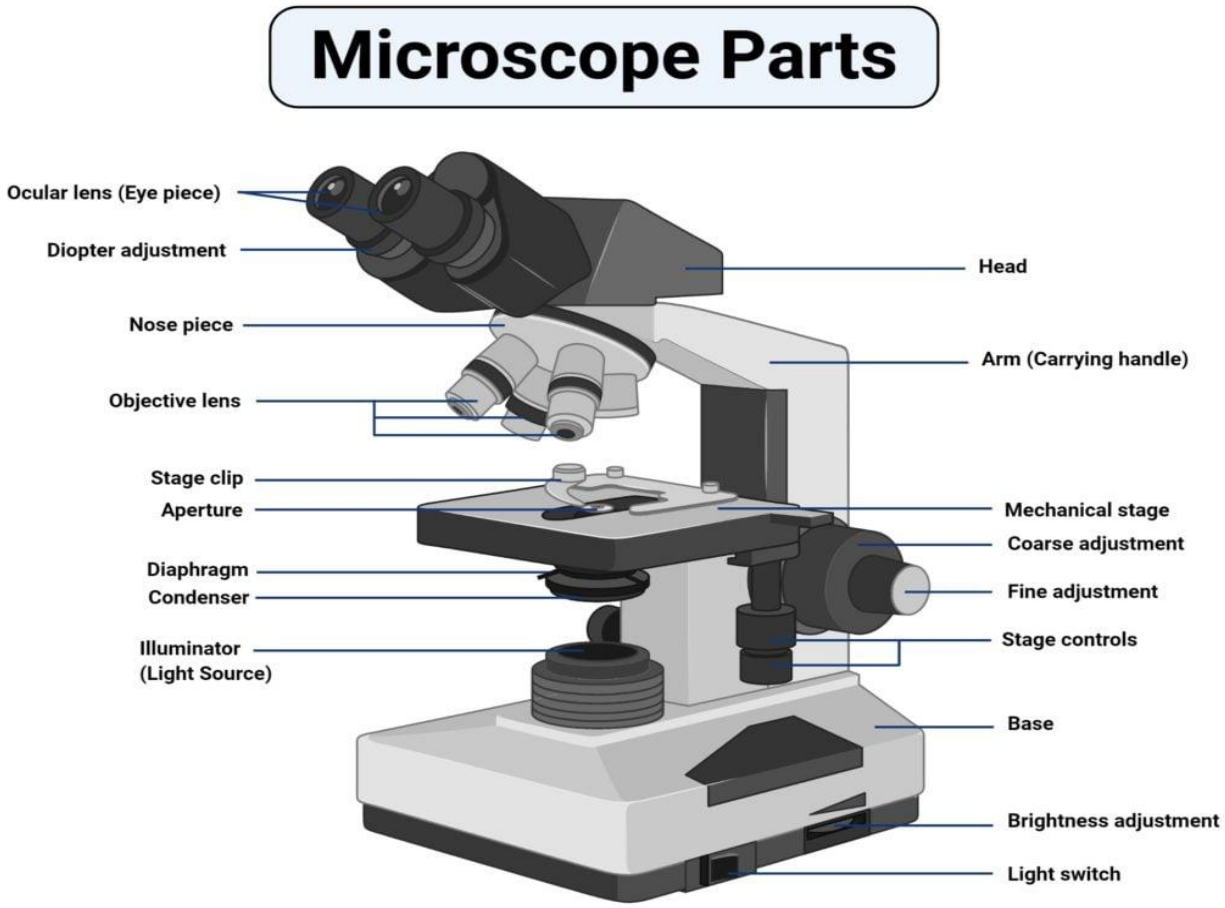


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

---

4. Samples Needed for Detection of Parasites

Different parasites require different clinical specimens depending on their habitat and life cycle.

Common Specimens Used:

1. **Stool**

   For intestinal protozoa and helminths

2. **Blood**

   For malaria, trypanosomiasis, filariasis

3. **Urine**

For *Schistosoma haematobium*

4. **Sputum**

   For *Paragonimus westermani*

5. **Tissue biopsy**

   For leishmaniasis, trichinosis

6. **Cerebrospinal fluid (CSF)**

   For *Trypanosoma brucei*

7. **Skin scraping**

   For cutaneous leishmaniasis

Figure 3: Types of clinical specimens used in parasitology

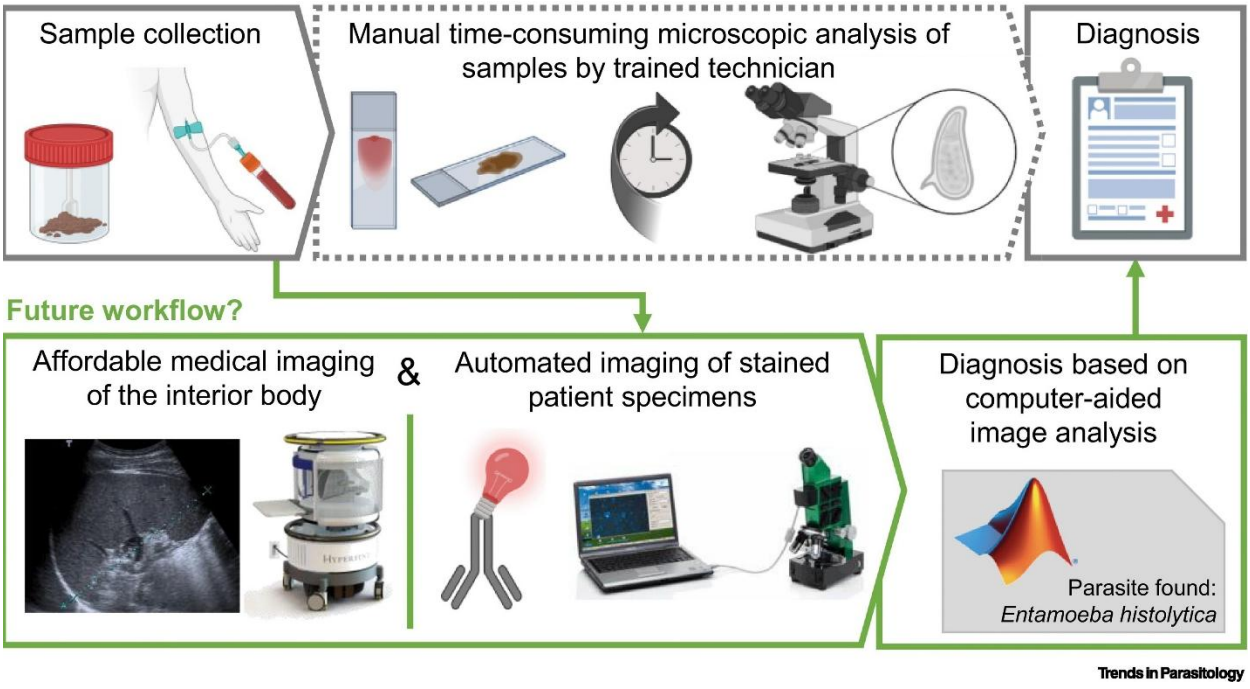
Lecture 2

Strategies for Diagnosis of Parasitic Infection

Collection and Transport of Stool Specimens

Factors Interfering with Stool Collection

Precautions During Specimen Collection



---

1. Strategies for Diagnosis of Parasitic Infection

The diagnosis of parasitic infections requires a **systematic approach** that combines clinical data with laboratory findings. No single method is sufficient for all parasites; therefore, multiple strategies are often used together.

Main Diagnostic Strategies:

A. Clinical Evaluation

- Patient history (travel, food, water, occupation)

- Symptoms such as diarrhea, anemia, fever, weight loss
- Exposure to contaminated food or water

Clinical findings alone are **not confirmatory** and must be supported by laboratory diagnosis.

B. Direct Parasitological Methods

These methods aim to **demonstrate the parasite itself**.

- Microscopic examination of specimens
- Detection of:
    - Ova
    - Larvae
    - Cysts
    - Trophozoites
    - Adult worms

This is the **most definitive method** of diagnosis.

C. Concentration Techniques

- Increase the number of parasites in the specimen
- Useful when parasites are present in low numbers
- Commonly used for stool samples

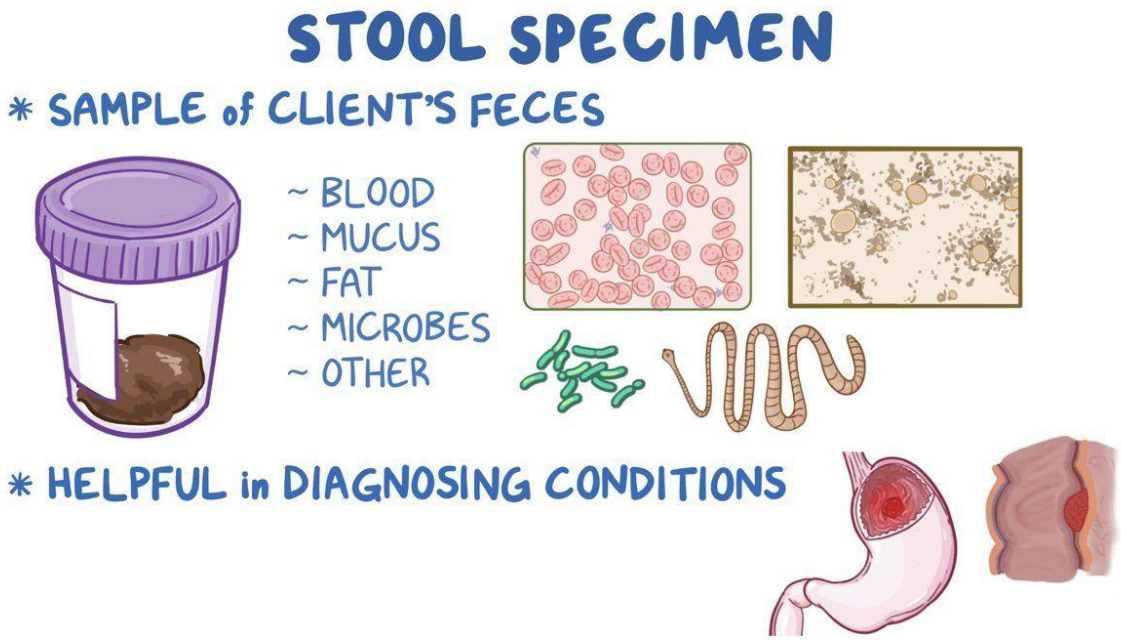
D. Immunological and Serological Methods

- Detection of antibodies or antigens
- Useful when parasites are difficult to demonstrate directly
- Examples: ELISA, rapid tests

E. Molecular Methods

- Detection of parasite DNA (PCR)
- Highly sensitive and specific
- Used mainly in research and reference laboratories

2. Collection and Transport of Stool Specimens for Enteric Pathogens



Stool examination is the **most common procedure** in diagnostic parasitology. Proper collection and transport are essential to avoid false results.

