Microbiology Lab
1st Term

PRACTICAL MEDICAL BACTERIOLOGY

BY

ASS. LECTURER

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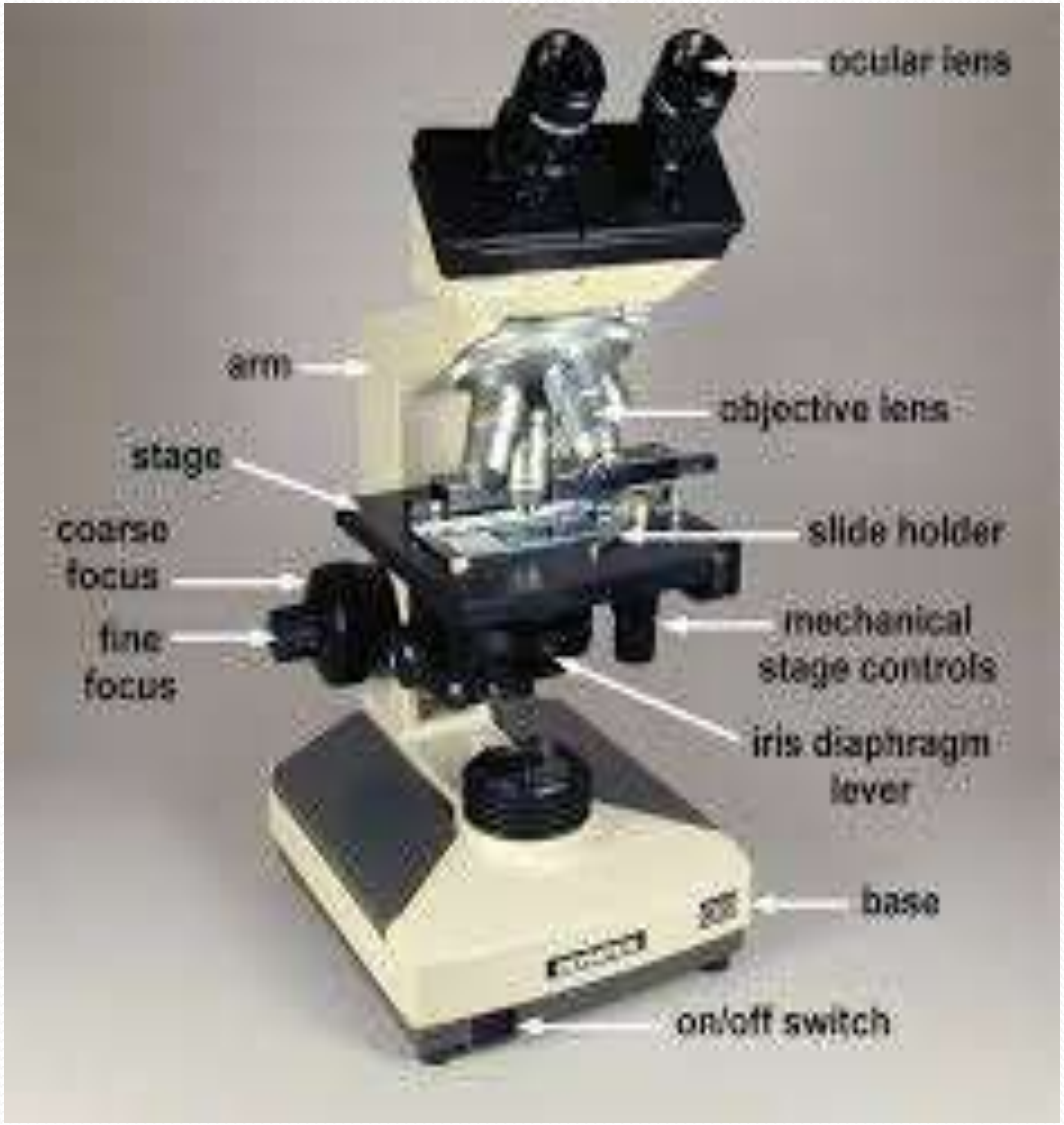
Light microscope



IDENTIFICATION METHOD

The most important task of a bacteriology is to identify the pathogens from the clinical sample so that appropriate treatment can be instituted.

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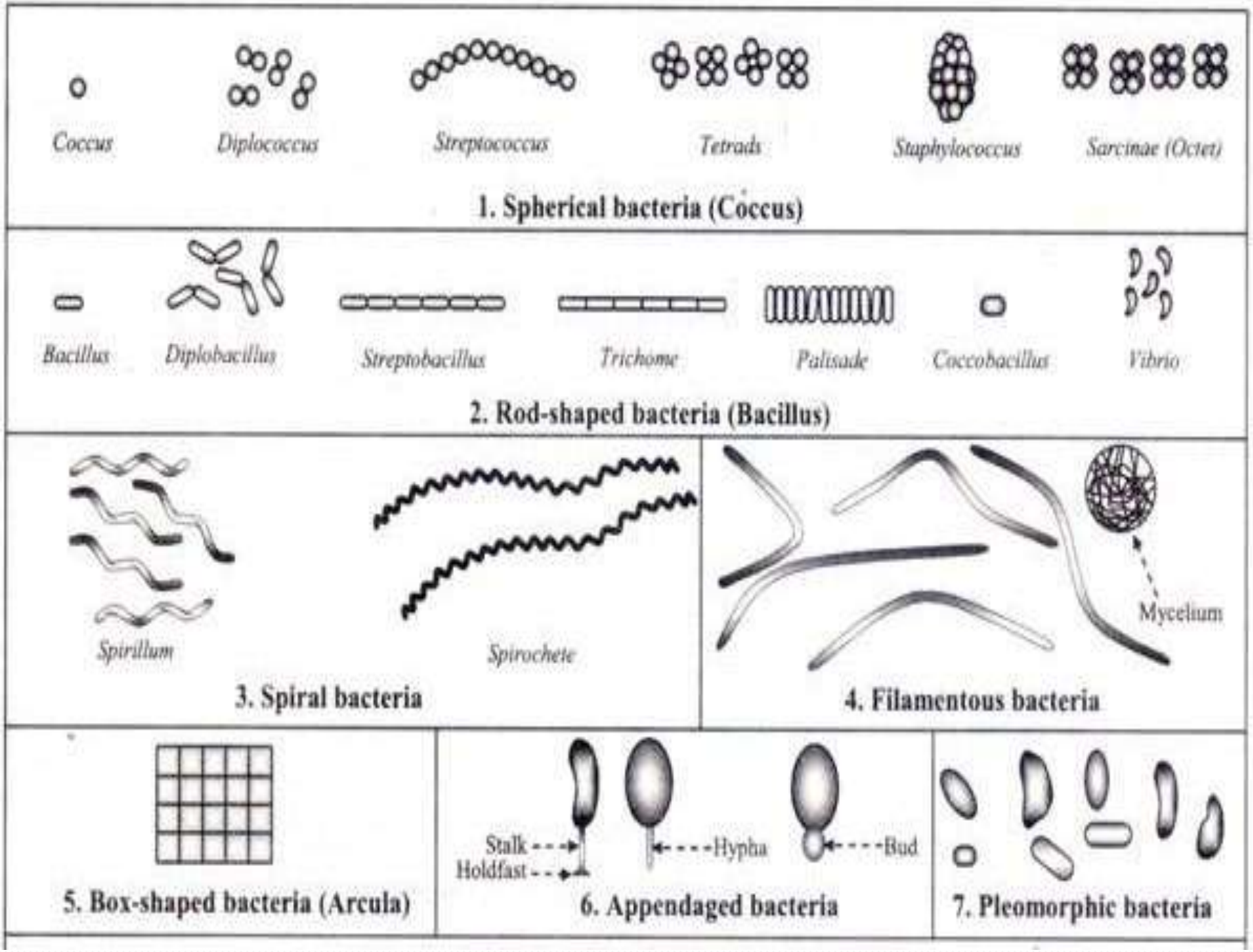
There are several methods to identified the different type of bacteria.

1. Isolation in pure form
2. Staining reaction
3. Morphology of bacterial colony
4. Cultural characteristics
5. Metabolism
6. Biochemical properties

Shape of Bacteria

- Bacteria display these basic shapes:

i. round- cocci, (from the Greek *kokkos* - a berry), sphere like shape
ii. rod shaped – bacilli (from the Latin *bacillus* - a stick or rod),
iii. spiral (quelled).
iv. Curved rod
v. Filamentous bacteria(long branching bacteria)

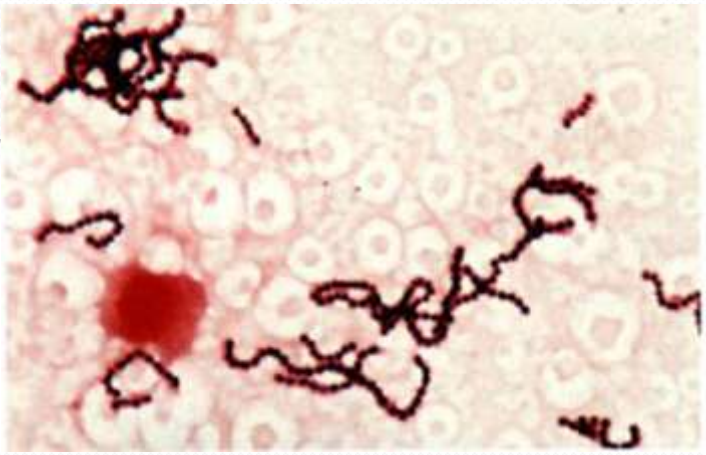

Coccus
Diplococcus
Streptococcus
Tetrads
Staphylococcus
Sarcinae (Octet)
1. Spherical bacteria (Coccus)
Bacillus
Diplobacillus
Streptobacillus
Trichome
Palisade
Coccobacillus
Vibrio
2. Rod-shaped bacteria (Bacillus)
Spirillum
Spirochete
3. Spiral bacteria
Mycelium
4. Filamentous bacteria
5. Box-shaped bacteria (Arcula)
Stalk
Holdfast
Hypha
Bud
6. Appendaged bacteria
7. Pleomorphic bacteria

Different Shapes and arrangement of Bacteria

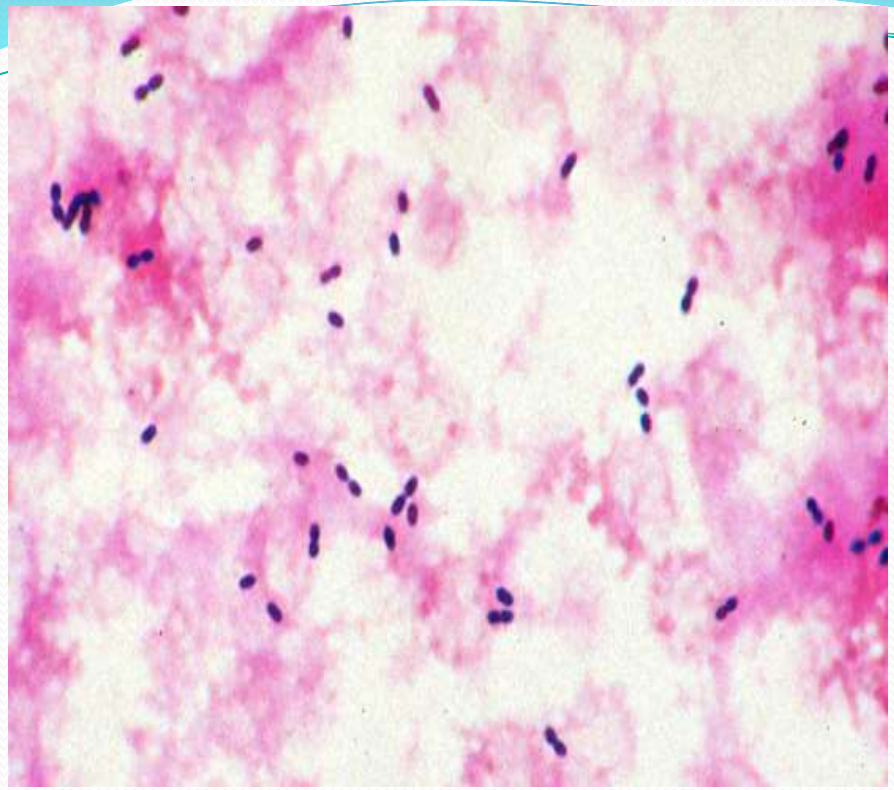
i. Coccus

Cluster arrangement

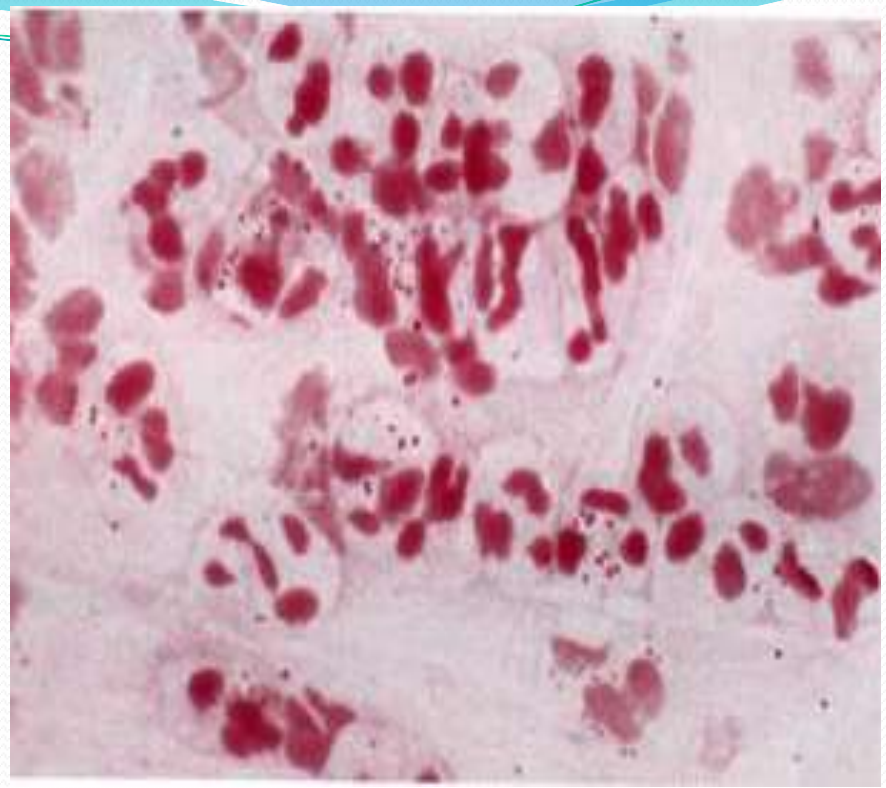
Staphylococcus



Chain like ***Streptococcus***



a



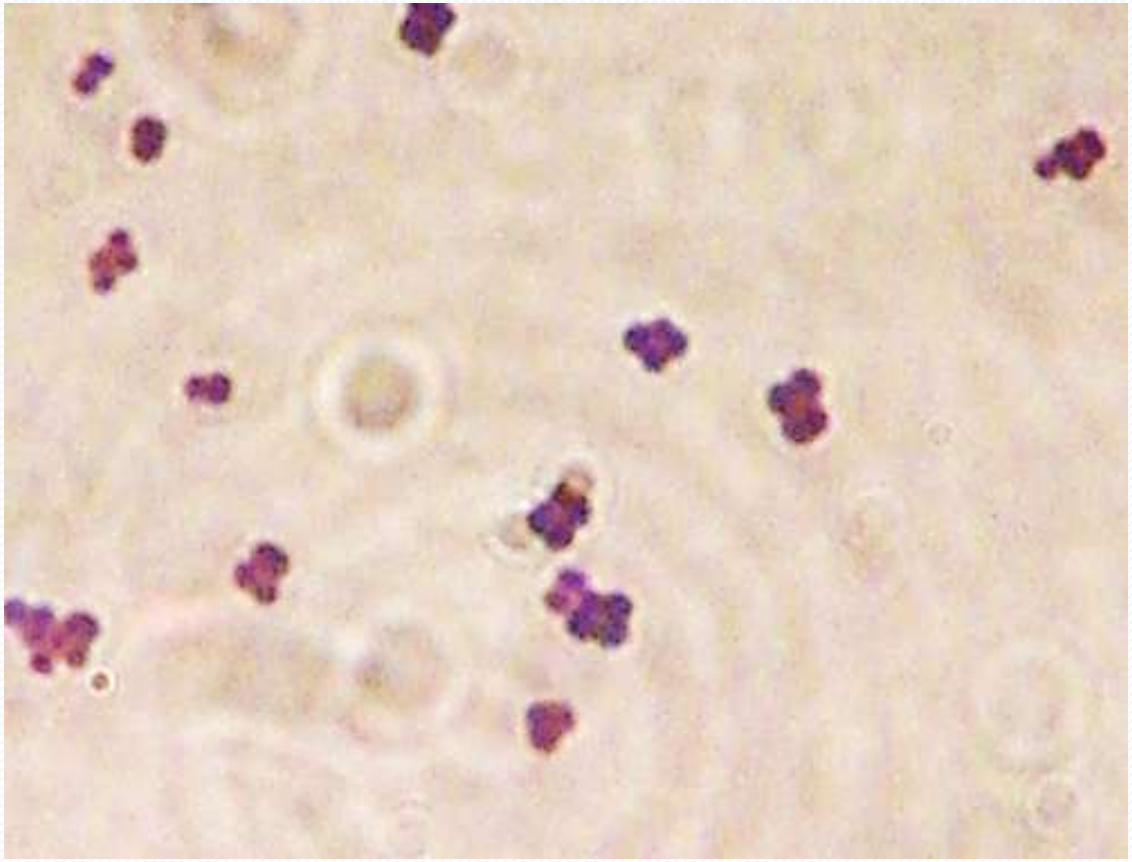
b

Coccus arranged in pairs

a- ***Streptococcus pneumoniae***

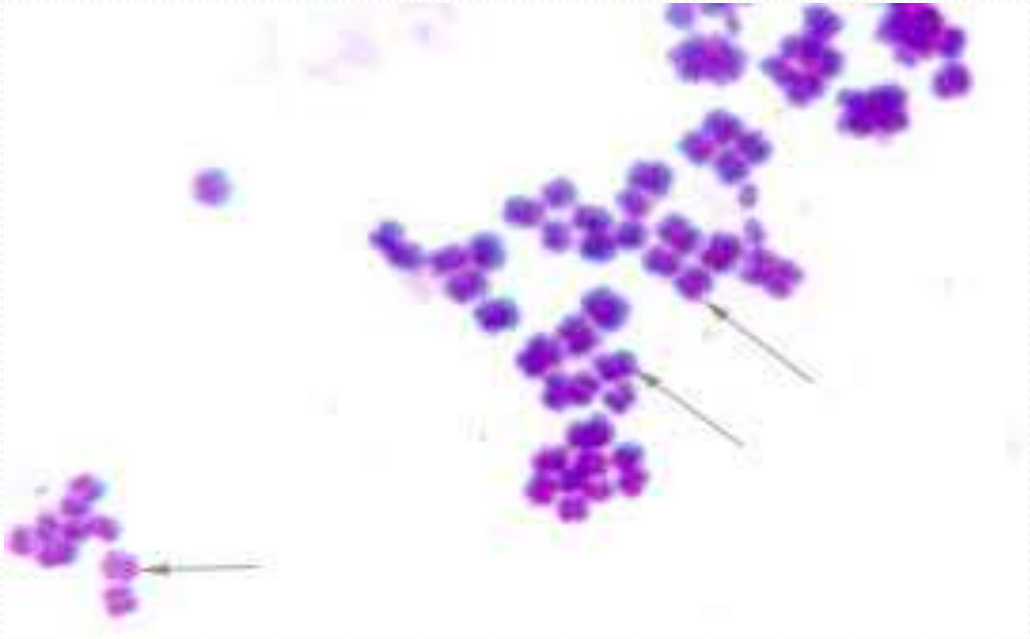
b- Neisseria gonorrhoeae

Coccus arranged in tetrads(four)



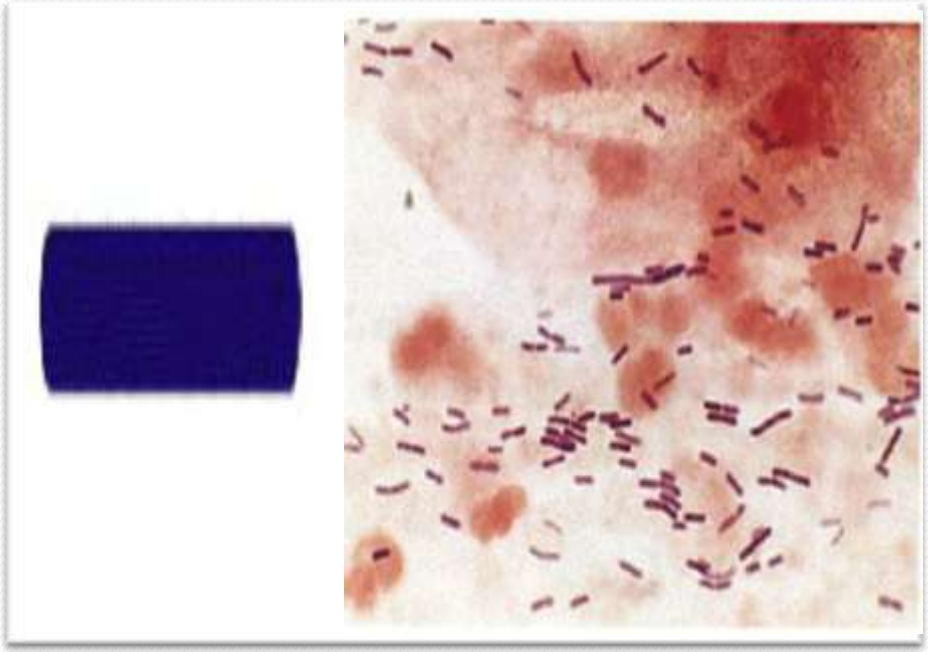
Micrococcus spp.

Package of eight(octet)

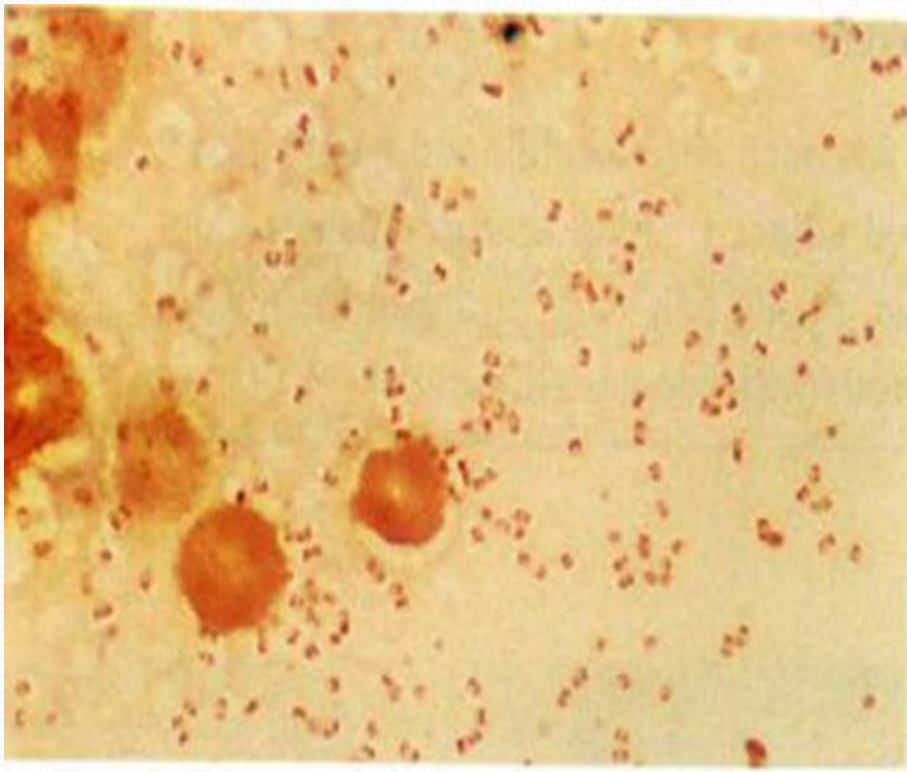


Sarcina spp.

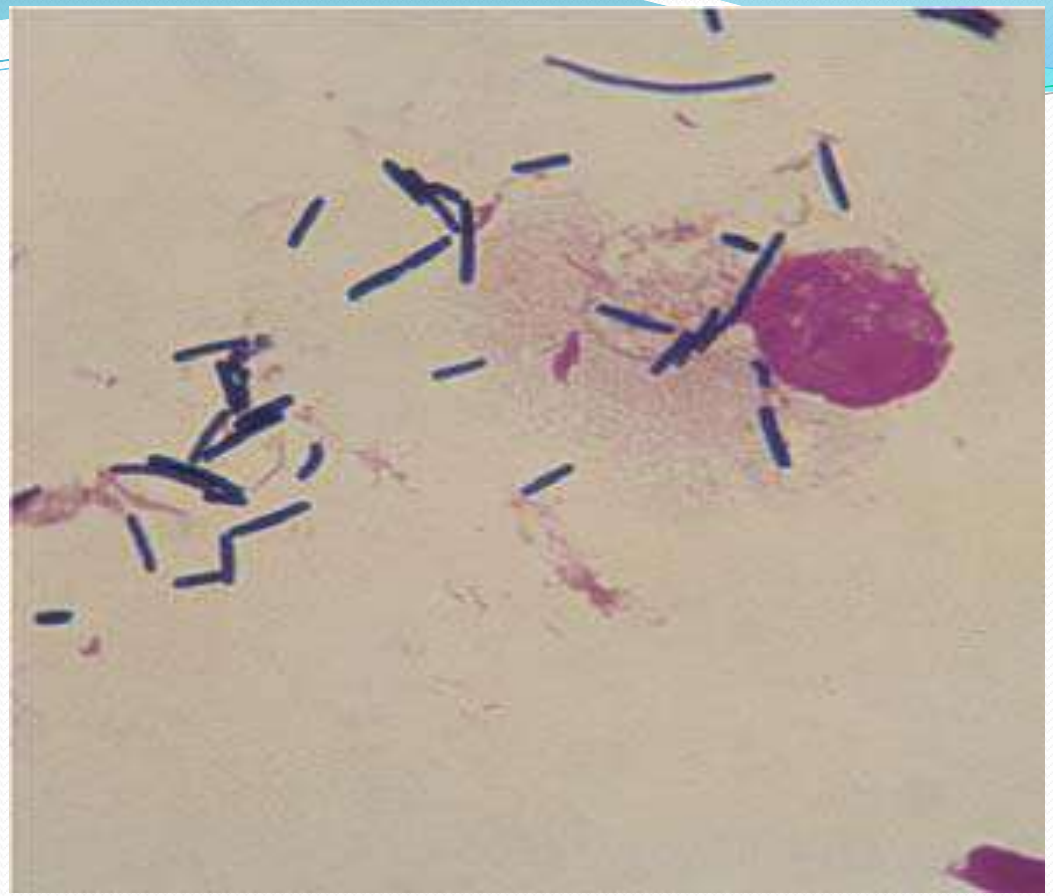
ii. Bacillus(rod shape)



Bacillus sp.

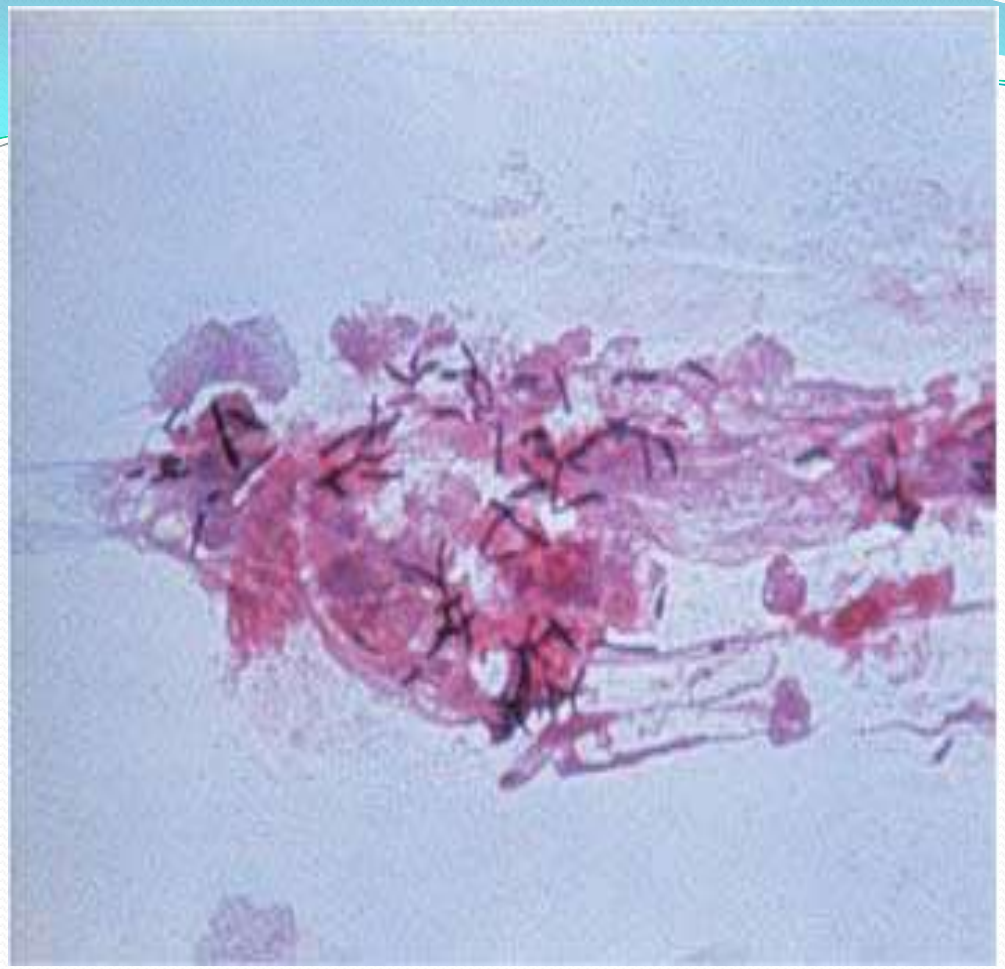


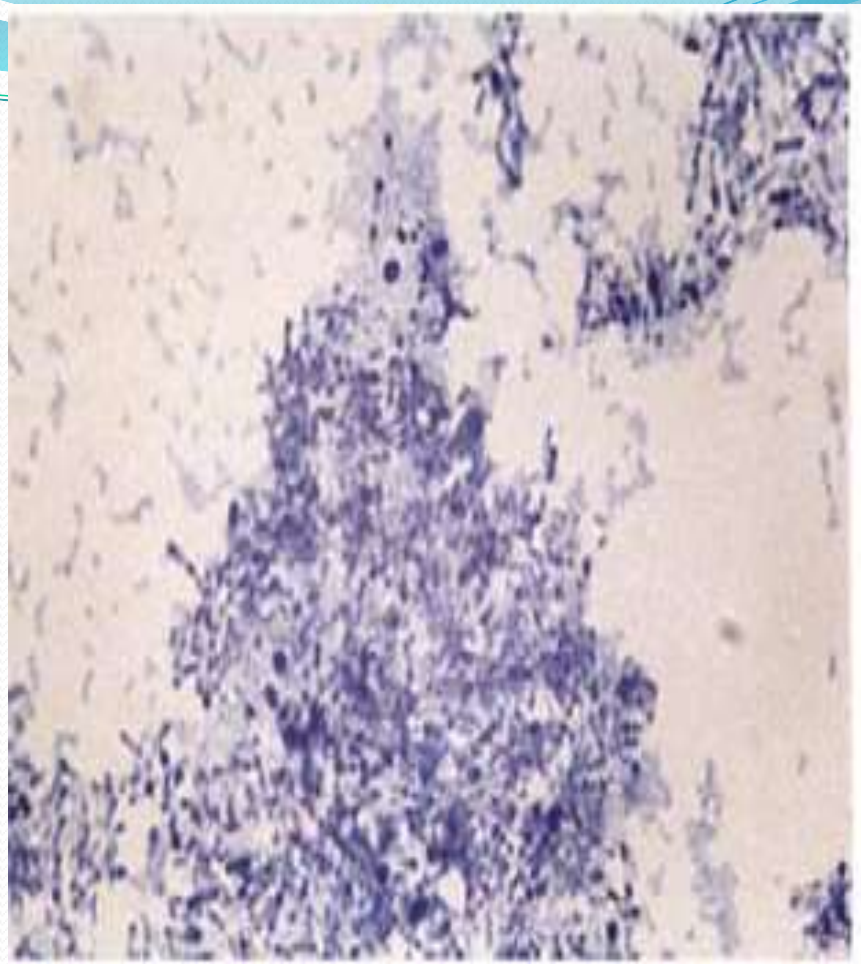
Escherichia(E.) coli
(Coccobacilli)



Bacilli shape(long rod)

Closteridium perfringens

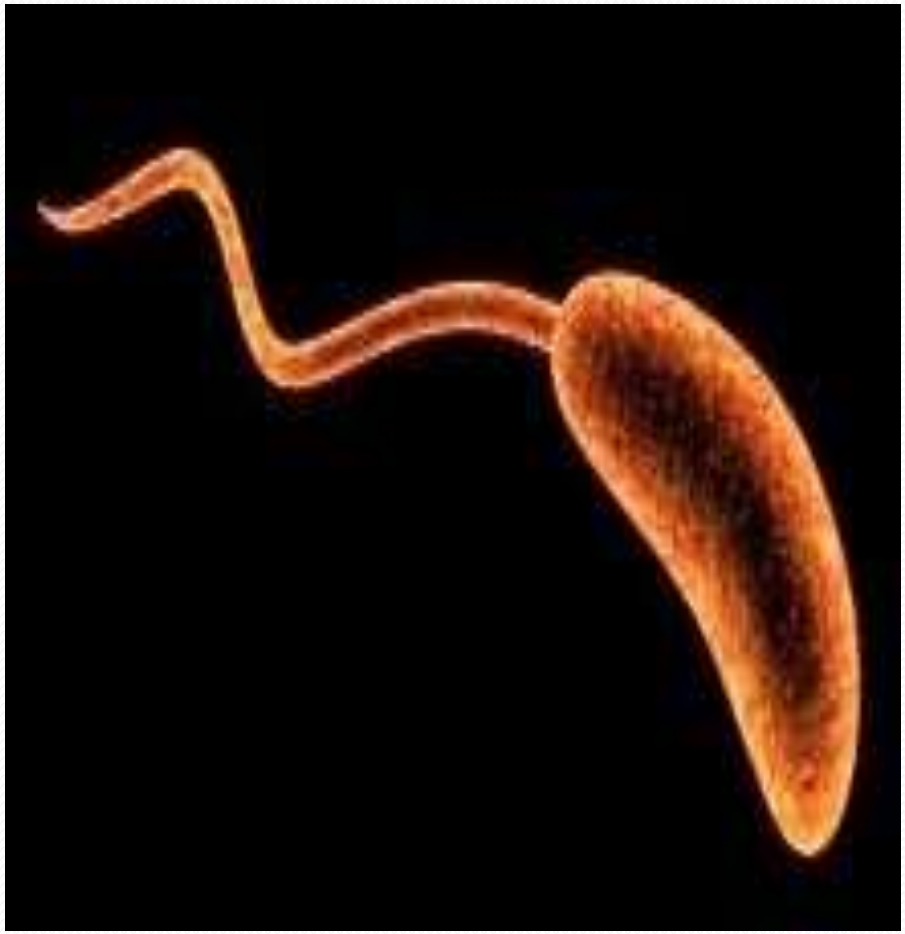




Corynebacterium diphtheriae(rod shape)

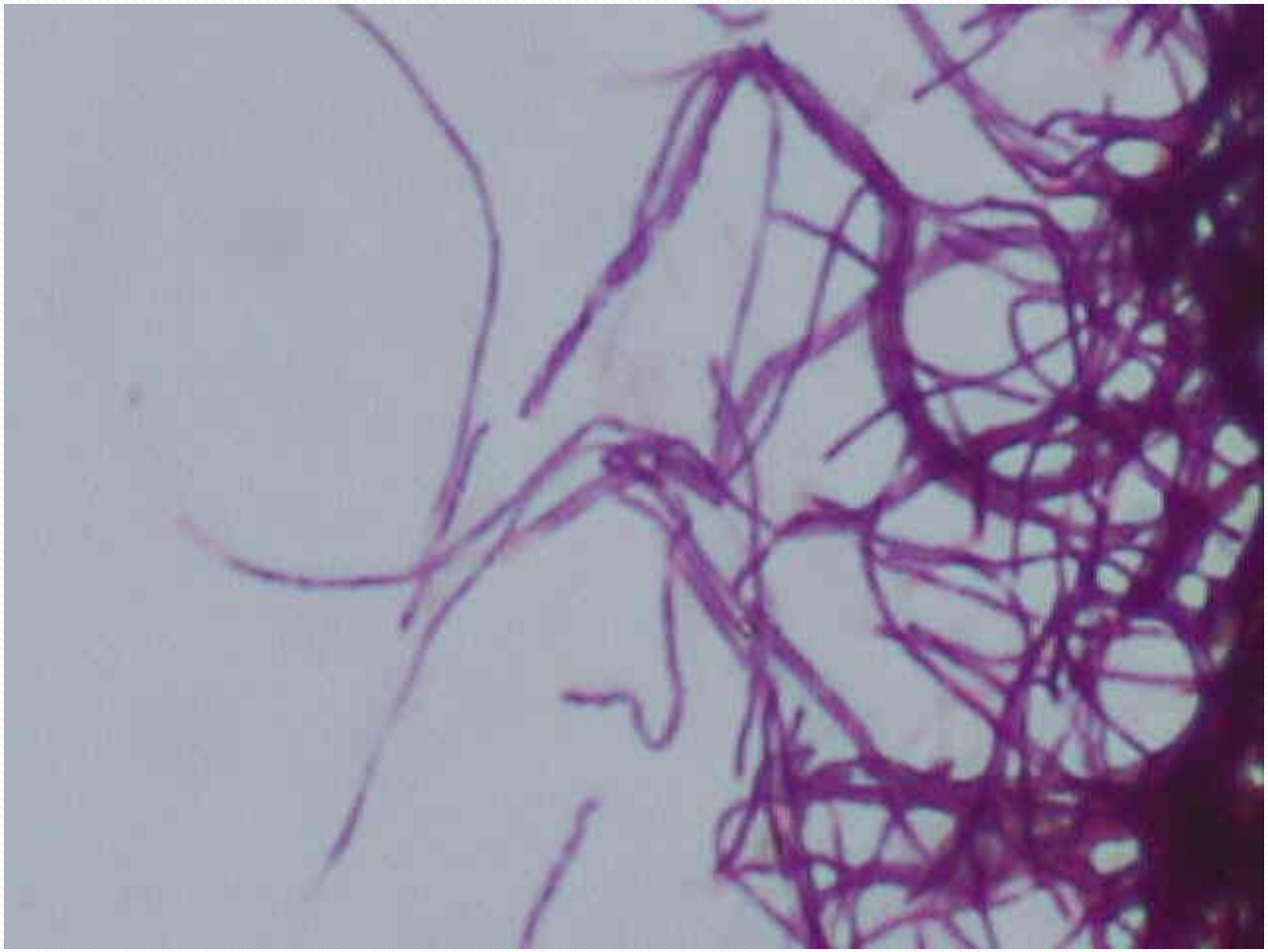
a- Gram stain b- Albert Stain

Curved Rod



Vibrio cholerae

Filamentous Bacteria



Streptomyces spp.

