Stool examination for intestinal parasitic infections.

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Objective of the lecture:

Students to learn:

1. Laboratory methods for the diagnosis of intestinal parasitic infections.
2. Methods of preserving stool samples for deferred parasitological examination.

Format of the lecture.

1. Macroscopic examination of stool
2. Microscopic examination of stool
3. Non-routine (Special laboratory diagnostic tests)
4. Staining of protozoan parasites
5. Diagnostic features of intestinal protozoa
6. Diagnostic features of intestinal helminths
7. Collection and preservation of stool specimens

Indications for stool examination*.

- Requested when investigating :

1. Infections (bacteria, fungus, virus, **parasites**).
2. GIT bleeding
3. Metabolic disorders.

Intestinal parasites.

1. Protozoa (single celled parasites)
2. Helminthes (worms)

Stages examined in stool samples:

1. **Protozoa**:

- Cyst (resistant stage)
- Trophozoite (fragile and motile stage)

2. **Helminthes:**

- Ova (eggs)
- Larva (immature worm)
- Adult worm (whole, segments)

Examination of stool.

1. Macroscopic (gross)
2. Microscopic

Macroscopic examination.

- Features examined:

i. **Consistency**
ii. **Composition**
iii. **Colour**
iv. **Presence of adult worms/segments.**

Consistency*:

- Physical status of the stool .
- Stool can be:

i. **Formed**
ii. **Semi-formed/unformed**
iii. **Liquid**

Formed stool

- Normal shape (More water has been reabsorbed and there are more cysts and few)

Semi-formed or unformed

- Soft and no regular shape -diarrhea

Liquid

- Note colour, flakes of mucus or blood present

Composition*.

i. **Blood and mucus**:

- ulceration or colitis
- E.g blood diarrhoea may be caused by bacteria/parasites

ii. **Bulky stool**:

- Malabsorption
- It signals a parasitic infection

Colour*.

i. **Pale yellowish**:

- Steatorrhoeic conditions
- Examples: Fat Malabsorption which occurs in certain parasite infections

ii. **Dark or black stools**:

- Examples: May be caused by drugs that contain iron, bismuth, certain foods e.g biscuits like Oreos

Adult worms.

i. **Whole adult worm:**

- *Ascaris* (roundworm)

ii. **Segments:**

- Tapeworm

Microscopic examination.

1. Direct (wet mount)
2. Concentration

Direct *

- **No staining**
- Observe **cellular exudate**, motile **trophozoites,** other **parasitic stages**, food, bacteria, yeast, fat globules.

Type of stool:

1. **All fresh** stools-semi formed/unformed, liquid (**less than 4 hrs** old after voiding).
2. Stool with **blood** and/ or **mucous (dysenteric stool)**.

- For **trophozoites** examine **unformed** stool as soon as possible (< **1hr** after voiding).

Procedure:

- Apply a sample of stool on a microscope slide
- Add a drop of normal saline
- Cover with coverslip
- Examine under the light microscope

Concentration.*

- **To increase** possibility of finding parasite stages (**eggs/ova**, **cysts**, **larvae**) in the stool **sample-too scanty** to be seen on Direct Microscopy.
- **Larger** volume of stool examined
- Trophozoites **destroyed** and cellular debris **distorted**- in most conc. methods.
- Does not work well on **liquid** stools
- Can be done on **preserved** stool.
- Should be performed on **every** stool examined.

Methods of concentration of stool.

1. Sedimentation
2. Flotation.

Sedimentation.

Chemical: **Formol-ether**

(low specific gravity).

Procedure.

1. Mix small portion of stool with formol-ether.
2. **Centrifuge**
3. Parasite stages at **bottom (sediment)**.
4. Debris on **top** (poured out)
5. Examine **sediment** on a microscope slide and covered with a coverslip under light microscopy.

Flotation.

Chemicals with **high specific gravity** (any of the following):

1. **Zinc sulphate**
2. Sodium chloride
3. Magnesium sulphate
4. Sucrose solution
5. Sodium dichromate
6. Sodium nitrate
7. Hyper saturated sugar solution
8. Magnesium sulphate + 5% Potassium Iodide

Procedure.