Stool Collection:

- Collect fresh stool in a **clean, dry, wide-mouthed container**
- The container must be free from:
  - Water
  - Urine
  - Disinfectants
- A portion containing mucus or blood is preferred

Time of Examination:

- Liquid stool: examine within **30 minutes**

- Formed stool: examine within **1 hour**
- Delayed examination may destroy trophozoites

Transport of Stool Specimens:

- If immediate examination is not possible:
  - Use preservatives (Formalin, PVA)
- Keep specimen at room temperature
- Avoid refrigeration unless specified

📌 Multiple stool samples (3 samples on different days) increase diagnostic accuracy.

---

3. Factors Interfering with Stool Collection

Several factors may interfere with the accurate diagnosis of intestinal parasites.

Major Interfering Factors:

1. **Drug Therapy**
    - Antibiotics
    - Antiparasitic drugs
    - Antacids
2. **Use of Laxatives or Enemas**
    - Dilutes stool
    - Washes away parasites
3. **Barium or Radiologic Contrast Media**
    - Interferes with microscopic examination
4. **Contamination**
    - Urine
    - Water
    - Soil
5. **Improper Storage**
    - Delay in examination
    - Exposure to heat or sunlight

---

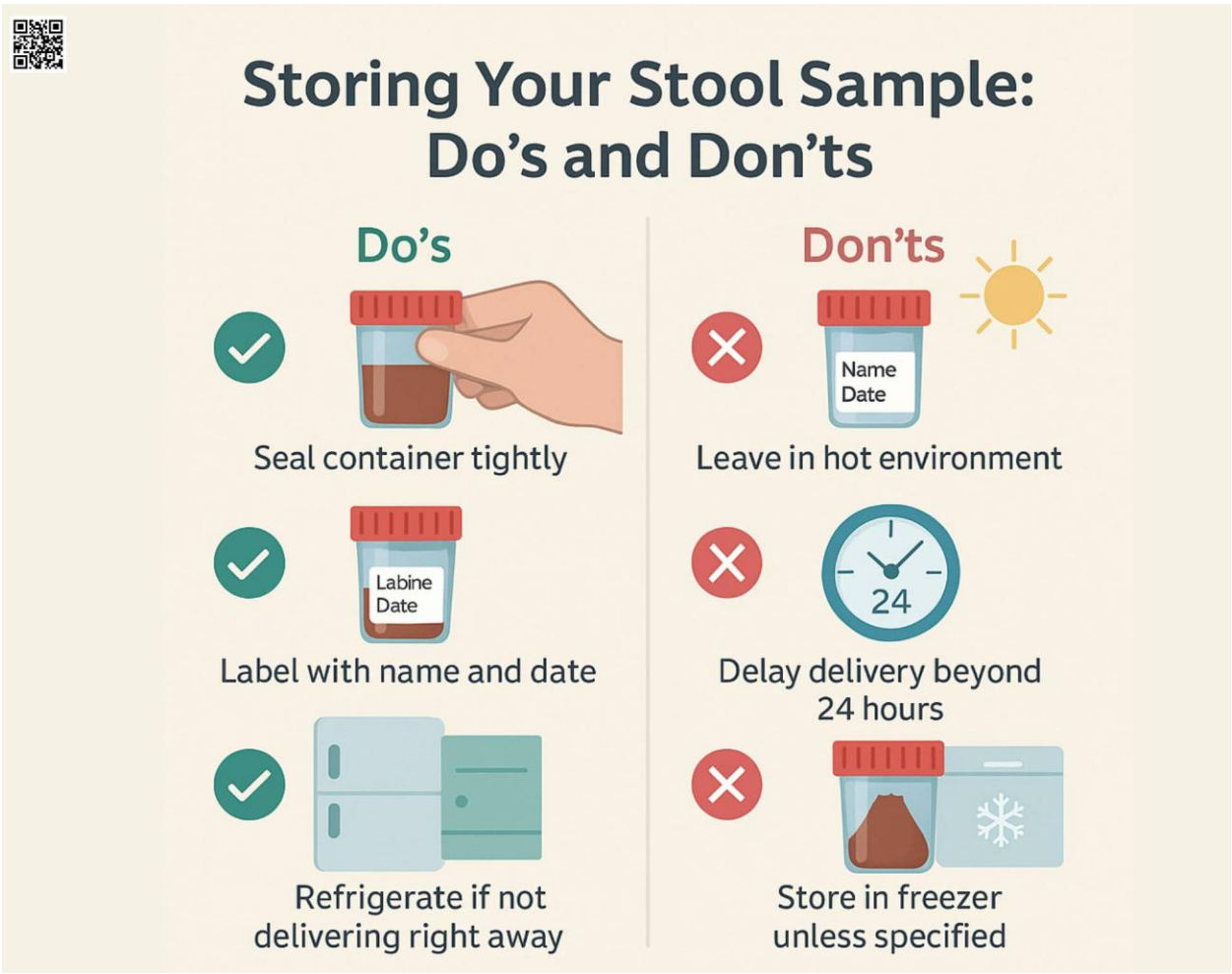
4. Precautions During Collection of Specimens

To ensure reliable laboratory results, the following precautions must be strictly followed:

Precautions:

- Collect stool before starting antiparasitic treatment
- Avoid contamination with urine or water
- Use labeled containers with patient information
- Ensure tight closure of the container
- Transport specimens promptly to the laboratory
- Use preservatives if delay is expected

Storing Your Stool Sample: Do's and Don'ts



Lecture 3

Examination of Stool Sample

Macroscopic and Microscopic Examination

Introduction

Stool examination is the **cornerstone of laboratory diagnosis** of intestinal parasitic infections. A properly examined stool sample can reveal the presence of **protozoa, helminths, ova, larvae, cysts, and trophozoites**.

Accurate diagnosis depends on:

- Proper collection
- Timely examination
- Correct examination technique
- Skilled microscopic observation

---

1. Macroscopic Examination of Stool

Macroscopic (gross) examination is the **first step** in stool analysis and provides valuable diagnostic clues even before microscopic examination.

Parameters Observed in Macroscopic Examination:

A. Consistency

- **Formed stool:** usually contains cysts and helminth eggs
- **Semi-formed stool:** may contain cysts and trophozoites
- **Watery stool:** commonly contains trophozoites

B. Color

- **Brown:** normal
- **Black:** possible bleeding (hookworm)
- **Greenish:** rapid intestinal transit
- **Pale or clay-colored:** biliary obstruction

C. Presence of Blood

- Indicates invasive intestinal parasites such as:
  - *Entamoeba histolytica*
  - *Trichuris trichiura*

D. Presence of Mucus

- Suggests intestinal irritation or infection
- Often associated with protozoal infections

E. Presence of Adult Worms or Segments

- Adult worms (e.g., *Ascaris lumbricoides*)
- Proglottids of tapeworms (*Taenia spp.*)

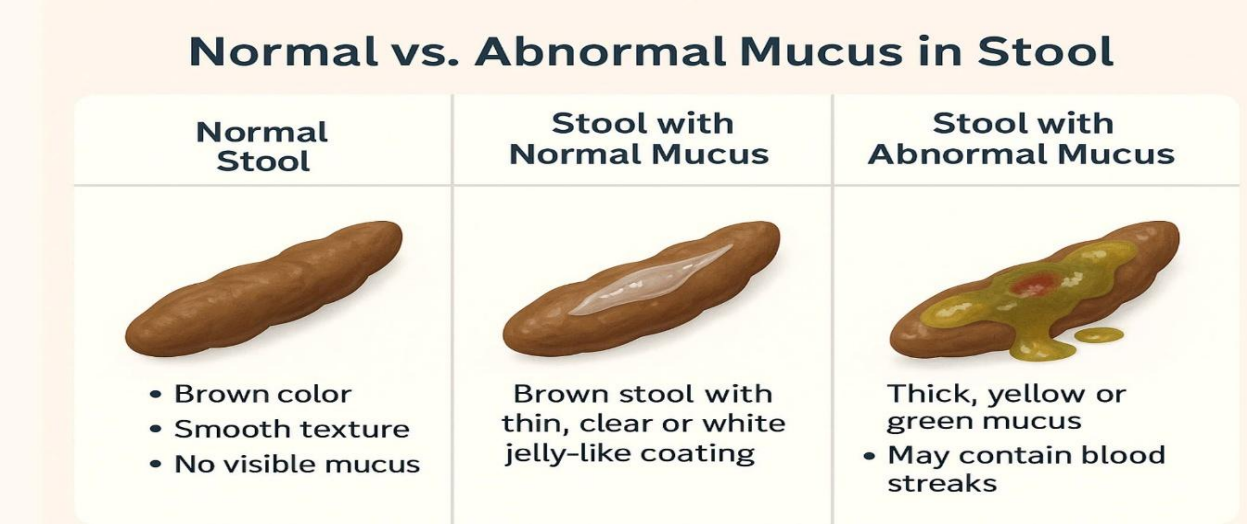


Figure 1: Macroscopic appearance of stool showing mucus and adult worms

---

2. Microscopic Examination of Stool

Microscopic examination aims to detect **microscopic stages of parasites**, which cannot be seen by naked eye.

Objectives:

- Detect ova, larvae, cysts, and trophozoites
- Identify parasite species
- Assess parasite load (semi-quantitative)

A. Direct Wet Mount Examination

This is the **simplest and most commonly used method**.

Types of Wet Mounts:

- Saline wet mount
- Iodine wet mount

(A detailed explanation of solutions will be covered in Lecture 4)

B. Procedure for Microscopic Examination

1. Place a small amount of stool on a clean glass slide
2. Add a drop of saline or iodine
3. Mix well to form a thin smear
4. Cover with a coverslip
5. Examine under microscope:
    - Low power (10×) to scan
    - High power (40×) for details

C. Findings in Microscopic Examination

Protozoa:

- **Cysts:** found in formed stool
- **Trophozoites:** found in liquid stool

Helminths:

- Eggs (ova)
- Larvae

D. Identification Criteria

Identification is based on:

- Size
- Shape
- Number of nuclei
- Internal structures
- Presence of operculum or spine (in helminth eggs)

📌 Accurate identification requires experience and repeated observation.