Spiral Shaped Bacteria

Spirillum volutans

Treponema pallidum

Staining reaction

a. The age of the culture is important. In older cultures , staining characteristics either vary or are not brought out well. Simple stains bring out the best morphology. Differential and special stains are necessary to bring out characteristics like: gram negative and gram positive bacteria, Acid fast and non acid fast , spirochetes, capsule and Flagella, etc.

Simple Stain

The simple stain can be used to determine cell shape, size, and arrangement. True to its name, the simple stain is a very simple staining procedure involving only one stain. You may choose from methylene blue, Gram safranin(RED), and Gram crystal violet.

Basic stains, such as methylene blue, Gram safranin, or Gram crystal violet are useful for staining most bacteria. These stains will readily give up a hydroxide ion or accept a hydrogen ion, which leaves the stain positively charged. Since the surface of most bacterial cells is negatively charged, these positively charged stains adhere readily to the cell surface.

Experimental Procedure

A. Wet Mount

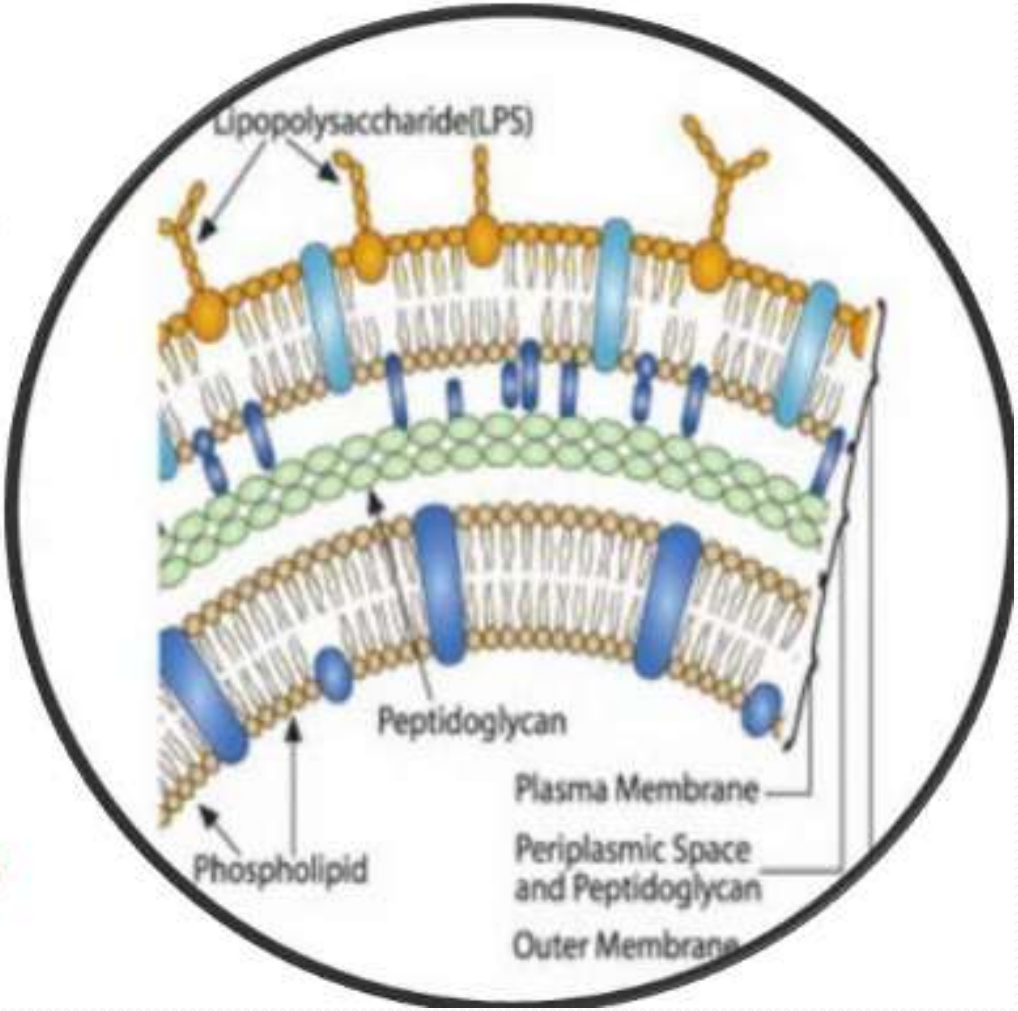
- *The wet mount is a preparation of a culture to observe motility (movement) or structure of microorganisms.*
- *Use a sterile inoculating loop to place a loopful of a motile bacillus culture on a slide. Cover immediately with a coverslip. Do not allow the preparation to dry out. Observe under the microscope. Draw a picture of what you see.*

B. Simple Stain

1. Place a loopful of ***Bacillus*** culture into a test tube of sterile distilled water to make a suspension of bacterial cells in the water. Place a loopful of this bacterial suspension on a clean slide. Allow the bacteria on the slide to air dry.
2. Heat fix the cells by passing the slide quickly through the flame of a Bunsen burner two or three times, with the glass surface exposed to the flame. Each pass should only be a second or two. The slide should not be so hot as to be uncomfortable to touch. (NOTE: your instructor will demonstrate this for you!)
3. Flood the slide with methylene blue stain for 60 seconds.
4. Rinse the slide with distilled water, blot it dry, and examine it under the microscope.
5. Draw what you observe.

Stains and Staining

- Bacteria are slightly negatively charged at pH 7.0
  - Basic dye stains bacteria
  - Acidic dye stains background
- Simple stain
  - Aqueous or alcohol solution of single basic dye



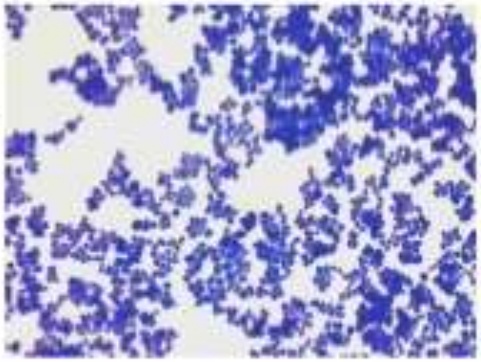
Simple Stains

Bacteria have nearly the same refractive index as water, therefore, when they are observed under a microscope they are opaque or nearly invisible to the naked eye.

Different types of staining methods are used to make the cells and their internal structures more visible under the light microscope.

Simple stains use one dye that stains the cell wall.

The cells are then visible against a light background.

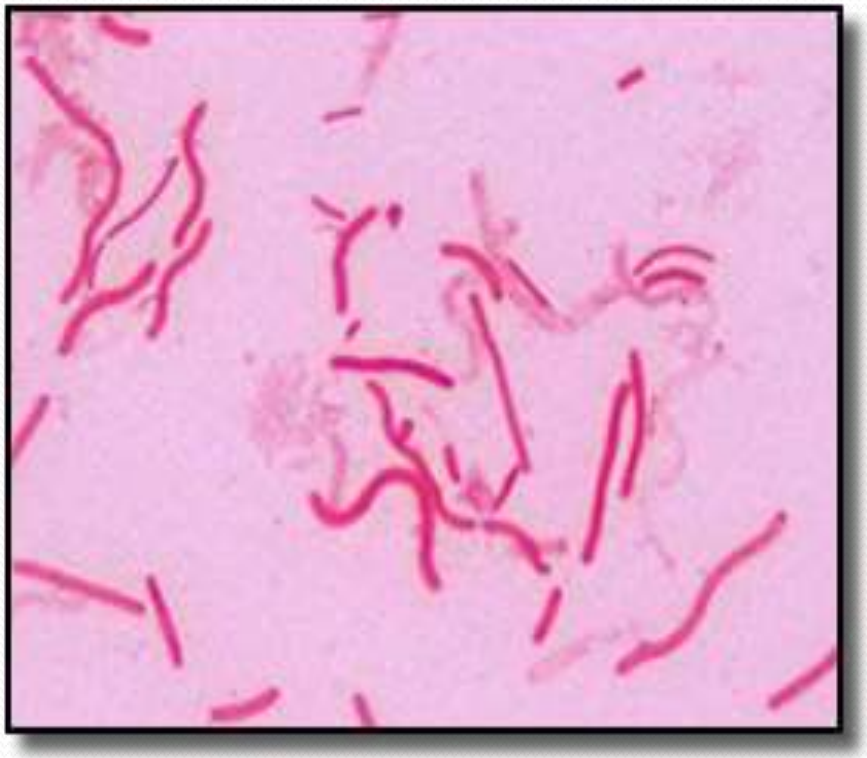


Steps:

1. **Place the slide on the staining rack.**
2. **Flood the slide with a basic stain: either crystal violet (1 min.), Safranin (2 min.), or Methylene blue (2 min.).**
3. **Wash the stain off the slide with deionized water.**
4. **Blot the slide with bibulous paper.**

Simple Staining

Drag the cursor over the images to read the description



Positive staining

Negative staining

a. Gram stain

a. Gram stain divides the bacteria into Gram positive & Gram negative.

The basic procedure goes like this:

i. Take a heat fixed bacterial smear.
ii. Flood the smear with CRYSTAL VIOLET for 30 second, then wash with water. [PRIMARY STAIN]
iii. Flood the smear with IODINE for 1 minute, then wash with water (mordant).
iv. Flood the smear with ETHANOL 95% for (15-30)sec, then wash with water. [DECOLORIZER]
v. Flood the smear with SAFRANIN for (60-80) second, then wash with water. [COUNTERSTAIN]
vi. Blot the smear, air dry and observe.

contd

- Examine under microscope

i. **Gram positive bacteria**- violet
ii. **Gram negative bacteria**- pink

b. Acid Fast Stain Ziehl-Neelsen method

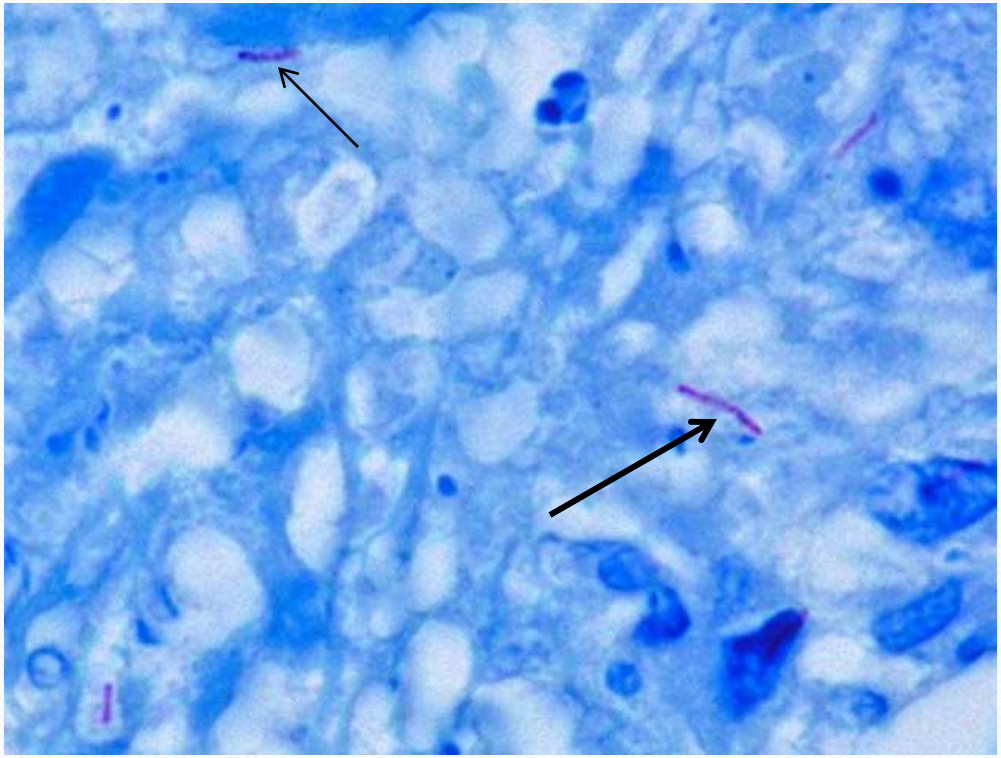
- Zn stain- which is divides the bacteria into Acid-Fast and non Acid-Fast.
- Principle: Some bacteria contain a waxy lipid, mycolic acid, in there cell wall. This lipid makes the cells more durable and is commonly associated with pathogens. Acid fast cell walls are so durable that the stain (carbol fuschin) must be driven into the cells with heat. All other cells will decolorize with this strong acid solvent, but acid fast bacteria will not. Other cells are then counterstained with methylene blue.

Procedure

i. Make a smear on the glass slide and allow to heat-fix.
ii. Flood the carbol fuchsin on the slide and & warm under the spirit lamp for 5 min until vapor
iii. Allow to cool and wash under tap water
iv. Decolorize by 20% solution of H_2SO_4 or a mix of acid – alcohol(3%HCL in 95% Ethanol)slowly drop wise until the dye no longer runs off from the smear for (10-30) sec.
v. Rinse with water.
vi. Counter stain with methelene blue for 2 minute.
vii. Wash under tap water and allow to dry.

contd

- Examine under microscope at oil immersion
- **Acid-Fast bacilli-** red
- **Background** -blue

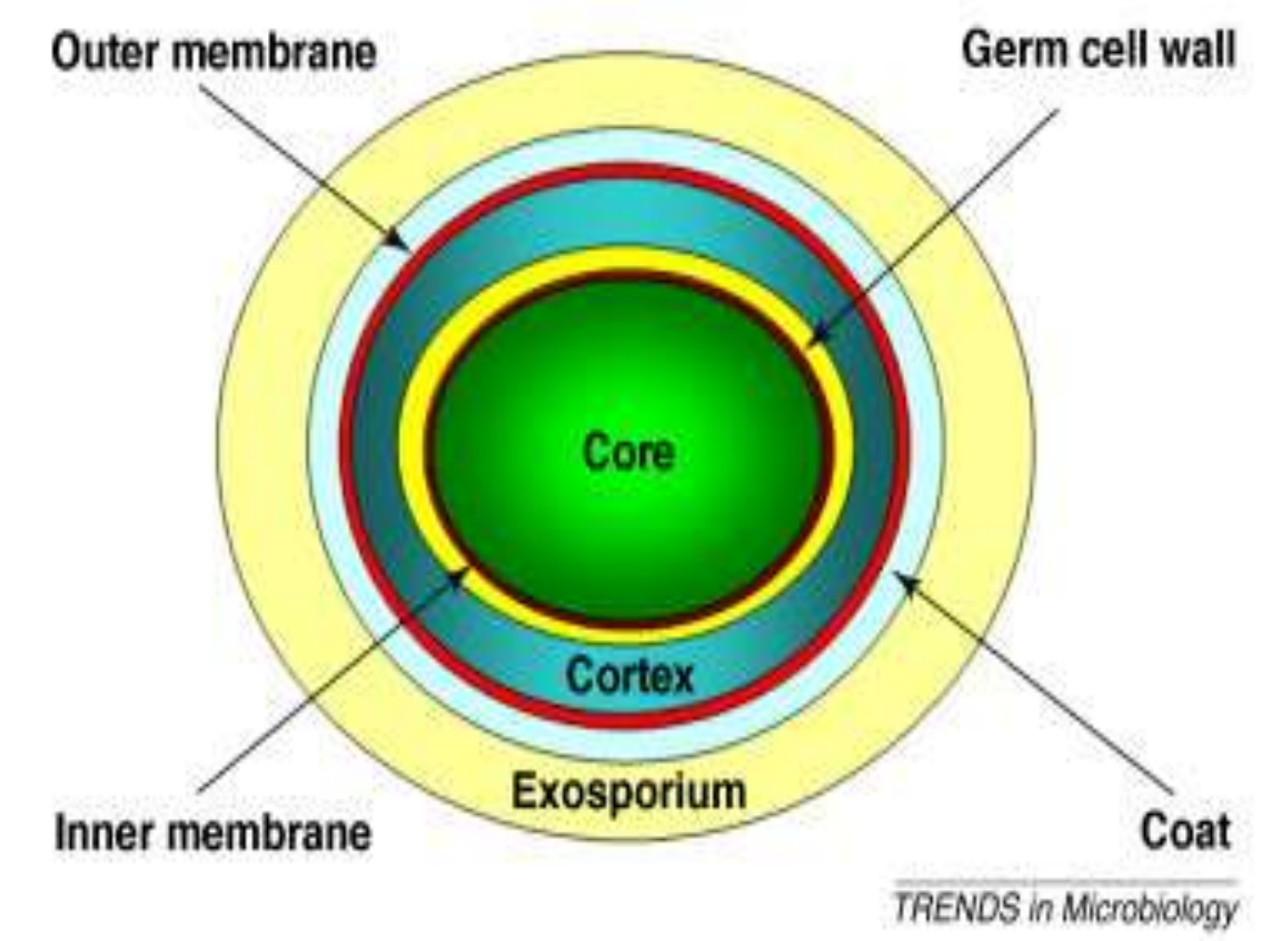


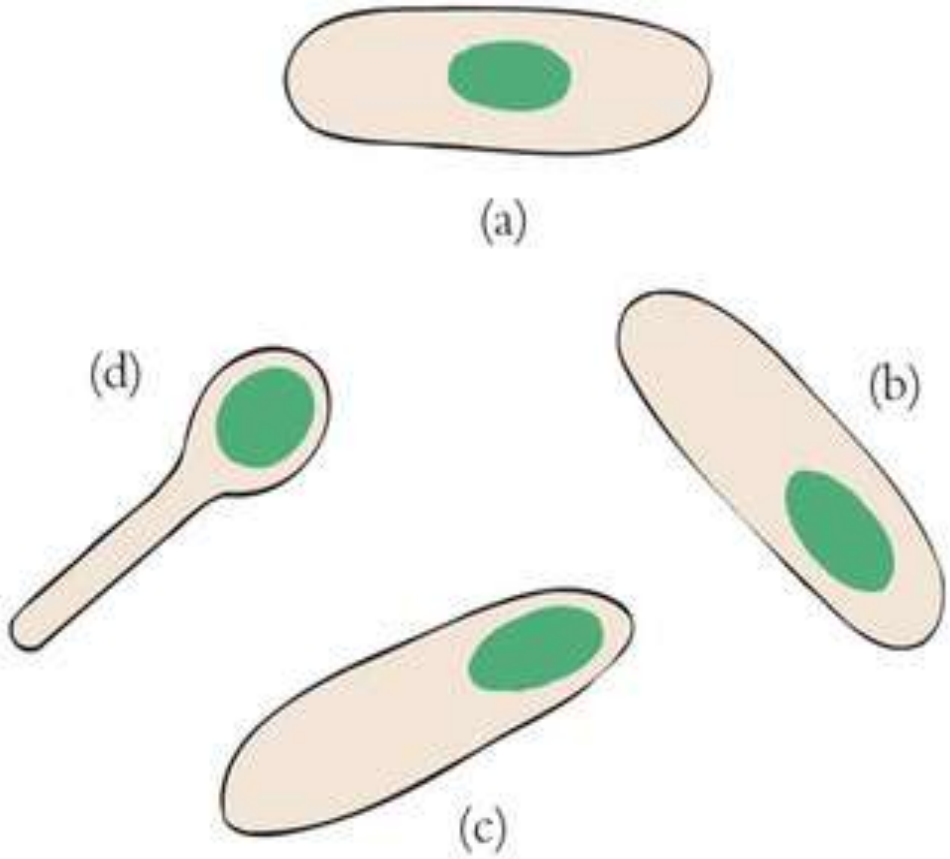
Mycobacterium tuberculosis

Bacterial Endospores

- Endospores are a dormant stage of some bacterium that allows it to survive conditions that would normally kill bacteria such as extreme drought or heat
- Endospores provide resistance against:
- drying
  - Low nutrient conditions
  - Radiation
  - High temperaturesand various chemical disinfectants

Structure of Bacterial Endospore





Location within the Parent cell

a- Central *Bacillus cereus*

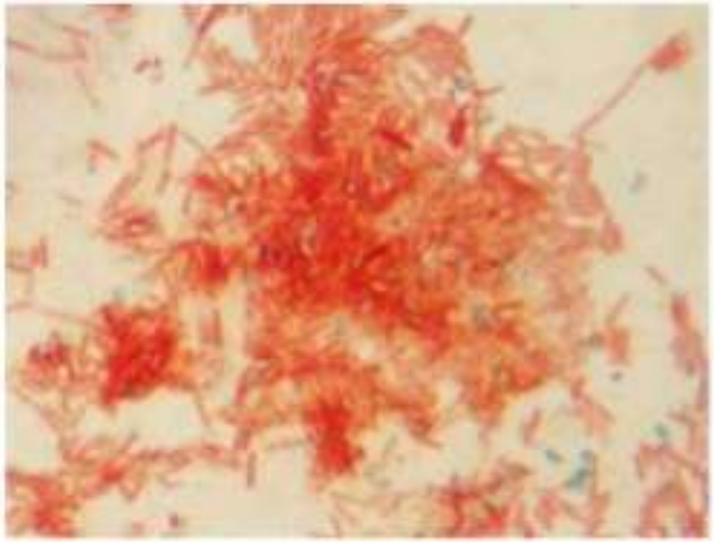
b- Sub terminal *B. subtilis*

c- Terminal *Closteridium sp.*

d- Bulging terminal *Cl. tetani*

Prokaryotes - Endospores

- Dormant, tough, non-reproductive structure produced by small number of bacteria.
- *Q: What is the function of endospores?*
- Resistant to radiation, desiccation, lysozyme, temperature, starvation, and chemical disinfectants.
- Endospores are commonly found in soil and water, where they may survive for very long periods of time.



An endospore stained bacterial smear of *Bacillus subtilis* showing endospores as green and vegetative cells as red.

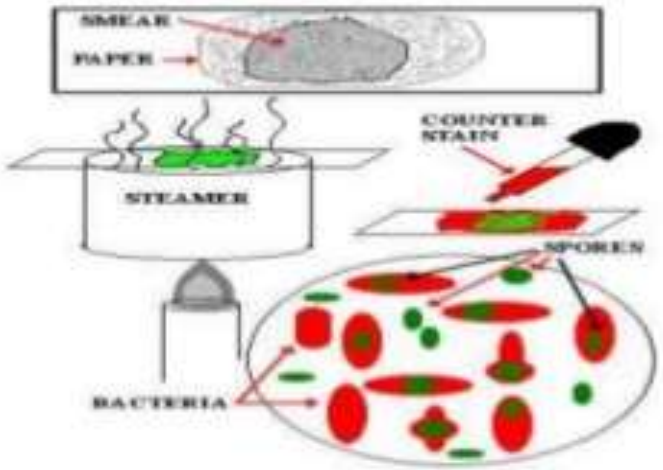


Image: *Bacillus subtilis*, SPO Science Image Library, Endospore stain from Dr. Ronald E. Hurlbert, Microbiology 101 lab manual

The Hanging Drop Slide and Bacterial Motility

Objective

To demonstrate : (a) form, (b) arrangement, and (c) motility of some micro–organisms, by means of hanging–drop slide preparations.

Materials

1. Culture (broth–24 hours).
   a) *Bacillus subtilis.*
   b) *Staphylococcus aureus.*
2. A hay infusion, stagnant water, rumen liquor, etc.
3. Concave (deep–well) slide, cover glasses, and Vaseline.

Procedure

1. With a toothpick, spread a small ring of Vaseline around the concavity of a depression slide (Figure 6a). Do not use too much Vaseline.
2. After thoroughly mixing one of the cultures, use the inoculating loop to aseptically place a small drop of one of the bacterial suspensions in the center of a cover slip (Figure 6b).
3. Lower the depression slide, with the concavity facing down, onto the cover slip so that the drop protrudes into the center of the concavity of the slide (Figure 6c). Press gently to form a seal.
4. Turn the hanging drop slide over (Figure 6d) and place on the stage of the microscope so that the drop is over the light hole.
5. Examine the drop by first locating its edge under low power and focusing on the drop. Switch to the high–dry objective and then, using immersion oil, to the 90 to 100X objective. In order to see the bacteria clearly, close the diaphragm as much as possible

for increased contrast. Note bacterial shape, size, arrangement, and motility. Be careful to distinguish between motility and Brownian movement.

6. Discard your cover slips and any contaminated slides in a container with disinfectant solution.

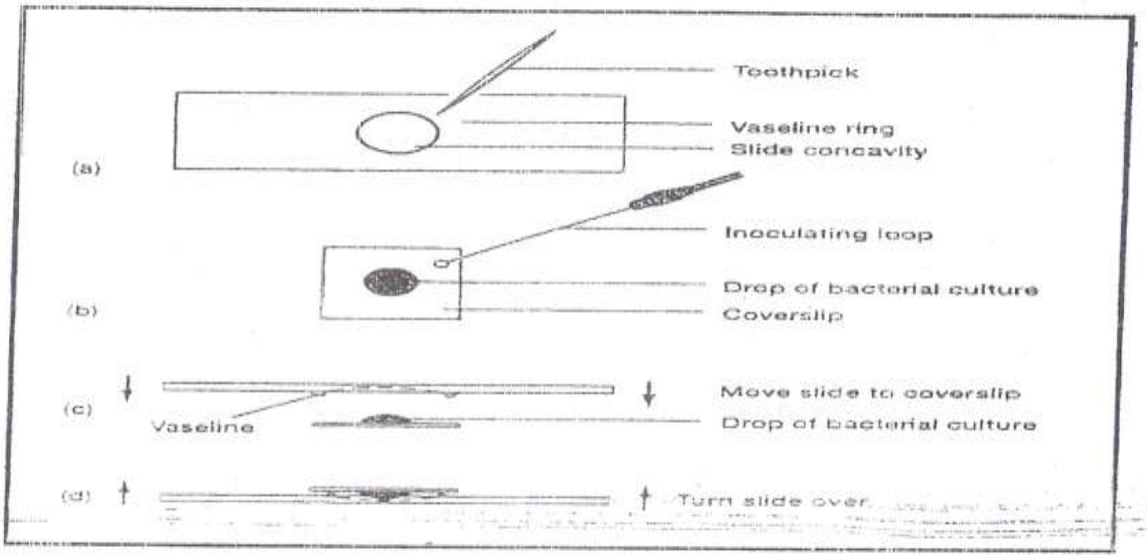
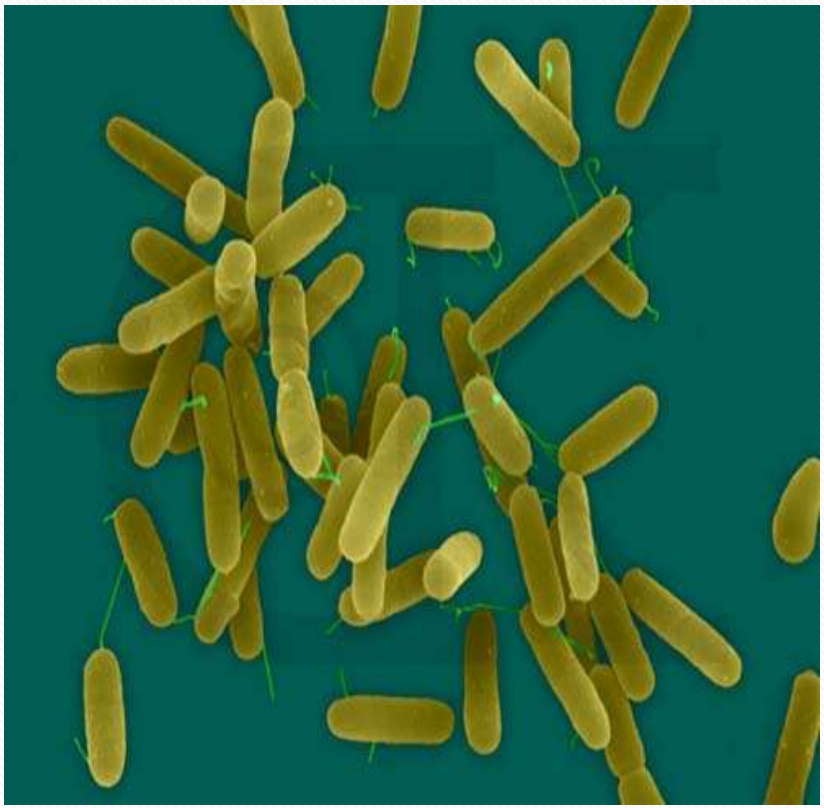


Figure (6): Preparation of a Hanging Drop Slide.

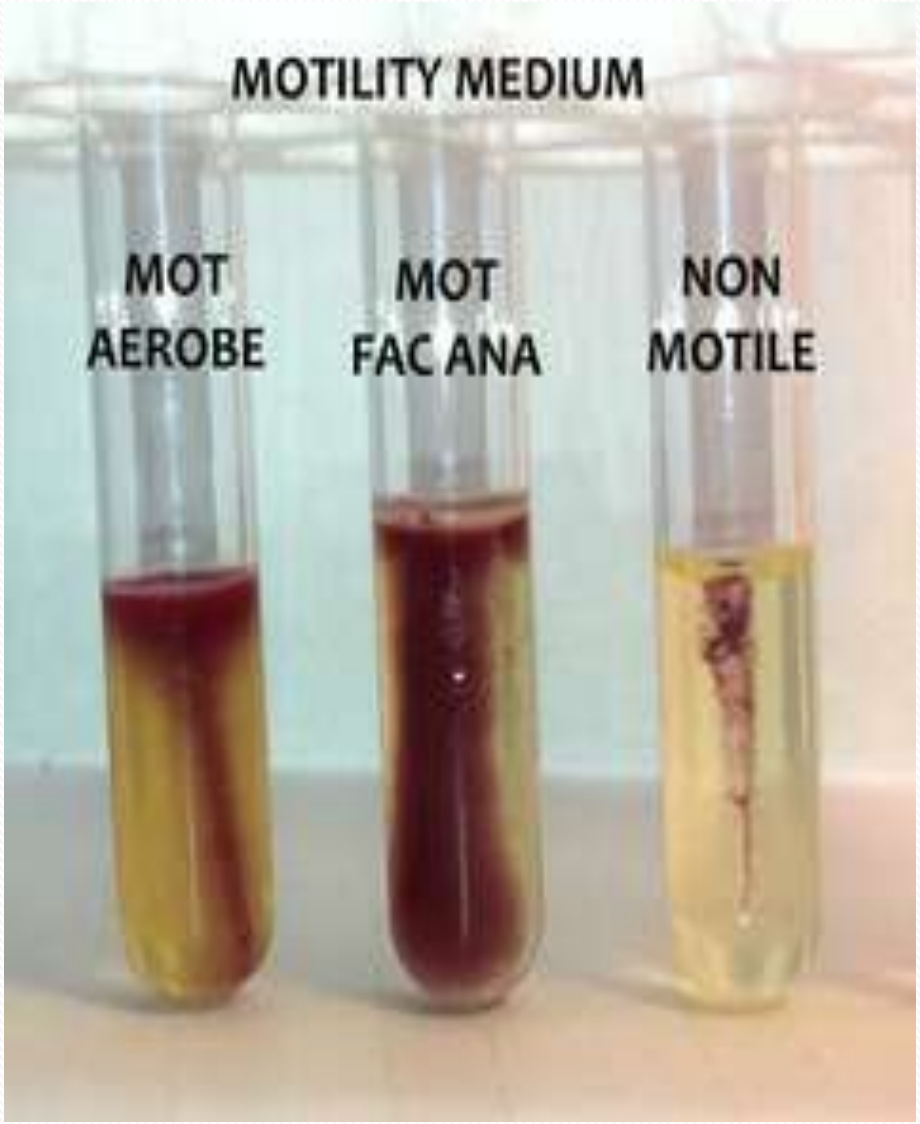
Flagella stain



Pseudomonas aeruginosa

3) Semi-Solid media Inoculation

- The most commonly used test for motility in microbiology lab.
- It depends on the ability of motile bacteria to move through semi-solid media.
- Ordinary solid media contain 1.5-2.0% Agar
- Semi solid media contain about 0.4% Agar


MOTILITY MEDIUM
MOT AEROBE
MOT FAC ANA
NON MOTILE

1. Isolation in pure form

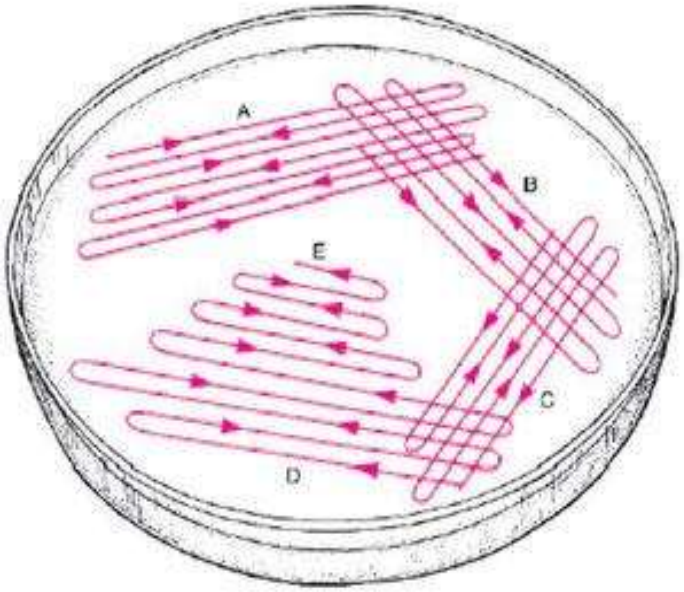
- Studies on the biochemical, antigenic and other characters of bacteria can be done only if the organism available in the pure form.

Technique:

a. Plating on solid culture media- clinical sample is streaked onto a solid medium (like: MacConkey agar, nutrient agar or blood agar) in such a way so as to ensure isolated discrete colonies.

b. Use of selective growth condition-most important example of this is the growth of anaerobic bacteria which will not take place in an environment having oxygen.

Pure culture Isolation Technique
Streak Plate Method



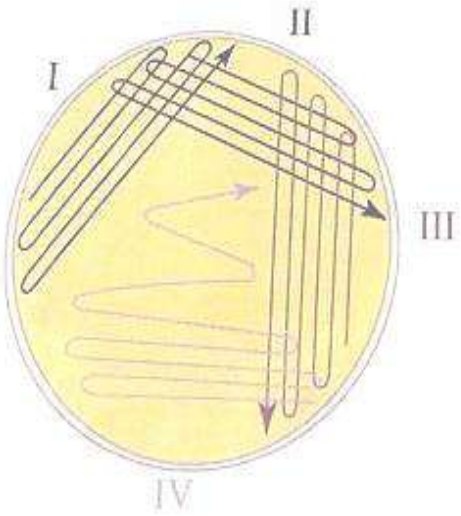


FIGURE 2-1 The quadrant method of streaking a plate for isolation. The agar surface is streaked as in I. After *flaming the loop*, the plate is rotated almost 90° and streaked as in II. The process is repeated for streaks III and IV.

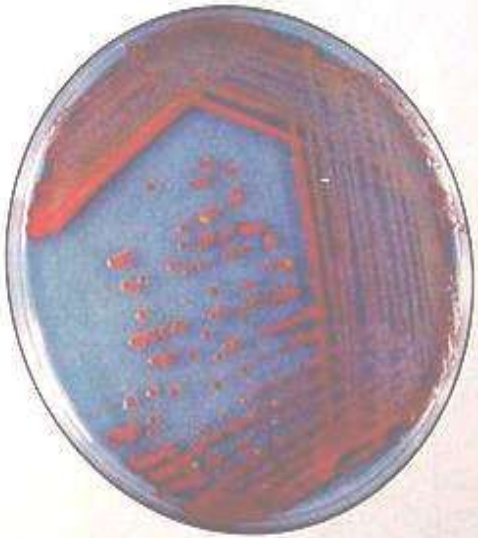


FIGURE 2-2 A streak plate of *Serratia marcescens* after incubation. Note the decreasing density of growth in the four streak patterns. On this plate, isolation is first obtained in the fourth streak. Cells from individual colonies may be transferred to sterile media to start pure cultures of each.

Culture Media

Culture media are used for recognition and identification (diagnosis) of microorganisms. The media are contained in plates (Petri dishes), in test tubes, flasks or screw capped bottles. **Used of media as :**

a- Simple or basal e.g., Nutrient agar, Nutrient broth.

b- Special-purpose media e.g., selective media, enriched media.

Types of culture media regarding their consisitency:

1- Liquid (fluid) media e.g., Nutrient broth, Peptone water.

2- Solid media, e.g., Nutrient agar, MacConkey agar.

3- Semisolid media : e.g., motility media.

Solid media as agar in special purpose as :

1. **Enriched media**, simple media enriched with substances e.g., added blood 5-10% added glucose 1-2%.
2. **Selective media** containing inhibitory substance as : e.g., bile salts, antibiotic, dyes,….etc., which favors the growth of the concerned microorganism and inhibit the growth of others, e.g., MacConkey agar, Bismuth Sulphate agar or SS agar.
3. **Differential media**, certain species produce characteristic growth that can easily recognized or can produce certain effects in the media, e.g., Triple sugar Iron agar (TSI), hemolytic and non-hemolytic species on blood agar.