1. Mix a portion of stool with chemical
2. **Centrifuge**
3. Debris at **bottom** of tube
4. Parasite stages **float**-touch microscope slide on top of tube
5. Examine float on microscope slide under light microscopy

Special laboratory diagnostic tests for <u>intestinal parasites</u>.*

- Non-routine tests.
- ***<u>Light Microscopy based:</u>***

i. **Kato-Katz method (eggs/g of stool).**
ii. **Cellulose tape (cellotape/scotch tape)** technique **(eggs/ova).**
iii. **Perianal morning wash (eggs, adult worms).**
iv. **Rectal snip/biopsy (eggs/ova).**
v. **Entero-capsule/string test (trophozoites, larva).**

Diagnostic features of protozoan parasites.*

Intestinal protozoa exist as:

1. **Trophozoites** (vegetative, motile)
2. **Cysts or oocyst** (protective wall)

- Both cysts and oocysts are the **infective** stage of protozoa

Features of the cyst.*

- Round/oval in shape.

1. **Size.**
2. **Nuclei.**
3. **Glycogen.**
4. **Chromatoid/chromidial bodies (bars/rods).**

Features of trophozoites.

i. **Size**
ii. **Organelles of movement** (flagella, cilia, pseudopodia)
iii. **Shape**
iv. **Nuclei** (number)
v. **Presence ingested red blood cells**

Staining of protozoa.*

- Stains for **diagnostic features** of **cysts** and **trophozoites** in stool specimens:

i. **Temporary.**

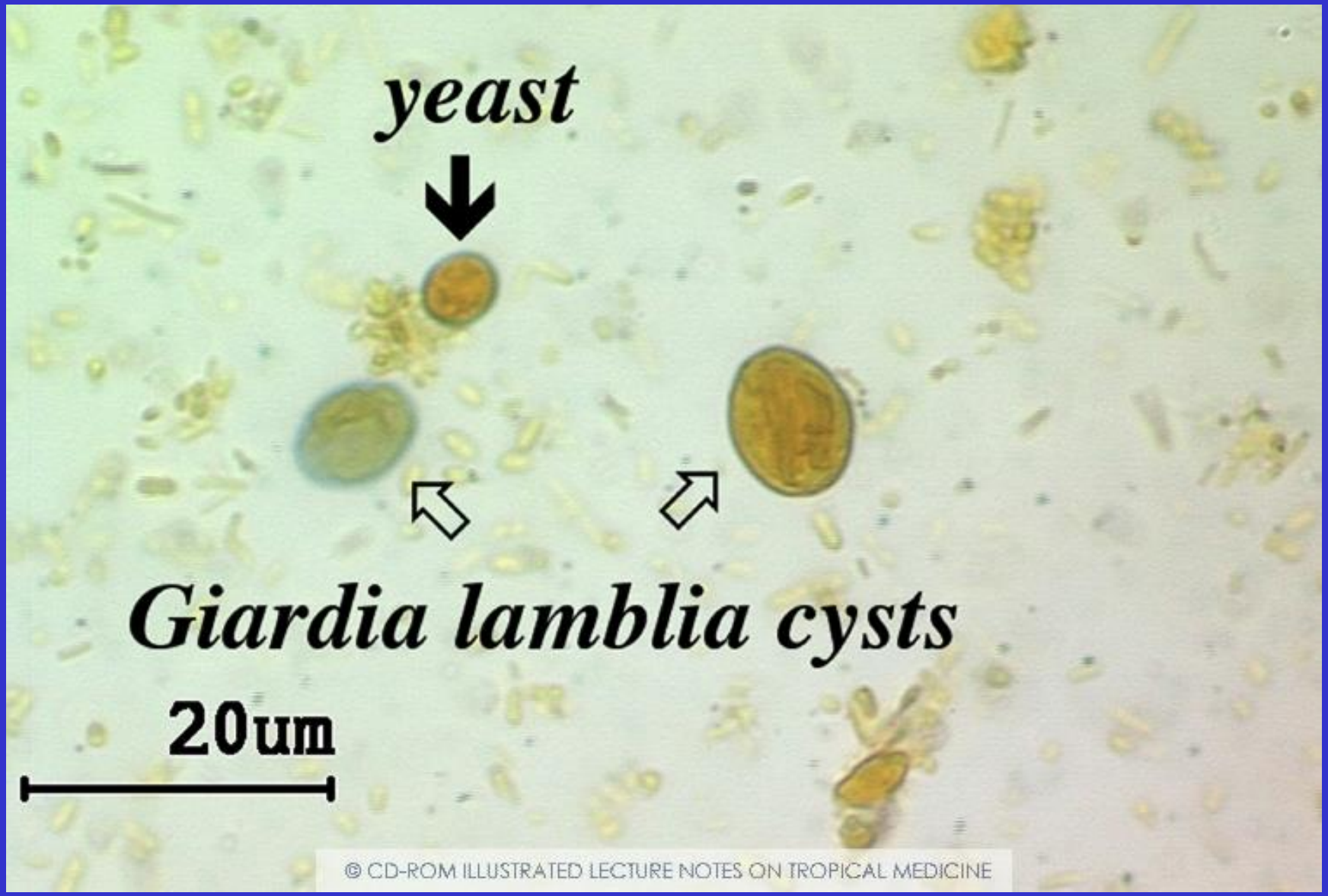
ii. **Permanent** .