E. Limitations of Direct Microscopy

- Low sensitivity in light infections
- May miss parasites present in small numbers
- Requires fresh specimens for motility observation

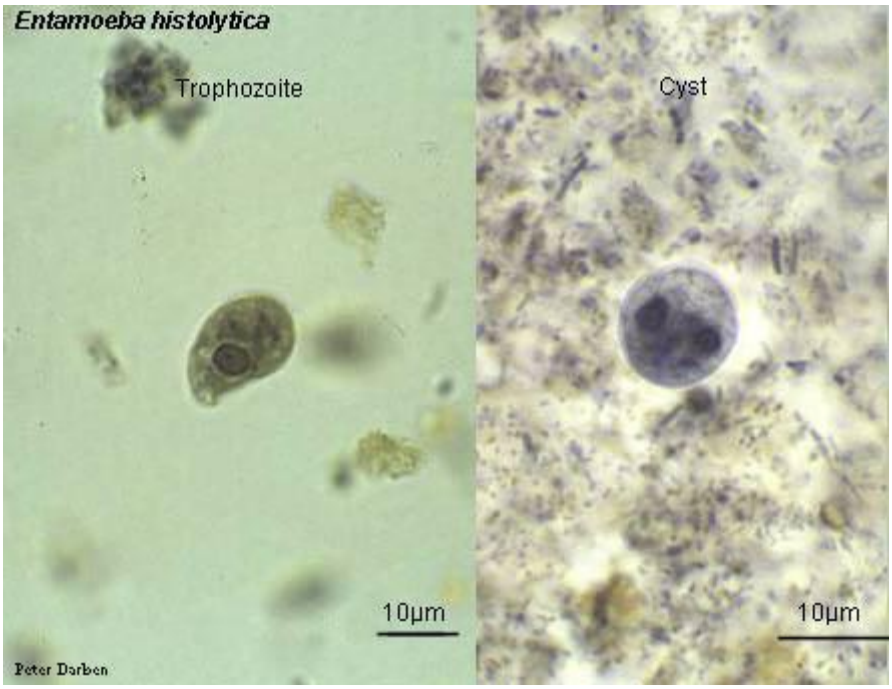


Figure : Intestinal and Luminal Protozoa

Importance of Stool Examination

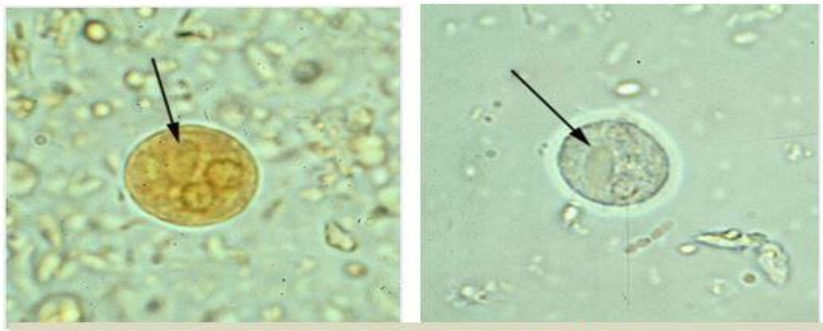
- Confirms diagnosis of intestinal parasitosis
- Guides appropriate treatment
- Helps monitor response to therapy
- Essential for epidemiological studies

Lecture 4

Preparation of Wet Mount Solutions

Saline – Iodine – Eosin Solutions

Advantages and Disadvantages of Each Solution



Cyst of E. histolytica/E. dispar in Wet Mount.
Left Side: Stained with Iodine
Right Side: Unstained preparation (Arrow- Notice the chromatoid body with blunt, rounded ends)
Image Source: DpDx

Introduction

Wet mount preparation is one of the **most important routine techniques** in diagnostic parasitology. It allows rapid detection of **protozoan trophozoites, cysts, helminth eggs, and larvae** in stool specimens.

The choice of solution used in wet mount preparation greatly affects:

- Visibility of parasites
- Preservation of morphology
- Observation of motility

---

1. Saline Wet Mount Solution

Definition

Saline wet mount uses **physiological saline (0.85% NaCl)** to examine stool samples.

Preparation of Saline Solution

- Sodium chloride (NaCl): **0.85 g**
- Distilled water: **100 mL**
- Mix well and store at room temperature

Procedure

1. Place a small amount of stool on a clean glass slide.
2. Add one drop of saline solution.
3. Mix gently to form a thin smear.
4. Cover with a coverslip.
5. Examine under microscope (10× and 40× objectives).

Advantages of Saline Wet Mount

- Preserves **motility of trophozoites**
- Maintains natural morphology of parasites
- Useful for detecting:
    - Motile trophozoites
    - Helminth eggs
    - Larvae

Disadvantages of Saline Wet Mount

- Poor visualization of internal structures
- Nuclei and cytoplasmic details are not clearly seen

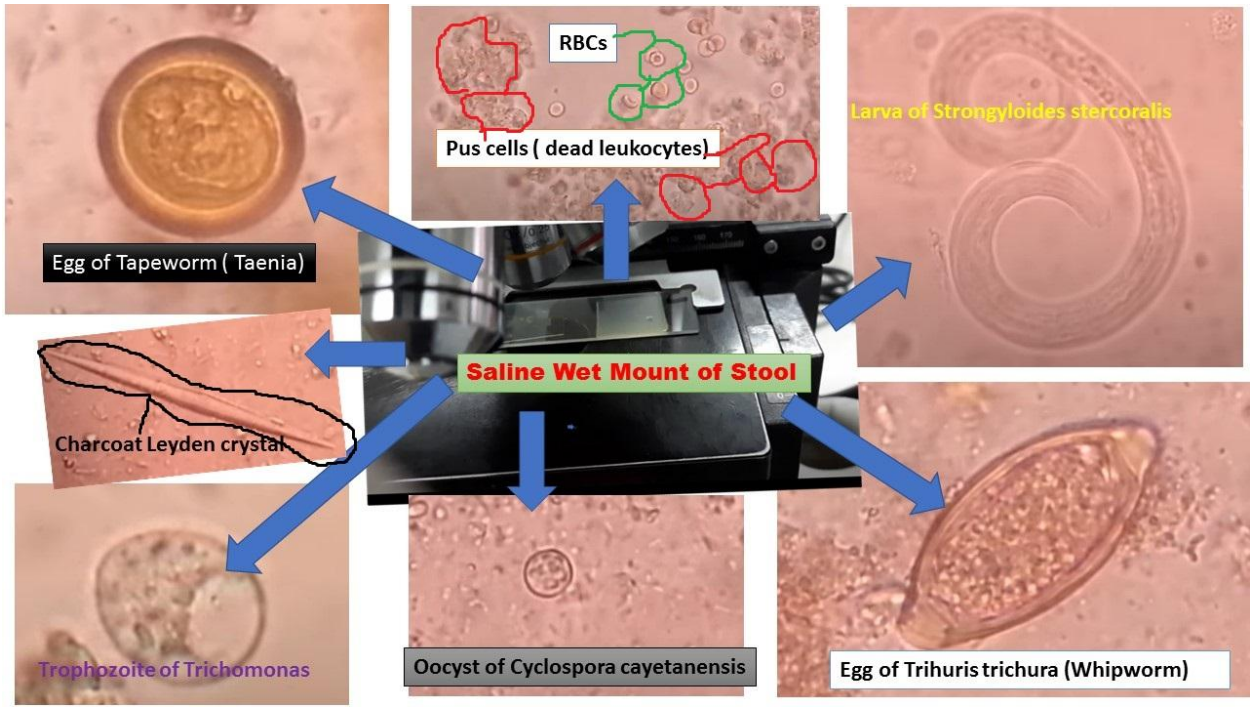


Figure : Saline Wet Mount for Stool

---

2. Iodine Wet Mount Solution

Definition

Iodine wet mount uses **diluted iodine solution** to stain stool samples, enhancing internal structures of parasites.

Preparation of Iodine Solution (Lugol's Iodine)

- Iodine: **1 g**
- Potassium iodide: **2 g**
- Distilled water: **100 mL**

(Usually diluted before use)

Procedure

1. Place stool sample on a slide.
2. Add one drop of iodine solution.
3. Mix gently.
4. Cover with a coverslip.
5. Examine microscopically.

Advantages of Iodine Wet Mount

- Enhances nuclear details
- Stains glycogen vacuoles
- Useful for identification of protozoan cysts

Disadvantages of Iodine Wet Mount

- Kills trophozoites (no motility)
- Causes shrinkage of organisms with time

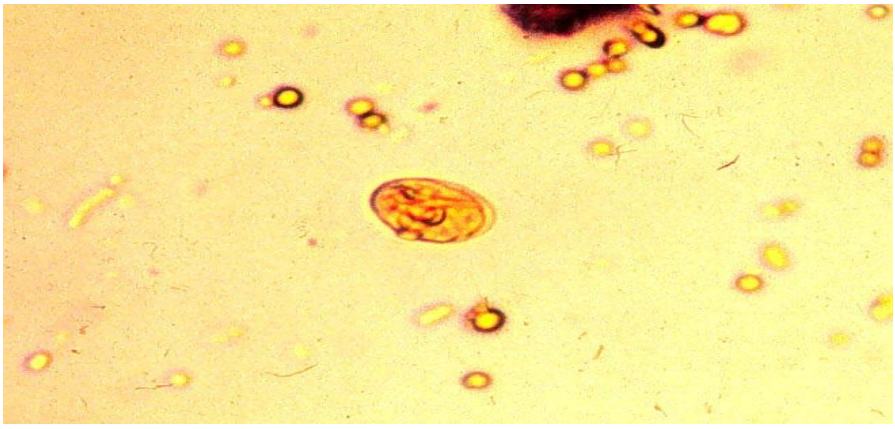
. 

Figure 2: Iodine wet mount showing protozoan CYST

---

3. Eosin Wet Mount Solution

Definition

Eosin is an acidic dye used occasionally in parasitology to differentiate **living and dead organisms**.

Preparation of Eosin Solution

- Eosin: **0.5% solution**
- Distilled water as solvent

Procedure

1. Mix stool sample with a drop of eosin.
2. Prepare wet mount.
3. Examine under microscope.

Advantages of Eosin Solution

- Dead organisms absorb eosin and appear stained
- Living organisms remain unstained

Disadvantages of Eosin Solution

- Limited use in routine diagnosis
- Poor visualization of internal structures

Best used for:
Viability assessment rather than identification.