Ordinary or Simple media

Nutrient Agar

Staphylococcus aureus

Streptococcus pyogens

Ordinary or Simple media

Nutrient Agar

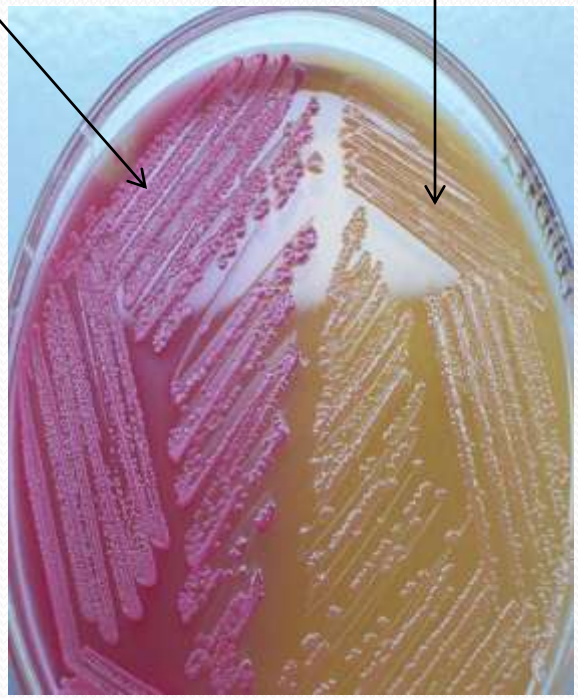
Bacillus subtilis

Proteus

Differential and selective Media
MacConkey Agar

Lactose fermenter (pink)

non lactose fermenter (pale or yellow)

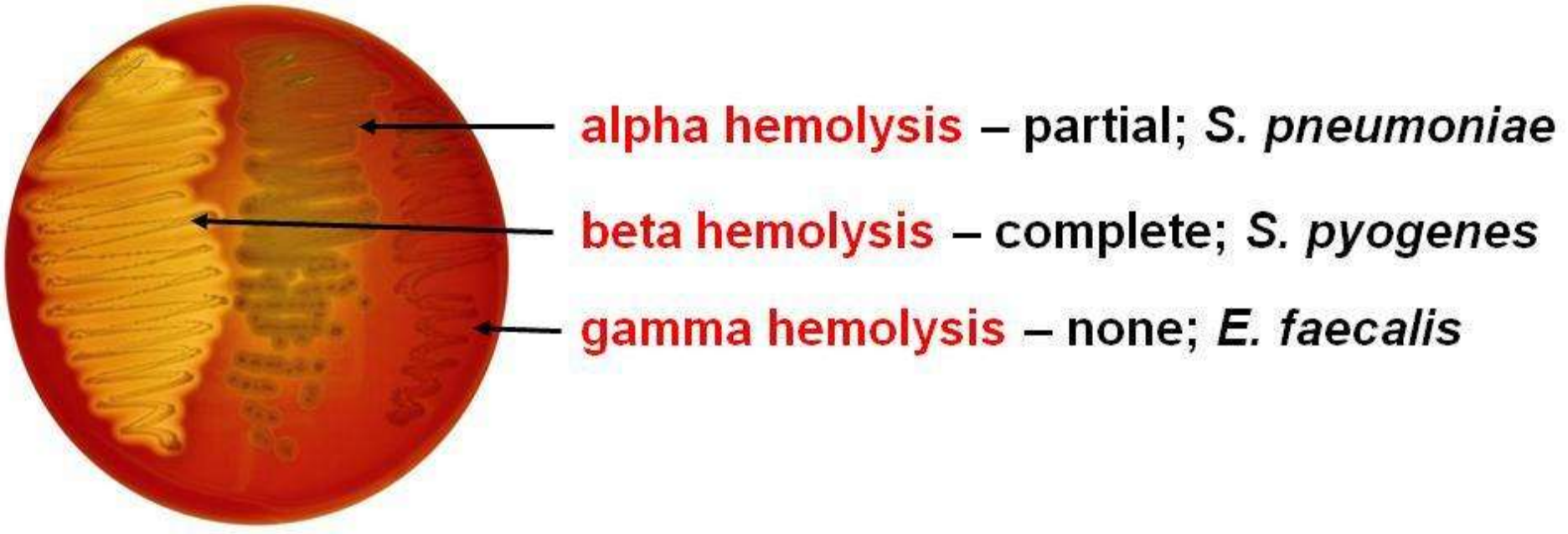


Enterobacter cloacae on MacConkey Agar:growth with pink colonies

Eschericia coli on MacConkey Agar:
growth, with pink colonies

Enrichment and Differential Media

BLOOD AGAR

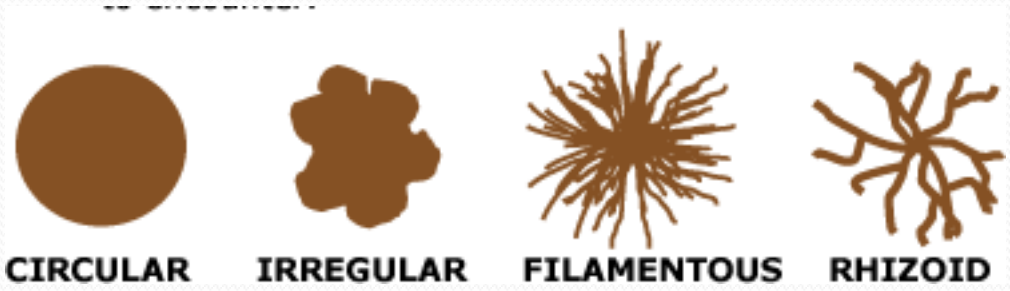


S: *Streptococcus*
E: *Enterococcus*

3. Morphology of the bacterial colony

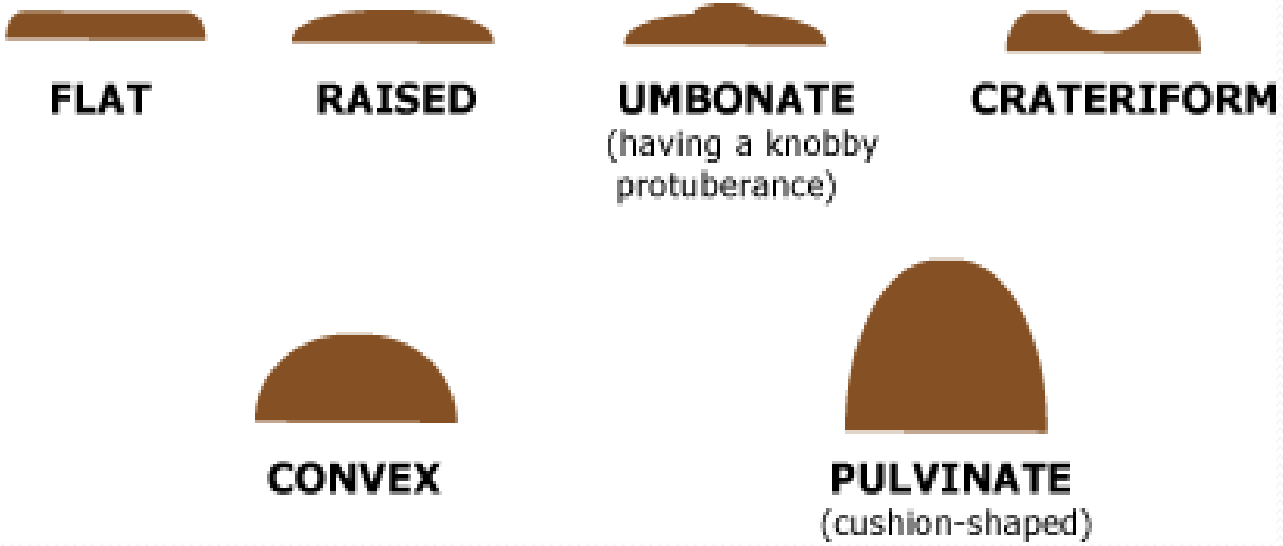
i. Shape: circular, irregular, radiate or rhizoid.
ii. Size: diameter in mm
iii. Elevation: flat, raised, low convex, dome shaped
iv. Margin: Entire, wavy, lobate, filiform
v. Surface: smooth, wavy, rough, granular, papillate, glistening etc.

Shape of the colony



Elevation of the colony

2. Elevation – This describes the "side view" of a colony. These are the most common.



Margins of the colony

3. Margin – The margin or edge of a colony (or any growth) may be an important characterisic in identifying an organisms. Several examples are shown below.

4. Cultural characteristics

These provide additional information for the identification of a bacterium.

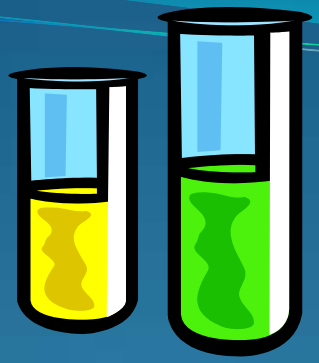
A. On solid medium the following characters are observed

i. Shape: circular, irregular, radiate or rhizoid.
ii. Size: The size of the colony can be a useful characteristic for identification. The diameter of a representative colony may be measured.
iii. Elevation:
iv. Margin: Entire, wavy, lobate, filiform
v. Surface: smooth, wavy, rough, granular, papillate, glistening etc.
vi. Size in mm
vii. Texture : dry, moist, mucoid, brittle, viscous, butyrous (buttery).
viii. Color : colorless, pink, black, red, bluish-green.

B. IN A FLUID MEDIUM FOLLOWING CHARACTERS ARE OBSERVED

i. Degree of growth- Absence, scanty, moderate, abundant etc.
ii. Present of turbidity and its nature.
iii. Presence of deposit and its character.
iv. Nature of surface growth.
v. Ease and disintegration and odor.

Growth in Liquid(broth) media



6.Media & Biochemical Tests

Laboratory Objectives

5.METABOLISM

To classify the differentiate species following aspects are studied

i. Requirement of oxygen
ii. The need of co2
iii. Capacity to form pigments
iv. Power of hemolysis

Tests To Know

- **Case Study Tests**
  - Catalase test
  - Coagulase test
  - Oxidase test
  - Urea hydrolysis
  - Lactose fermentation
  - Sucrose fermentation
  - Glucose fermentation & gas production
  - Triple sugar iron(TSI) test
  - Indole
  - Methyl Red/Voges Proskauer
  - Citrate
  - H_2S production in SIM
  - Growth and reactions on differentia and selective media
    Mannitol salt agar (MSA)

ACTION OF DYES AND ANTIBIOTICS

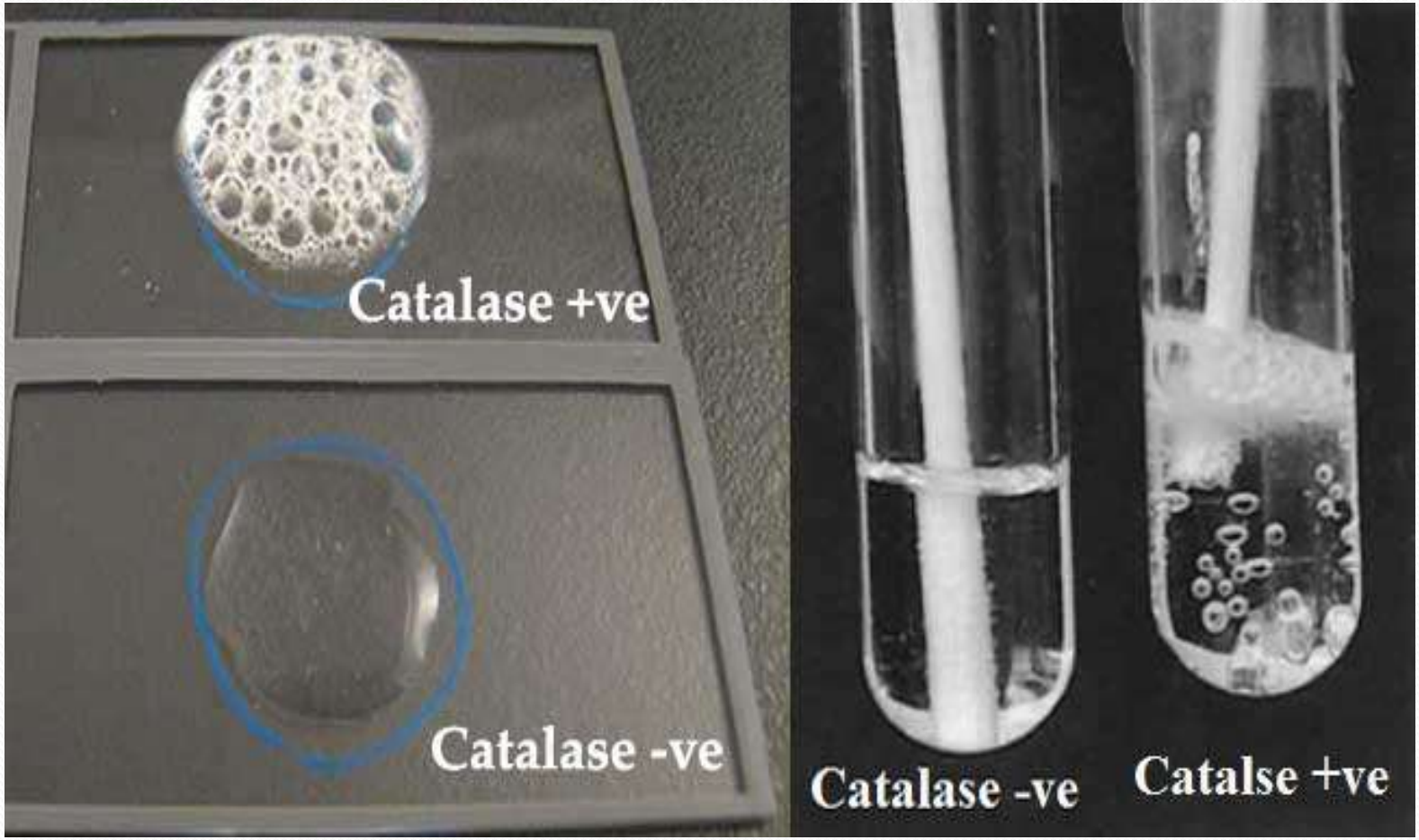
Antibiotic sensitivity test

Catalase test

- Used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.
- PRINCIPLE:
  - Catalase act as a catalyst in the breakdown of **hydrogen peroxide to oxygen and water**. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hrs old.

Catalase test

- slide method(left)
- tube method(right



Coagulase

- Property it tests for: This tests for the bacteria's ability to clot blood plasma using the enzyme coagulase.
- Media and Reagents: This media contains rabbit plasma dissolved in buffer.
- How to Perform Test: Inoculate rabbit plasma with one single colony. Break up colony and stir until blended in plasma. Incubate at 37 degrees C for 24 hours.

Coagulase Results

- Reading Results:
  - If the organism is has coagulase it will clump the plasma.
  - If the organism does not have coagulase it will not clump the plasma.

Slide test (clumping factor)

Presumptive needs confirming with the tube test

Tube test (free coagulase)

Check tubes at ½, 1, 2 & 4 hrs and overnight

Oxidase Test

Discriminates organisms that can produce cytochrome oxidase which catalyzes the transfer of electrons from reduced cytochrome c in the electron transport chain to molecular oxygen.

Test uses NNNN-tetramethyl-p-phenylenediamine (Oxidase Reagent) as an artificial electron acceptor: when oxidized it is colorless, when reduced it turns purple

*Look for color change on the bacteria in the filter paper! (The reagent will turn light purple(violet) when exposed to oxygen in the air)

Urea Hydrolysis

- Property it tests for: This test is done to determine a bacteria's ability to hydrolyze urea to make ammonia using the enzyme urease.
- Media and Reagents Used: Urea broth contains a yeast extract, monopotassium phosphate, disodium phosphate, urea, and phenol red indicator.
- How to Perform Test: Inoculate Urea broth with inoculating loop.
- Reading Results: Urea broth is a yellow-orange color. The enzyme urease will be used to hydrolyze urea to make ammonia. If ammonia is made, the broth turns a bright pink color, and is positive. If test is negative, broth has no color change and no ammonia is made.

Urease Test

A- Slant Agar

A

B- Urea Broth

B

Triple Sugar Iron (TSI) Fermentation Test

Basic Principle

Purpose

Used to differentiate and identify pathogenic Gram-negative enteric bacteria

TSI Medium

Contains three types of sugars: glucose (0.1%), lactose (1%) and sucrose (1%)
Also contains phenol red (pH indicator) and ferrous ammonium sulfate
Original control of the medium is red - due to alkaline pH

Results

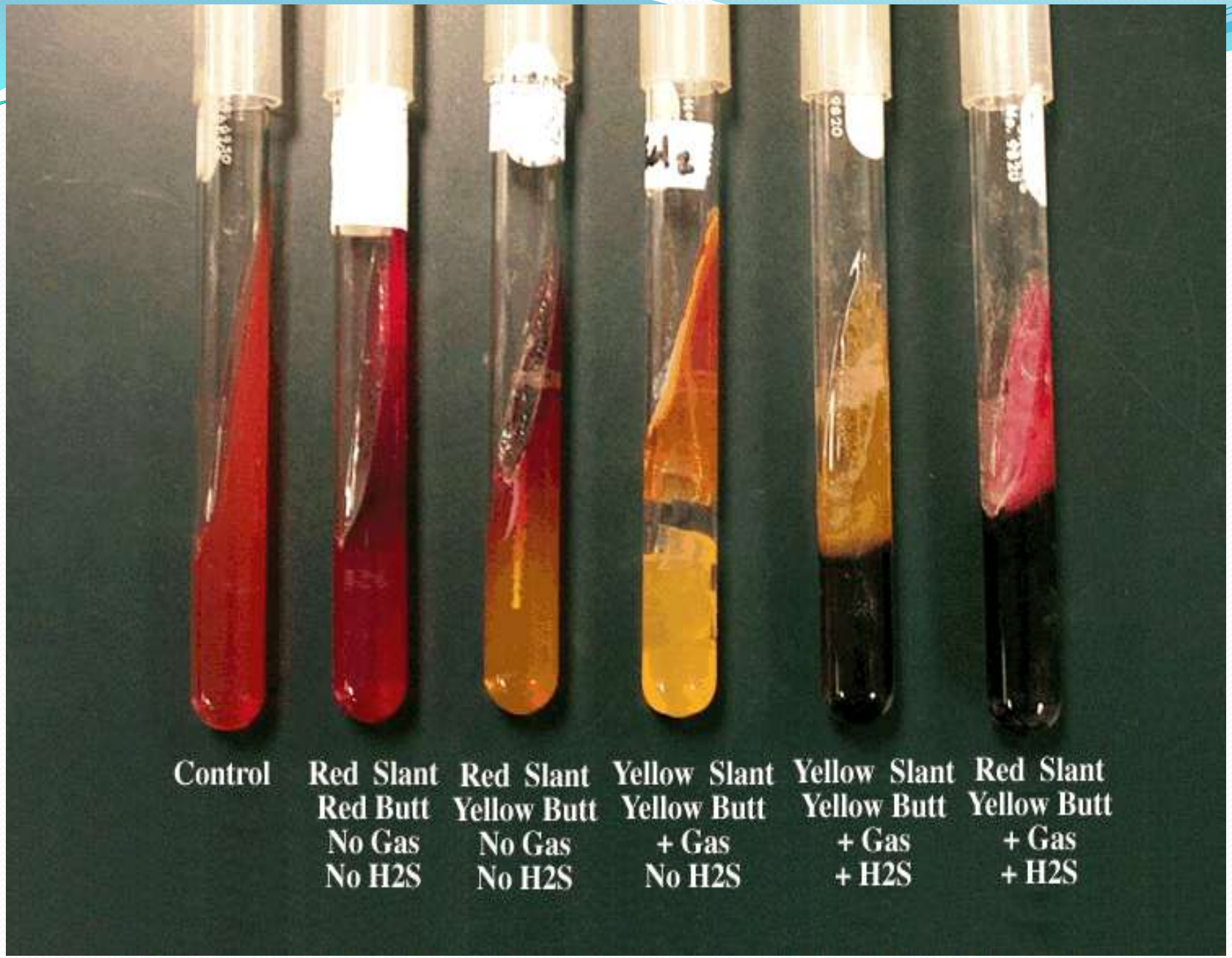
If no fermentation --> medium remains red

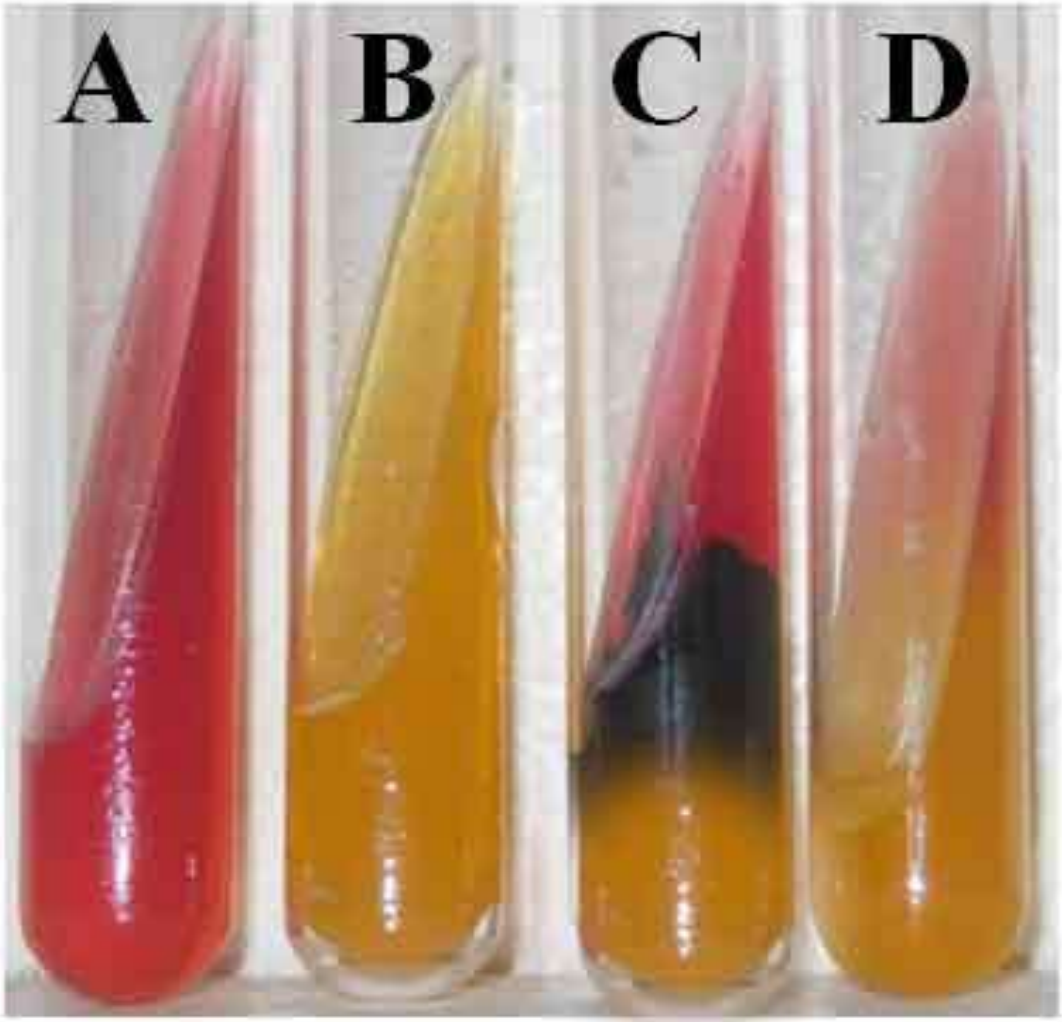
If the bacteria ferment all three sugars --> acids are produced --> entire medium turns yellow

If the bacterium ferments only glucose --> acids are produced --> if the cap is loose --> acids are oxidized --> medium slant becomes red again

If gases such as CO_2 or H_2 released during fermentation --> bubbles collect in the medium

If H_2S is produced --> reacts with ferrous ammonium sulfate in the medium --> black ferrous sulfide precipitate formed at the bottom of the medium (black butt)


Control
Red Slant
Red Butt
No Gas
No H2S
Red Slant
Yellow Butt
No Gas
No H2S
Yellow Slant
Yellow Butt
+ Gas
No H2S
Yellow Slant
Yellow Butt
+ Gas
+ H2S
Red Slant
Yellow Butt
+ Gas
+ H2S



A) ***Psuedomonas aeroginosa*:** Gluc (-), Lac/Suc (-), H_2S (-)

B) ***Escherichia coli*:** Gluc (+), Lac/Suc (+), H_2S (-)

C) ***Salmonella typhimurium*:** Gluc (+), Lac/Suc (-), H_2S (+)

D) ***Shigella boydii*:** Gluc (+), Lac/Suc (-), H_2S (-)

Some example of Triple Sugar Iron (TSI) Agar Reactions

Name of the organisms	Slant	Butt	Gas	H_2S
Escherichia, Klebsiella, Enterobacter	Acid (A)	Acid (A)	Pos (+)	Neg (-)
Shigella, Serratia	Alkaline (K)	Acid (A)	Neg (-)	Neg (-)
Salmonella, Proteus	Alkaline (K)	Acid (A)	Pos (+)	Pos (+)
Pseudomonas	Alkaline (K)	Alkaline (K)	Neg (-)	Neg (-)

Lactose Fermentation

- Property it tests for: This tests for the bacteria's ability to ferment lactose.
- Media and Reagents Used: Lactose broth contains beef extract, gelatin peptone, and lactose. A phenol red indicator is added to indicate acid production from fermentation.
- How to Perform Test: Inoculate lactose broth with inoculating loop.
- **Results**
  - A positive result is yellow after indicator is added (indicating lactose fermentation)
  - A negative result will have no color change or will be reddish.

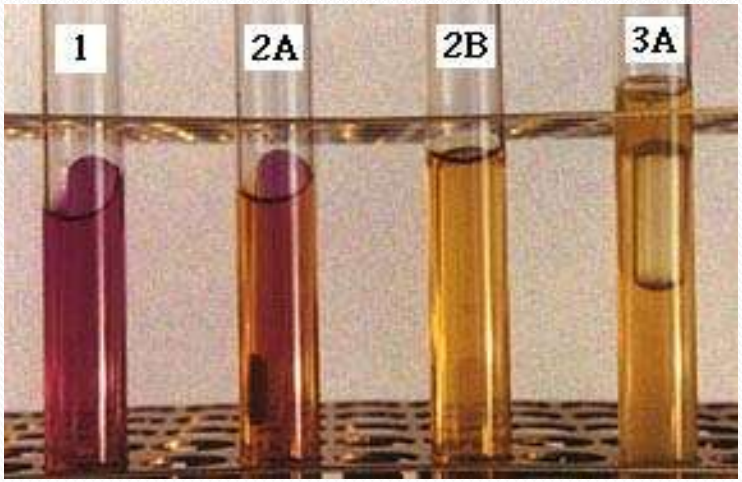
Sucrose Fermentation

- Property it tests for: This test is done to help differentiate species of the family *Enterobacteriaceae*. This tests for the bacteria's ability to ferment sucrose and production of acid end-product
- Media and Reagents Used: Sucrose broth contains beef extract, gelatin peptone, and sucrose. Phenol red indicator is added to indicate an acid end-product.
- How to Perform Test: Inoculate sucrose broth with inoculating loop.
- Results
  - A positive result is yellow after indicator is added (indicating sucrose fermentation)
  - A negative result has no color change or is reddish.

Glucose Fermentation & Gas Production

- Property it tests for: This test is done to help differentiate species of the family *Enterobacteriaceae*. This tests for the bacteria's ability to ferment glucose and produce gas and/or an acid end-product..
- Media and Reagents Used: Glucose broth contains beef extract, gelatin peptone, and glucose. A phenol red indicator is added to indicate an acid end-product. A Durham tube is added to indicate gas production.
- How to Perform Test: Inoculate broth with inoculating loop.
- Results
  - A positive result for acid is yellow after indicator is added (indicating glucose fermentation)
  - A positive result for gas is a bubble in the Durham tube.
  - A completely negative result has no color change or reddish color and no bubble.

Sugar Fermentation Tests

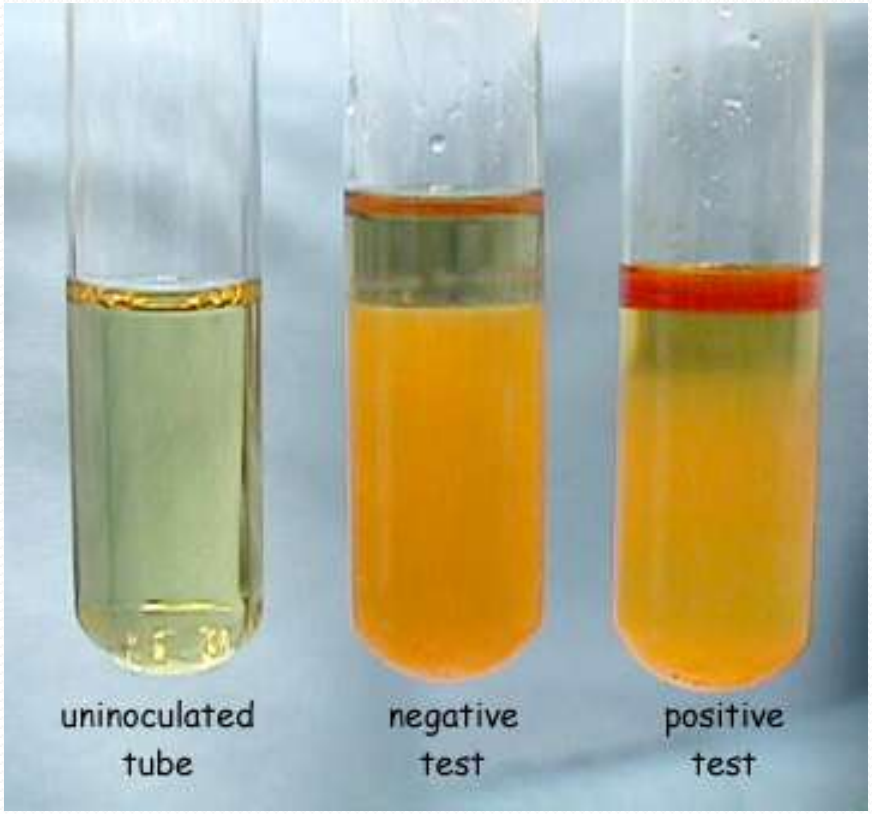


Tube 1: Negative acid /Negative gas
Tube 2A: Must incubate longer (ambiguous result)
Tube 2B: Positive acid /Negative gas
Tube 3A: Positive acid/ Positive gas

Indole Test

- Property it tests for: This test is performed to help differentiate species of the family *Enterobacteriaceae*.
- Media and Reagents Used: Tryptone broth contains tryptophan. Kovac's reagent—contains hydrochloric acid, dimethylaminobenzaldehyde, and amyl alcohol—yellow in color.
- How to Perform Test: Inoculate Tryptone broth with inoculating loop.
- Reading Results: Kovac's reagent reacts with indole and creates a red color at the top part of the test tube.

Indole

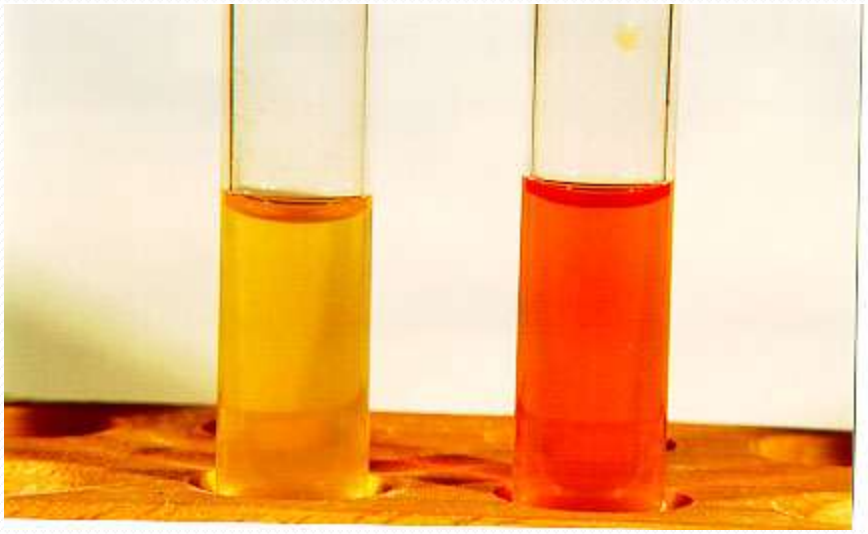


Methyl Red/Voges Proskauer (MR/VP)

- Properties these test for: Both tests are used to differentiate species of the family *Enterobacteriaceae*.
- Media and Reagents Used:
  - Glucose Broth
  - Methyl Red indicator for acid
  - Voges Proskauer reagents—A: 5% Alpha-Naphthol, & ethanol, B: Potassium Hydroxide, & Deionized Water.
- How to Perform Tests: Inoculate 2 glucose broths with inoculating loop. After 48 hours of incubation, add a few drops of MR to one tube, and VP reagents to the other tube.
  - MR—tests for acid end products from glucose fermentation.
  - VP—tests for acetoin production from glucose fermentation.

MR/VP continued

- Reading Results:
  - MR— a + result is red (indicating pH below 6) and a – result is yellow (indicating no acid production)
  - VP—A + result is red after VP reagents are added (indicating the presence of acetoin) and a – result is no color change.



Methyl Red: left – and right +

VP: left + and right –

Citrate Utilization test

Inoculation method: streak and stab slant with needle

Contains: citrate as sole carbon source, ammonium salts as sole nitrogen source, bromthymol blue pH indicator: neutral pH = green, alkaline = prussian blue. Media; Simmon's citrate agar.Discriminates organisms that can produce citrase to metabolize citrate into oxaloacetate and pyruvate. These organisms are forced to utilize ammonium salts as the nitrogen source producing alkaline ammonia waste.

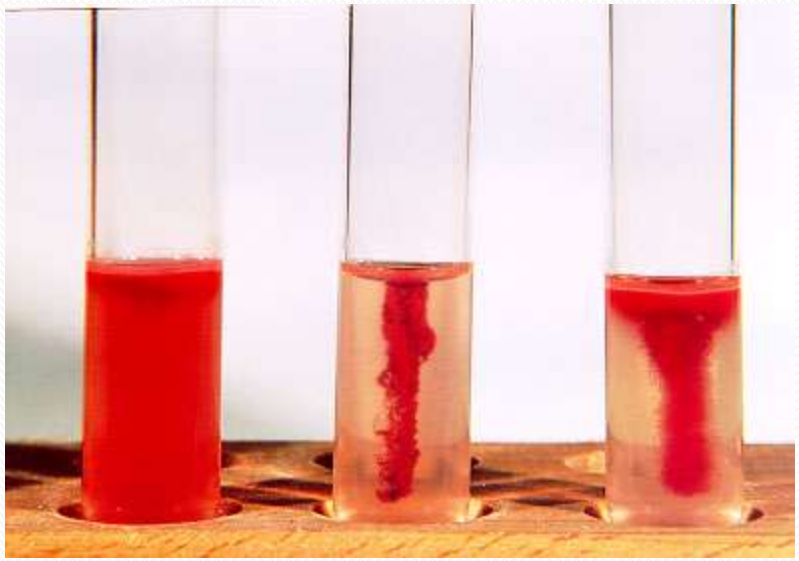
Results: Prussian blue slant and or butt = positive for citrase production

Green = negative for citrase production

Motility Test

- Property it tests for: This test is done to help differentiate species of bacteria that are motile.
- Media and Reagents Used: Motility media contains tryptose, sodium chloride, agar, and a color indicator.
- How to Perform Test: Stab motility media with inoculating needle.
- Reading Results: If bacteria is motile, there will be growth going out away from the stab line, and test is positive. If bacteria is not motile, there will only be growth along the stab line. A colored indicator can be used to make the results easier to see.

Motility



From left to right:

\+ − +

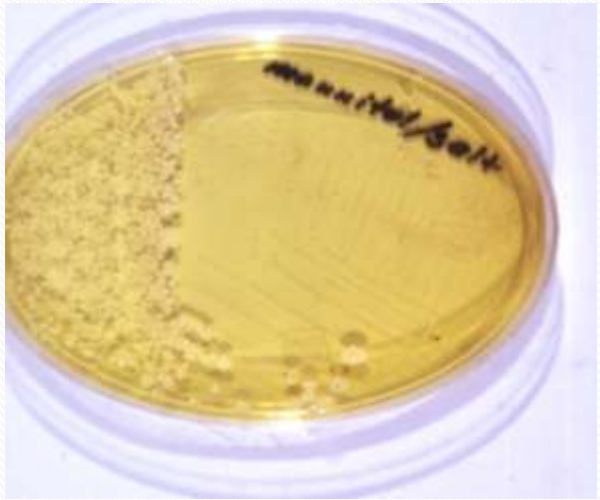
Mannitol Salt Agar (MSA)

- Property it tests for: This tests for the bacteria's ability to tolerate 7% salt concentration and ferment mannitol. The media is selective because it selects for salt tolerant bacteria.
- Media and Reagents: MSA media contains nutrient agar, mannitol, 7% sodium chloride and phenol red indicator.
- How to Perform Test: Inoculate an MSA plate using streak plate method and incubate 24-48 hours.

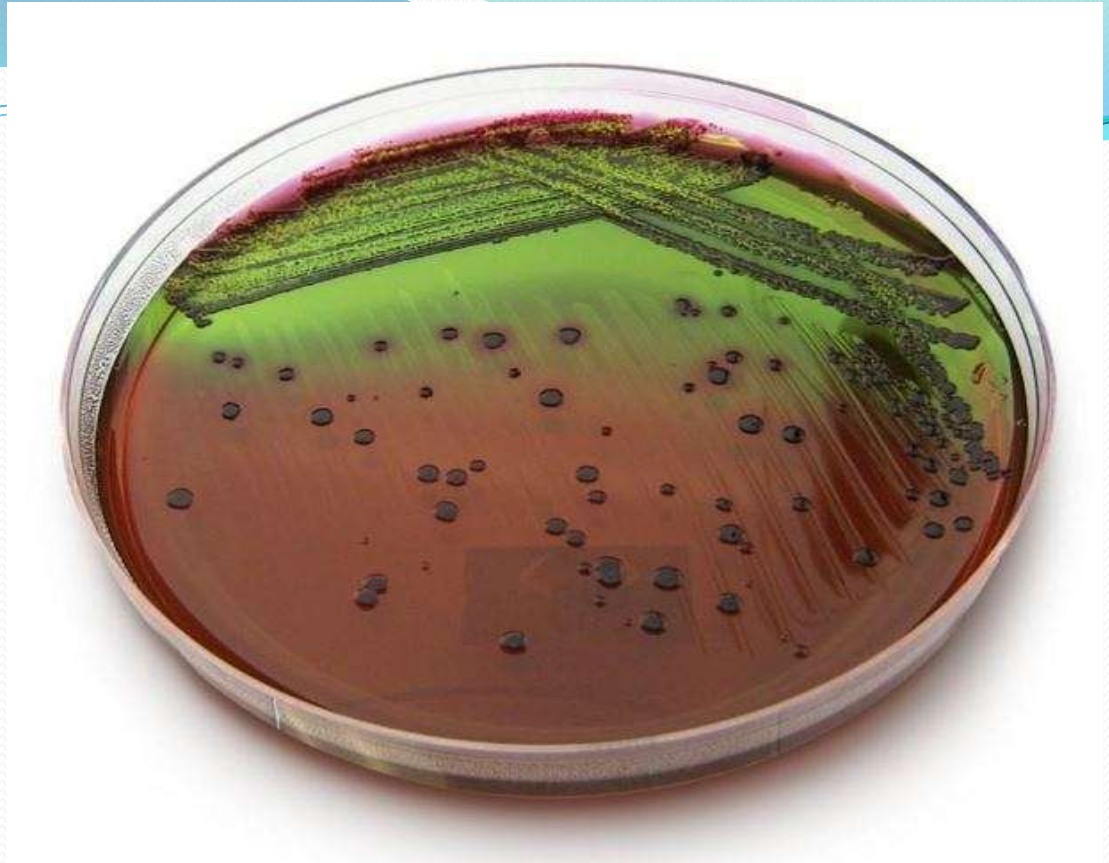
MSA Results

- Reading Results:
  - If the organism is tolerant to salt it will grow.
  - If the organism is not tolerant to salt it will not grow.
  - If the salt tolerant organism can ferment mannitol then there will be yellow zones around the colonies.
  - If the salt tolerant organism cannot ferment mannitol then the media will remain pink.