Temporary stains.*

i. Lugol’s Iodine
ii. Burrow’s stain
iii. Acridine orange
iv. Eosin
v. Sargeaunt

Permanent stains.

i. **Giemsa**
ii. **Trichome** (Modified Gomori)
iii. **Modified Ziehl neelsen**


yeast
Giardia lamblia cysts
20um
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Diagnostic features of intestinal worms*.

1. **Larva and eggs (ova):**

i. Size

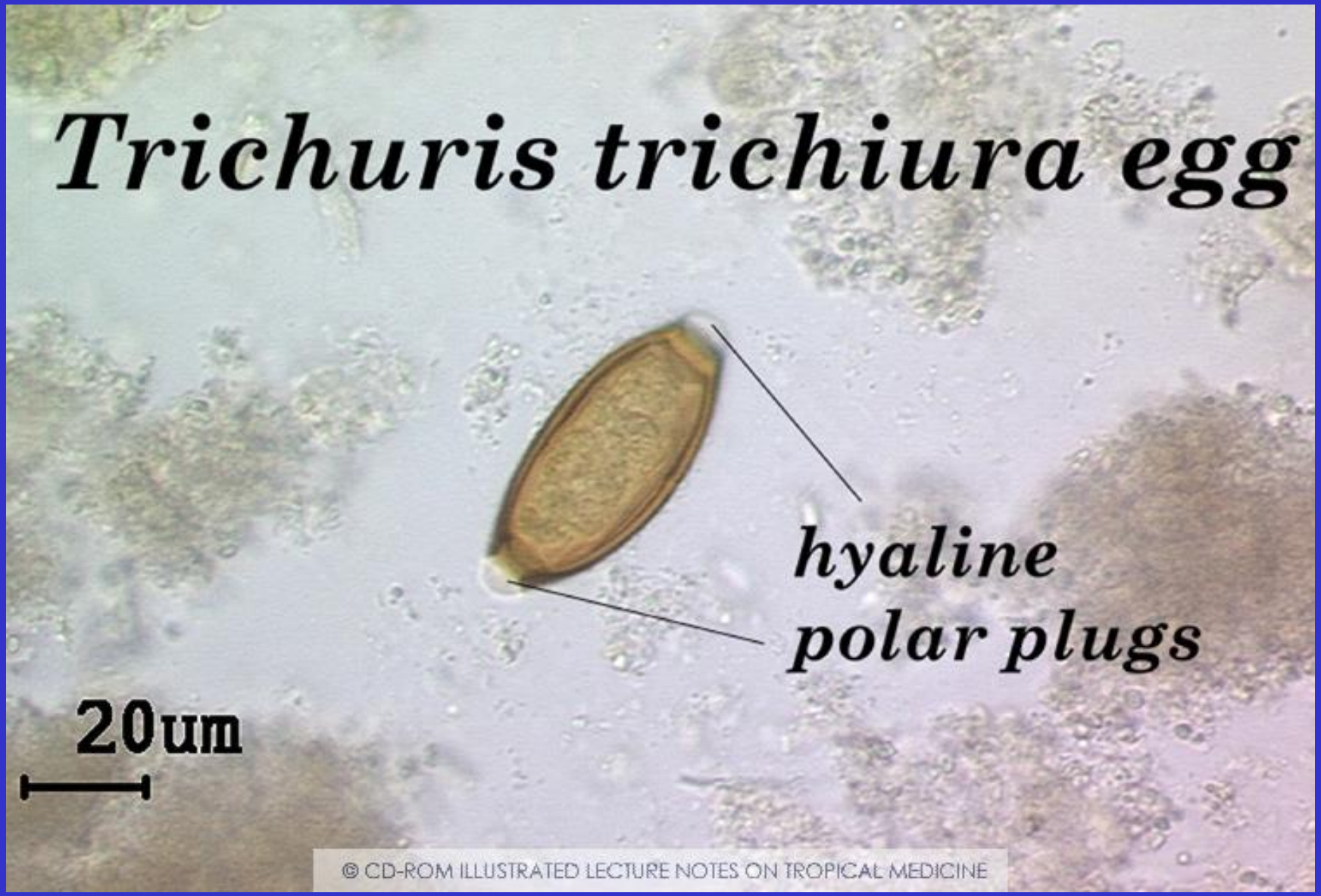
ii. Shape

iii. Surface appearance

2. **Adult worms:**

i. Segments

ii. Shape (whole)-round/flat.


Trichuris trichiura egg
hyaline
polar plugs
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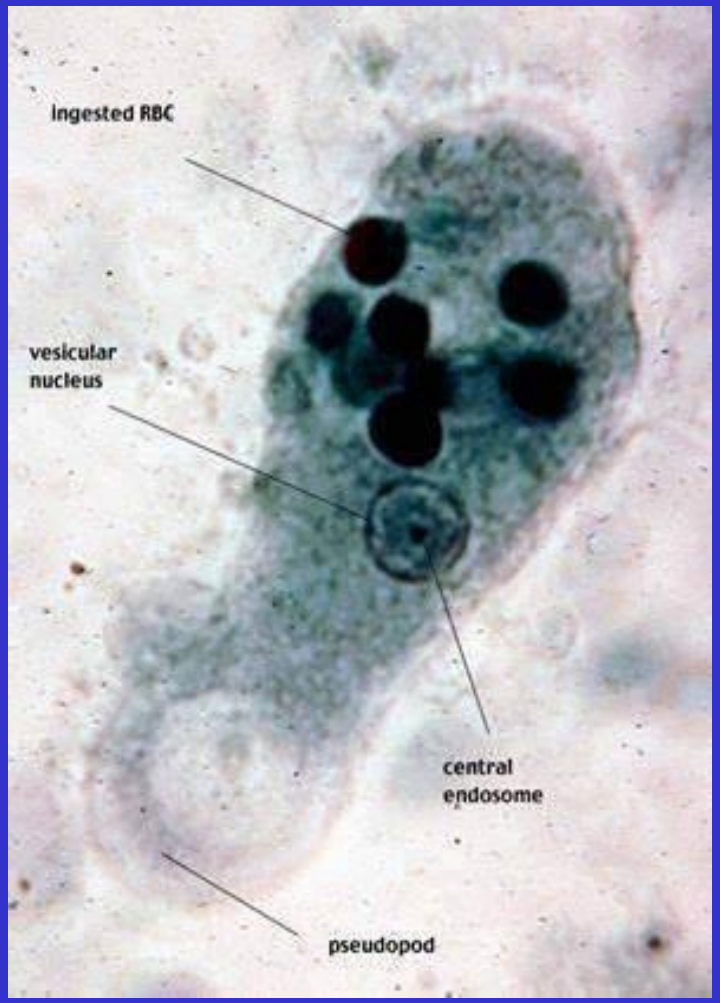
Entamoeba histolytica **trophozoite**



Collection and preservation of stool specimens.*

- Stool samples not examined soon as passage
- To prevent alteration of characteristic morphology of different stages of parasites.
- **Methods**

i. **Temporary**
ii. **Semi permanent**
iii. **Permanent**

Temporary preservation

- **Refrigeration** at **3-5^{0}C** in a **closed container.**
- Preserves protozoan **cysts** for **several days.**

Semi permanent preservation*.

1. Formalin (5%)
2. Polyvinyl alcohol (PVA)
3. Merthiolate-Iodine-Formalin (MIF).

- All preserve for several months

Formalin

- All helminthes stages & protozoan cysts)

PVA

- Protozoan trophozoites & cysts

MIF.

- All stages of helminthes & protozoa

Permanent preservation.*

- For **protozoan cysts** and **trophozoites.**
- Faecal smears **stained** with:

i. **Iron-hematoxylin**

ii. **Trichome**

Type of specimen

1. Fresh faecal specimen
2. PVA preserved (not formalin)

FIN