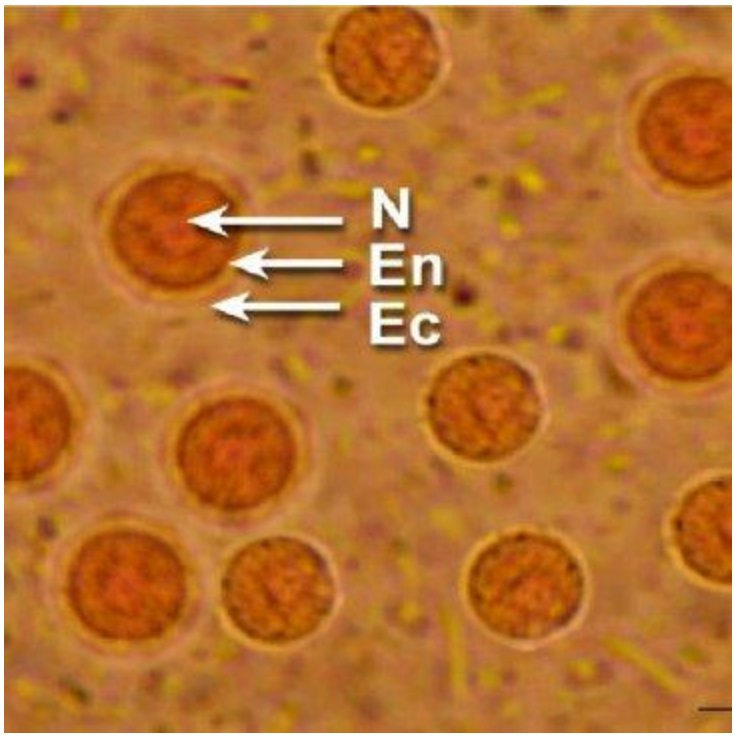
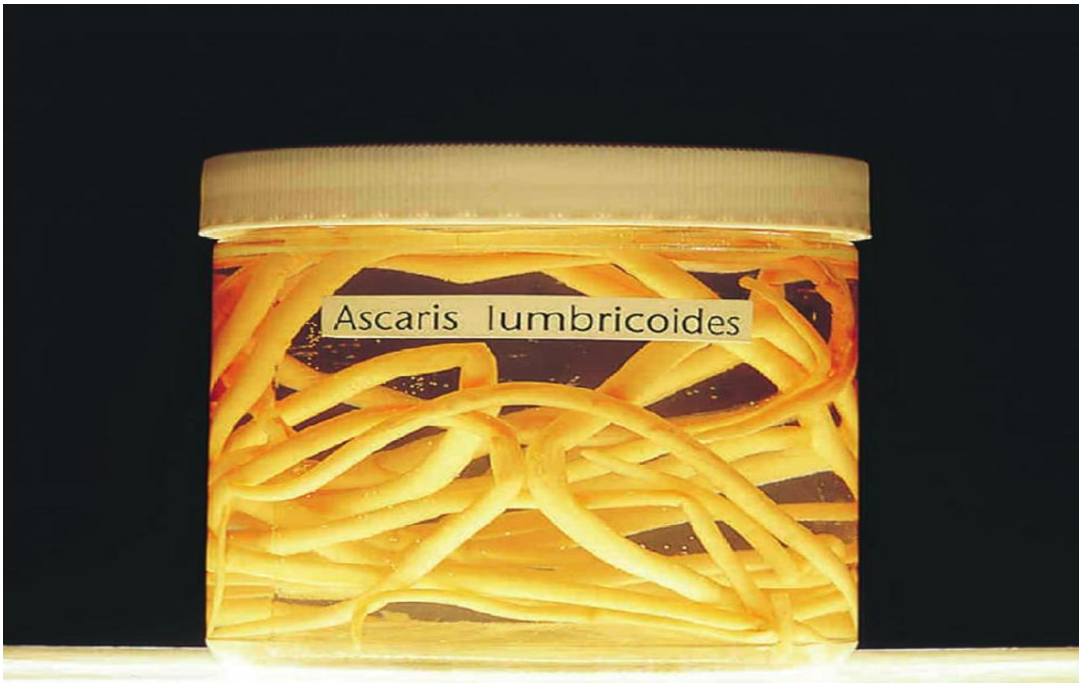


Figure 3: Stained Acanthamoeba cysts with iodine wet mount stain. The cysts appeared yellowish-brown with well-defined ectocyst (Ec) and endocyst (En) and central nucleus (N) (×400)

Lecture 5

Preparation of Preservatives and Fixatives for Stool Specimens

Formalin (5–10%) – PVA – Schaudinn's Fixative



Introduction

In diagnostic parasitology, preservatives and fixatives are used to **maintain the morphology of parasites** when immediate examination of stool specimens is not possible. They are essential for:

- Preserving protozoan cysts and trophozoites
- Maintaining helminth eggs and larvae
- Allowing delayed examination
- Preparing permanent stained smears

The choice of preservative depends on:

- Type of parasite
- Diagnostic method used
- Duration of specimen storage

1. Formalin Solution (5–10%)

Definition

Formalin is an aqueous solution of **formaldehyde** used widely as a preservative for stool specimens in parasitology laboratories.

Preparation of Formalin Solution

- Formaldehyde (37–40%):
  - 5 mL for 5% formalin
  - 10 mL for 10% formalin
- Distilled water: to make **100 mL**

Mix well in a fume hood.

Principle of Action

Formalin kills parasites while **preserving their external morphology**, especially helminth eggs and larvae.

Uses of Formalin

- Preservation of stool specimens
- Concentration techniques (Formalin–Ether method)
- Detection of helminth eggs and larvae
- Long-term storage of samples

Advantages of Formalin

- Preserves eggs and larvae very well
- Suitable for concentration methods
- Inexpensive and widely available
- Long shelf life

Disadvantages of Formalin

- Poor preservation of protozoan trophozoites
- Not suitable for permanent stained smears
- Toxic and irritating to skin and eyes

- **Best used for:**
Helminth diagnosis and concentration procedures.

---

2. PVA (Polyvinyl Alcohol) Fixative

Definition

PVA is a fixative used mainly for **preserving protozoan trophozoites and cysts** for preparation of permanent stained smears.

Composition of PVA Fixative

- Polyvinyl alcohol
- Alcohol (ethanol)
- Acetic acid
- Fixative agent

(Some formulations may contain mercury compounds)

Principle of Action

PVA fixes parasites to the glass slide and preserves **fine cytological details**, especially nuclei and cytoplasm.

Uses of PVA

- Preservation of stool specimens for:

    - Trichrome stain
    - Iron hematoxylin stain
- Permanent slide preparation
- Detailed protozoan identification

Advantages of PVA

- Excellent preservation of protozoan morphology
- Ideal for permanent staining
- Provides clear nuclear details

Disadvantages of PVA

- Not suitable for concentration techniques
- Toxic if mercury-containing
- Requires special disposal procedures

---

3. Schaudinn’s Fixative

Definition

Schaudinn’s fixative is a **strong fixative** used mainly for preserving protozoa for detailed morphological study.

Composition of Schaudinn’s Fixative

- Mercuric chloride
- Absolute alcohol
- Acetic acid

Principle of Action

This fixative rapidly penetrates cells and preserves **internal structures**, especially nuclear details.

Uses of Schaudinn's Fixative

- Fixation of protozoan trophozoites
- Preparation of permanent stained slides
- Research and reference laboratories

Advantages of Schaudinn's Fixative

- Excellent preservation of protozoan morphology
- Superior nuclear detail
- High-quality permanent preparations

Disadvantages of Schaudinn's Fixative

- Highly toxic (contains mercury)
- Requires careful handling
- Special waste disposal required

Comparison of Preservatives and Fixatives

Fixative	Best For	Concentration	Permanent Stain	Toxicity
Formalin	Helminth eggs & larvae	Yes	No	Moderate
PVA	Protozoa	No	Yes	Moderate
Schaudinn's	Protozoa (detail)	No	Yes	High

Lecture 6

Laboratory Diagnosis of Enteric Protozoa

Routine Methods Used in the Parasitology Laboratory

Introduction

Enteric protozoa are unicellular parasites that inhabit the **intestinal tract** of humans and are a major cause of diarrheal diseases worldwide. Accurate laboratory diagnosis is essential for:

- Proper treatment
- Prevention of complications
- Control of transmission

Common enteric protozoa include:

- *Entamoeba histolytica*
- *Giardia lamblia*
- *Balantidium coli*
- *Cryptosporidium spp.*

Stages Detected in Stool Samples

Enteric protozoa are usually detected in one or both of the following stages:

- **Trophozoite:**
  - Motile, feeding stage
  - Found mainly in liquid or diarrheic stool
- **Cyst:**
  - Infective, resistant stage
  - Found mainly in formed stool

Routine Laboratory Methods Used for Diagnosis

---

1. Direct Microscopic Examination

Principle

Direct microscopic examination aims to **demonstrate protozoa directly** in stool samples using wet mount preparations.

Procedure

- Prepare saline and iodine wet mounts
- Examine under low and high power magnification

Findings

- Motile trophozoites in saline mounts
- Cysts with nuclear details in iodine mounts

Advantages

- Simple and rapid
- Low cost
- Can detect motility

Disadvantages

- Low sensitivity in light infections
- Requires fresh specimens

---

2. Concentration Techniques

Principle

Concentration techniques increase the likelihood of detecting protozoa by **separating parasites from fecal debris**.

Commonly Used Methods

- Formalin–Ether sedimentation
- Zinc sulfate flotation

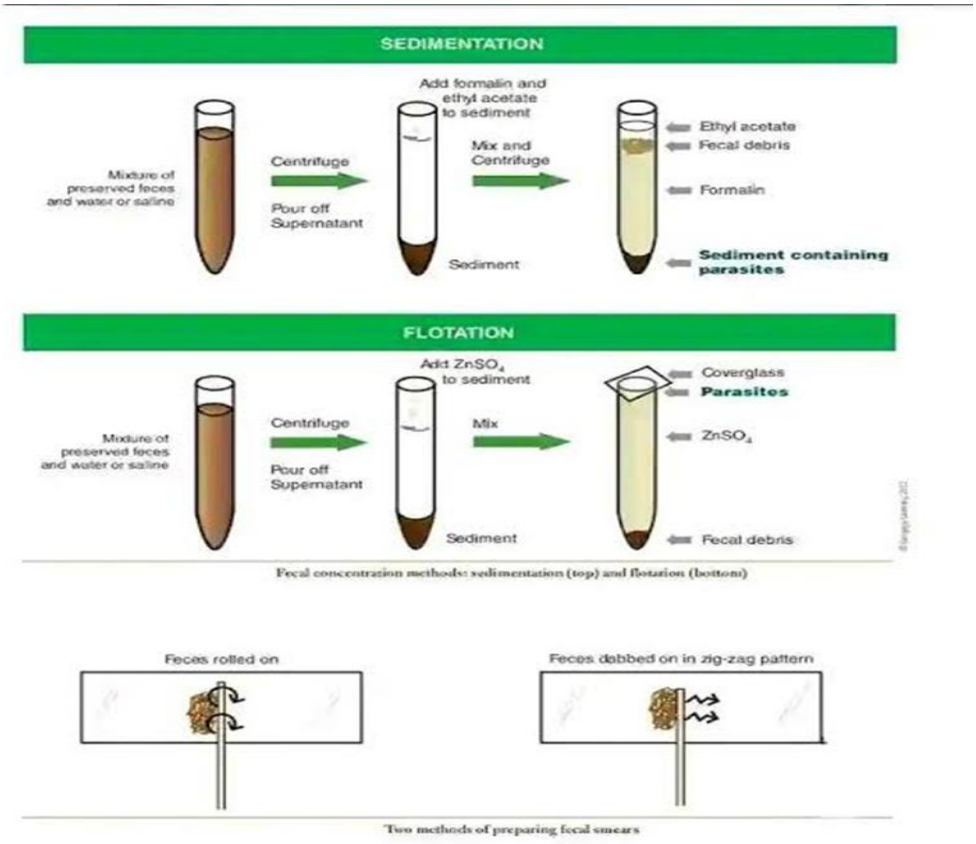
Advantages

- Higher sensitivity than direct examination
- Useful in low parasite burden

Disadvantages

- May distort trophozoites
- Requires more processing time

.



Fecal concentration methods: sedimentation (top) and flotation (bottom)

Two methods of preparing fecal smears

---

3. Permanent Stained Smears

Principle

Permanent stains allow **detailed study of protozoan morphology**, especially nuclear structures.