Growth with no mannitol fermentation.

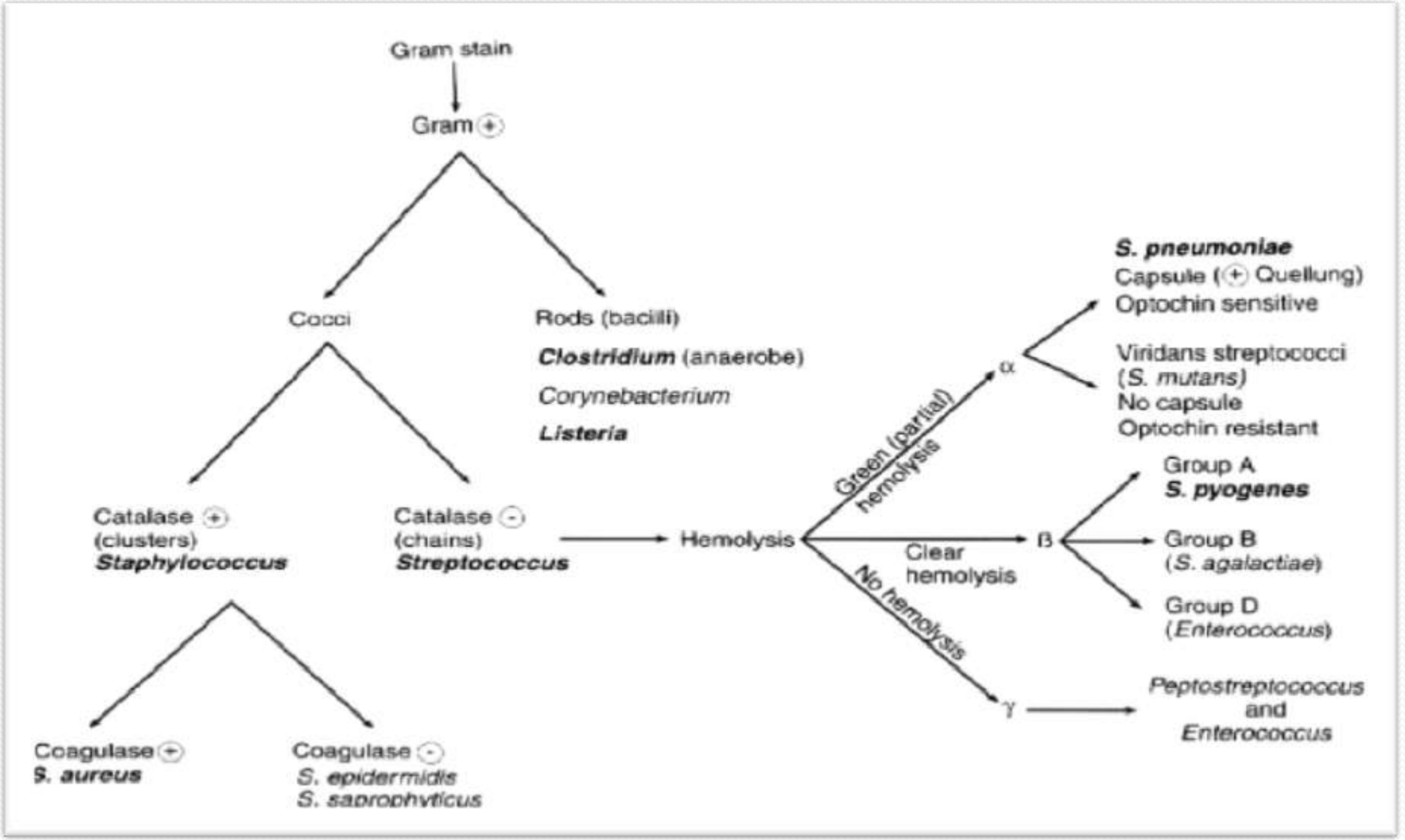


Growth with + mannitol fermentation.

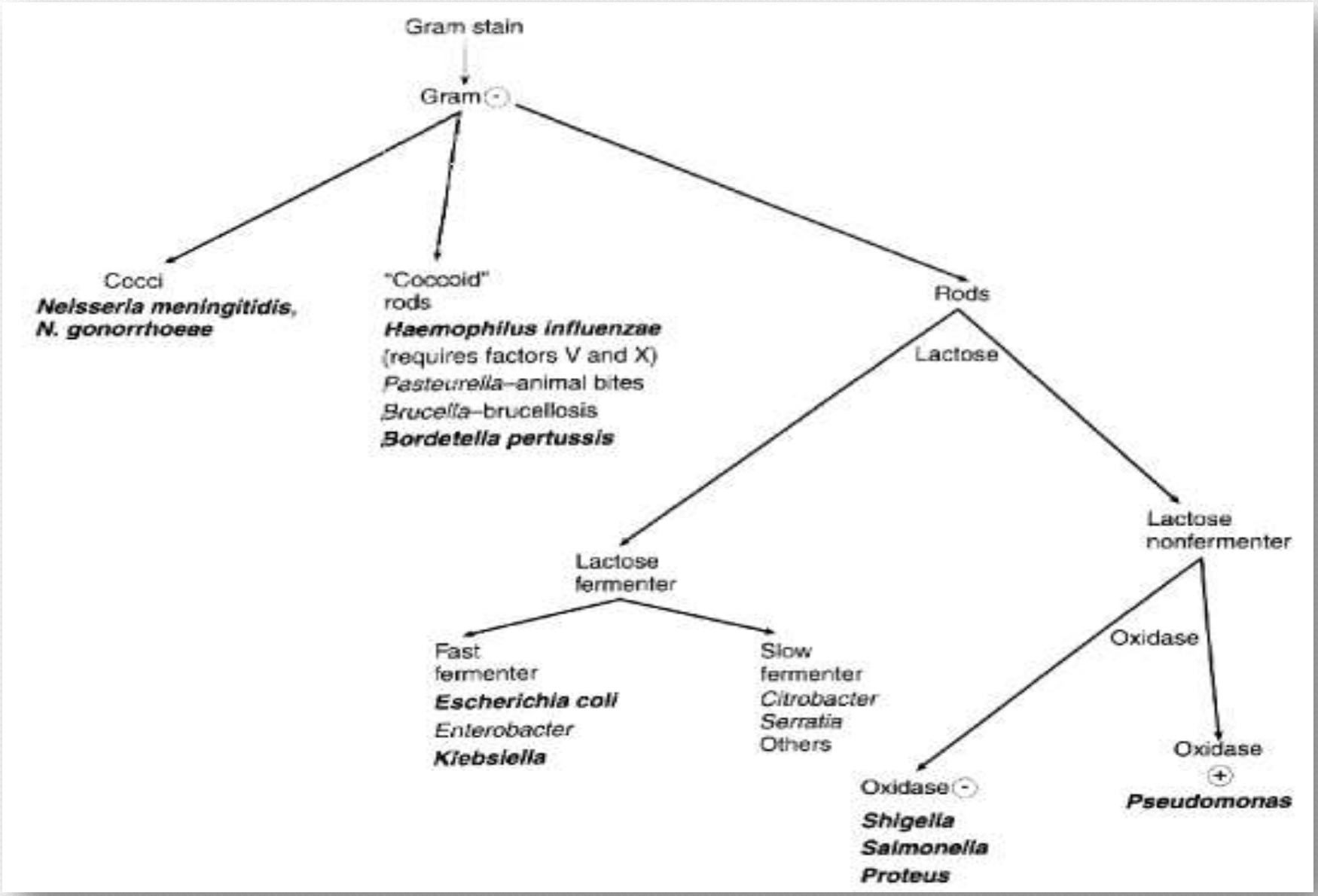


- **Eosin Methylene Blue Agar is a both selective and differential culture medium. It is selective culture medium for gram-negative bacteria due to the presence of two Dyes(EOSIN& METHYLENE BLUE). Colored colonies in Eosin Methylene Blue (EMB) Agar are Lactose fermenter. Colorless colonies in Eosin Methylene Blue (EMB) Agar are Non lactose fermenter. *E. coli* colonies have a characteristic green sheen. It is commonly used for the isolation and differentiation of coliforms and fecal coliforms.**

Gram positive flowchart



Gram negative flowchart



Key identification characteristics for Enterobacteriaceae

1.

GENUS/SPECIES	Fermentation of				Gas	MR	VP	Indole	Citrate	Urease	H2S	Motility
	G	L	S	M								
Escherichia coli	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)
Shiegella	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-/+)	(-)	(-)	(-)	(-)
Shiegella sonnei	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Salmonella	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(+)
Klebsiella Pneumo.	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(-)	(-)
Enterobacter	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Serratia	(+)	(+)	(-)	(+)	(+)	(-/+)	(+)	(-)	(+)	(-)	(-)	(+)
Proteus	(+)	(-)	(-)	(+)	(-/+)	(+)	(-)	(+)	(-/+)	(+)	(+)	(+)
morganella	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Yersinia	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-/+)	(-)	(-/+)	(-)	(+)

G: Glucose, L:Lactose, S:Sucrose, M: Manitol, MR: Methyl Red, VP: Voges Proskauer

DYES

I. ANILINE

II. ACRIDINE

- Used as skin & wound antiseptics
- Bacteriostatic on high dilution but low bactericidal

ANILINE DYES:

- Eg: brilliant green, malachite green & crystal violet
- More active against Gram +ve than Gram –ve
- No action against tubercle bacilli

MODE OF ACTION:

- React with the acid in the cell

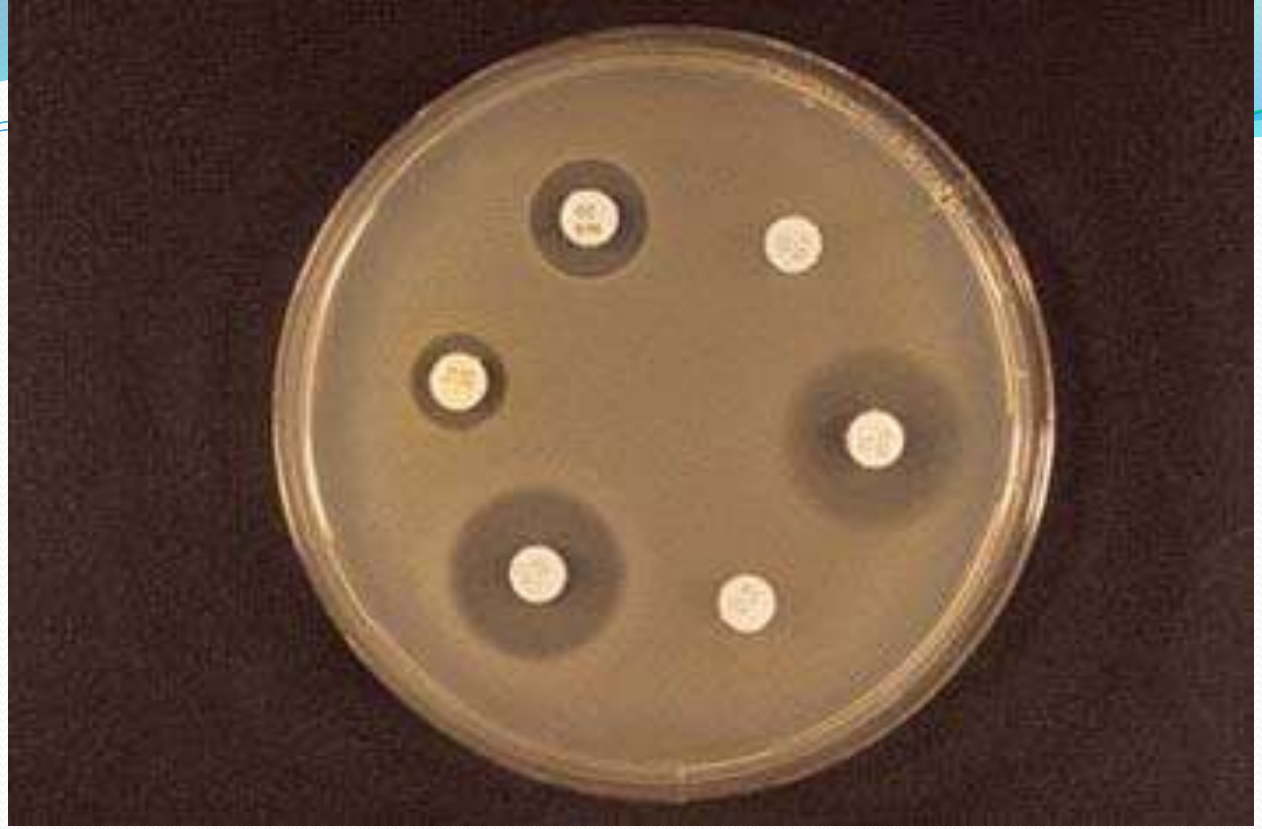
Used in microbiology labs as selective agents in culture media

ACRIDINE DYES

- Active against Gram +ve than Gram –ve
- ex: proflavine, acriflavine, euflavine & aminarine

MODE OF ACTION:

- Impair the DNA complex of organism & destroys the reproductive capacity of cell.



Antibiotic sensitivity test: also known as the “disk diffusion test” is used to test the resistance of a bacterial culture to various anti-infective agents. The method provides a basis for classification of a bacterial strain as “susceptible,” “resistant,” or “intermediate” according to the dimension of the inhibition zone

Thank you

Microbiology Lab
1st Term

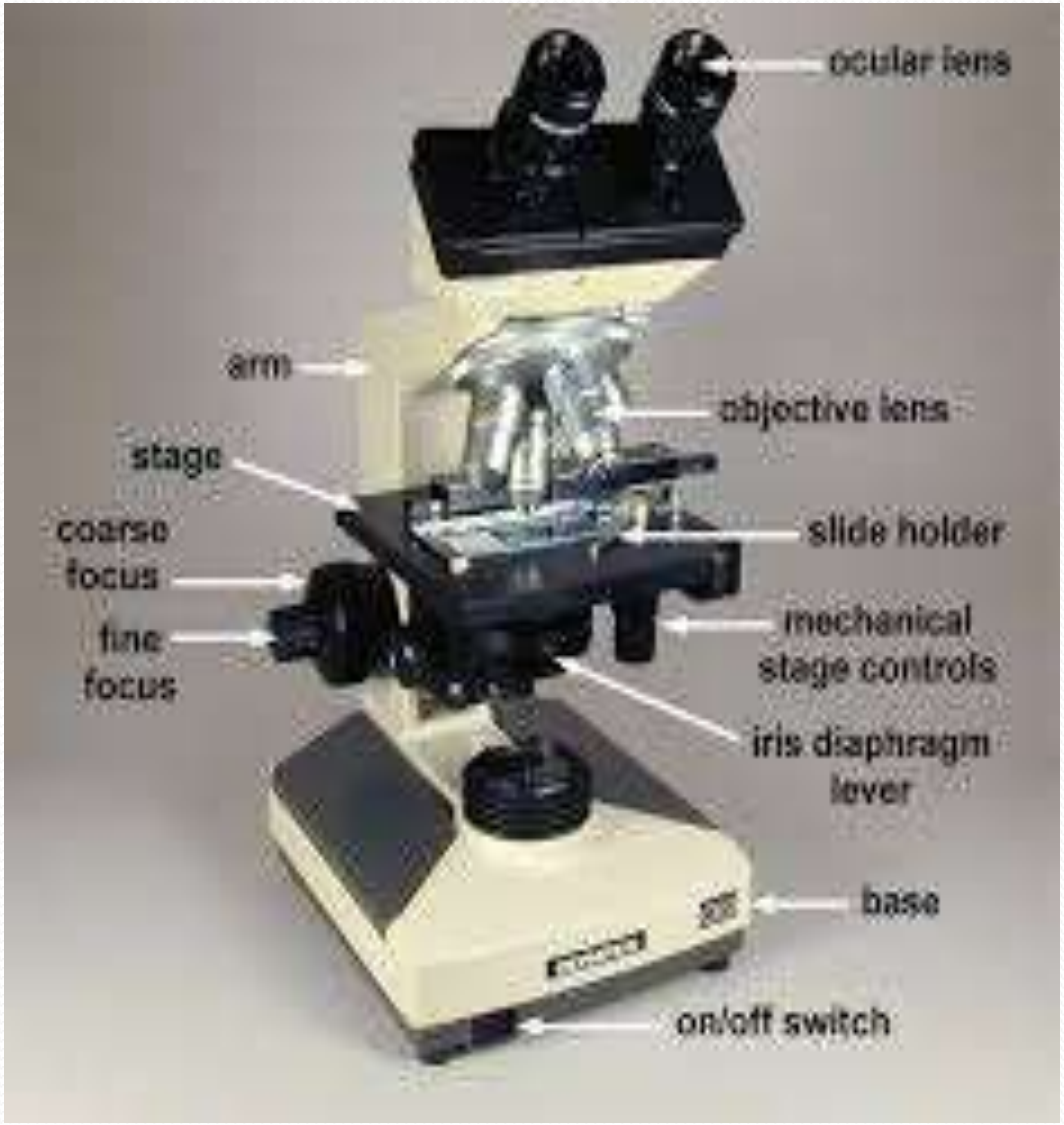
PRACTICAL MEDICAL BACTERIOLOGY

BY

LECTURER

HANAN IBRAHEEM

Light microscope



IDENTIFICATION METHOD

The most important task of a bacteriology is to identify the pathogens from the clinical sample so that appropriate treatment can be instituted.

contd

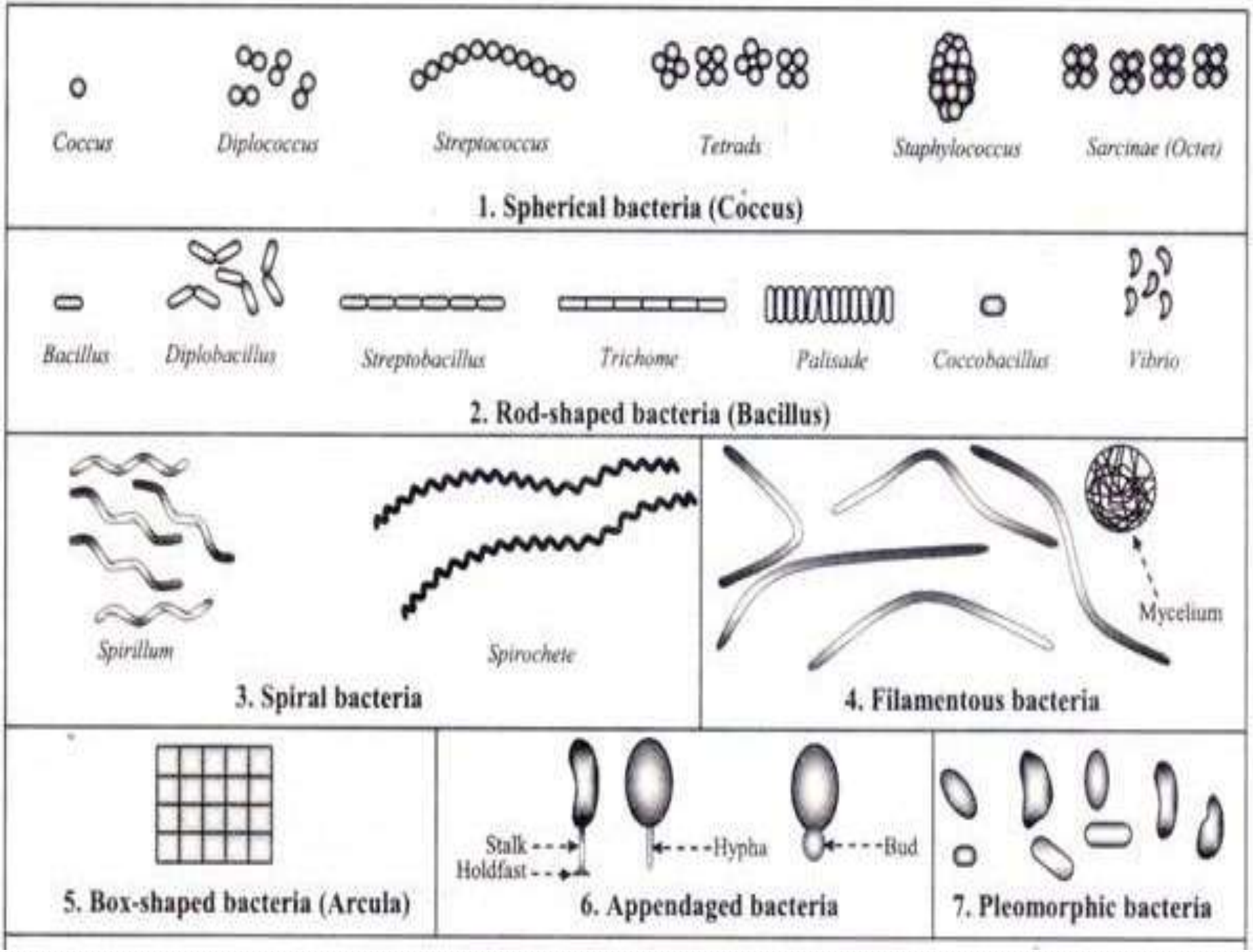
There are several methods to identified the different type of bacteria.

1. Isolation in pure form
2. Staining reaction
3. Morphology of bacterial colony
4. Cultural characteristics
5. Metabolism
6. Biochemical properties

Shape of Bacteria

- Bacteria display these basic shapes:

i. round- cocci, (from the Greek *kokkos* - a berry), sphere like shape
ii. rod shaped – bacilli (from the Latin *bacillus* - a stick or rod),
iii. spiral (quelled).
iv. Curved rod
v. Filamentous bacteria(long branching bacteria)

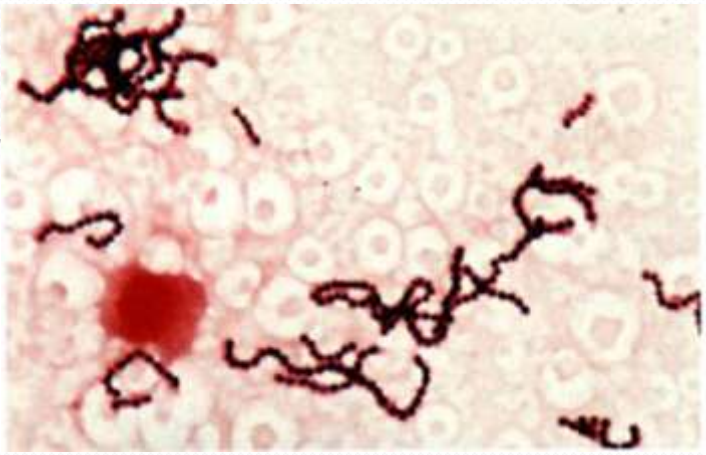

Coccus
Diplococcus
Streptococcus
Tetrads
Staphylococcus
Sarcinae (Octet)
1. Spherical bacteria (Coccus)
Bacillus
Diplobacillus
Streptobacillus
Trichome
Palisade
Coccobacillus
Vibrio
2. Rod-shaped bacteria (Bacillus)
Spirillum
Spirochete
3. Spiral bacteria
Mycelium
4. Filamentous bacteria
5. Box-shaped bacteria (Arcula)
Stalk
Holdfast
Hypha
Bud
6. Appendaged bacteria
7. Pleomorphic bacteria

Different Shapes and arrangement of Bacteria

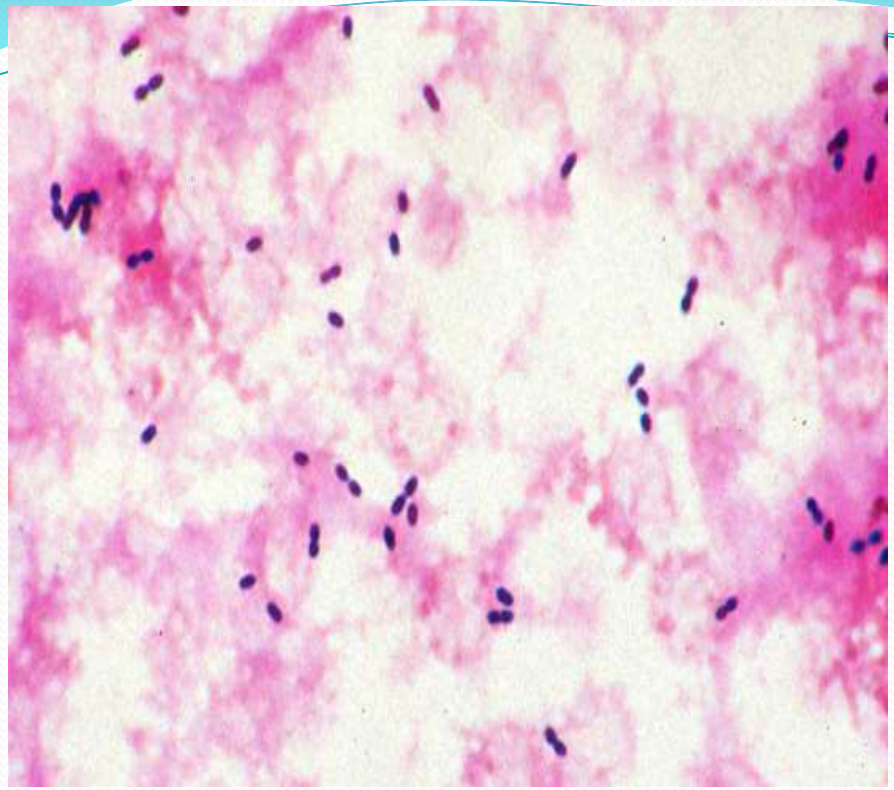
i. Coccus

Cluster arrangement

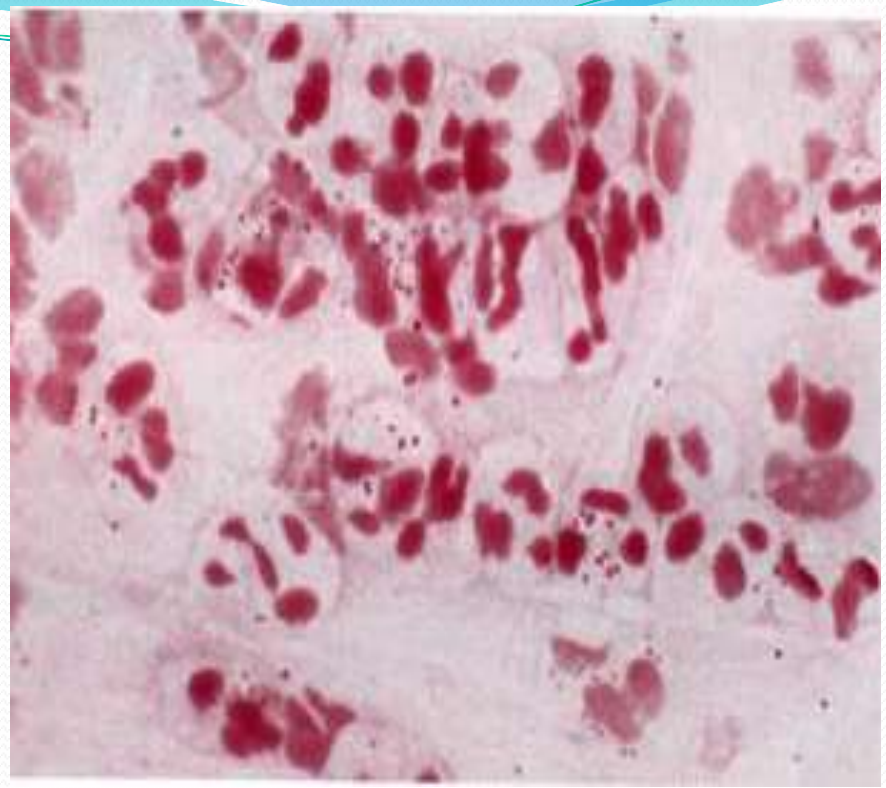
Staphylococcus



Chain like ***Streptococcus***



a



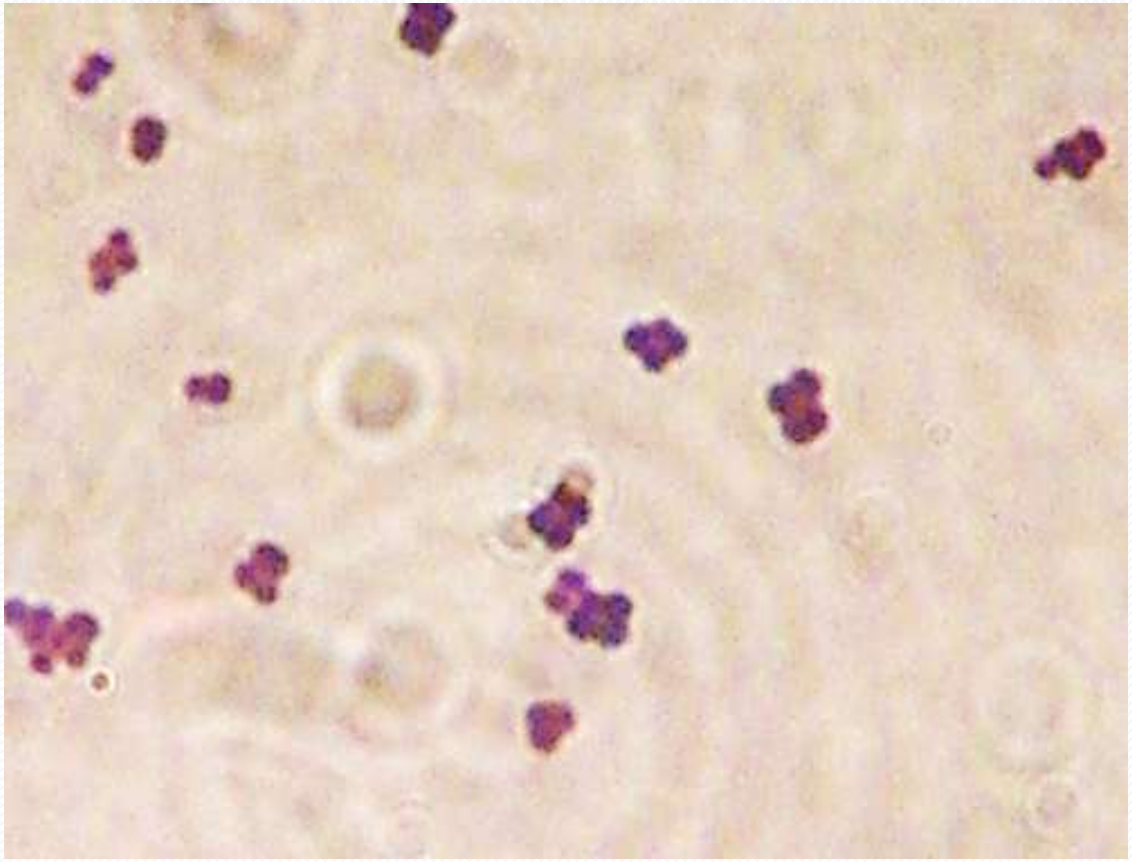
b

Coccus arranged in pairs

a- ***Streptococcus pneumoniae***

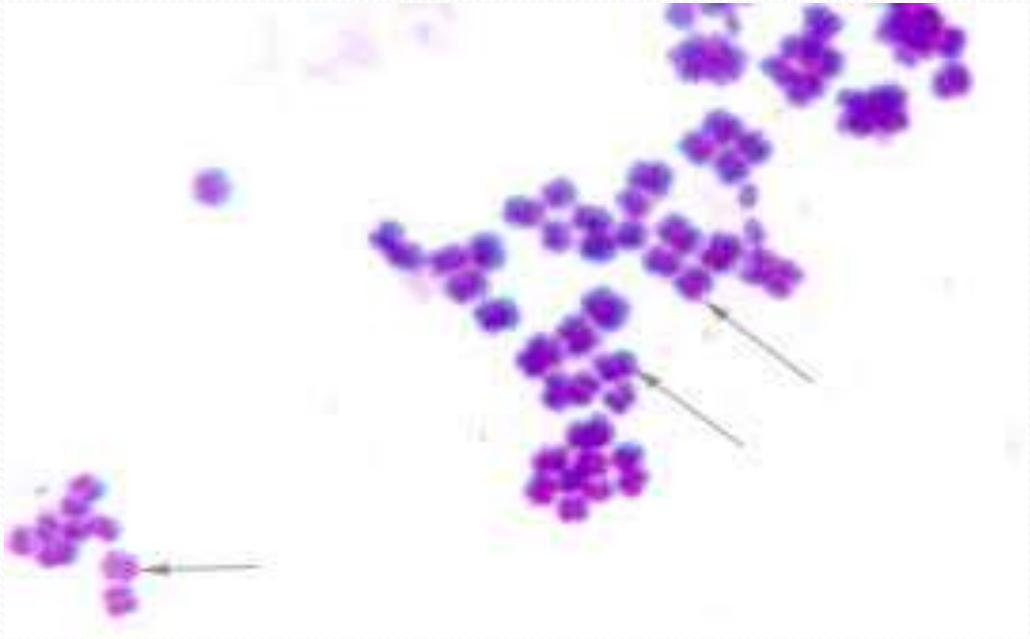
b- Neisseria gonorrhoeae

Coccus arranged in tetrads(four)



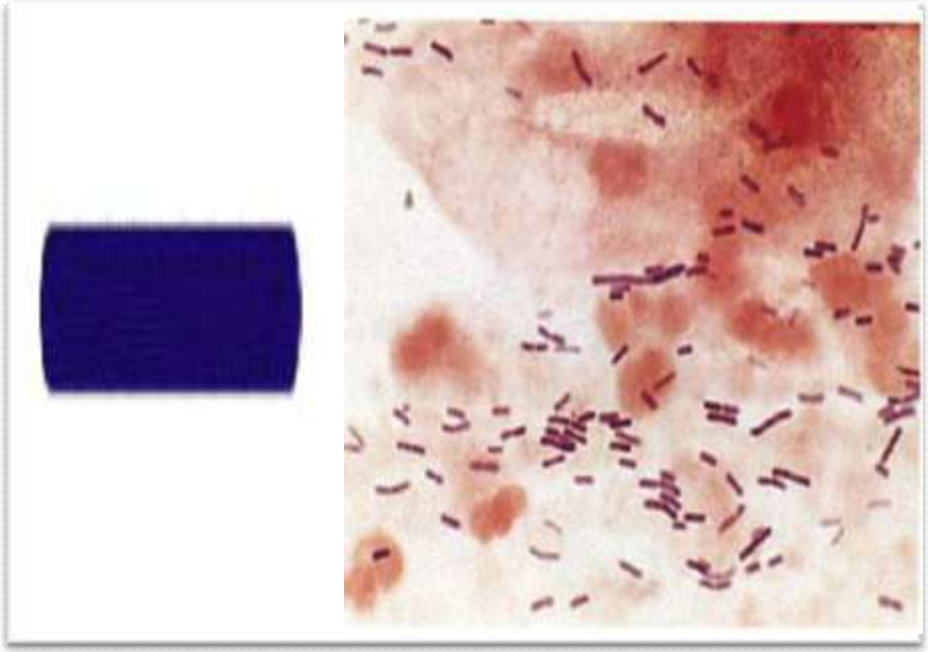
Micrococcus spp.

Package of eight(octet)

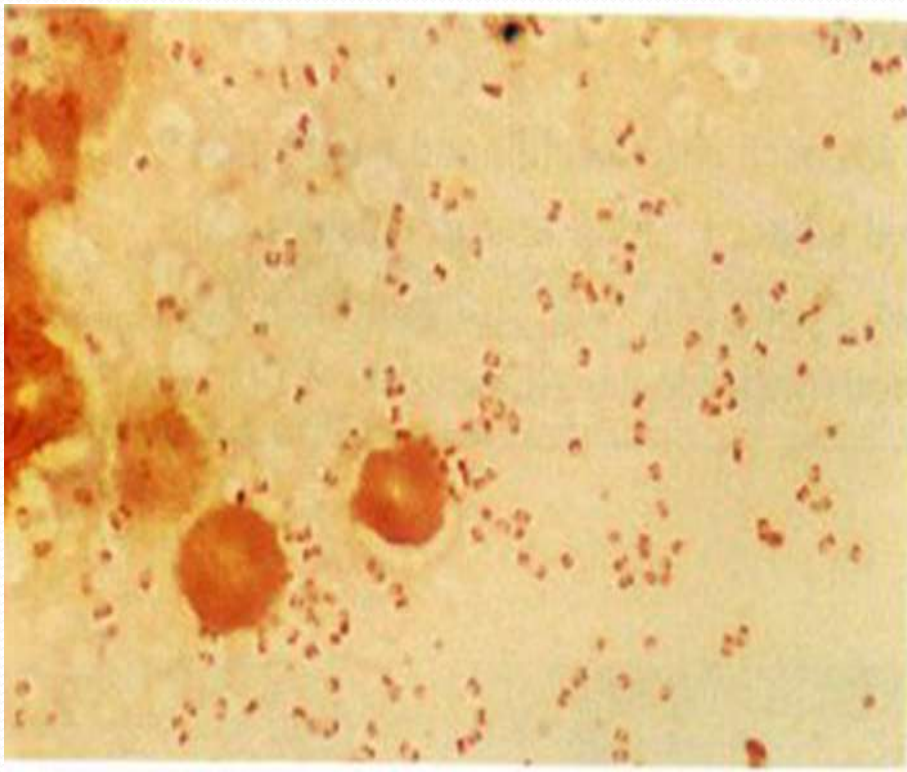


Sarcina spp.

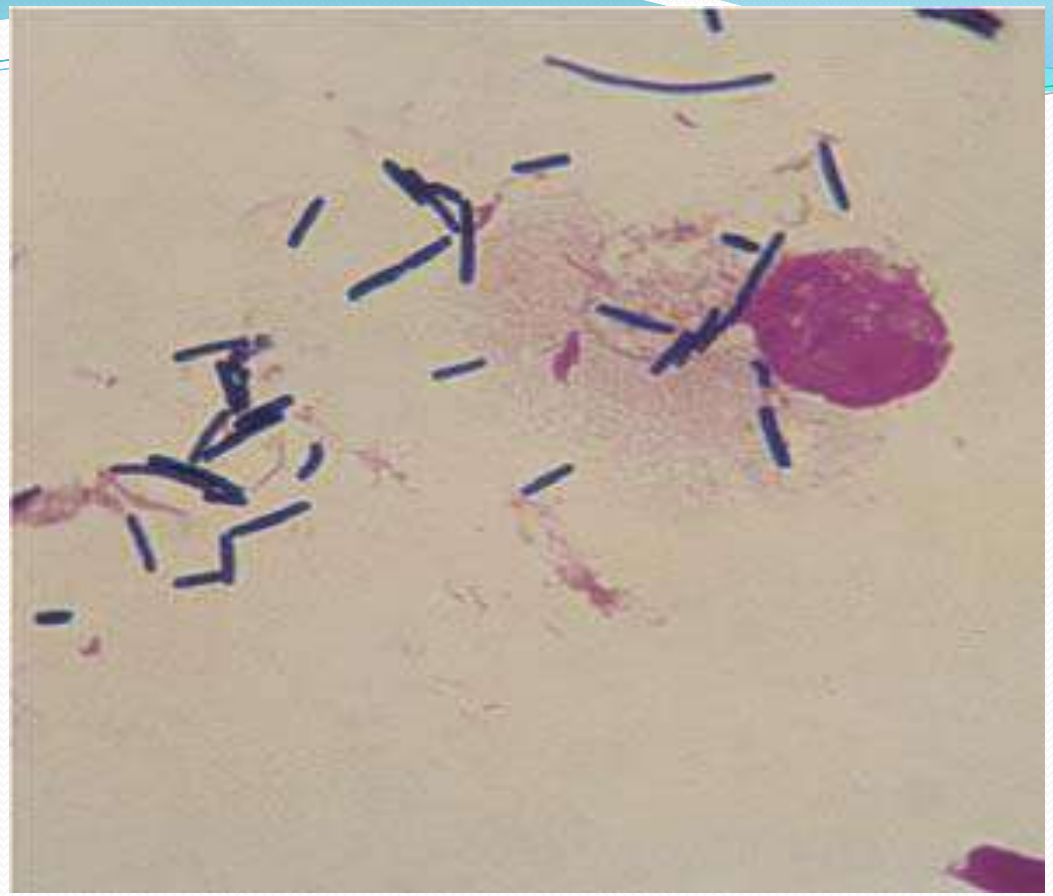
ii. Bacillus(rod shape)



Bacillus sp.