Common Stains Used

- Trichrome stain
- Iron hematoxylin stain

Advantages

- Excellent visualization of internal structures
- Permanent record for teaching and reference

Disadvantages

- Time-consuming
- Requires fixation (PVA or Schaudinn's)

---

4. Antigen Detection Methods

Principle

Detects **specific protozoan antigens** in stool samples using immunological techniques.

Commonly Used Tests

- ELISA
- Immunochromatographic assays

Advantages

- High sensitivity and specificity
- Useful when microscopy is inconclusive

Disadvantages

- Higher cost
- Requires special kits

5. Molecular Methods

Principle

Detection of protozoan DNA using PCR-based techniques.

Advantages

- Highly sensitive and specific
- Can differentiate pathogenic and non-pathogenic species

Disadvantages

- Expensive
- Requires advanced laboratory facilities

Lecture 7

Preparation of Buffered Methylene Blue (BMB) Stain

Detection of Amoebic Trophozoites

Introduction

Buffered Methylene Blue (BMB) stain is a **simple differential stain** used in diagnostic parasitology laboratories for the **detection and identification of amoebic trophozoites**, especially *Entamoeba histolytica*, in fresh stool specimens.

This stain is particularly useful because it:

- Enhances nuclear and cytoplasmic details
- Allows rapid examination
- Is easy to prepare and apply

Importance of BMB Stain

- Helps differentiate **amoebic trophozoites** from other intestinal cells
- Enhances visualization of:
  - Nucleus
  - Cytoplasm
  - Ingested red blood cells (diagnostic for *E. histolytica*)
- Useful in routine laboratory diagnosis of amoebiasis

📌 **Key diagnostic feature:**
The presence of **ingested RBCs inside trophozoites** confirms *Entamoeba histolytica*.

Principle of Buffered Methylene Blue Stain

Methylene blue is a **basic dye** that stains acidic cellular components such as:

- Nucleus
- Cytoplasmic structures

The buffering system maintains an optimal pH, allowing:

- Clear differentiation of nuclear details
- Proper staining of protozoan structures without distortion

Preparation of Buffered Methylene Blue (BMB) Stain

Reagents Required:

- Methylene blue
- Phosphate buffer (pH 6.8)
- Distilled water

Preparation Steps:

1. Dissolve **0.3 g methylene blue** in **100 mL distilled water**.
2. Add phosphate buffer to maintain pH at **6.8**.
3. Mix well and filter if necessary.
4. Store in a dark bottle at room temperature.

Procedure for Staining Stool Specimens

1. Place a small amount of fresh stool on a clean glass slide.
2. Add one drop of buffered methylene blue stain.
3. Mix gently to form a thin smear.
4. Cover with a coverslip.
5. Examine immediately under the microscope:
    - Low power (10×) for scanning
    - High power (40×) for details

Microscopic Findings

Amoebic Trophozoites:

- Cytoplasm appears **light blue**
- Nucleus stains **deep blue**
- Ingested red blood cells appear as **dark round bodies**

Advantages of BMB Stain

- Rapid and simple technique
- Enhances nuclear and cytoplasmic details
- Useful for fresh stool specimens
- Low cost and easy to prepare

Disadvantages of BMB Stain

- Not suitable for permanent slides
- Limited use in preserved specimens
- Requires fresh stool for best results

Comparison with Other Stains

Stain	Use	Slide Type
BMB	Amoebic trophozoites	Temporary
Trichrome	Protozoa morphology	Permanent
Iron hematoxylin	Nuclear details	Permanent

Diagnostic Significance

- BMB stain is especially helpful in **acute amoebic dysentery**
- Detection of trophozoites with ingested RBCs is **pathognomonic**
- Supports rapid clinical decision-making

Lecture 8

Concentration Methods in Diagnostic Parasitology

Purpose and Types of Concentration Methods

Introduction

In many parasitic infections, parasites may be present in stool specimens in **small numbers**, making them difficult to detect by direct microscopic examination. Concentration methods are laboratory techniques designed to **increase the number of parasites per microscopic field** by separating them from fecal debris.

These methods are especially important for:

- Light infections
- Intermittent shedding of parasites
- Routine laboratory diagnosis

Purpose of Using Concentration Methods

The main purposes of concentration techniques include:

1. **Increasing Sensitivity**
    - Improves detection of ova, cysts, and larvae
    - Reduces false-negative results
2. **Removal of Fecal Debris**
    - Provides clearer microscopic fields
    - Facilitates parasite identification
3. **Detection of Low Parasite Load**
    - Useful when parasites are few in number
4. **Routine Screening**
    - Commonly used in diagnostic laboratories

Principle of Concentration Methods

Concentration techniques are based on differences in:

- **Specific gravity**
- **Size**
- **Weight**

between parasites and fecal debris.

Depending on the method, parasites are either:

- Concentrated in the **sediment**
- Or float on the **surface** of a solution

Types of Concentration Methods

Concentration methods are classified into **two main types**:

---

1. Sedimentation Methods

Principle

Sedimentation methods concentrate parasites by allowing them to **settle at the bottom** of the container due to gravity or centrifugation.

Common Sedimentation Techniques:

- Formalin–Ether method
- Formalin–Ethyl acetate method

Formalin–Ether Sedimentation Technique

Procedure:

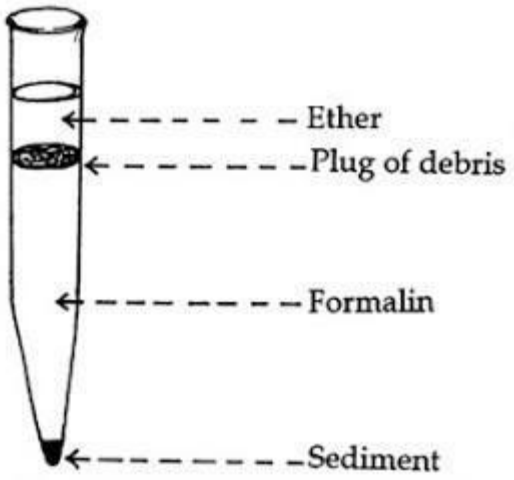
1. Mix stool sample with 10% formalin.
2. Filter through gauze to remove large debris.
3. Add ether to the filtrate.
4. Shake and centrifuge.
5. Four layers are formed:
    - Ether (top)
    - Debris plug
    - Formalin
    - Sediment (contains parasites)
6. Discard supernatant and examine sediment microscopically.

Advantages of Sedimentation Methods:

- Detects most parasites
- Effective for:
    - Helminth eggs
    - Protozoan cysts
    - Larvae
- High sensitivity

Disadvantages of Sedimentation Methods:

- Fecal debris may remain
- Ether is flammable and toxic



Formal ether sedimentation technique

2. Flotation Methods

Principle

Flotation methods are based on floating parasites in a solution with **higher specific gravity** than the parasites.

Common Flotation Techniques:

- Zinc sulfate flotation
- Saturated salt flotation

Zinc Sulfate Flotation Technique

Procedure:

1. Mix stool with zinc sulfate solution.
2. Centrifuge or allow to stand.
3. Parasites float to the surface.
4. Collect surface layer for microscopic examination.

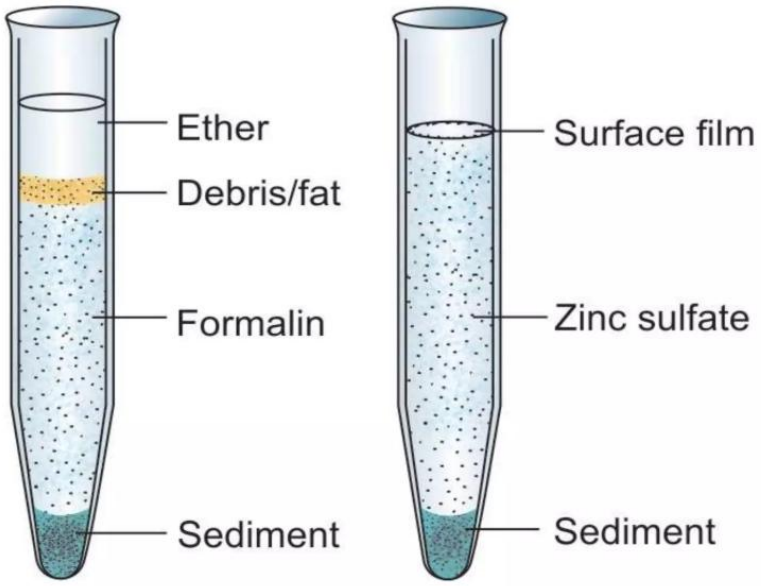
Advantages of Flotation Methods:

- Cleaner preparation
- Less fecal debris
- Good for protozoan cysts

Disadvantages of Flotation Methods:

- Heavy eggs may not float
- Some parasites may be distorted

Formol-ether sedimentation technique (schematic diagram)
zinc sulfate flotation concentration technique (schematic) diagram;



Lecture 9

Artifacts Found in Fecal Specimens

Kato–Katz Method

Kato Katz technique

Kato Katz technique is used for qualitative and semi-quantitative diagnosis of intestinal helminthic infestations; causedby Ascaris lumbricoides, Trichuris trichiura, hookworm and especially Schistosoma spp.

WHO has recommended Kato Katz technique in areas with moderate to high transmission rates of soil transmitted helminths (i.e. where the proportion of infected individuals is >20– >50%) or intestinal schistosomiasis (>10–50%). Where the prevalence of soil transmitted helminths (STH) is <20%, the specificity of this technique makes it less aappropriat and more sensitive tools should be used

Principle

People infected with STH or intestinal schistosomes pass the eggs of the worms through their faeces. In the Kato-Katz technique faeces are pressed through a mesh screen to remove large particles. A portion of sieved sample is then transferred to the hole of a template on a slide. After filling the hole, the template is removed and the remaining sample is covered with a piece of cellophane soaked in glycerol. The glycerol clears the faecal material from around the eggs. The eggs are then counted and the number calculated per gram of faeces

Materials

1_Kato-set (Template with hole, screen, nylon or plastic, plastic spatula)

2_Newspaper or glazed tile

3_Microscope slides

4_Cellophane as cover slip, soaked in Glycerol-malachite green or or glycerol-methylene blue solution.

5_Fresh stool

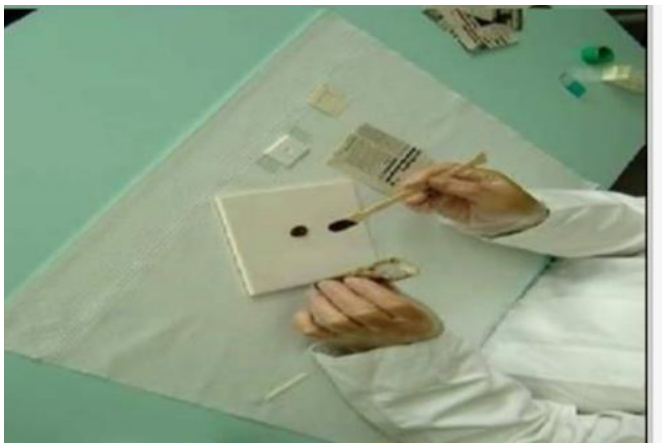
6_Gloves

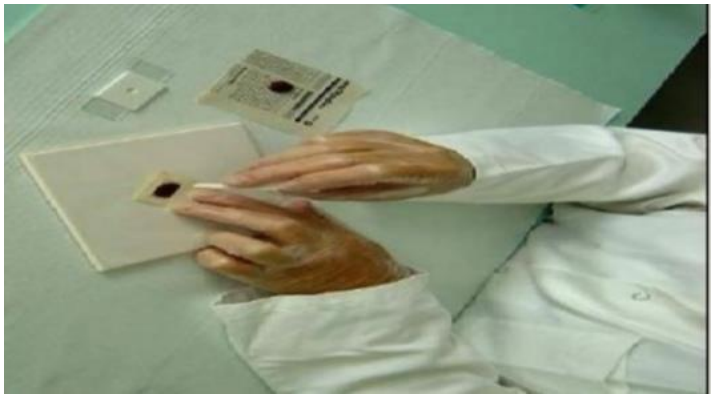
Procedure of Katz-Kato Technique

Label a glass slide with the sample number and then place a plastic **template** on top of it. Place a small amount of the faecal sample on a newspaper and press a piece of nylon screen on top. Using a spatula, scrape the sieved faecal material through the screen so that only the debris remains. Scrape up some of the sieved faeces to fill the hole in the template, avoiding air bubbles and levelling the faeces off to remove any excess. Carefully lift off the template and place it in a bucket of water mixed with concentrated detergent so that it can be reused. Place one piece of the

cellophane, which has been soaked overnight in methylene blue glycerol solution, over the faecal sample. Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle.Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip.If done well, it should be possible to read newspaper print through the stool smear. Place the slide with the cellophane upwards.

Note: If hookworm is present in the area the slide should be read within 30–60 minutes. After that time, the hookworm eggs disappear





Examination and Results ▪

Place the slide under a microscope and examine the whole area in a systematic zigzag pattern. ▪ Record the number and the type of each egg of each species on a recording form alongside the sample number. ▪ Finally, multiply the

number of eggs by the appropriate number (see inlet-information of the kato-set) to give the number of eggs per gram (epg) – the standard measurement to assess the intensity of infection

Advantages

• Best method to identify eggs of Ascaris, hookworms and S. mansoni .

• Allows both identification and quantification of those eggs so it is easy to calculate the worm burden in a patient .

• It is easy to perform and to transfer madepreparations .

• Therefore, it is good for field studies as the preparation can be done in the field then transferred to the equipped reference laboratory

Disadvantages:

Not suitable for liquid stool (not for diarrheal samples) Suitable only for stool specimens that contain at least 20 egg/gram of feces .

Lecture 10

Application of Immunological Methods in Diagnosis of Parasites

Detection of Antibodies in Serum (ELISA)

Introduction

Immunological methods are essential in modern parasitology for **detecting host immune responses** to parasitic infections. These methods are particularly useful when:

- Parasites are difficult to detect directly
- Infection is extra-intestinal
- Early or chronic infections need diagnosis

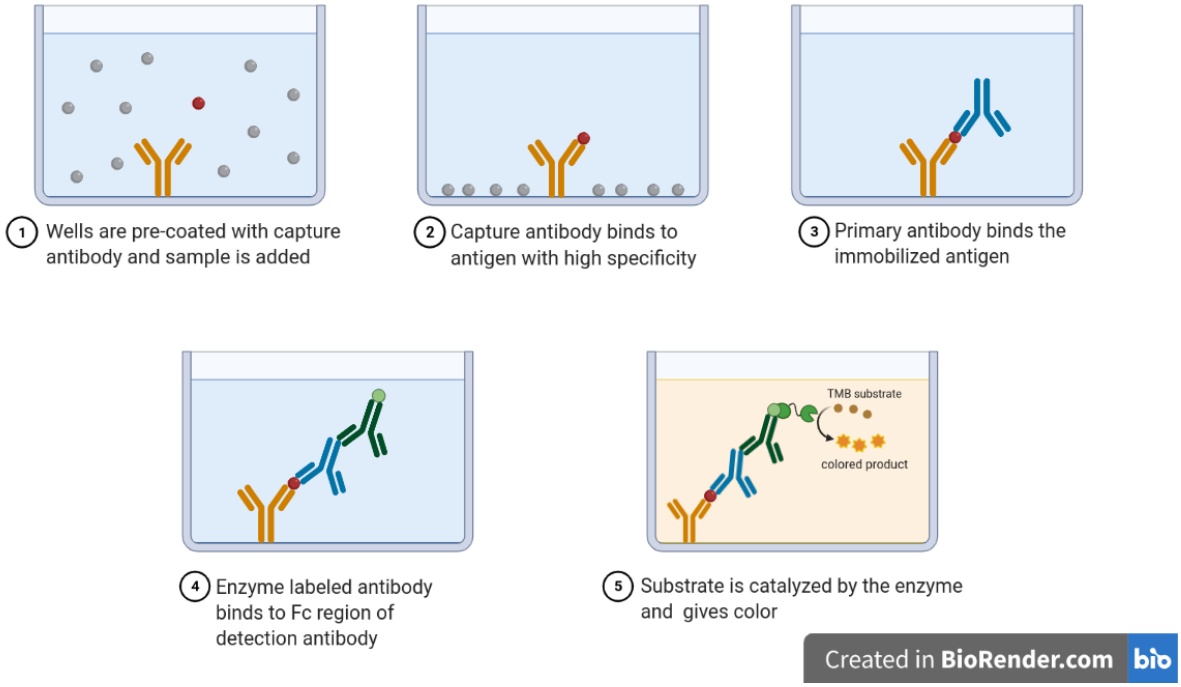
One of the most widely used immunological techniques is **Enzyme-Linked Immunosorbent Assay (ELISA)**, which can detect either **antibodies** or **antigens**.

---

1. Principle of Antibody Detection by ELISA

ELISA detects **specific antibodies (IgG, IgM, or IgA)** produced by the host in response to parasitic infection.

- Parasite antigen is coated on a microtiter plate
- Patient serum is added; if antibodies are present, they bind to the antigen
- Secondary enzyme-linked antibody binds to the primary antibody
- Substrate is added, producing a color change
- Color intensity is proportional to the antibody concentration



---

2. Types of ELISA for Parasites

A. Indirect ELISA

- Detects antibodies in the patient serum
- Commonly used for:
  - *Entamoeba histolytica*
  - *Toxoplasma gondii*
  - *Giardia lamblia*

B. Capture (Sandwich) ELISA

- Detects parasite antigens in serum or stool
- More sensitive for acute infection

C. Competitive ELISA

- Measures antibody competition with labeled antibodies
- Useful for differentiating closely related parasites

3. Procedure of Indirect ELISA

1. Coat microplate wells with parasite antigen
2. Block non-specific binding sites with blocking buffer
3. Add patient serum; incubate to allow binding
4. Wash to remove unbound antibodies
5. Add enzyme-labeled secondary antibody
6. Wash again
7. Add substrate; color develops if antibodies are present
8. Measure optical density using a spectrophotometer

---

4. Interpretation of Results

- **Positive result:** Color change above cut-off value
- **Negative result:** No significant color change
- **High OD values:** Indicate high antibody levels

---

5. Advantages of Immunological Methods

- High sensitivity and specificity
- Useful for infections with low parasite load
- Rapid and suitable for large-scale screening
- Applicable to extra-intestinal parasites

---

6. Disadvantages

- Expensive and requires laboratory equipment
- Cross-reactivity may occur with related parasites
- Antibody levels may persist after treatment

---

7. Applications in Enteric Protozoa

- Detection of **anti-Entamoeba histolytica antibodies** in serum
- Useful when trophozoites are not present in stool

- Supports diagnosis of **invasive amoebiasis**

Lecture 11

Detection of Antigens in Stool (Rapid Diagnostic Tests (RDTs)

Immunological Diagnosis for Enteric Protozoa and Visceral Leishmaniasis

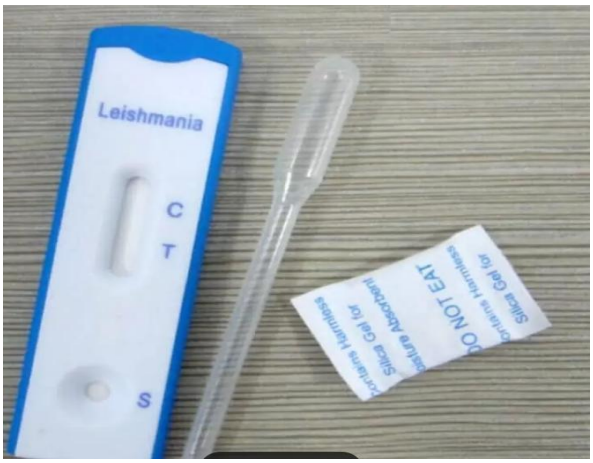
---

Introduction

In addition to antibody detection, **antigen detection in stool** and **rapid immunodiagnostic tests** play a vital role in parasitology, especially when:

- Parasites are difficult to observe microscopically
- Rapid results are required
- Screening of large populations is needed

Rapid Diagnostic Tests (RDTs)



Principle

RDTs are **immunochromatographic strip tests** that provide **quick qualitative results** for parasite detection.

- Based on **antibody-antigen reactions**
- Color band appears if parasite antigen is present

Examples of RDTs

- *Giardia lamblia* stool test
- *Entamoeba histolytica* stool test
- RDT for **visceral leishmaniasis (VL)** detecting rK39 antigen