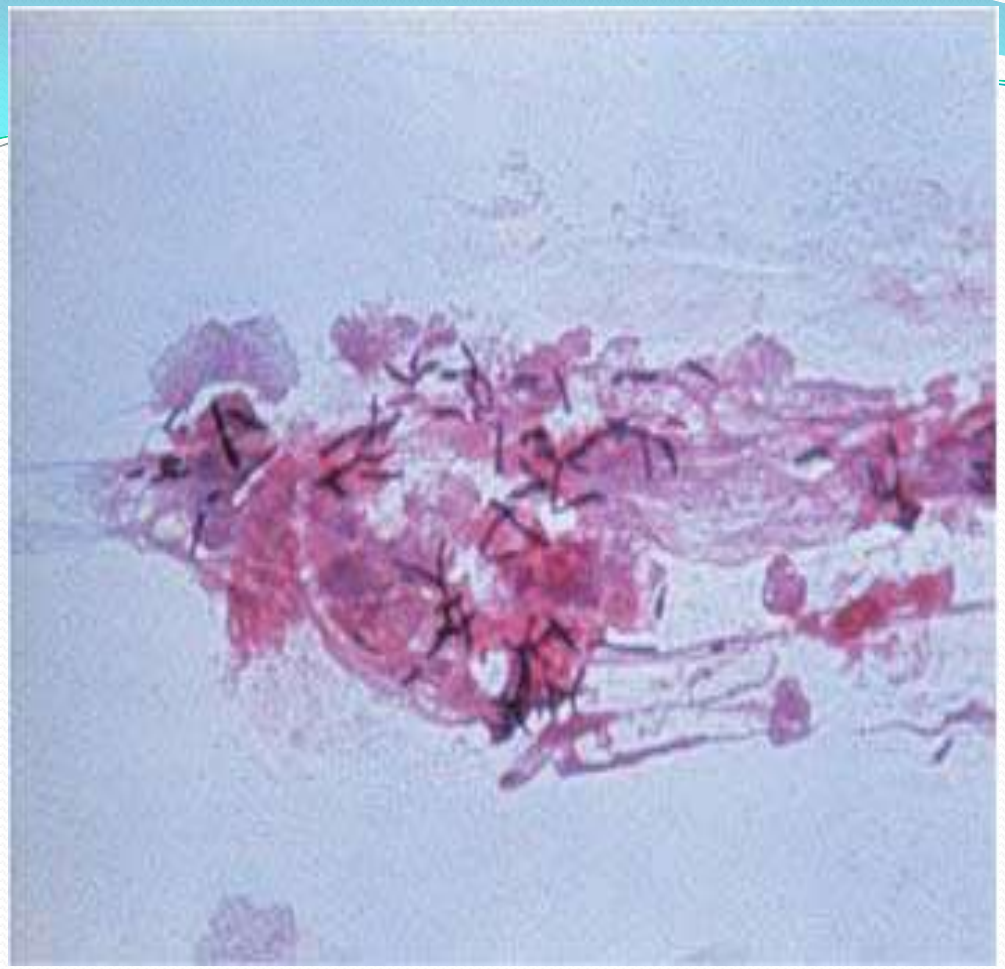


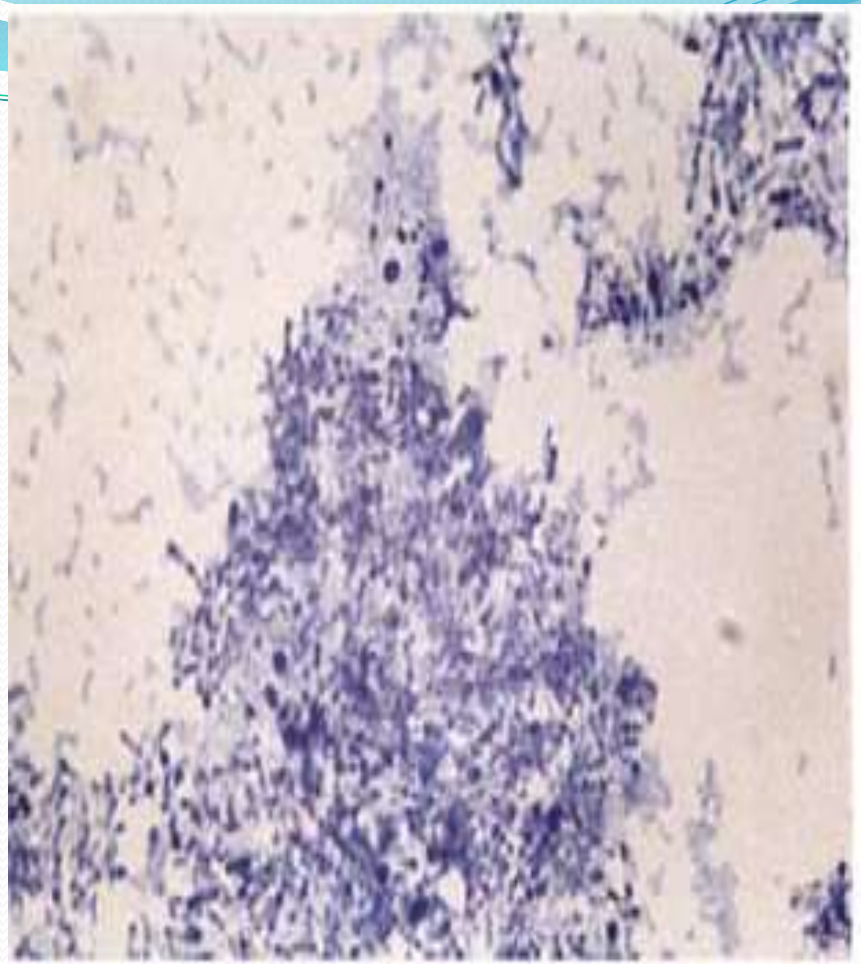
Escherichia(E.) coli
(Coccobacilli)



Bacilli shape(long rod)

Closteridium perfringens

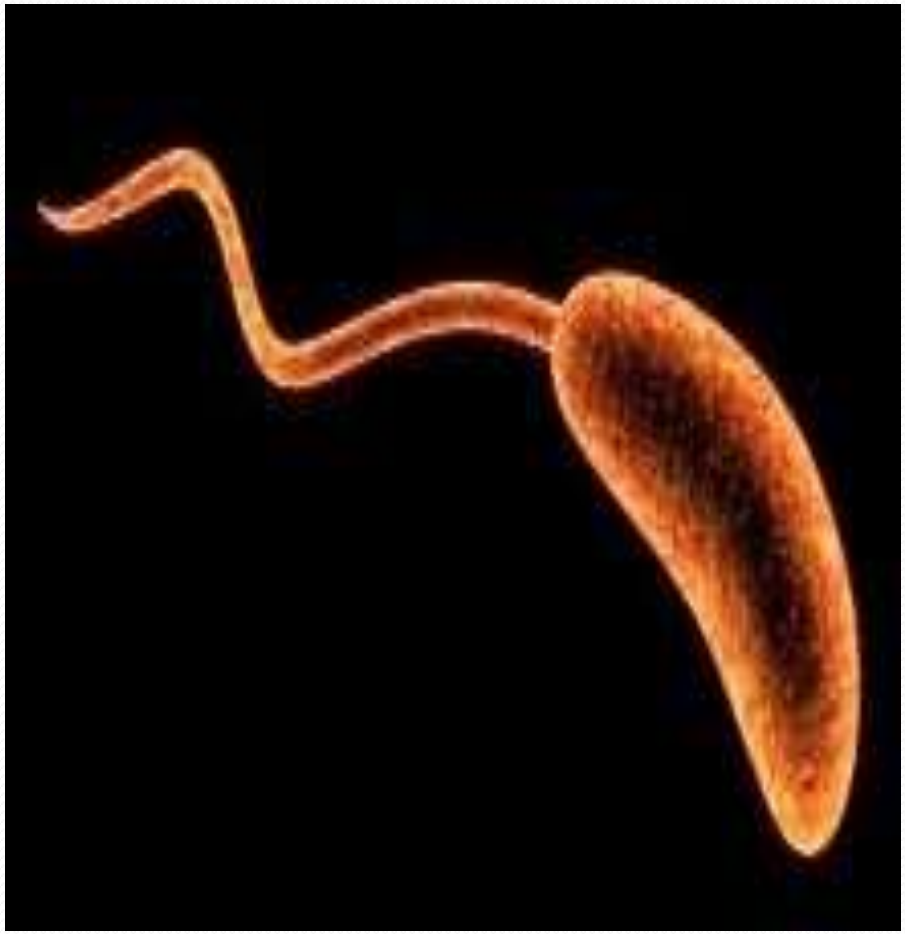




Corynebacterium diphtheriae(rod shape)

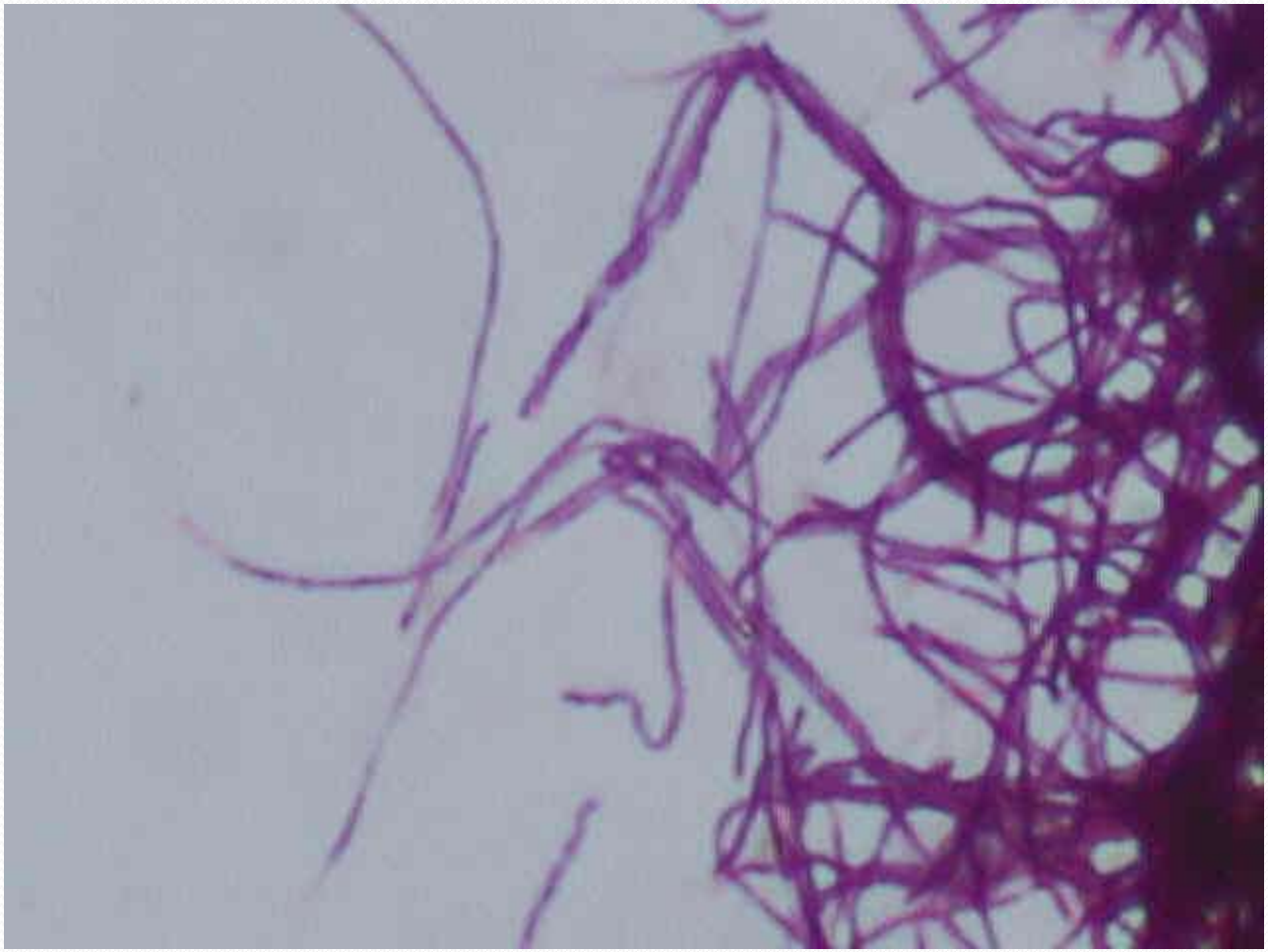
a- Gram stain b- Albert Stain

Curved Rod



Vibrio cholerae

Filamentous Bacteria



Streptomyces spp.

Spiral Shaped Bacteria

Spirillum volutans

Treponema pallidum

Staining reaction

a. The age of the culture is important. In older cultures , staining characteristics either vary or are not brought out well. Simple stains bring out the best morphology. Differential and special stains are necessary to bring out characteristics like: gram negative and gram positive bacteria, Acid fast and non acid fast , spirochetes, capsule and Flagella, etc.

Simple Stain

The simple stain can be used to determine cell shape, size, and arrangement. True to its name, the simple stain is a very simple staining procedure involving only one stain. You may choose from methylene blue, Gram safranin(RED), and Gram crystal violet.

Basic stains, such as methylene blue, Gram safranin, or Gram crystal violet are useful for staining most bacteria. These stains will readily give up a hydroxide ion or accept a hydrogen ion, which leaves the stain positively charged. Since the surface of most bacterial cells is negatively charged, these positively charged stains adhere readily to the cell surface.

Experimental Procedure

A. Wet Mount

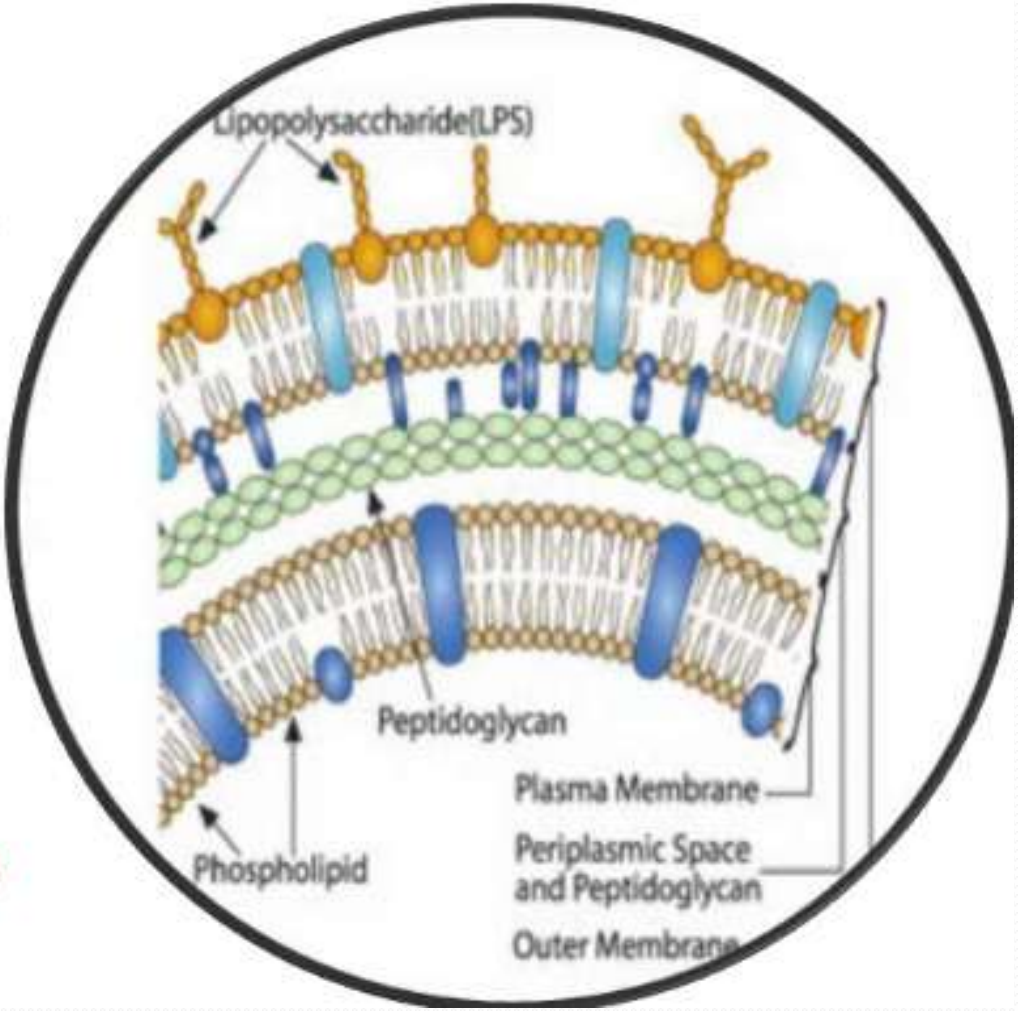
- *The wet mount is a preparation of a culture to observe motility (movement) or structure of microorganisms.*
- *Use a sterile inoculating loop to place a loopful of a motile bacillus culture on a slide. Cover immediately with a coverslip. Do not allow the preparation to dry out. Observe under the microscope. Draw a picture of what you see.*

B. Simple Stain

1. Place a loopful of ***Bacillus*** culture into a test tube of sterile distilled water to make a suspension of bacterial cells in the water. Place a loopful of this bacterial suspension on a clean slide. Allow the bacteria on the slide to air dry.
2. Heat fix the cells by passing the slide quickly through the flame of a Bunsen burner two or three times, with the glass surface exposed to the flame. Each pass should only be a second or two. The slide should not be so hot as to be uncomfortable to touch. (NOTE: your instructor will demonstrate this for you!)
3. Flood the slide with methylene blue stain for 60 seconds.
4. Rinse the slide with distilled water, blot it dry, and examine it under the microscope.
5. Draw what you observe.

Stains and Staining

- Bacteria are slightly negatively charged at pH 7.0
  - Basic dye stains bacteria
  - Acidic dye stains background
- Simple stain
  - Aqueous or alcohol solution of single basic dye



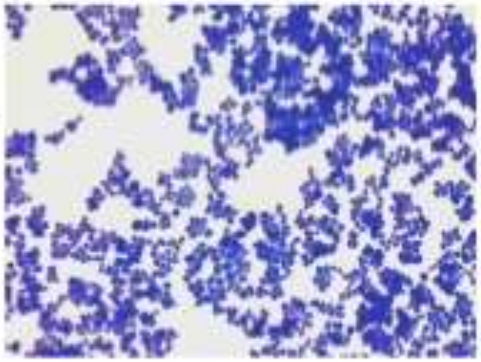
Simple Stains

Bacteria have nearly the same refractive index as water, therefore, when they are observed under a microscope they are opaque or nearly invisible to the naked eye.

Different types of staining methods are used to make the cells and their internal structures more visible under the light microscope.

Simple stains use one dye that stains the cell wall.

The cells are then visible against a light background.

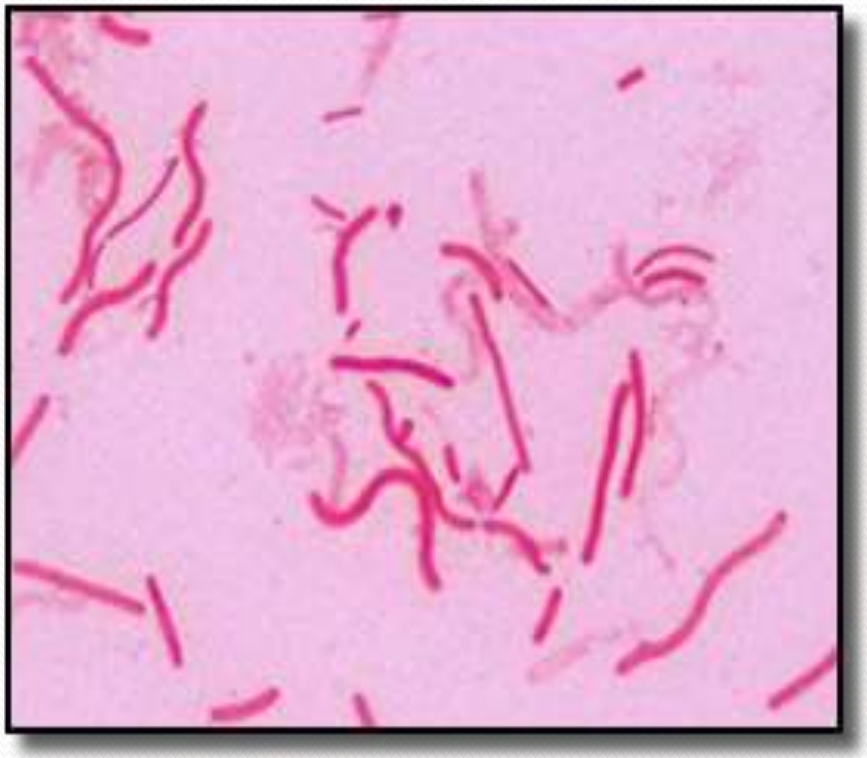


Steps:

1. **Place the slide on the staining rack.**
2. **Flood the slide with a basic stain: either crystal violet (1 min.), Safranin (2 min.), or Methylene blue (2 min.).**
3. **Wash the stain off the slide with deionized water.**
4. **Blot the slide with bibulous paper.**

Simple Staining

Drag the cursor over the images to read the description



Positive staining

Negative staining

a. Gram stain

a. Gram stain divides the bacteria into Gram positive & Gram negative.

The basic procedure goes like this:

i. Take a heat fixed bacterial smear.
ii. Flood the smear with CRYSTAL VIOLET for 30 second, then wash with water. [PRIMARY STAIN]
iii. Flood the smear with IODINE for 1 minute, then wash with water (mordant).
iv. Flood the smear with ETHANOL 95% for (15-30)sec, then wash with water. [DECOLORIZER]
v. Flood the smear with SAFRANIN for (60-80) second, then wash with water. [COUNTERSTAIN]
vi. Blot the smear, air dry and observe.

contd

- Examine under microscope

i. **Gram positive bacteria**- violet
ii. **Gram negative bacteria**- pink

b. Acid Fast Stain Ziehl-Neelsen method

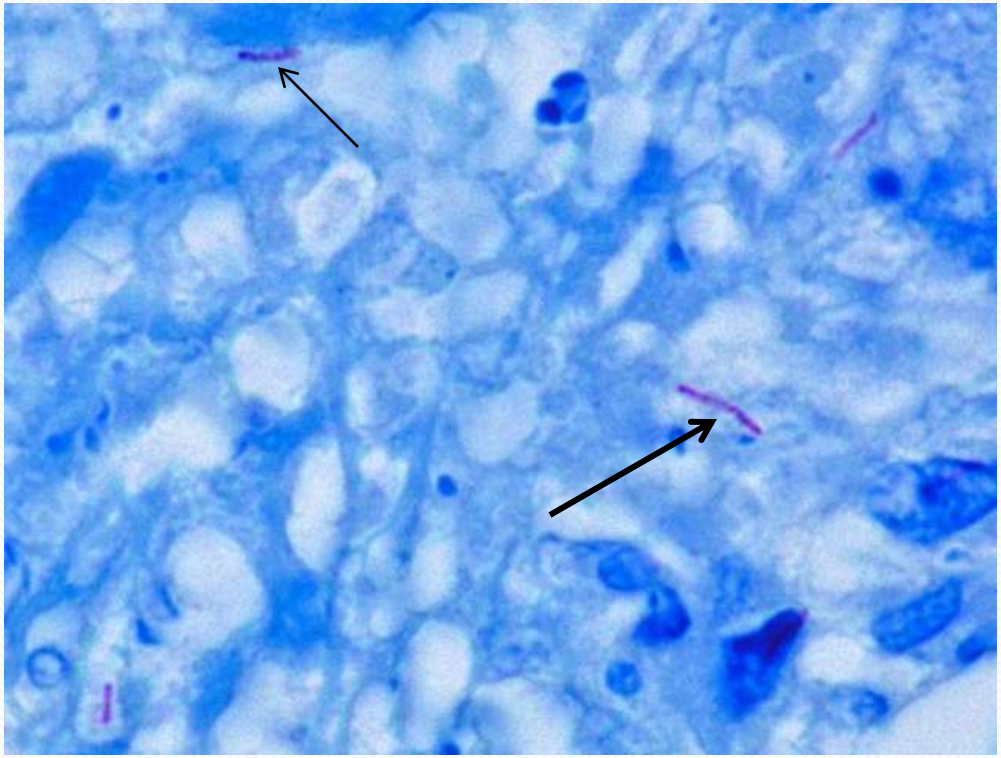
- Zn stain- which is divides the bacteria into Acid-Fast and non Acid-Fast.
- Principle: Some bacteria contain a waxy lipid, mycolic acid, in there cell wall. This lipid makes the cells more durable and is commonly associated with pathogens. Acid fast cell walls are so durable that the stain (carbol fuschin) must be driven into the cells with heat. All other cells will decolorize with this strong acid solvent, but acid fast bacteria will not. Other cells are then counterstained with methylene blue.

Procedure

i. Make a smear on the glass slide and allow to heat-fix.
ii. Flood the carbol fuchsin on the slide and & warm under the spirit lamp for 5 min until vapor
iii. Allow to cool and wash under tap water
iv. Decolorize by 20% solution of H_2SO_4 or a mix of acid – alcohol(3%HCL in 95% Ethanol)slowly drop wise until the dye no longer runs off from the smear for (10-30) sec.
v. Rinse with water.
vi. Counter stain with methelene blue for 2 minute.
vii. Wash under tap water and allow to dry.

contd

- Examine under microscope at oil immersion
- **Acid-Fast bacilli-** red
- **Background** -blue

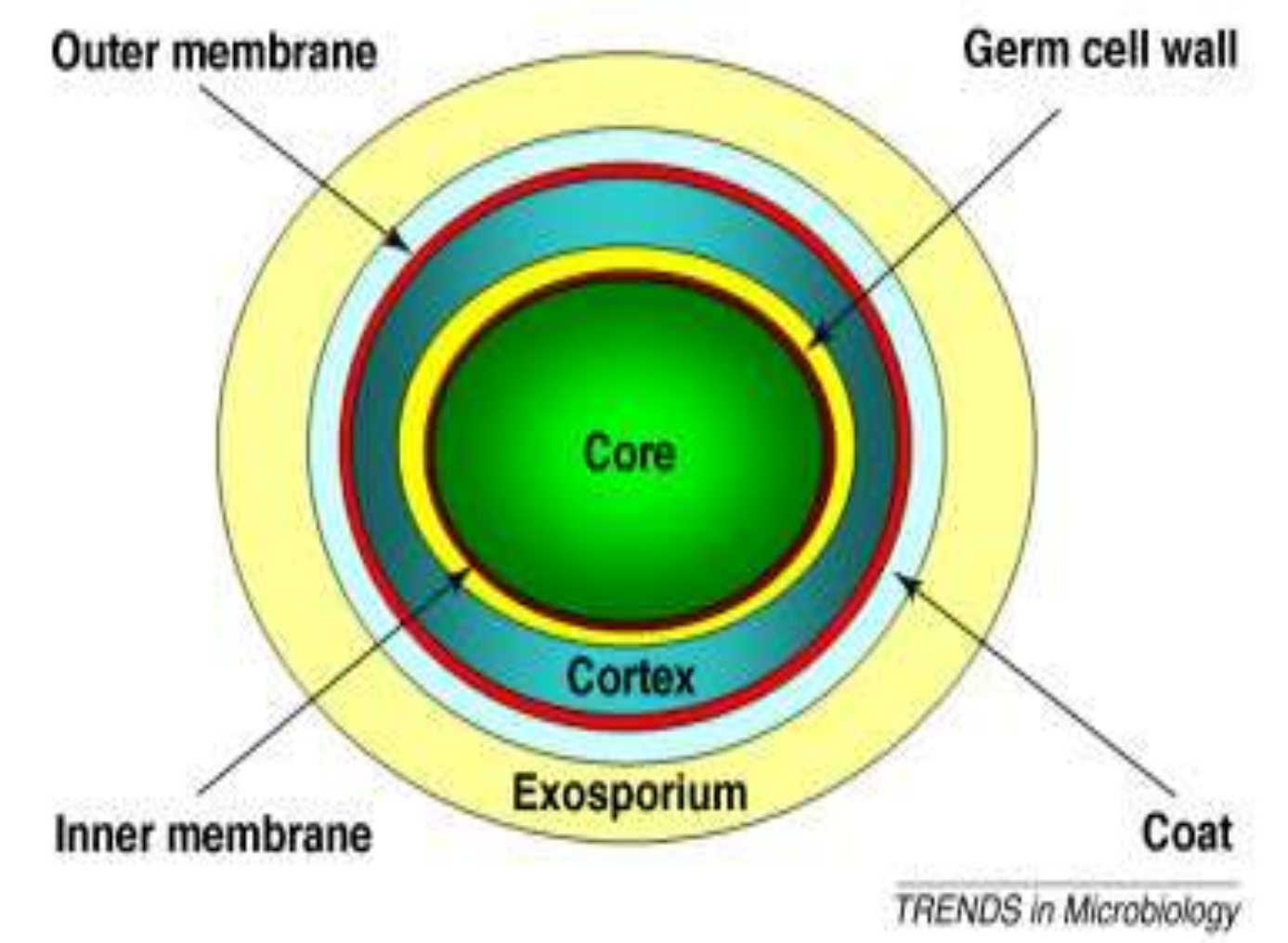


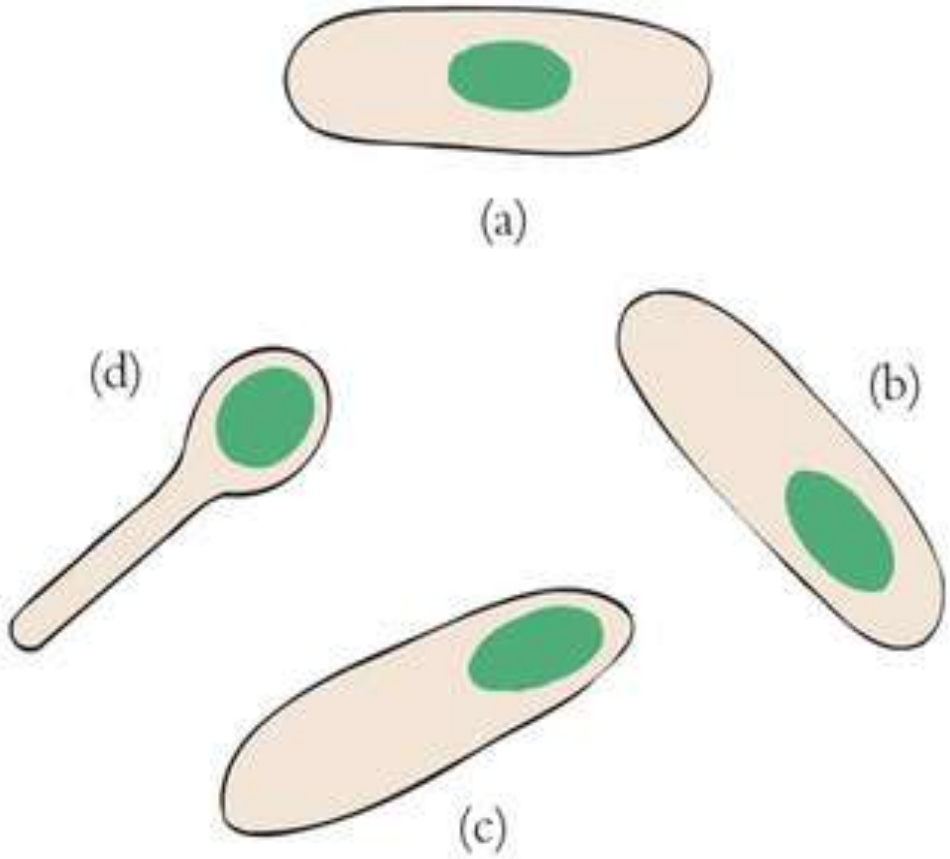
Mycobacterium tuberculosis

Bacterial Endospores

- Endospores are a dormant stage of some bacterium that allows it to survive conditions that would normally kill bacteria such as extreme drought or heat
- Endospores provide resistance against:
- drying
  - Low nutrient conditions
  - Radiation
  - High temperaturesand various chemical disinfectants

Structure of Bacterial Endospore





Location within the Parent cell

a- Central *Bacillus cereus*

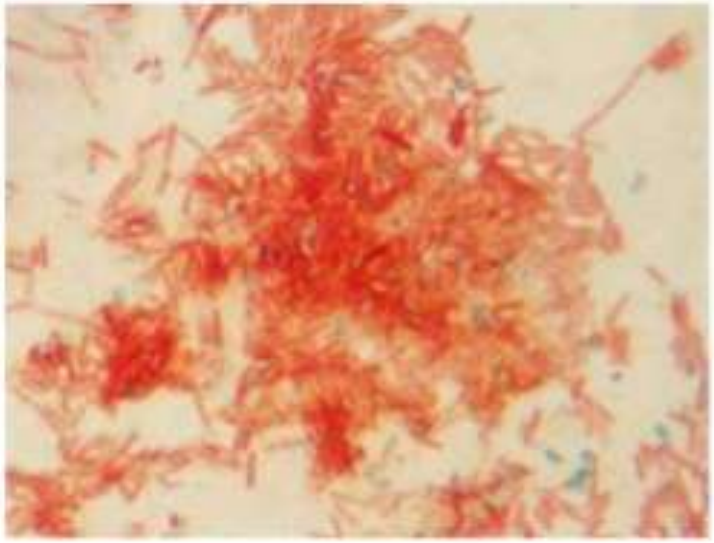
b- Sub terminal *B. subtilis*

c- Terminal *Closteridium sp.*

d- Bulging terminal *Cl. tetani*

Prokaryotes - Endospores

- Dormant, tough, non-reproductive structure produced by small number of bacteria.
- *Q: What is the function of endospores?*
- Resistant to radiation, desiccation, lysozyme, temperature, starvation, and chemical disinfectants.
- Endospores are commonly found in soil and water, where they may survive for very long periods of time.



An endospore stained bacterial smear of *Bacillus subtilis* showing endospores as green and vegetative cells as red.

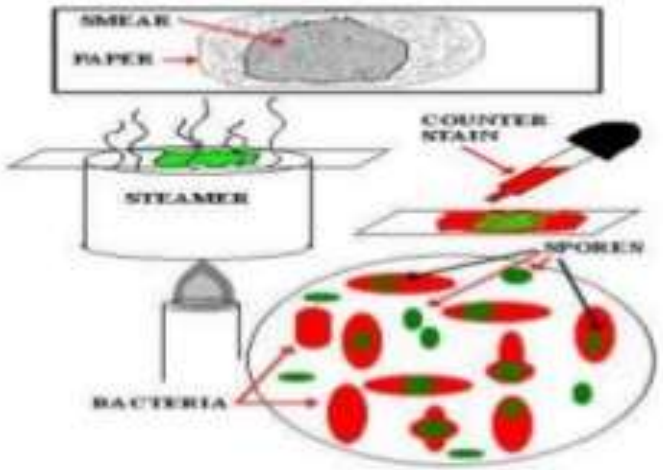


Image: *Bacillus subtilis*, SPO Science Image Library, Endospore stain from Dr. Ronald E. Hurlbert, Microbiology 101 lab manual

The Hanging Drop Slide and Bacterial Motility

Objective

To demonstrate : (a) form, (b) arrangement, and (c) motility of some micro–organisms, by means of hanging–drop slide preparations.

Materials

1. Culture (broth–24 hours).
   a) *Bacillus subtilis.*
   b) *Staphylococcus aureus.*
2. A hay infusion, stagnant water, rumen liquor, etc.
3. Concave (deep–well) slide, cover glasses, and Vaseline.

Procedure

1. With a toothpick, spread a small ring of Vaseline around the concavity of a depression slide (Figure 6a). Do not use too much Vaseline.
2. After thoroughly mixing one of the cultures, use the inoculating loop to aseptically place a small drop of one of the bacterial suspensions in the center of a cover slip (Figure 6b).
3. Lower the depression slide, with the concavity facing down, onto the cover slip so that the drop protrudes into the center of the concavity of the slide (Figure 6c). Press gently to form a seal.
4. Turn the hanging drop slide over (Figure 6d) and place on the stage of the microscope so that the drop is over the light hole.
5. Examine the drop by first locating its edge under low power and focusing on the drop. Switch to the high–dry objective and then, using immersion oil, to the 90 to 100X objective. In order to see the bacteria clearly, close the diaphragm as much as possible

for increased contrast. Note bacterial shape, size, arrangement, and motility. Be careful to distinguish between motility and Brownian movement.