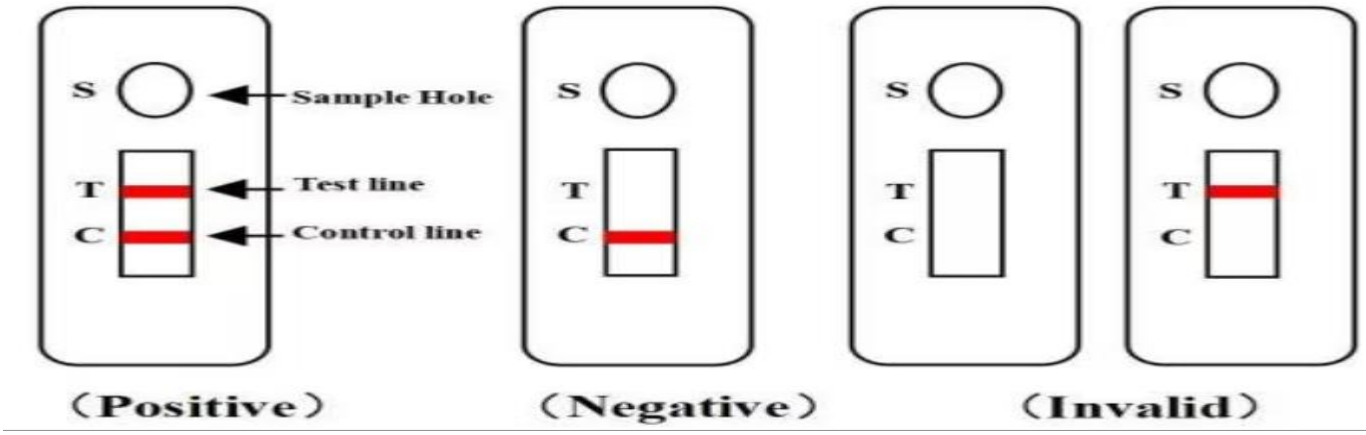
---

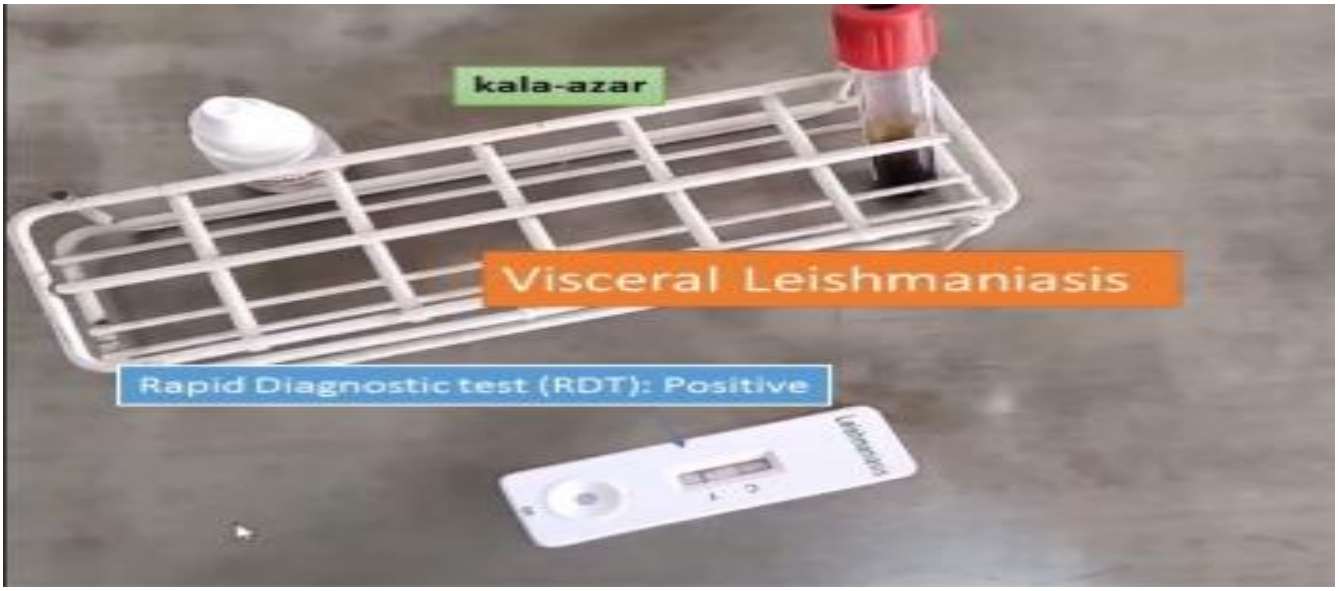
Procedure

1. Collect stool or blood sample
2. Apply sample to test strip
3. Add buffer
4. Observe results in 10–20 minutes

Interpretation:

- Single control line = negative
- Control + test line = positive





Advantages

- Rapid (results in 10–20 min)
- Easy to perform, minimal training
- Useful in field or remote areas

Disadvantages

- Qualitative, not quantitative
- False positives possible due to cross-reactivity
- Less sensitive than ELISA in some cases

3. Diagnostic Significance

- Useful for **early and rapid diagnosis**
- Reduces dependence on skilled microscopists
- Supports **control programs** for endemic areas
- RDTs for visceral leishmaniasis are critical in resource-limited regions

Lecture 12

Laboratory Diagnosis of Intestinal Coccidia

Example: *Cryptosporidium parvum*

Modified Ziehl–Neelsen Stain

Introduction

Intestinal coccidia are protozoan parasites that infect the **intestinal epithelium** and cause **diarrheal diseases**, especially in immunocompromised patients. Key coccidian parasites include:

- *Cryptosporidium parvum*
- *Cyclospora cayetanensis*
- *Cystoisospora belli*

These parasites are **small, intracellular, and difficult to detect** using routine stool microscopy. Specialized staining techniques, such as the **Modified Ziehl–Neelsen (MZN) stain**, are used for identification.

Principle of Modified Ziehl–Neelsen (MZN) Stain

- MZN stain is an **acid-fast staining method**
- Coccidian oocysts retain the **carbol fuchsin dye** even after decolorization with acid-alcohol
- Background stool material is counterstained with methylene blue
- Results in **red oocysts against a blue background**

Materials Required

- Clean glass slides
- Carbol fuchsin solution
- Acid-alcohol (1% HCl in ethanol)
- Methylene blue counterstain
- Immersion oil (for high-power observation)

- Stool specimens (fresh or preserved)

Procedure

1. Place a small amount of stool on a clean slide.
2. Prepare a thin smear and air-dry.
3. Fix smear with methanol for 5 minutes.
4. Flood smear with **carbol fuchsin** and heat gently (do not boil).
5. Allow stain to act for 10–15 minutes.
6. Rinse gently with water.
7. Decolorize with **acid-alcohol** for 1–2 minutes.
8. Rinse with water.
9. Counterstain with **methylene blue** for 30–60 seconds.
10. Rinse, air-dry, and examine under microscope (100× oil immersion).

Microscopic Findings

- **Coccidian oocysts** appear **bright red**
- Background stool material appears **blue**
- Oocyst sizes:
    - *Cryptosporidium parvum*: 4–6 µm
    - *Cyclospora*: 8–10 µm
    - *Cystoisospora*: 20–30 µm

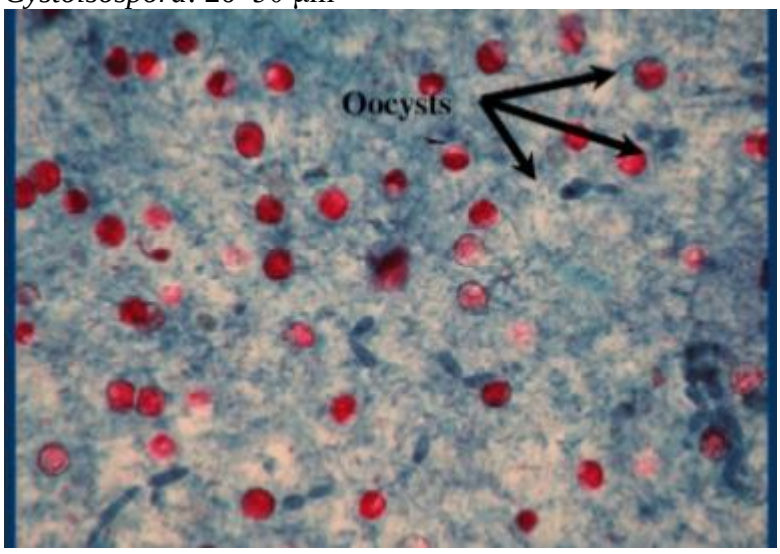


Figure 1: *Cryptosporidium parvum* oocysts stained with Modified Ziehl–Neelsen

Advantages

- Specific for acid-fast coccidian oocysts
- Easy to perform in routine labs
- Preserves oocyst morphology for identification

Disadvantages

- Requires careful staining to avoid over- or under-decolorization
- Less sensitive in low parasite load
- Cannot differentiate species without size or molecular methods

Diagnostic Significance

- Confirms coccidial infection in stool
- Important for immunocompromised patients (HIV/AIDS)
- Guides treatment and public health interventions

Lecture 13

Direct and Indirect Diagnosis of *Toxoplasma gondii*

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects humans and animals. Infection is usually **asymptomatic**, but can cause severe disease in:

- Pregnant women (congenital toxoplasmosis)
- Immunocompromised patients (HIV/AIDS, organ transplant)

Diagnosis can be achieved through **direct detection of the parasite** or **indirect serological methods**.

---

1. Direct Diagnosis

A. Detection in Tissue or Body Fluids

- Trophozoites and cysts can be found in:
  - Tissue biopsies
  - Cerebrospinal fluid (CSF)
  - Amniotic fluid

B. Techniques

1. **Microscopy**
   - Staining tissue sections (H&E, Giemsa)
   - Detection of tachyzoites or tissue cysts
2. **PCR (Polymerase Chain Reaction)**
   - Detects *T. gondii* DNA
   - Highly sensitive and specific
   - Used in CSF, amniotic fluid, or blood

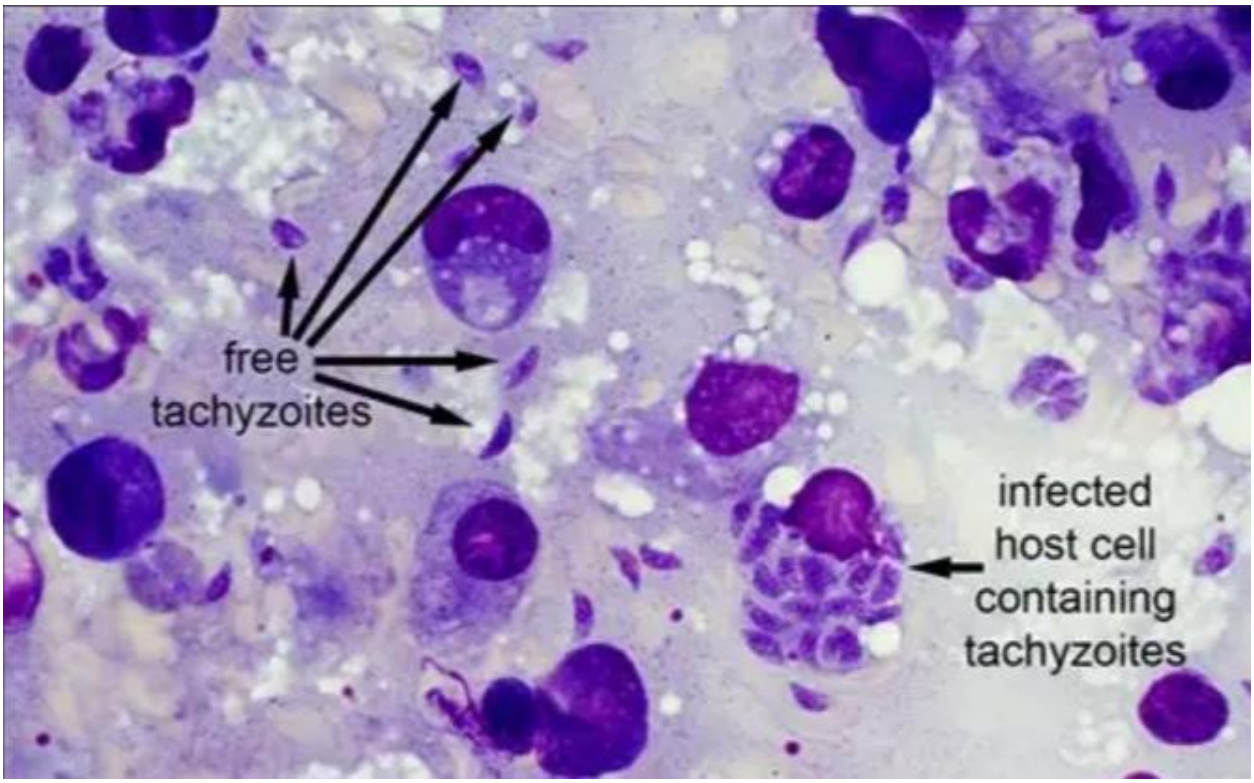


Figure 1: Tachyzoites of *Toxoplasma gondii* in tissue section*)*

---

2. Indirect Diagnosis (Serological Methods)

Principle

Indirect diagnosis relies on detection of **host antibodies** produced against *T. gondii*:

- IgM: Indicates **recent infection**
- IgG: Indicates **past or chronic infection**

Common Serological Tests

1. **ELISA (Enzyme-Linked Immunosorbent Assay)**
    - Detects IgG and IgM antibodies
    - Quantitative and widely used
2. **Indirect Fluorescent Antibody (IFA) Test**
    - Detects antibodies using fluorescent-labeled anti-human IgG/IgM
    - Useful for confirmatory testing
3. **Sabin-Feldman Dye Test**

- Gold standard
- Detects complement-fixing antibodies
- Requires live tachyzoites (limited use)

Interpretation

Antibody	Significance
IgM positive, IgG negative	Recent infection
IgM positive, IgG positive	Recent infection or persistent IgM
IgG positive, IgM negative	Past infection, immunity
Both negative	No prior exposure

---

3. Diagnostic Significance

- Direct detection confirms **active infection**
- Serological testing is widely used for **screening pregnant women**
- PCR is crucial in **immunocompromised patients** and prenatal diagnosis

---

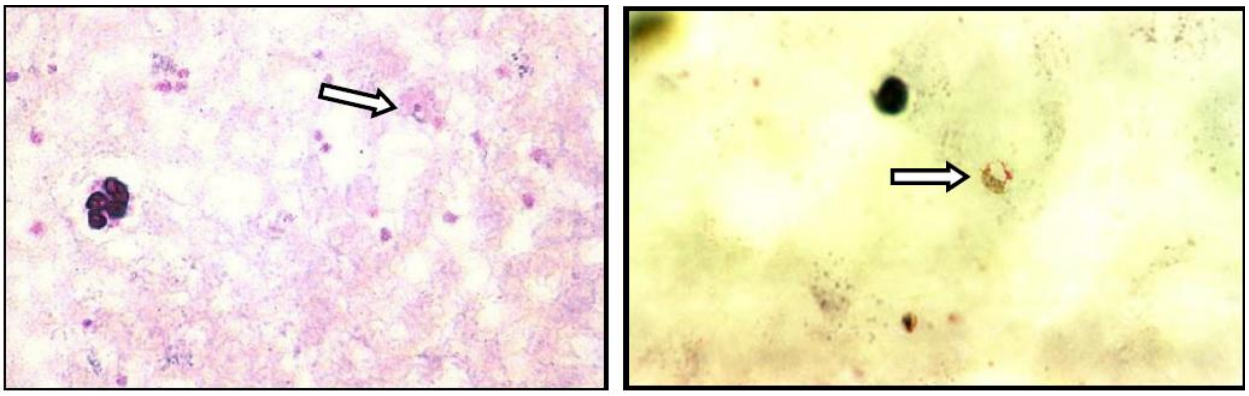
4. Advantages and Disadvantages

Method	Advantages	Disadvantages
Direct (Microscopy)	Definitive	Requires tissue/fluid, low sensitivity
PCR	Highly sensitive & specific	Expensive, specialized lab needed
ELISA / IFA	Easy, quantitative, widely available	Cannot always differentiate acute vs chronic
Sabin-Feldman Dye Test	Gold standard	Requires live parasites, limited use

---

Lecture 14

Methods of Laboratory Diagnosis: Thick and Thin Blood Smears



Introduction

Blood-borne parasites, particularly **Plasmodium species (malaria)**, **Trypanosoma**, and **microfilariae**, require specialized laboratory techniques for accurate diagnosis. The main methods include **thick and thin blood smears**, as well as modern rapid diagnostic techniques like QBC and RDTs.

---

1. Thick and Thin Blood Smears

Principle

- **Thick smear**: Concentrates blood to detect **low parasite density**
- **Thin smear**: Preserves morphology for **species identification**

Materials Required

- Microscope slides
- Sterile lancet or finger-prick device
- Giemsa stain
- Immersion oil
- Microscope

Preparation of Thick Smear

1. Place a **drop of blood** (~2–3 µL) in the center of a slide.
2. Spread into a **circular area ~1 cm diameter**.
3. Air-dry completely.
4. Fix with methanol if necessary (for some stains).
5. Stain with **Giemsa** for 10–20 minutes.
6. Examine under **100× oil immersion**.

Purpose: Detect presence of parasites, especially in low-density infections.

Preparation of Thin Smear

1. Place a **small drop of blood** near one end of the slide.
2. Spread with another slide at a 30–45° angle to form a **feathered edge**.
3. Air-dry and fix with **methanol**.
4. Stain with **Giemsa**.
5. Examine under **100× oil immersion**.

Purpose: Determine species of parasite and estimate parasitemia.

Interpretation

- **Thick smear**: Count number of parasites per high-power field
- **Thin smear**: Identify species based on morphology of parasites (size, shape, and chromatin)

Figure: Thick and thin blood smear comparison
0

Lecture 15

Laboratory Diagnosis: Quantitative Buffy Coat (QBC), Rapid Diagnostic Tests (RDTs), and Non-Microscopic Methods

---

Introduction

Laboratory diagnosis of parasitic infections in blood relies on **both microscopic and non-microscopic techniques**. Modern approaches include:

- **Quantitative Buffy Coat (QBC) test**
- **Rapid Diagnostic Tests (RDTs)**
- Other immunological or molecular tests

These techniques complement **thick and thin blood smears** to provide accurate, rapid, and field-applicable diagnosis.

---

1. Quantitative Buffy Coat (QBC) Test

Principle

- Blood is collected in a **capillary tube containing fluorescent dye**
- Centrifugation separates the **buffy coat**, where parasites concentrate
- Fluorescent parasites are detected under UV microscopy

Advantages

- Rapid (few minutes)
- Detects low parasitemia
- Easy to perform with minimal sample volume

Disadvantages

- Cannot easily identify parasite species
- Requires specialized fluorescent microscope

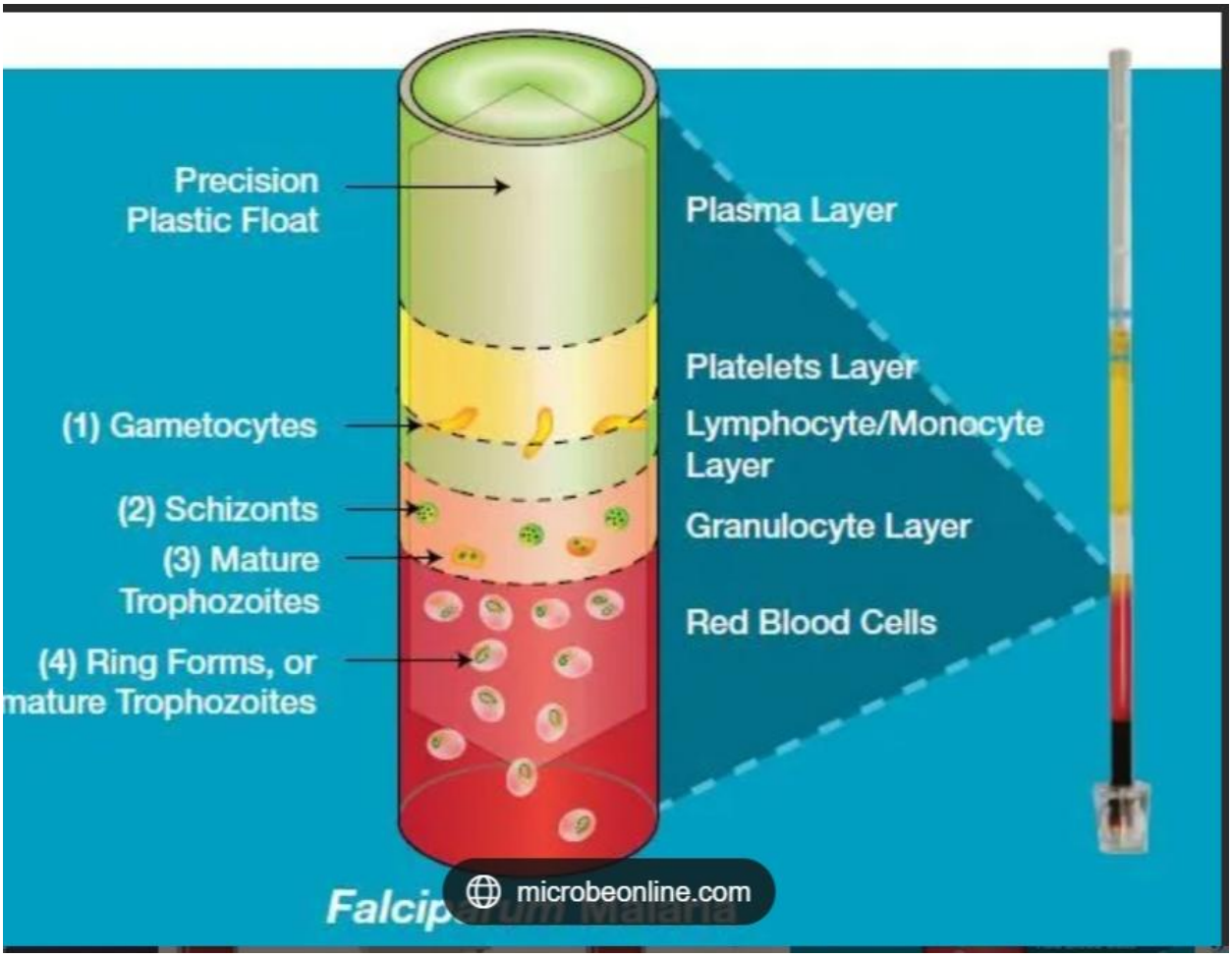


Figure: Buffy coat showing fluorescent malaria parasites

---

2. Rapid Diagnostic Tests (RDTs)

---

3. Non-Microscopic Methods

Examples

- **ELISA** for parasite antigen detection in blood
- **PCR-based molecular methods** for DNA detection
- **Immunofluorescence assays**

---

4. Integration of Diagnostic Methods

Method	Sensitivity	Specificity	Field Use	Parasite Identification
Thick smear	High	Moderate	Requires microscope	Species identification possible
Thin smear	Moderate	High	Laboratory	Morphology and species
QBC	High	Moderate	Limited	Difficult
RDT	Moderate	High	Excellent	Limited species info
ELISA / PCR	High	High	Laboratory	Confirmatory

References

1- Helminthology ,D.D MORO Abadan Iran 2020