6. Discard your cover slips and any contaminated slides in a container with disinfectant solution.

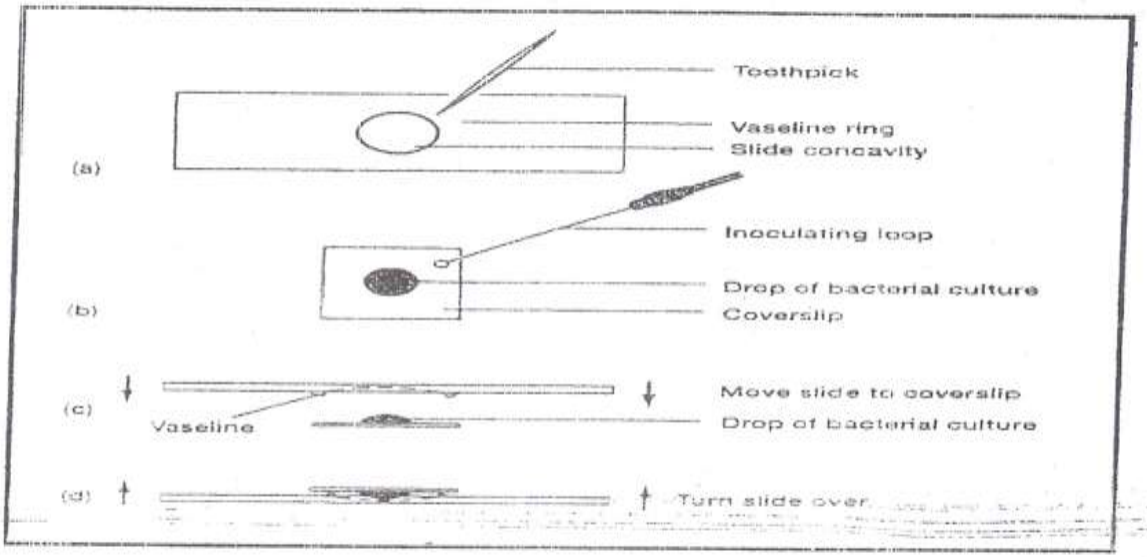
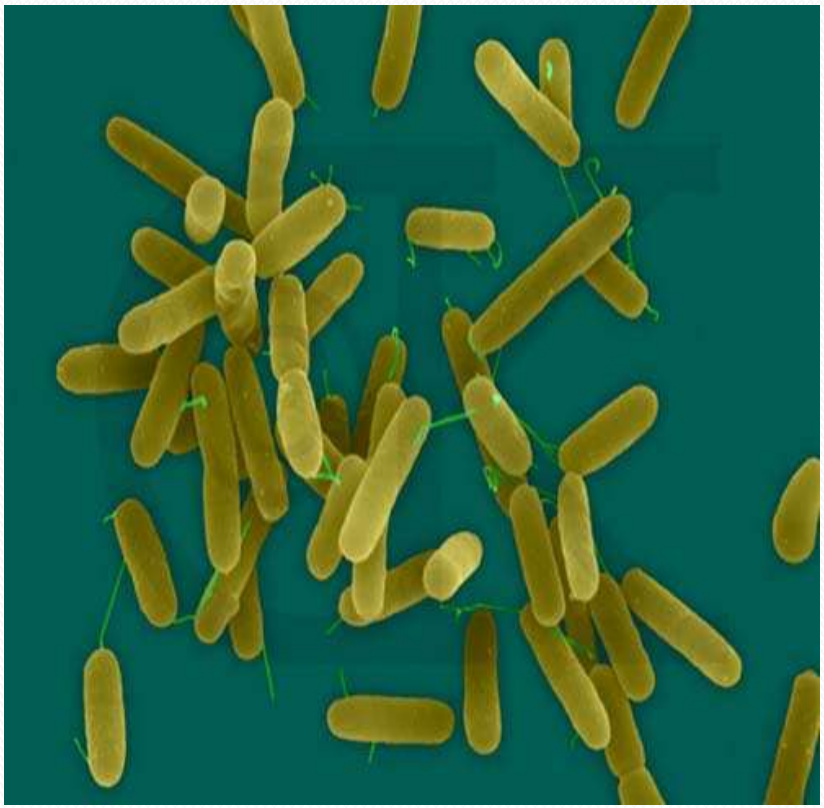


Figure (6): Preparation of a Hanging Drop Slide.

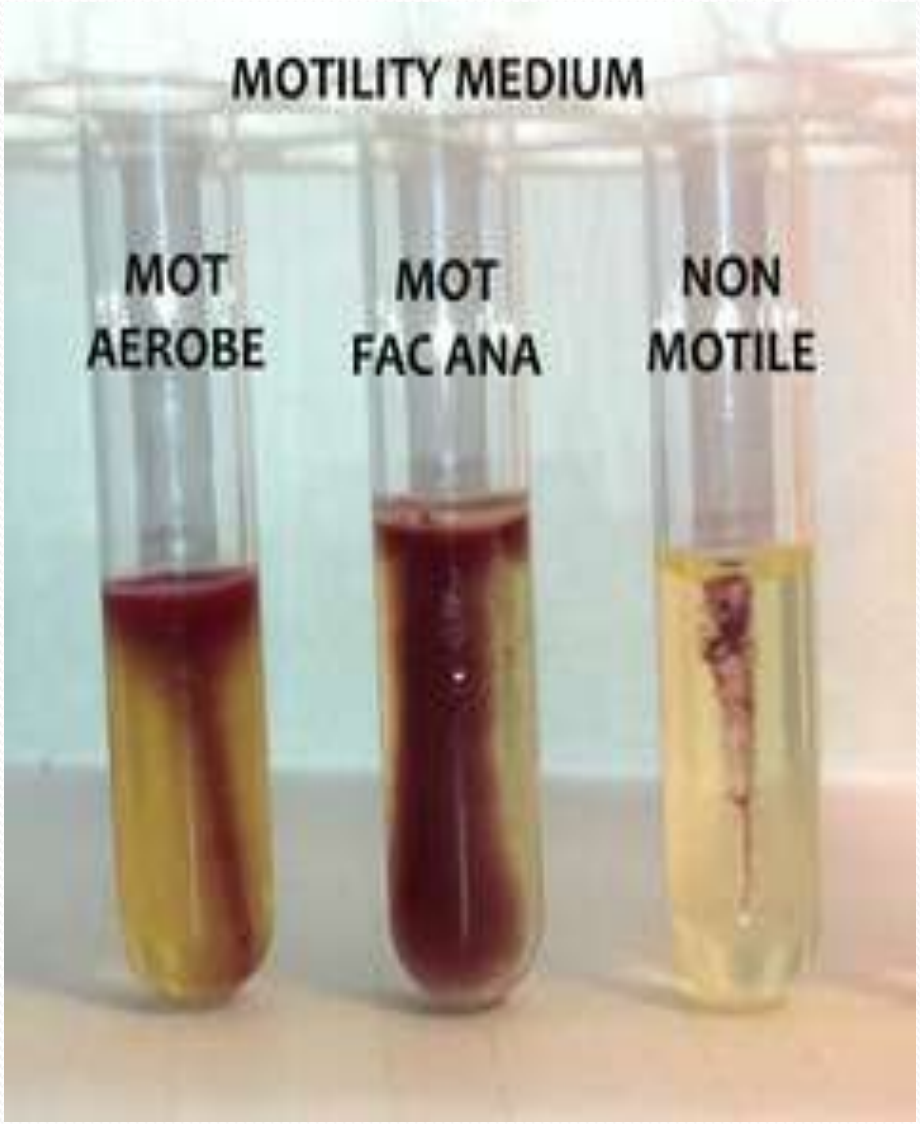
Flagella stain



Pseudomonas aeruginosa

3) Semi-Solid media Inoculation

- The most commonly used test for motility in microbiology lab.
- It depends on the ability of motile bacteria to move through semi-solid media.
- Ordinary solid media contain 1.5-2.0% Agar
- Semi solid media contain about 0.4% Agar


MOTILITY MEDIUM
MOT AEROBE
MOT FAC ANA
NON MOTILE

1. Isolation in pure form

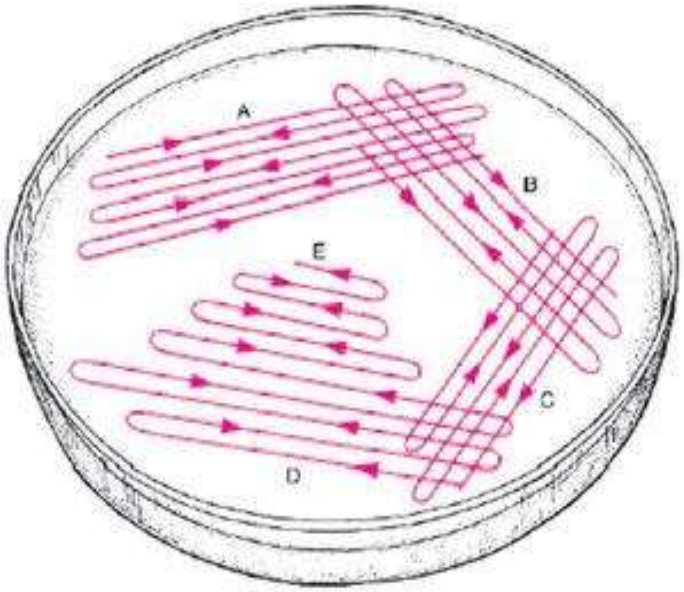
- Studies on the biochemical, antigenic and other characters of bacteria can be done only if the organism available in the pure form.

Technique:

a. Plating on solid culture media- clinical sample is streaked onto a solid medium (like: MacConkey agar, nutrient agar or blood agar) in such a way so as to ensure isolated discrete colonies.

b. Use of selective growth condition-most important example of this is the growth of anaerobic bacteria which will not take place in an environment having oxygen.

Pure culture Isolation Technique
Streak Plate Method



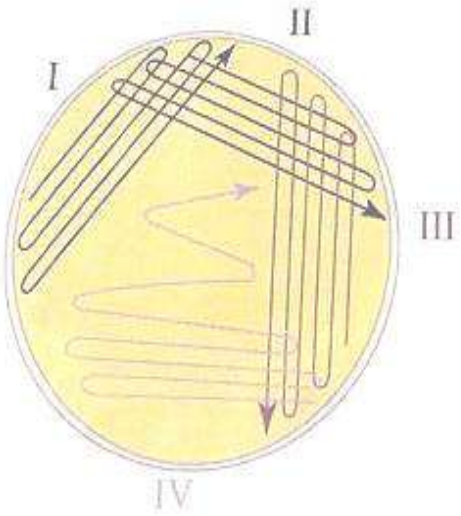


FIGURE 2-1 The quadrant method of streaking a plate for isolation. The agar surface is streaked as in I. After *flaming the loop*, the plate is rotated almost 90° and streaked as in II. The process is repeated for streaks III and IV.

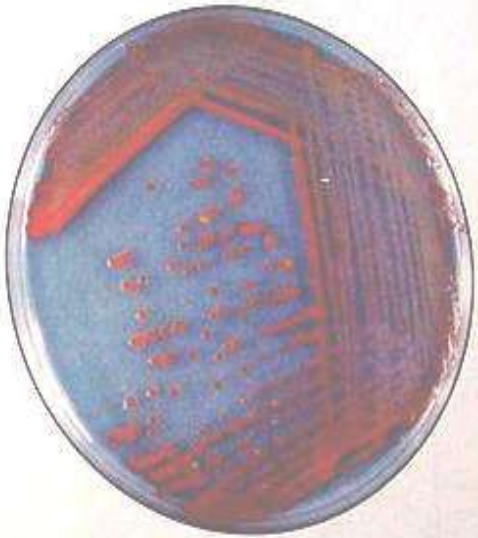


FIGURE 2-2 A streak plate of *Serratia marcescens* after incubation. Note the decreasing density of growth in the four streak patterns. On this plate, isolation is first obtained in the fourth streak. Cells from individual colonies may be transferred to sterile media to start pure cultures of each.

Culture Media

Culture media are used for recognition and identification (diagnosis) of microorganisms. The media are contained in plates (Petri dishes), in test tubes, flasks or screw capped bottles. **Used of media as :**

a- Simple or basal e.g., Nutrient agar, Nutrient broth.

b- Special-purpose media e.g., selective media, enriched media.

Types of culture media regarding their consisitency:

1- Liquid (fluid) media e.g., Nutrient broth, Peptone water.

2- Solid media, e.g., Nutrient agar, MacConkey agar.

3- Semisolid media : e.g., motility media.

Solid media as agar in special purpose as :

1. **Enriched media**, simple media enriched with substances e.g., added blood 5-10% added glucose 1-2%.
2. **Selective media** containing inhibitory substance as : e.g., bile salts, antibiotic, dyes,….etc., which favors the growth of the concerned microorganism and inhibit the growth of others, e.g., MacConkey agar, Bismuth Sulphate agar or SS agar.
3. **Differential media**, certain species produce characteristic growth that can easily recognized or can produce certain effects in the media, e.g., Triple sugar Iron agar (TSI), hemolytic and non-hemolytic species on blood agar.

Ordinary or Simple media

Nutrient Agar

Staphylococcus aureus

Streptococcus pyogens

Ordinary or Simple media

Nutrient Agar

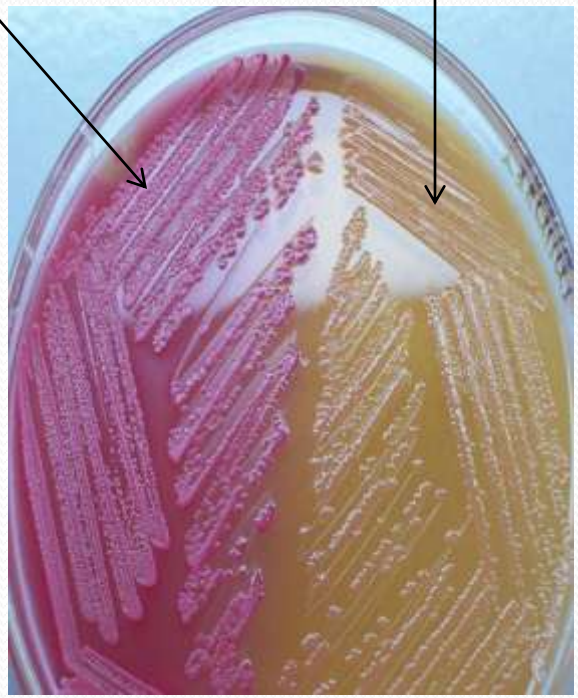
Bacillus subtilis

Proteus

Differential and selective Media
MacConkey Agar

Lactose fermenter (pink)

non lactose fermenter (pale or yellow)

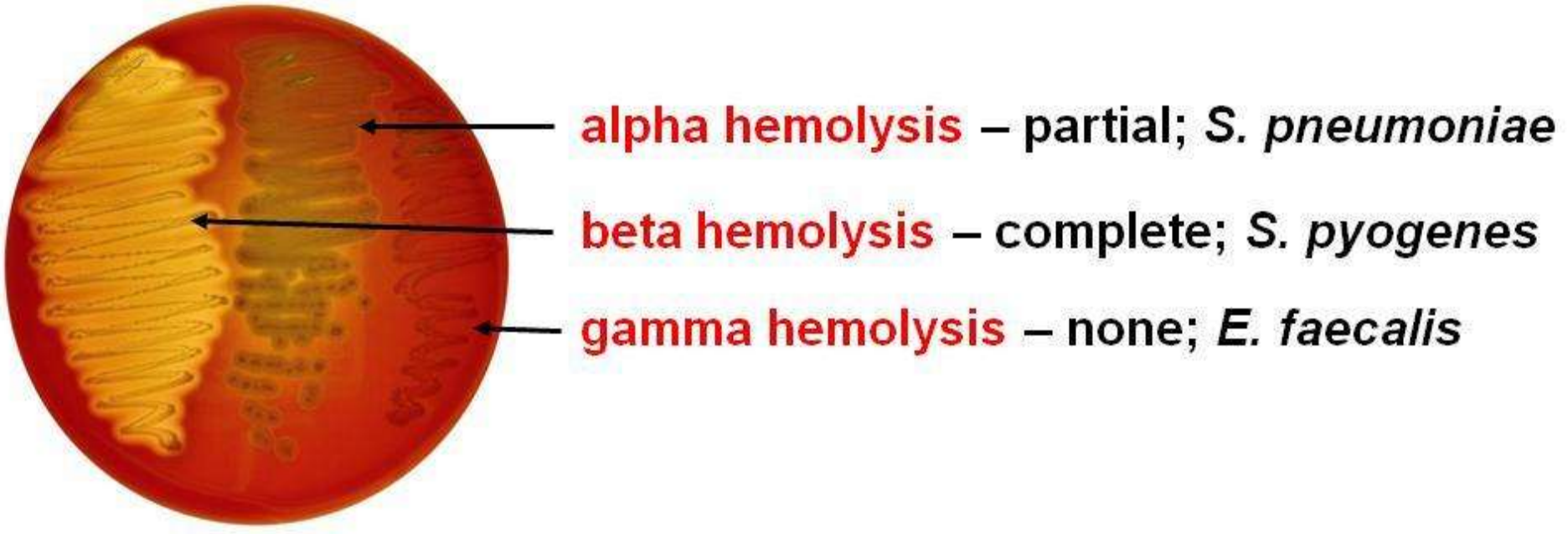


Enterobacter cloacae on MacConkey Agar:growth with pink colonies

Eschericia coli on MacConkey Agar:
growth, with pink colonies

Enrichment and Differential Media

BLOOD AGAR

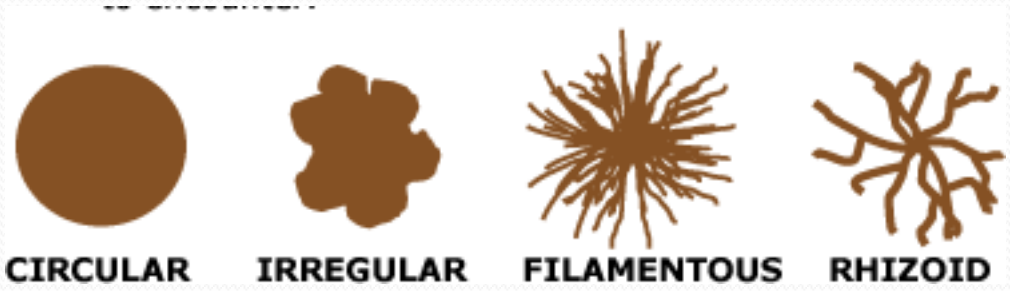


S: *Streptococcus*
E: *Enterococcus*

3. Morphology of the bacterial colony

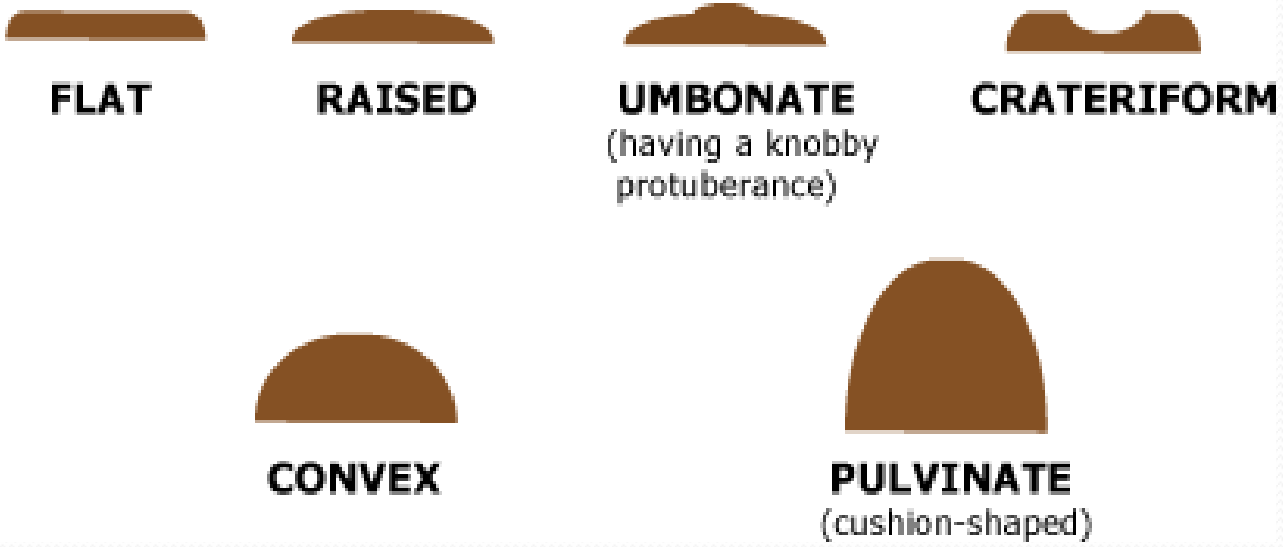
i. Shape: circular, irregular, radiate or rhizoid.
ii. Size: diameter in mm
iii. Elevation: flat, raised, low convex, dome shaped
iv. Margin: Entire, wavy, lobate, filiform
v. Surface: smooth, wavy, rough, granular, papillate, glistening etc.

Shape of the colony



Elevation of the colony

2. Elevation – This describes the "side view" of a colony. These are the most common.



Margins of the colony

3. Margin – The margin or edge of a colony (or any growth) may be an important characterisic in identifying an organisms. Several examples are shown below.

4. Cultural characteristics

These provide additional information for the identification of a bacterium.

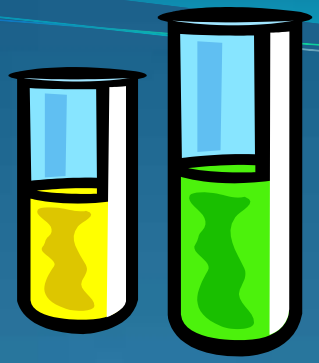
A. On solid medium the following characters are observed

i. Shape: circular, irregular, radiate or rhizoid.
ii. Size: The size of the colony can be a useful characteristic for identification. The diameter of a representative colony may be measured.
iii. Elevation:
iv. Margin: Entire, wavy, lobate, filiform
v. Surface: smooth, wavy, rough, granular, papillate, glistening etc.
vi. Size in mm
vii. Texture : dry, moist, mucoid, brittle, viscous, butyrous (buttery).
viii. Color : colorless, pink, black, red, bluish-green.

B. IN A FLUID MEDIUM FOLLOWING CHARACTERS ARE OBSERVED

i. Degree of growth- Absence, scanty, moderate, abundant etc.
ii. Present of turbidity and its nature.
iii. Presence of deposit and its character.
iv. Nature of surface growth.
v. Ease and disintegration and odor.

Growth in Liquid(broth) media



6.Media & Biochemical Tests

Laboratory Objectives

5.METABOLISM

To classify the differentiate species following aspects are studied

i. Requirement of oxygen
ii. The need of co2
iii. Capacity to form pigments
iv. Power of hemolysis

Tests To Know

- **Case Study Tests**
  - Catalase test
  - Coagulase test
  - Oxidase test
  - Urea hydrolysis
  - Lactose fermentation
  - Sucrose fermentation
  - Glucose fermentation & gas production
  - Triple sugar iron(TSI) test
  - Indole
  - Methyl Red/Voges Proskauer
  - Citrate
  - H_2S production in SIM
  - Growth and reactions on differentia and selective media
    Mannitol salt agar (MSA)

ACTION OF DYES AND ANTIBIOTICS

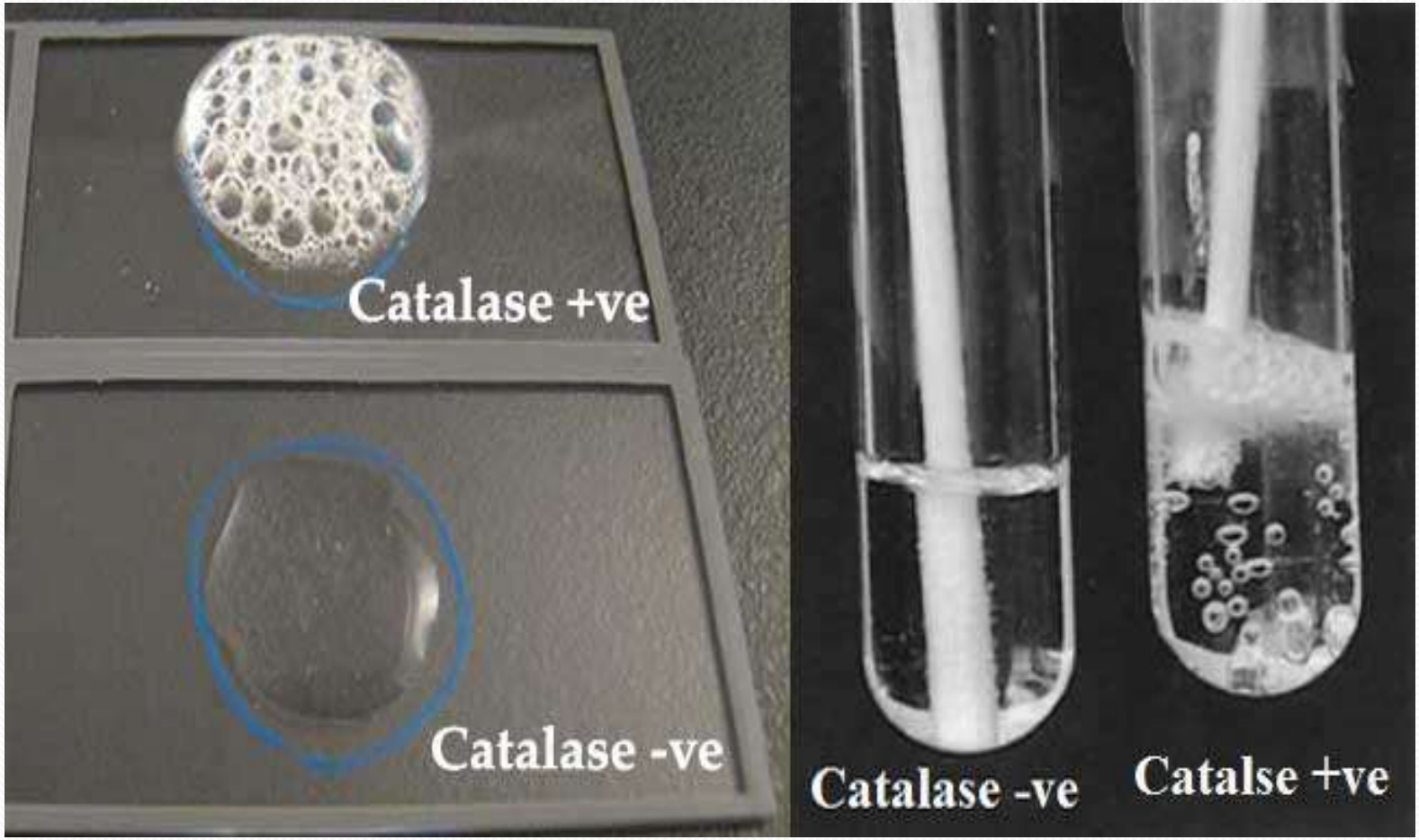
Antibiotic sensitivity test

Catalase test

- Used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.
- PRINCIPLE:
  - Catalase act as a catalyst in the breakdown of **hydrogen peroxide to oxygen and water**. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hrs old.

Catalase test

- slide method(left)
- tube method(right



Coagulase

- Property it tests for: This tests for the bacteria's ability to clot blood plasma using the enzyme coagulase.
- Media and Reagents: This media contains rabbit plasma dissolved in buffer.
- How to Perform Test: Inoculate rabbit plasma with one single colony. Break up colony and stir until blended in plasma. Incubate at 37 degrees C for 24 hours.

Coagulase Results

- Reading Results:
  - If the organism is has coagulase it will clump the plasma.
  - If the organism does not have coagulase it will not clump the plasma.

Slide test (clumping factor)

Presumptive needs confirming with the tube test

Tube test (free coagulase)

Check tubes at ½, 1, 2 & 4 hrs and overnight

Oxidase Test

Discriminates organisms that can produce cytochrome oxidase which catalyzes the transfer of electrons from reduced cytochrome c in the electron transport chain to molecular oxygen.

Test uses NNNN-tetramethyl-p-phenylenediamine (Oxidase Reagent) as an artificial electron acceptor: when oxidized it is colorless, when reduced it turns purple

*Look for color change on the bacteria in the filter paper! (The reagent will turn light purple(violet) when exposed to oxygen in the air)

Urea Hydrolysis

- Property it tests for: This test is done to determine a bacteria's ability to hydrolyze urea to make ammonia using the enzyme urease.
- Media and Reagents Used: Urea broth contains a yeast extract, monopotassium phosphate, disodium phosphate, urea, and phenol red indicator.
- How to Perform Test: Inoculate Urea broth with inoculating loop.
- Reading Results: Urea broth is a yellow-orange color. The enzyme urease will be used to hydrolyze urea to make ammonia. If ammonia is made, the broth turns a bright pink color, and is positive. If test is negative, broth has no color change and no ammonia is made.

Urease Test

A- Slant Agar

A

B- Urea Broth

B

Triple Sugar Iron (TSI) Fermentation Test

Basic Principle

Purpose

Used to differentiate and identify pathogenic Gram-negative enteric bacteria

TSI Medium

Contains three types of sugars: glucose (0.1%), lactose (1%) and sucrose (1%)
Also contains phenol red (pH indicator) and ferrous ammonium sulfate
Original control of the medium is red - due to alkaline pH

Results

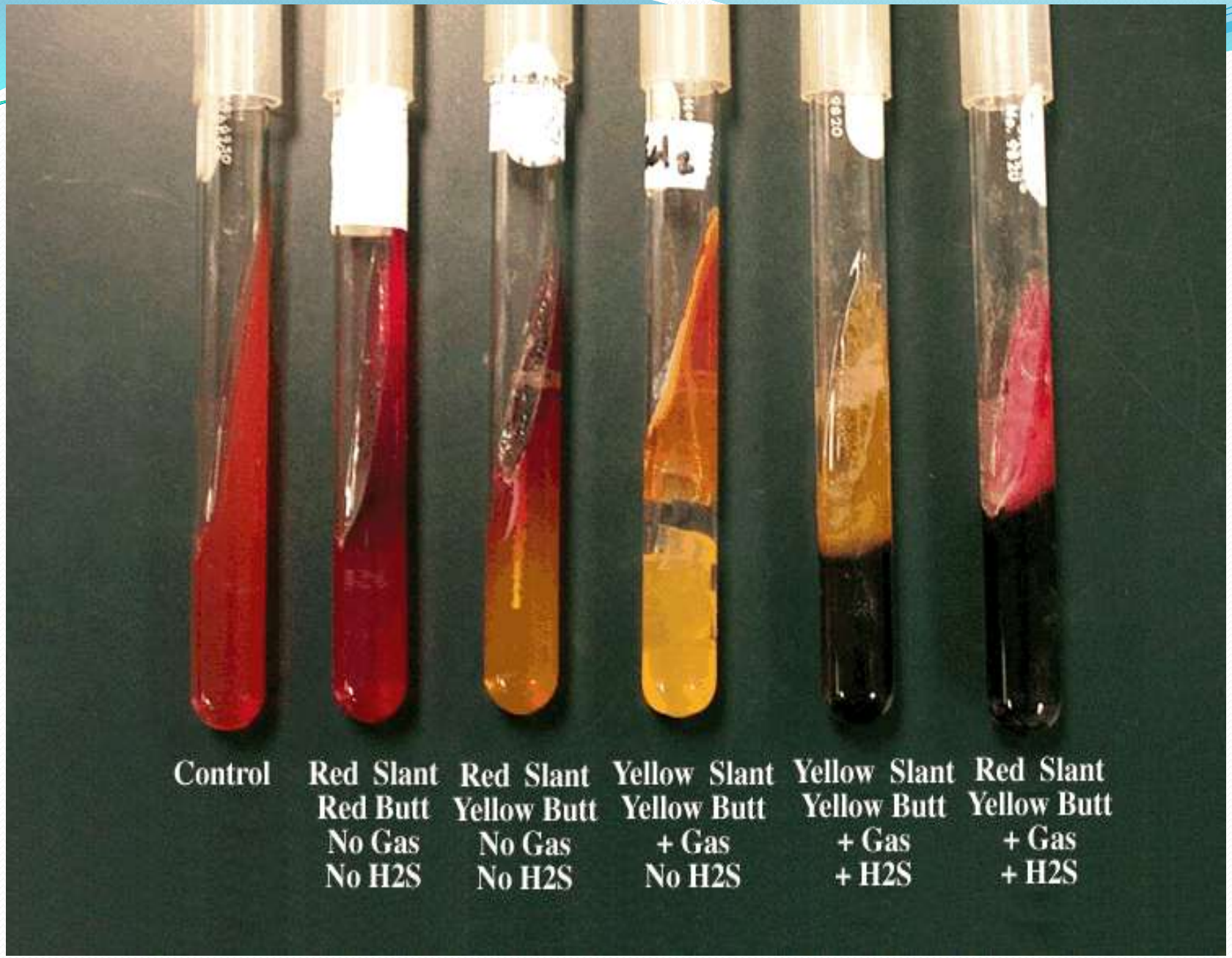
If no fermentation --> medium remains red

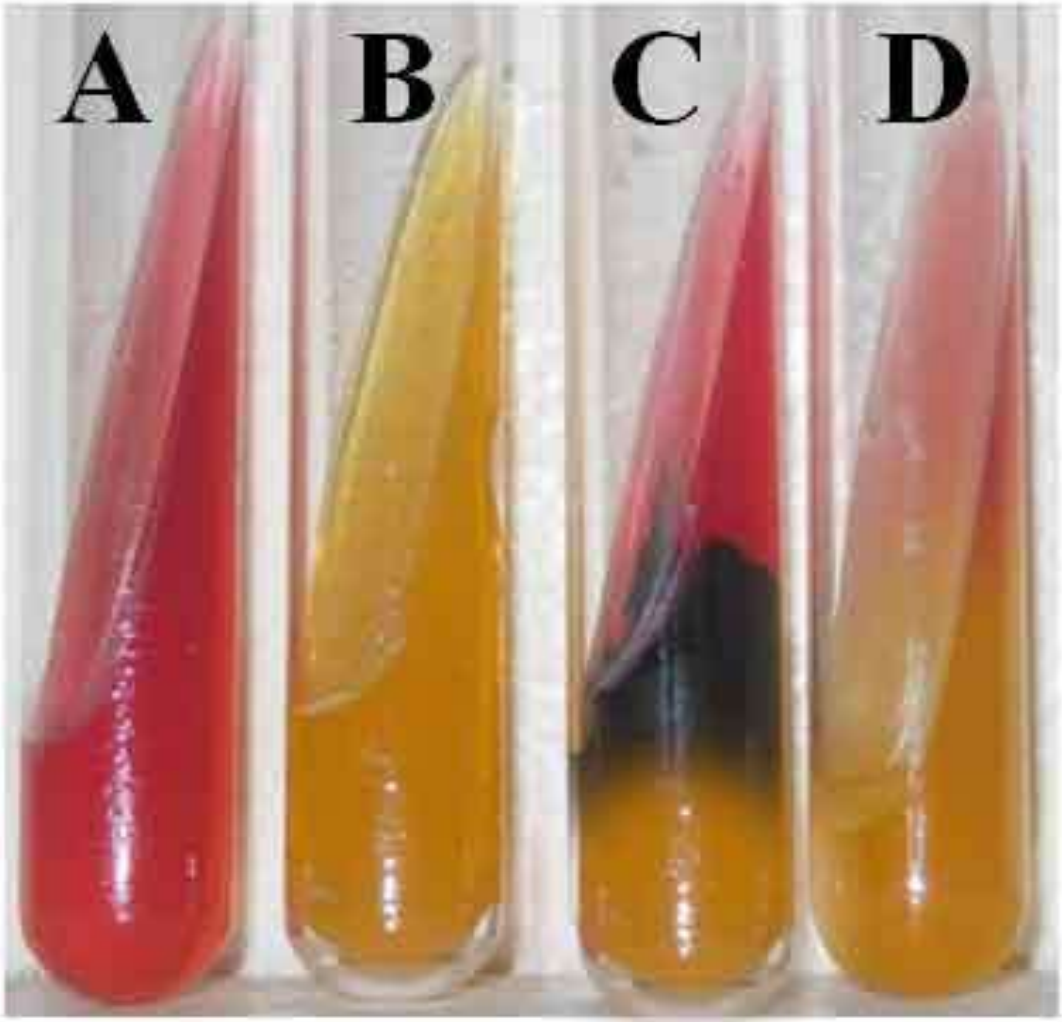
If the bacteria ferment all three sugars --> acids are produced --> entire medium turns yellow

If the bacterium ferments only glucose --> acids are produced --> if the cap is loose --> acids are oxidized --> medium slant becomes red again

If gases such as CO_2 or H_2 released during fermentation --> bubbles collect in the medium

If H_2S is produced --> reacts with ferrous ammonium sulfate in the medium --> black ferrous sulfide precipitate formed at the bottom of the medium (black butt)


Control
Red Slant
Red Butt
No Gas
No H2S
Red Slant
Yellow Butt
No Gas
No H2S
Yellow Slant
Yellow Butt
+ Gas
No H2S
Yellow Slant
Yellow Butt
+ Gas
+ H2S
Red Slant
Yellow Butt
+ Gas
+ H2S



A) ***Psuedomonas aeroginosa*:** Gluc (-), Lac/Suc (-), H_2S (-)

B) ***Escherichia coli*:** Gluc (+), Lac/Suc (+), H_2S (-)

C) ***Salmonella typhimurium*:** Gluc (+), Lac/Suc (-), H_2S (+)

D) ***Shigella boydii*:** Gluc (+), Lac/Suc (-), H_2S (-)

Some example of Triple Sugar Iron (TSI) Agar Reactions

Name of the organisms	Slant	Butt	Gas	H_2S
Escherichia, Klebsiella, Enterobacter	Acid (A)	Acid (A)	Pos (+)	Neg (-)
Shigella, Serratia	Alkaline (K)	Acid (A)	Neg (-)	Neg (-)
Salmonella, Proteus	Alkaline (K)	Acid (A)	Pos (+)	Pos (+)
Pseudomonas	Alkaline (K)	Alkaline (K)	Neg (-)	Neg (-)

Lactose Fermentation

- Property it tests for: This tests for the bacteria's ability to ferment lactose.
- Media and Reagents Used: Lactose broth contains beef extract, gelatin peptone, and lactose. A phenol red indicator is added to indicate acid production from fermentation.
- How to Perform Test: Inoculate lactose broth with inoculating loop.
- **Results**
  - A positive result is yellow after indicator is added (indicating lactose fermentation)
  - A negative result will have no color change or will be reddish.

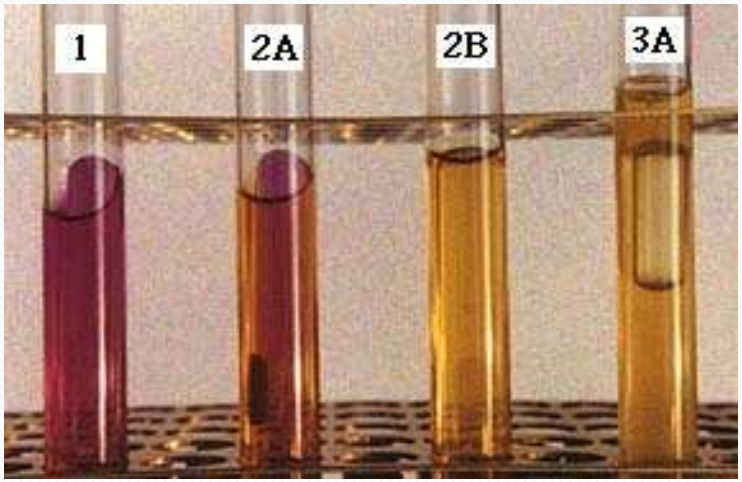
Sucrose Fermentation

- Property it tests for: This test is done to help differentiate species of the family *Enterobacteriaceae*. This tests for the bacteria's ability to ferment sucrose and production of acid end-product
- Media and Reagents Used: Sucrose broth contains beef extract, gelatin peptone, and sucrose. Phenol red indicator is added to indicate an acid end-product.
- How to Perform Test: Inoculate sucrose broth with inoculating loop.
- Results
  - A positive result is yellow after indicator is added (indicating sucrose fermentation)
  - A negative result has no color change or is reddish.

Glucose Fermentation & Gas Production

- Property it tests for: This test is done to help differentiate species of the family *Enterobacteriaceae*. This tests for the bacteria's ability to ferment glucose and produce gas and/or an acid end-product..
- Media and Reagents Used: Glucose broth contains beef extract, gelatin peptone, and glucose. A phenol red indicator is added to indicate an acid end-product. A Durham tube is added to indicate gas production.
- How to Perform Test: Inoculate broth with inoculating loop.
- Results
  - A positive result for acid is yellow after indicator is added (indicating glucose fermentation)
  - A positive result for gas is a bubble in the Durham tube.
  - A completely negative result has no color change or reddish color and no bubble.

Sugar Fermentation Tests

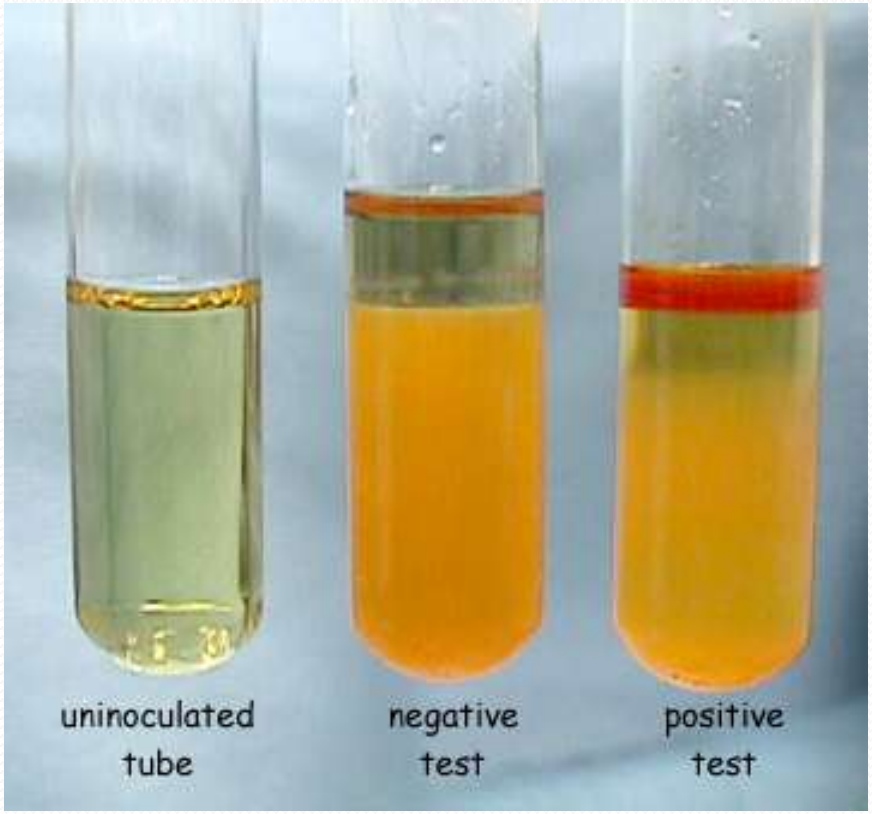


Tube 1: Negative acid /Negative gas
Tube 2A: Must incubate longer (ambiguous result)
Tube 2B: Positive acid /Negative gas
Tube 3A: Positive acid/ Positive gas

Indole Test

- Property it tests for: This test is performed to help differentiate species of the family *Enterobacteriaceae*.
- Media and Reagents Used: Tryptone broth contains tryptophan. Kovac's reagent—contains hydrochloric acid, dimethylaminobenzaldehyde, and amyl alcohol—yellow in color.
- How to Perform Test: Inoculate Tryptone broth with inoculating loop.
- Reading Results: Kovac's reagent reacts with indole and creates a red color at the top part of the test tube.

Indole

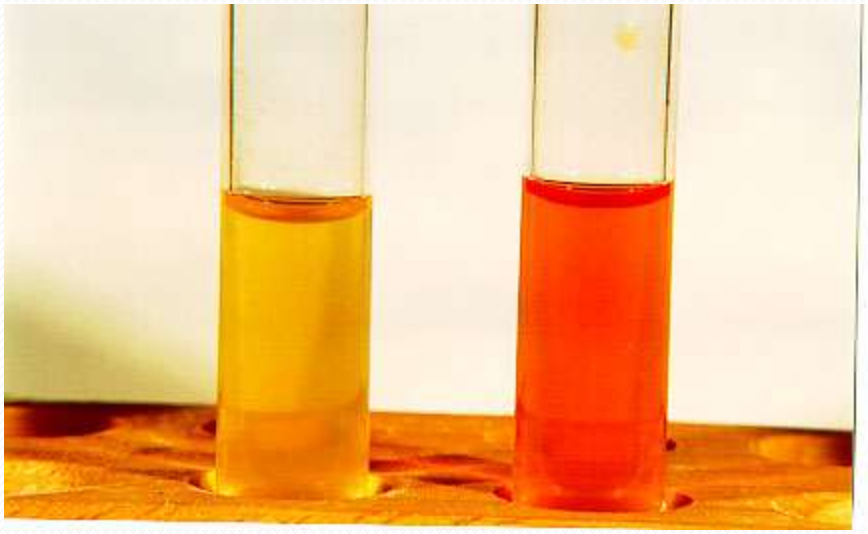


Methyl Red/Voges Proskauer (MR/VP)

- Properties these test for: Both tests are used to differentiate species of the family *Enterobacteriaceae*.
- Media and Reagents Used:
  - Glucose Broth
  - Methyl Red indicator for acid
  - Voges Proskauer reagents—A: 5% Alpha-Naphthol, & ethanol, B: Potassium Hydroxide, & Deionized Water.
- How to Perform Tests: Inoculate 2 glucose broths with inoculating loop. After 48 hours of incubation, add a few drops of MR to one tube, and VP reagents to the other tube.
  - MR—tests for acid end products from glucose fermentation.
  - VP—tests for acetoin production from glucose fermentation.

MR/VP continued

- Reading Results:
  - MR— a + result is red (indicating pH below 6) and a – result is yellow (indicating no acid production)
  - VP—A + result is red after VP reagents are added (indicating the presence of acetoin) and a – result is no color change.



Methyl Red: left – and right +

VP: left + and right –

Citrate Utilization test

Inoculation method: streak and stab slant with needle

Contains: citrate as sole carbon source, ammonium salts as sole nitrogen source, bromthymol blue pH indicator: neutral pH = green, alkaline = prussian blue. Media; Simmon's citrate agar.Discriminates organisms that can produce citrase to metabolize citrate into oxaloacetate and pyruvate. These organisms are forced to utilize ammonium salts as the nitrogen source producing alkaline ammonia waste.

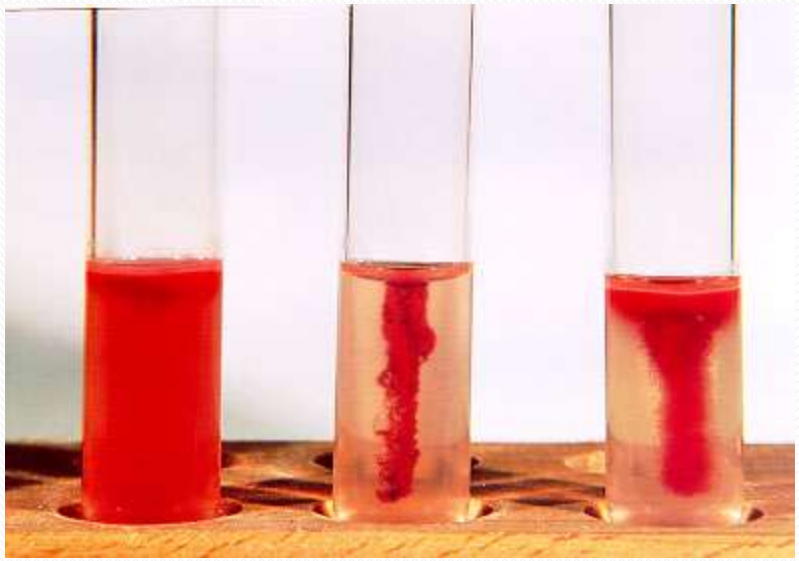
Results: Prussian blue slant and or butt = positive for citrase production

Green = negative for citrase production

Motility Test

- Property it tests for: This test is done to help differentiate species of bacteria that are motile.
- Media and Reagents Used: Motility media contains tryptose, sodium chloride, agar, and a color indicator.
- How to Perform Test: Stab motility media with inoculating needle.
- Reading Results: If bacteria is motile, there will be growth going out away from the stab line, and test is positive. If bacteria is not motile, there will only be growth along the stab line. A colored indicator can be used to make the results easier to see.

Motility



From left to right:

\+ − +

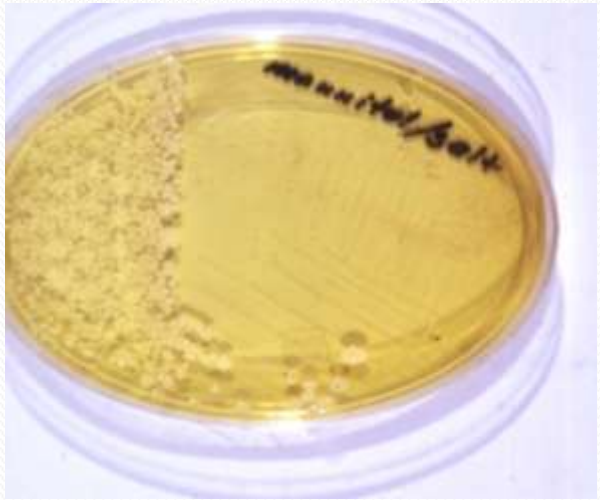
Mannitol Salt Agar (MSA)

- Property it tests for: This tests for the bacteria's ability to tolerate 7% salt concentration and ferment mannitol. The media is selective because it selects for salt tolerant bacteria.
- Media and Reagents: MSA media contains nutrient agar, mannitol, 7% sodium chloride and phenol red indicator.
- How to Perform Test: Inoculate an MSA plate using streak plate method and incubate 24-48 hours.

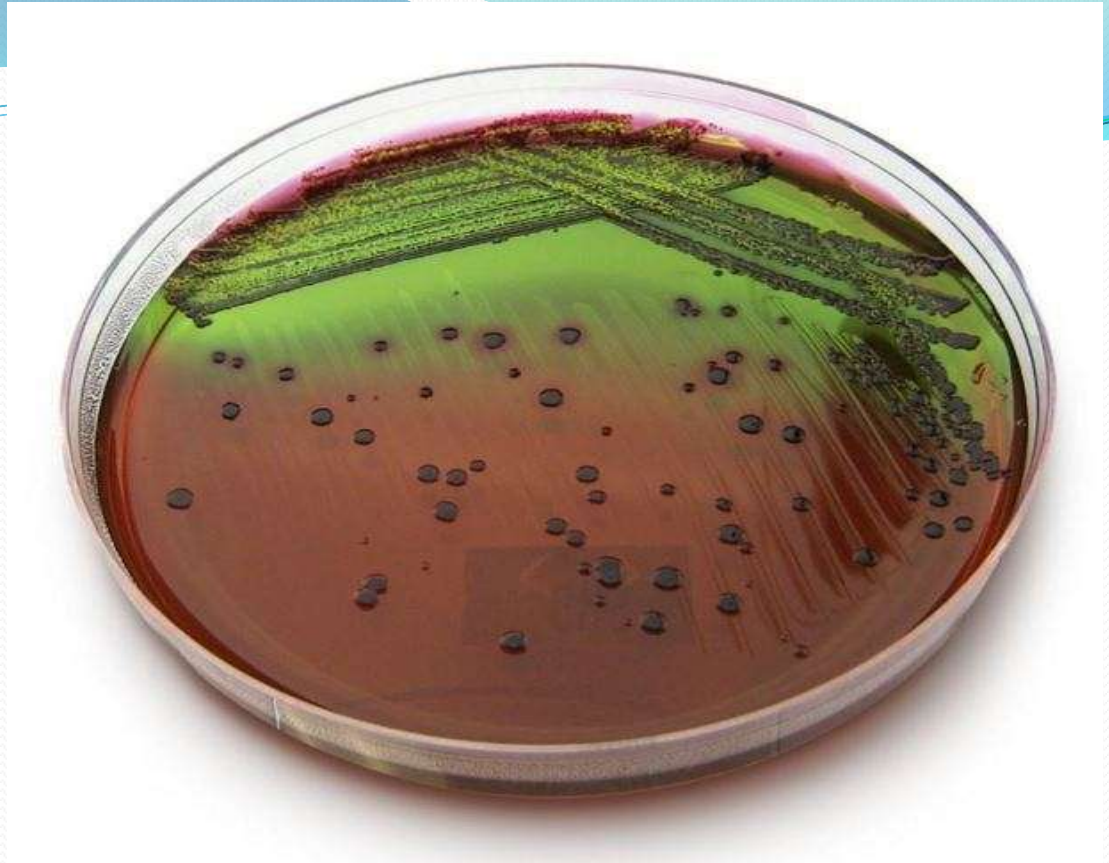
MSA Results

- Reading Results:
  - If the organism is tolerant to salt it will grow.
  - If the organism is not tolerant to salt it will not grow.
  - If the salt tolerant organism can ferment mannitol then there will be yellow zones around the colonies.
  - If the salt tolerant organism cannot ferment mannitol then the media will remain pink.

Growth with no mannitol fermentation.

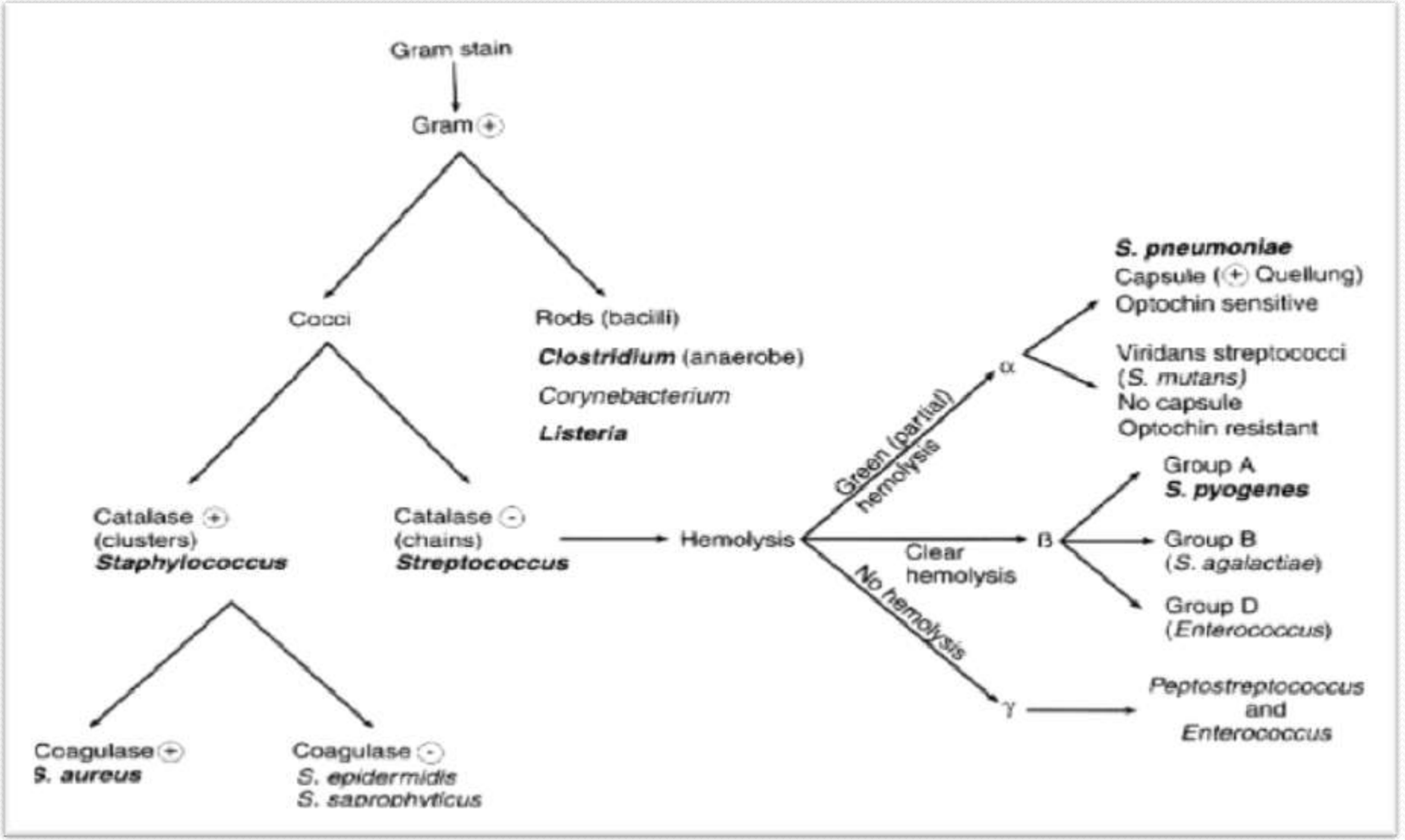


Growth with + mannitol fermentation.

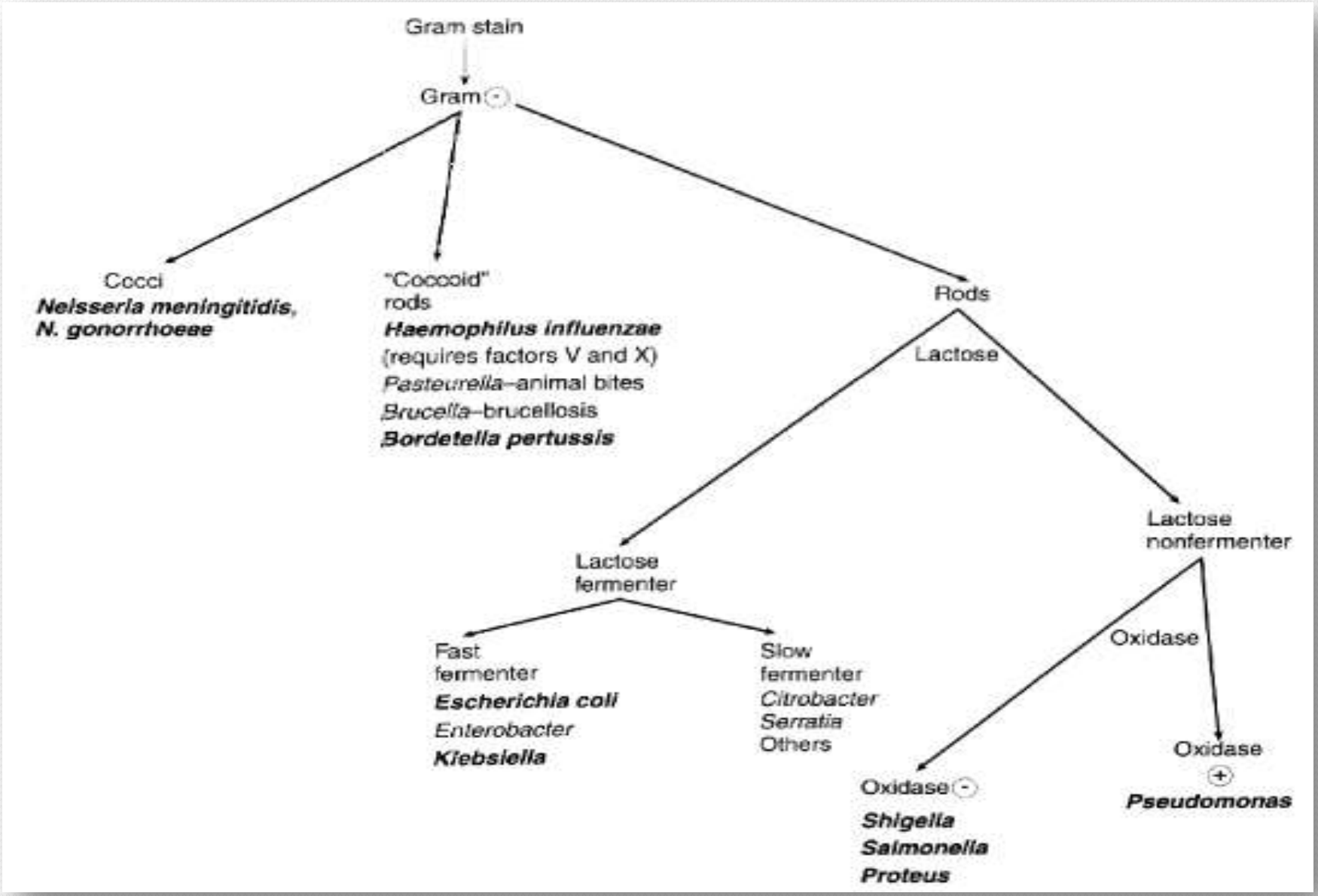


- **Eosin Methylene Blue Agar is a both selective and differential culture medium. It is selective culture medium for gram-negative bacteria due to the presence of two Dyes(EOSIN& METHYLENE BLUE). Colored colonies in Eosin Methylene Blue (EMB) Agar are Lactose fermenter. Colorless colonies in Eosin Methylene Blue (EMB) Agar are Non lactose fermenter. *E. coli* colonies have a characteristic green sheen. It is commonly used for the isolation and differentiation of coliforms and fecal coliforms.**

Gram positive flowchart



Gram negative flowchart



Key identification characteristics for Enterobacteriaceae

1.

GENUS/SPECIES	Fermentation of				Gas	MR	VP	Indole	Citrate	Urease	H2S	Motility
	G	L	S	M								
Escherichia coli	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)
Shiegella	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-/+)	(-)	(-)	(-)	(-)
Shiegella sonnei	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Salmonella	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(+)
Klebsiella Pneumo.	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(-)	(-)
Enterobacter	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Serratia	(+)	(+)	(-)	(+)	(+)	(-/+)	(+)	(-)	(+)	(-)	(-)	(+)
Proteus	(+)	(-)	(-)	(+)	(-/+)	(+)	(-)	(+)	(-/+)	(+)	(+)	(+)
morganella	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Yersinia	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-/+)	(-)	(-/+)	(-)	(+)

G: Glucose, L:Lactose, S:Sucrose, M: Manitol, MR: Methyl Red, VP: Voges Proskauer

DYES

I. ANILINE

II. ACRIDINE

- Used as skin & wound antiseptics
- Bacteriostatic on high dilution but low bactericidal

ANILINE DYES:

- Eg: brilliant green, malachite green & crystal violet
- More active against Gram +ve than Gram –ve
- No action against tubercle bacilli

MODE OF ACTION:

- React with the acid in the cell

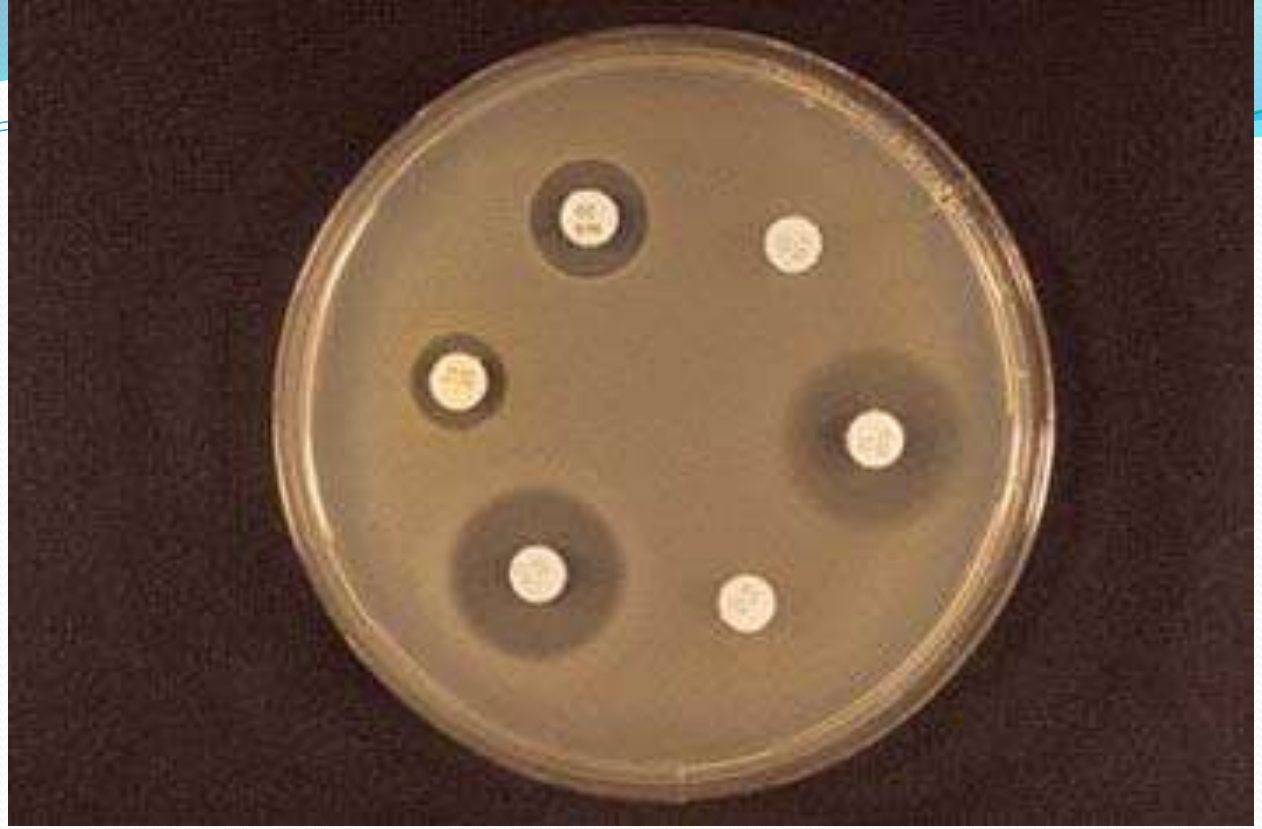
Used in microbiology labs as selective agents in culture media

ACRIDINE DYES

- Active against Gram +ve than Gram –ve
- ex: proflavine, acriflavine, euflavine & aminarine

MODE OF ACTION:

- Impair the DNA complex of organism & destroys the reproductive capacity of cell.



Antibiotic sensitivity test: also known as the “disk diffusion test” is used to test the resistance of a bacterial culture to various anti-infective agents. The method provides a basis for classification of a bacterial strain as “susceptible,” “resistant,” or “intermediate” according to the dimension of the inhibition zone

Thank you

Syllabus of Human Parasitology

Phylum: Protozoa

Class: **Amoeba**

Intestinal and oral amoeba

Entamoeba hystolytica, *E. coli, Endolimux nana, Iodomoeba butschilli, Entamoeba gingivalis*

Class: **Flagellates**

Intestinal flagellates

Giardia lambilia, Chilomastics mesnelli

Genital tract flagellates

Trichomonas vaginalis

Blood and tissues flagellates

Leishamnia tropica, L. donovani, L. mexicana brasielensis

Trypanosoma gambiense, T. rhodesiense, T. cruzi

Class: **Ciliates**

Balantidium coli

Class: **Sporozoa**

Plasmodium vivax, P. falciparum, P. ovale, P. malariae

Class: **Coccidia**

Toxoplasma gondii

Phyllum: Platyhelminthes (FLAT WORMS)

Class: **Cestoda(es) TAPE WORMS**

Taenia saginata, T. solium, Hymenolepis nana,

Ecchinococcus granulosus, E. multilocularis

Class: **Trematoda(es)**

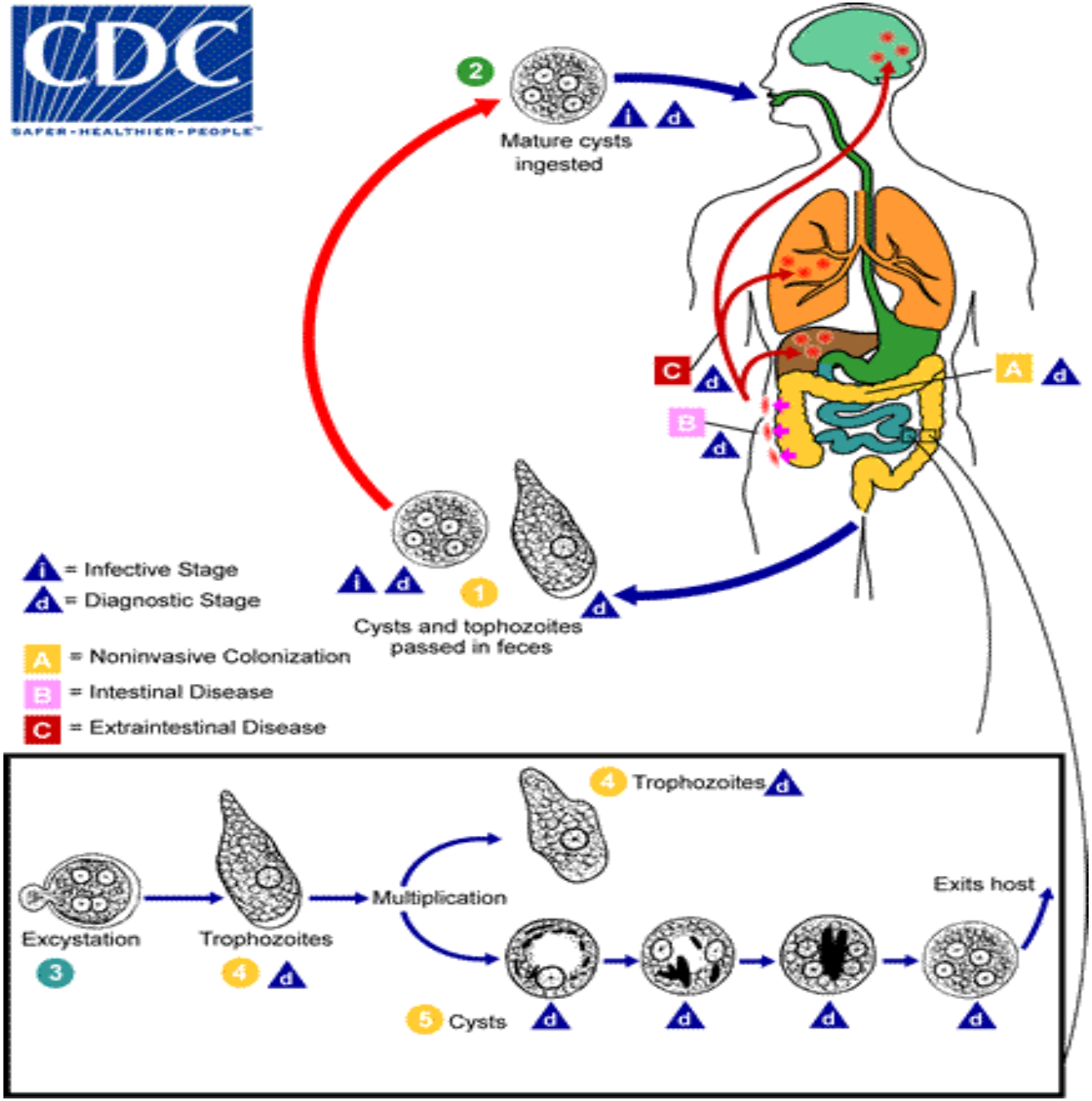
Schistosoma mansoni, S. japonicum, S. haematobium

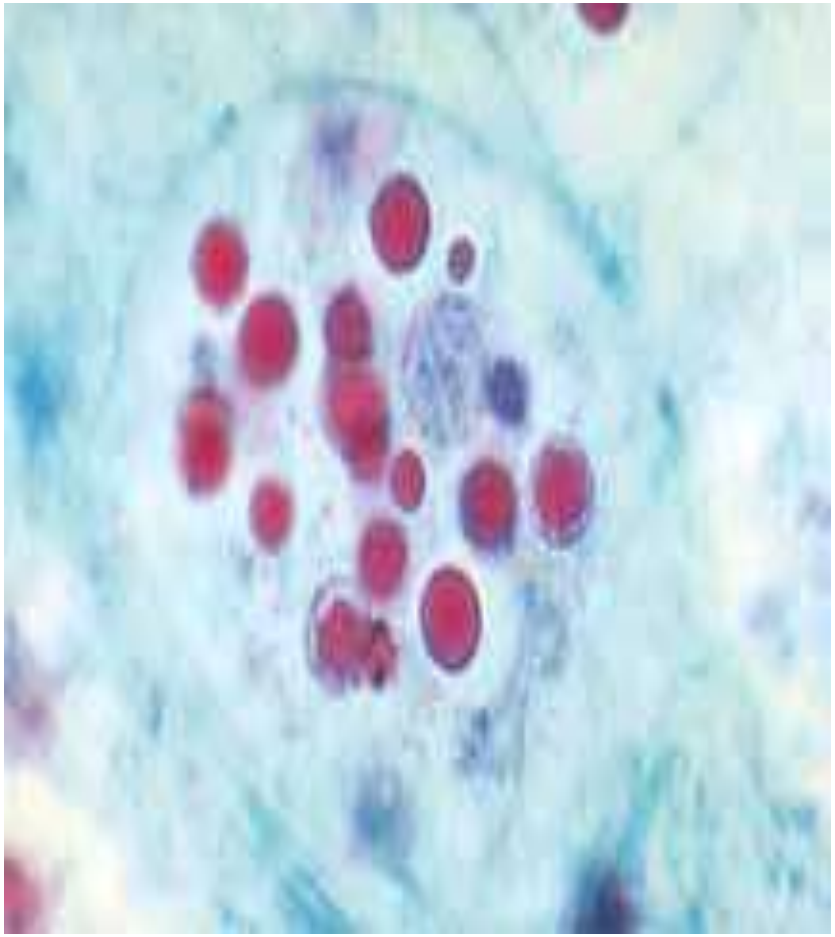
Phyllum: Nematohelminthes(Nematoda) ROUND WORMS

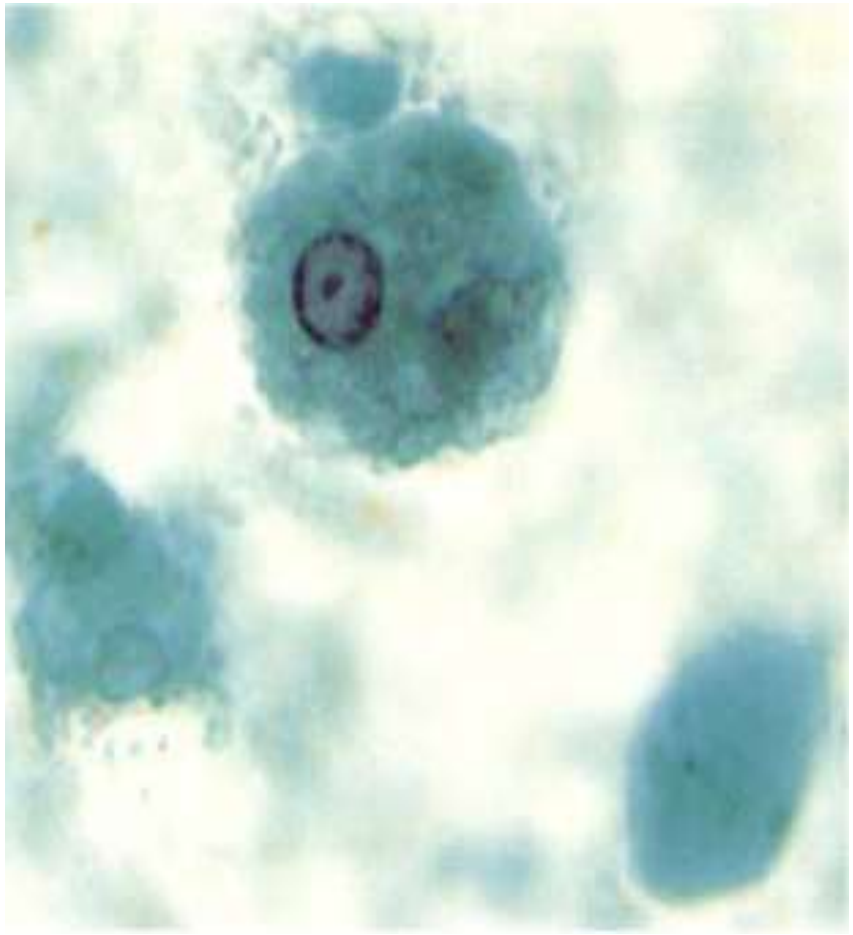
Trichuris trichuira, Enterobius vermicularis, Ancylostoma duodenale, Ascaris lumbricoides

Phyllum : Acanthocephala

Phyllum: Arthropoda(es)

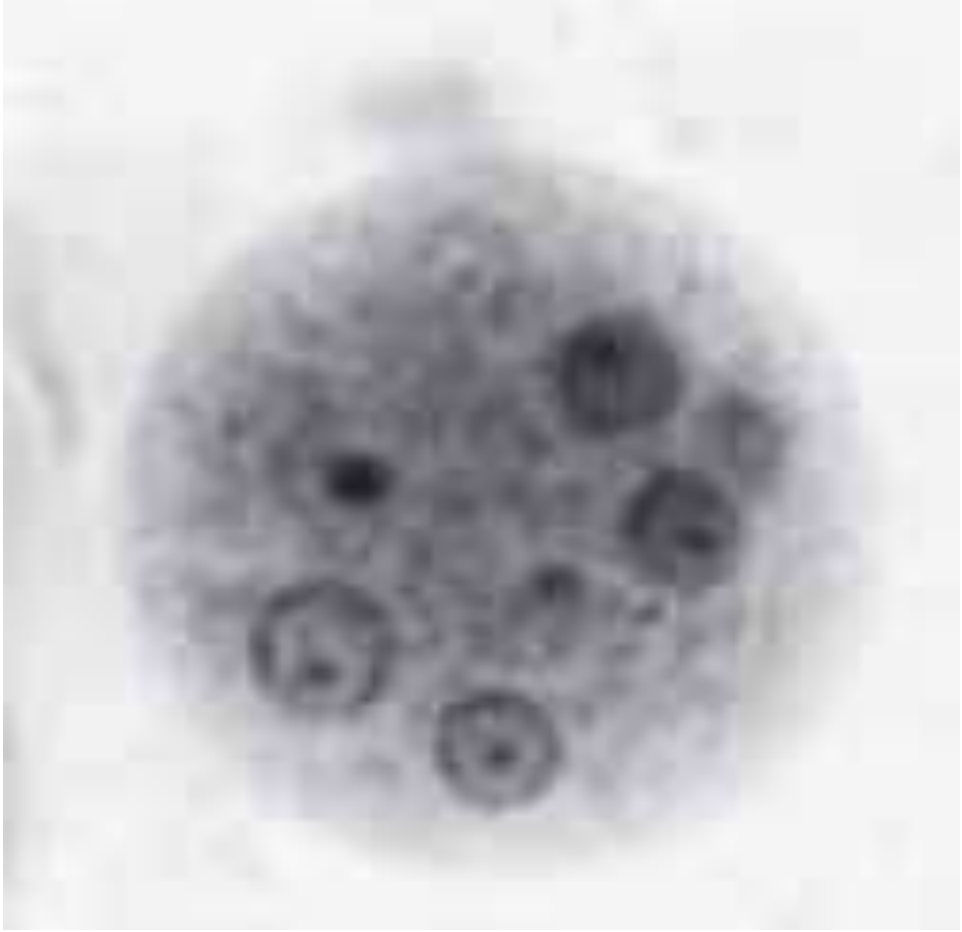

CDC
SAFER·HEALTHIER·PEOPLE™
2
Mature cysts ingested
i
d
C
d
A
d
B
d
i
d
1
Cysts and tophozoites passed in feces
d
i = Infective Stage
d = Diagnostic Stage
A = Noninvasive Colonization
B = Intestinal Disease
C = Extraintestinal Disease
4 Trophozoites d
Multiplication
Exits host
Excystation
3
Trophozoites
4 d
5 Cysts
d
d
d
d



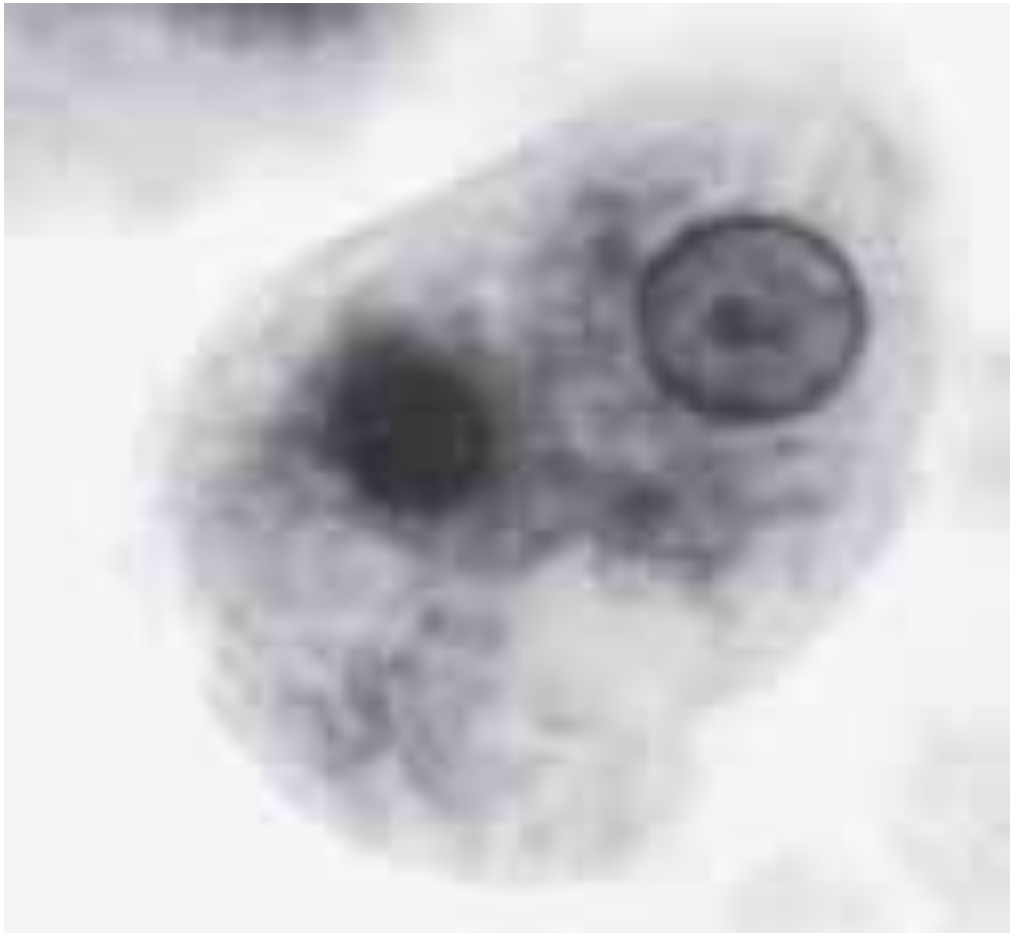


1 2

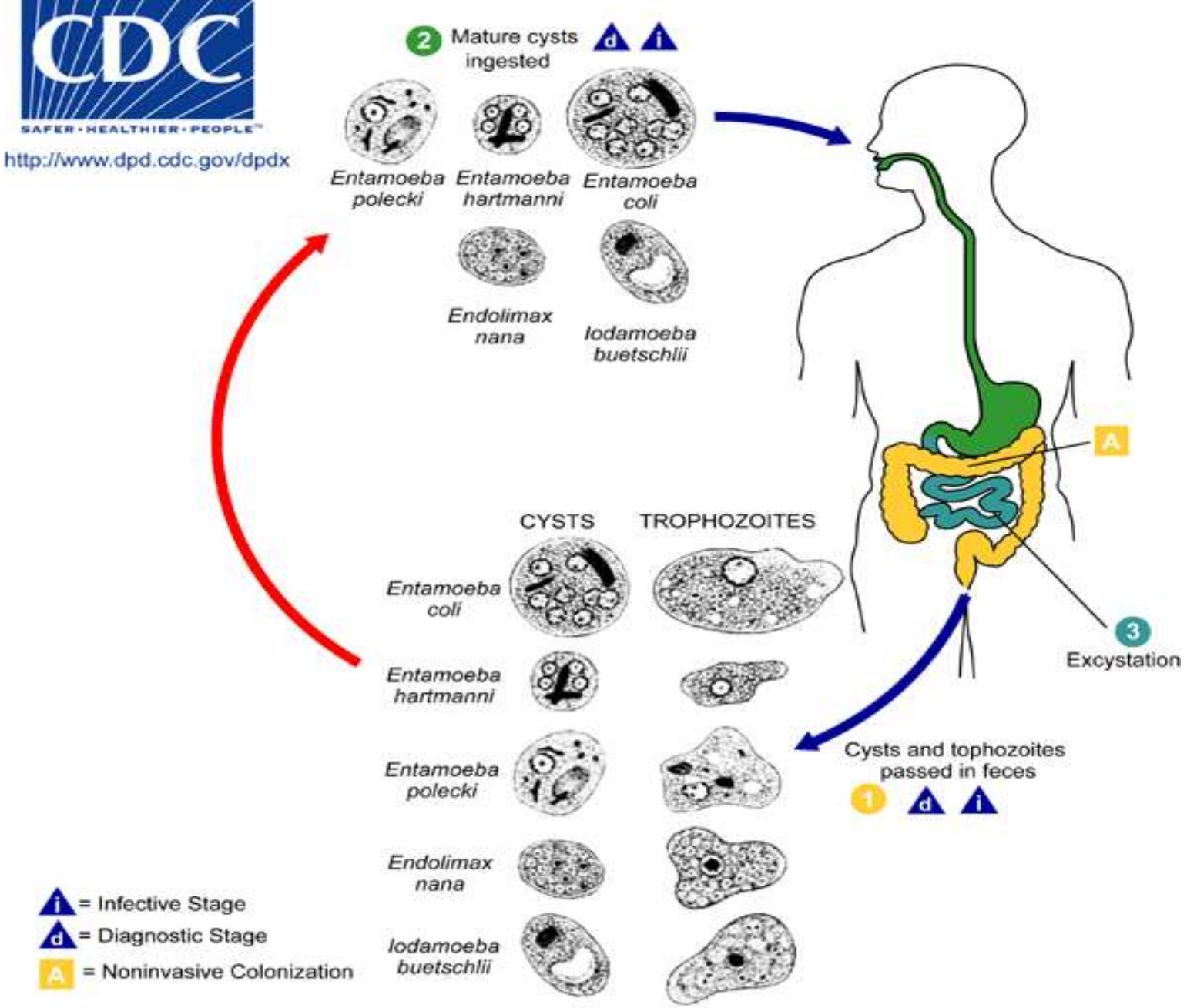
Entamoeba histolytica trophozoite 1-containing ingested red blood cells 2-stained with trichrome stain



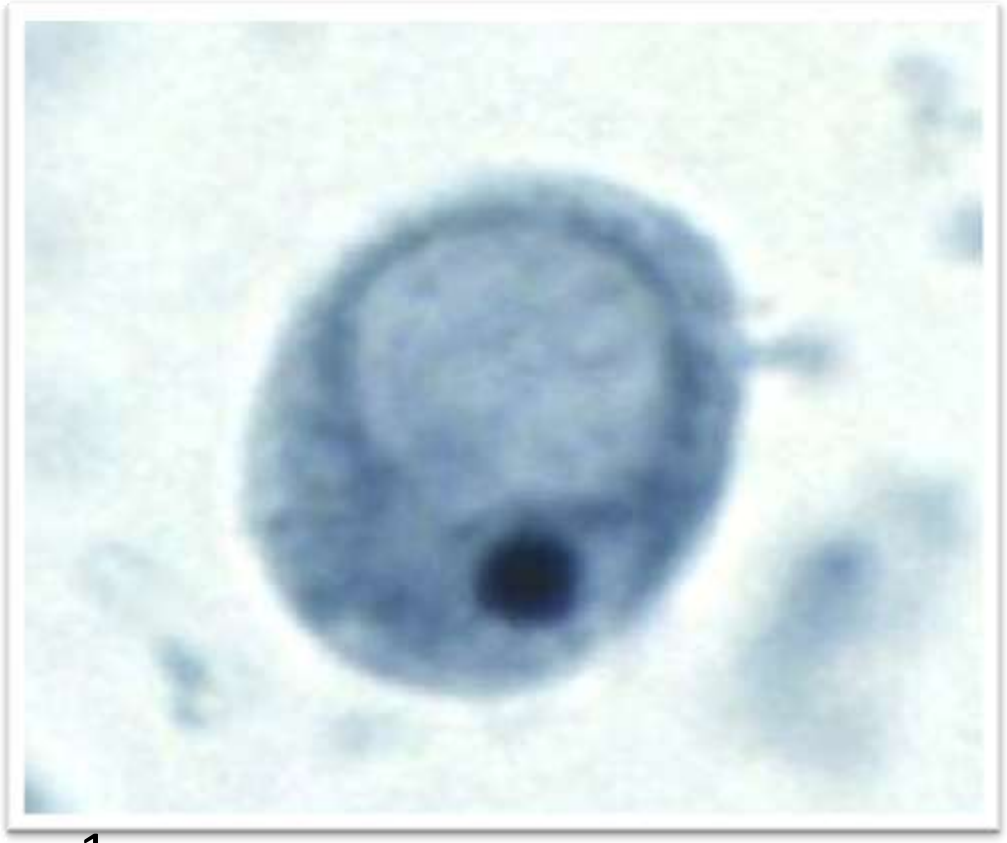
Mature Cyst of *Entamoeba histolytica*



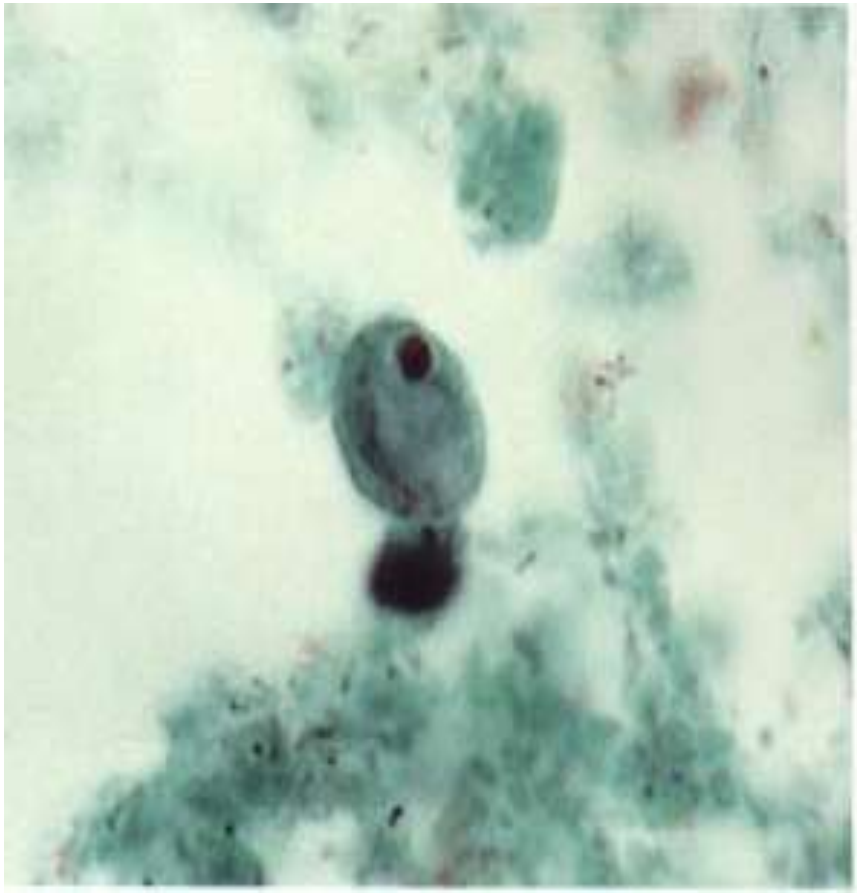
Trophozoite of *Entamoeba hystolytica*



Life Cycle of Commensal Intestinal Amoebas



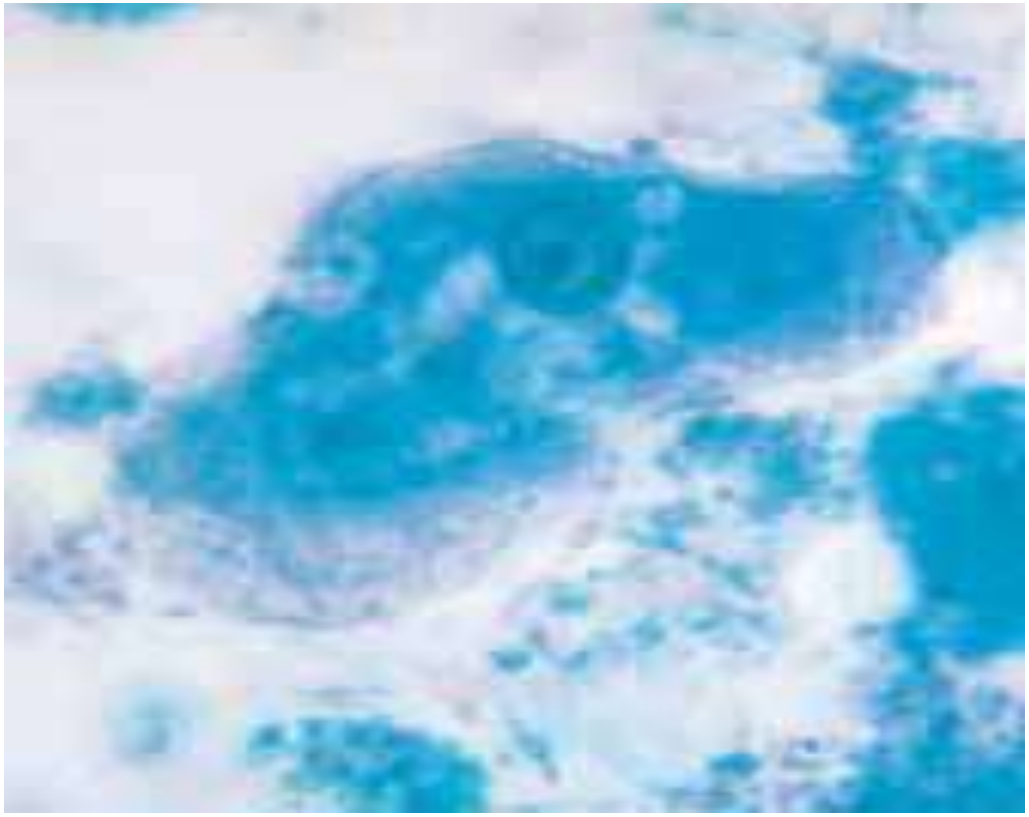
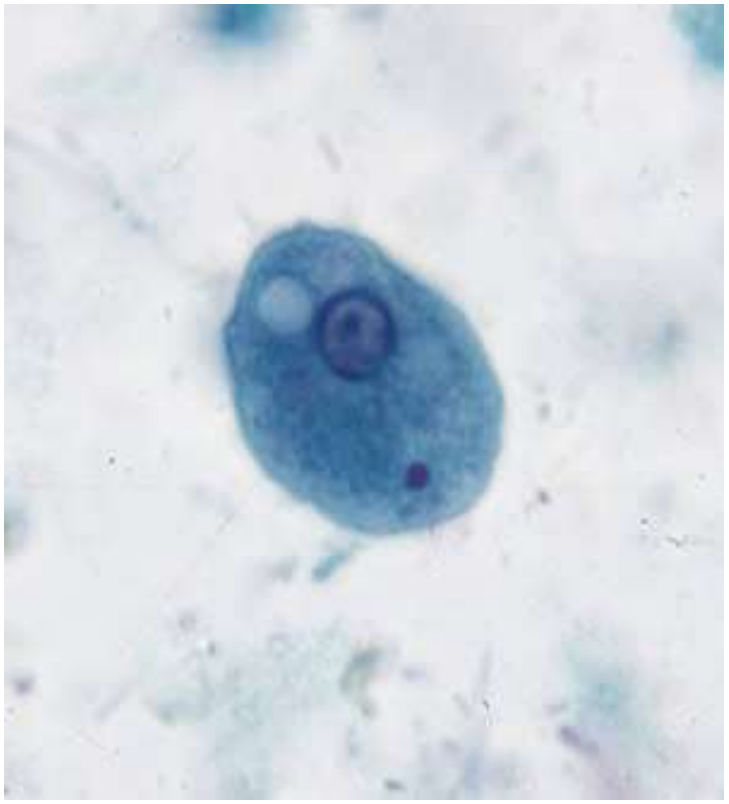
1



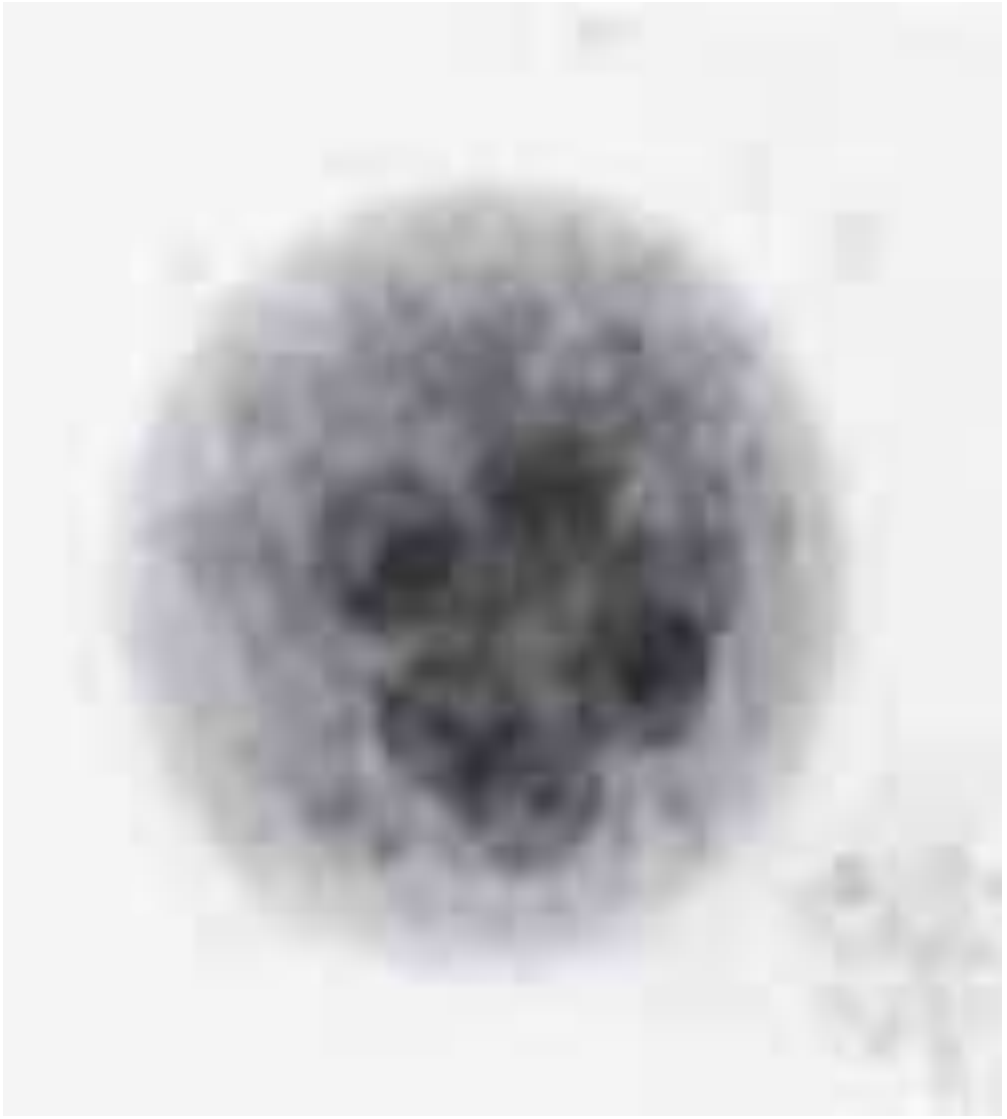
2

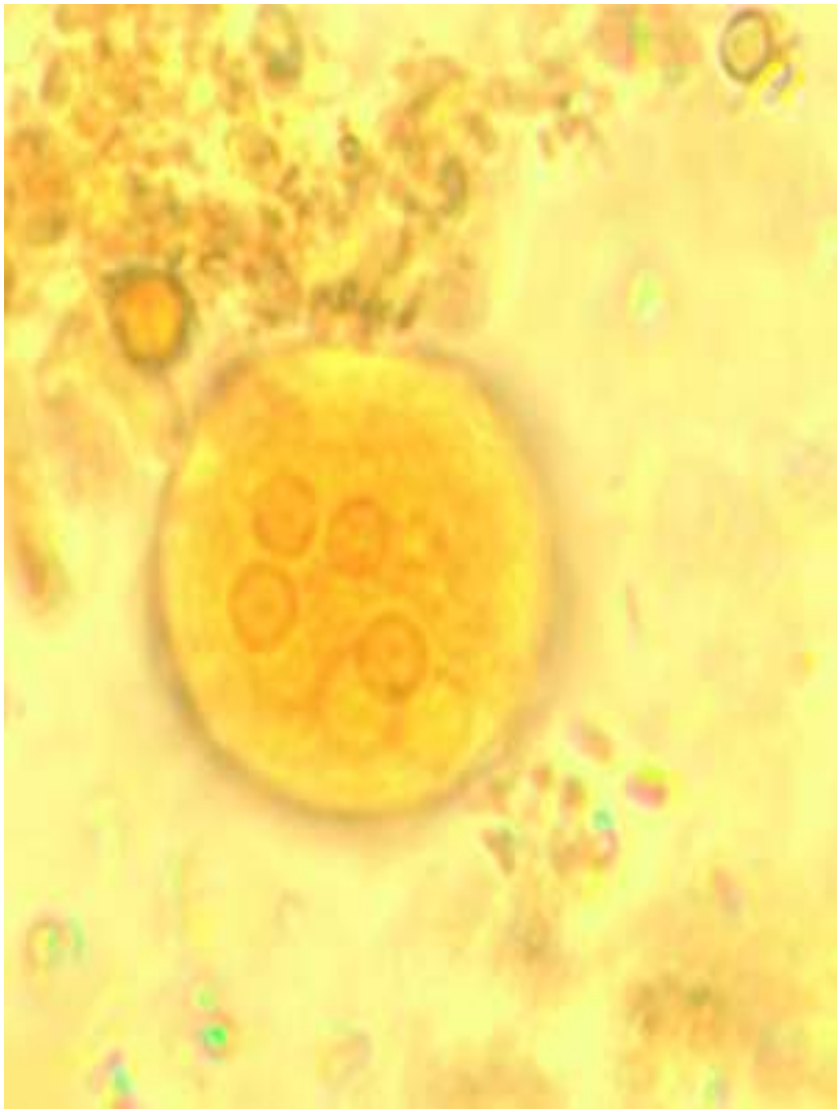
Iodomoeba butschlii 1- Cyst

2- Trophozoite

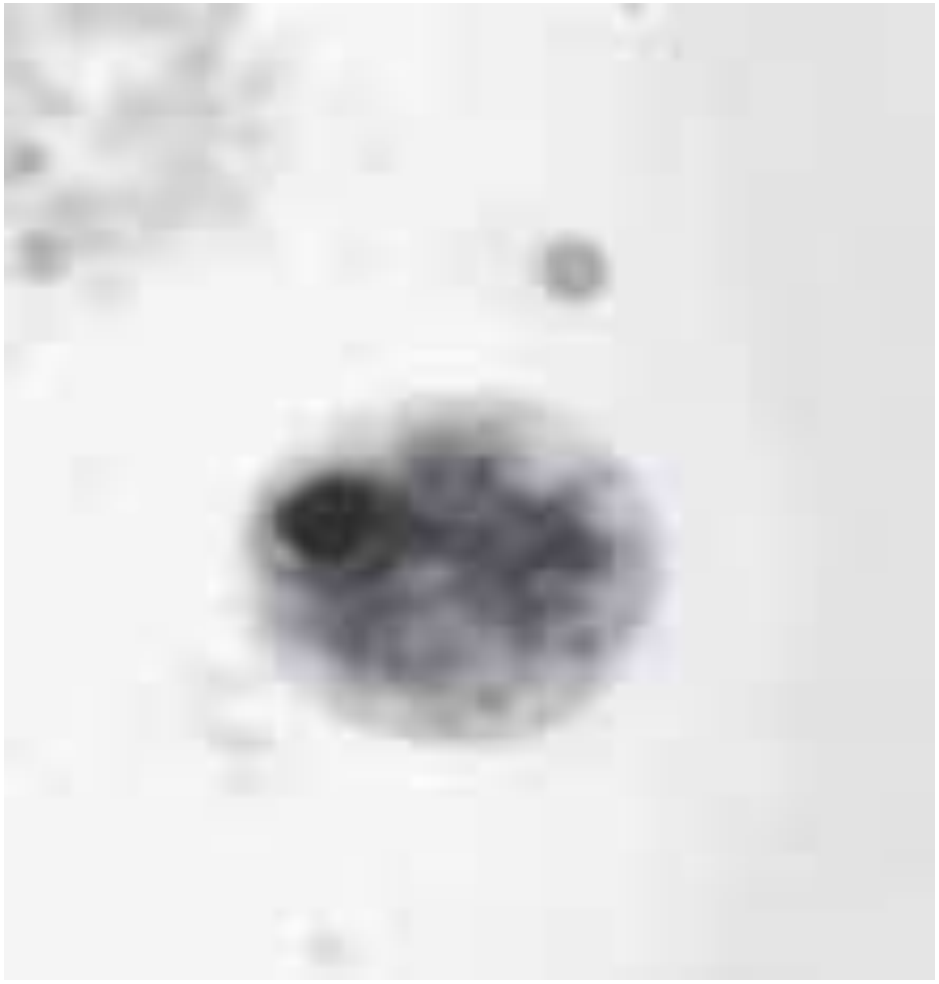



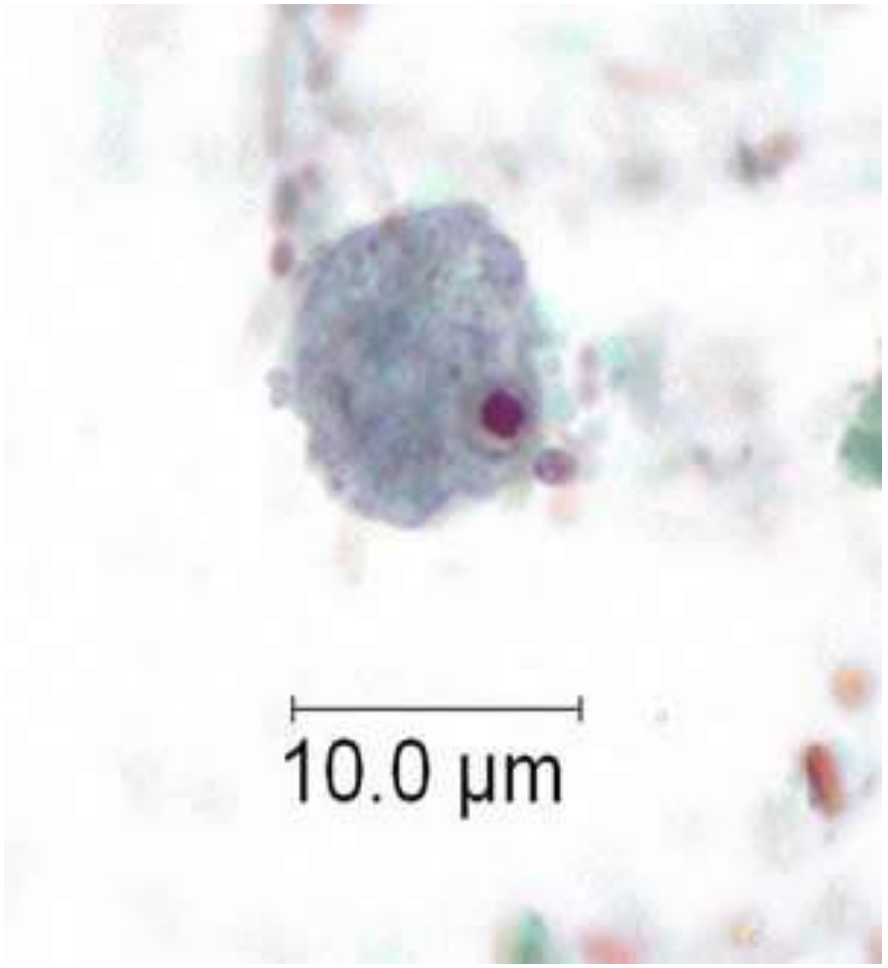
Entamoeba coli trophozoite



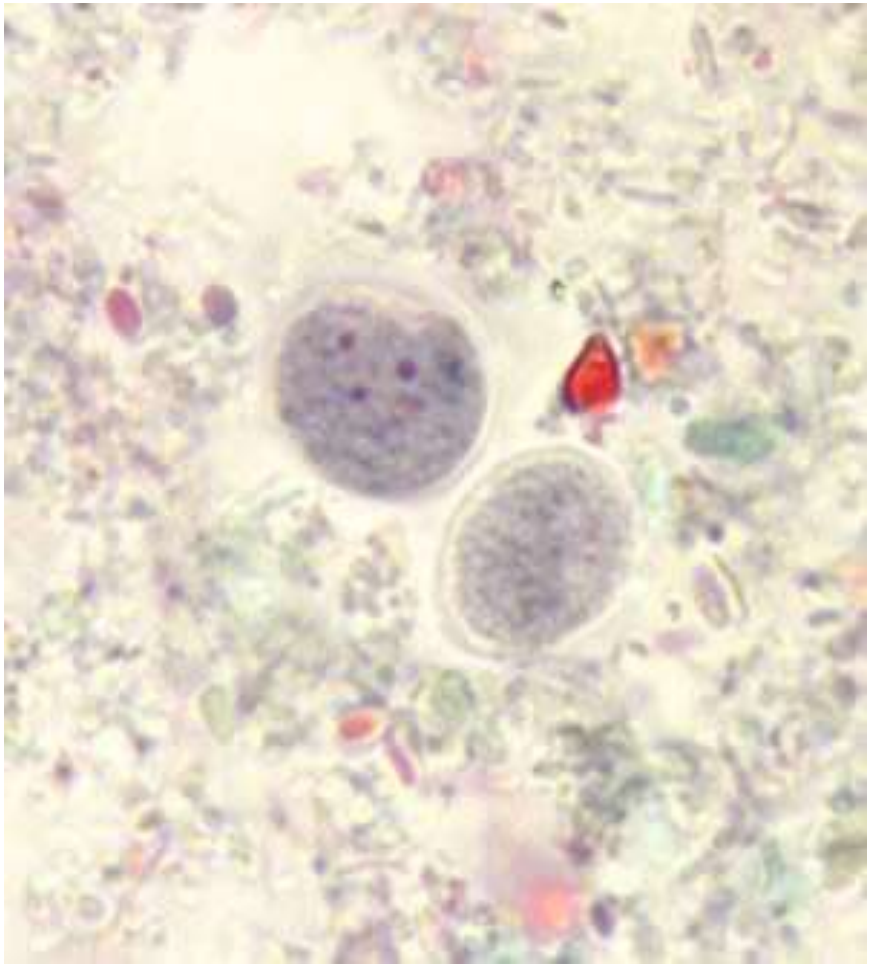


Cyst of *Entamoeba coli*



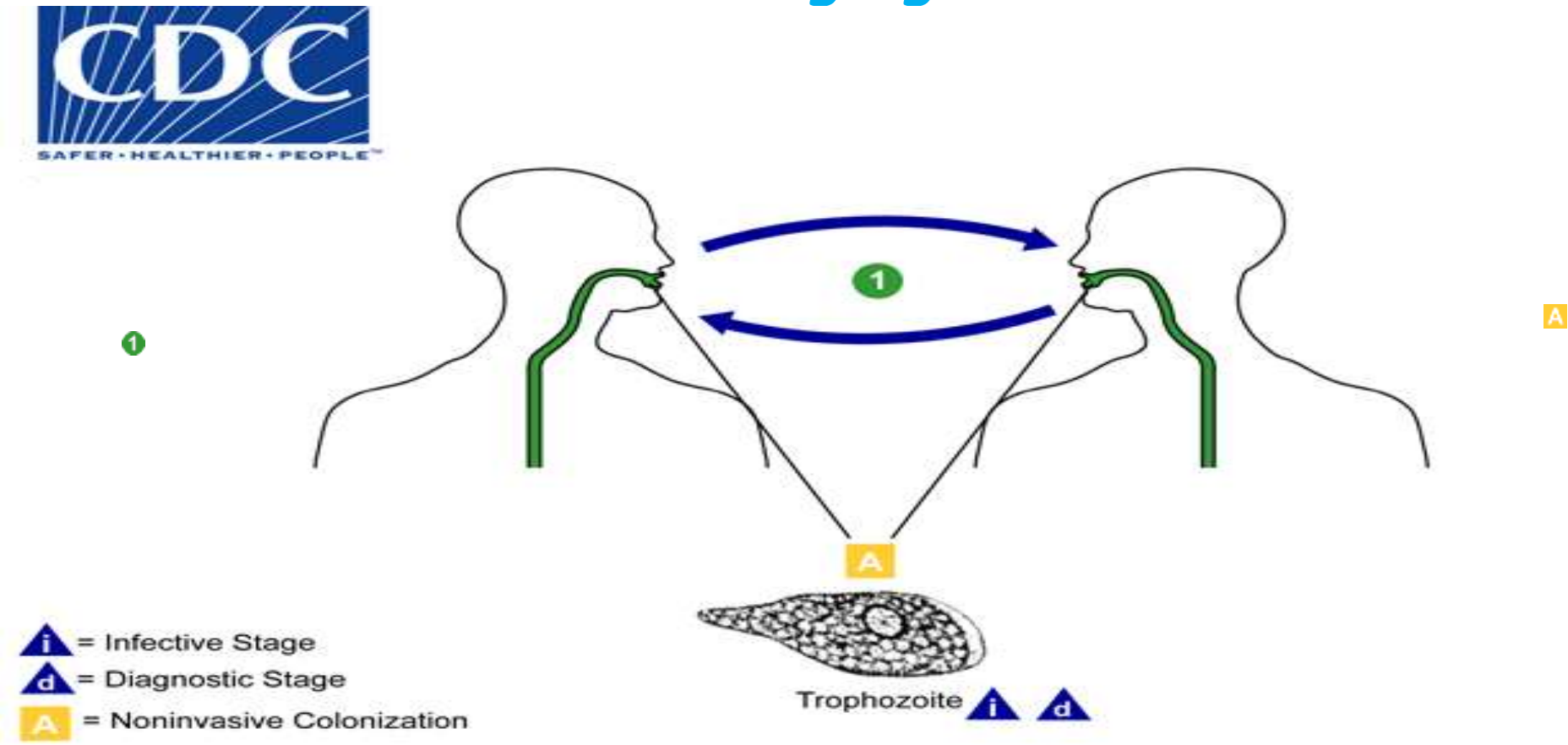


Trophozoite of *Endolimax nana*

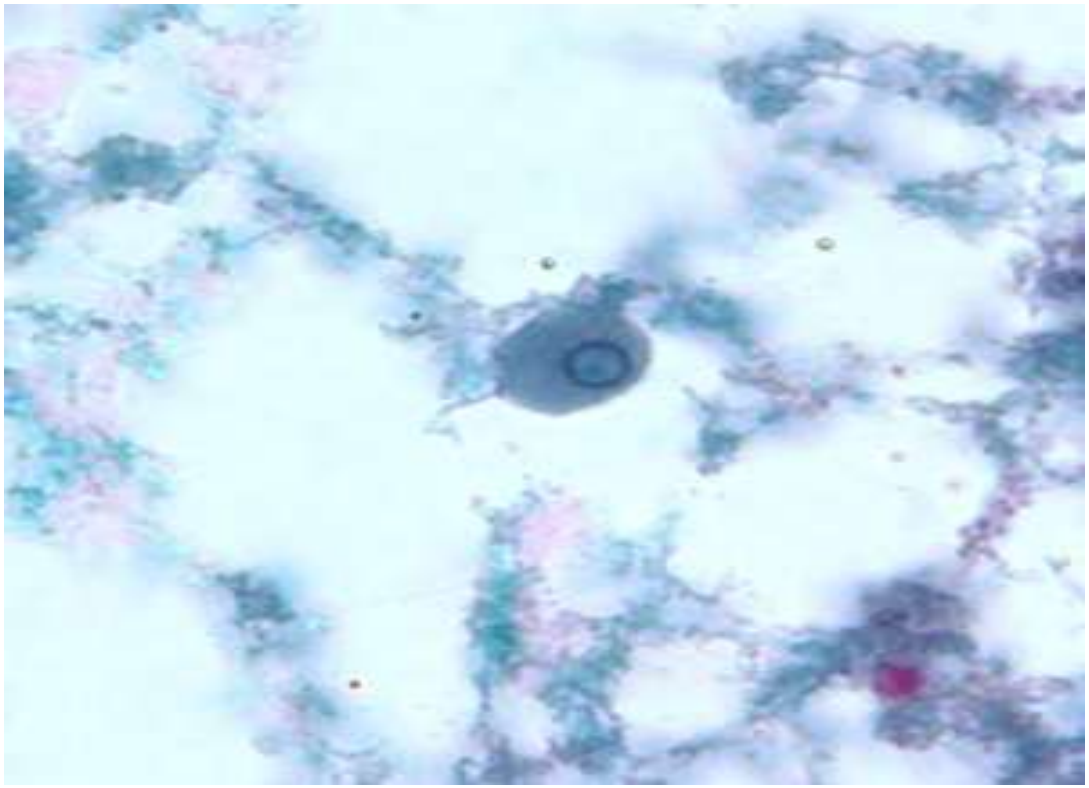


Cyst of *Endolimax nana*

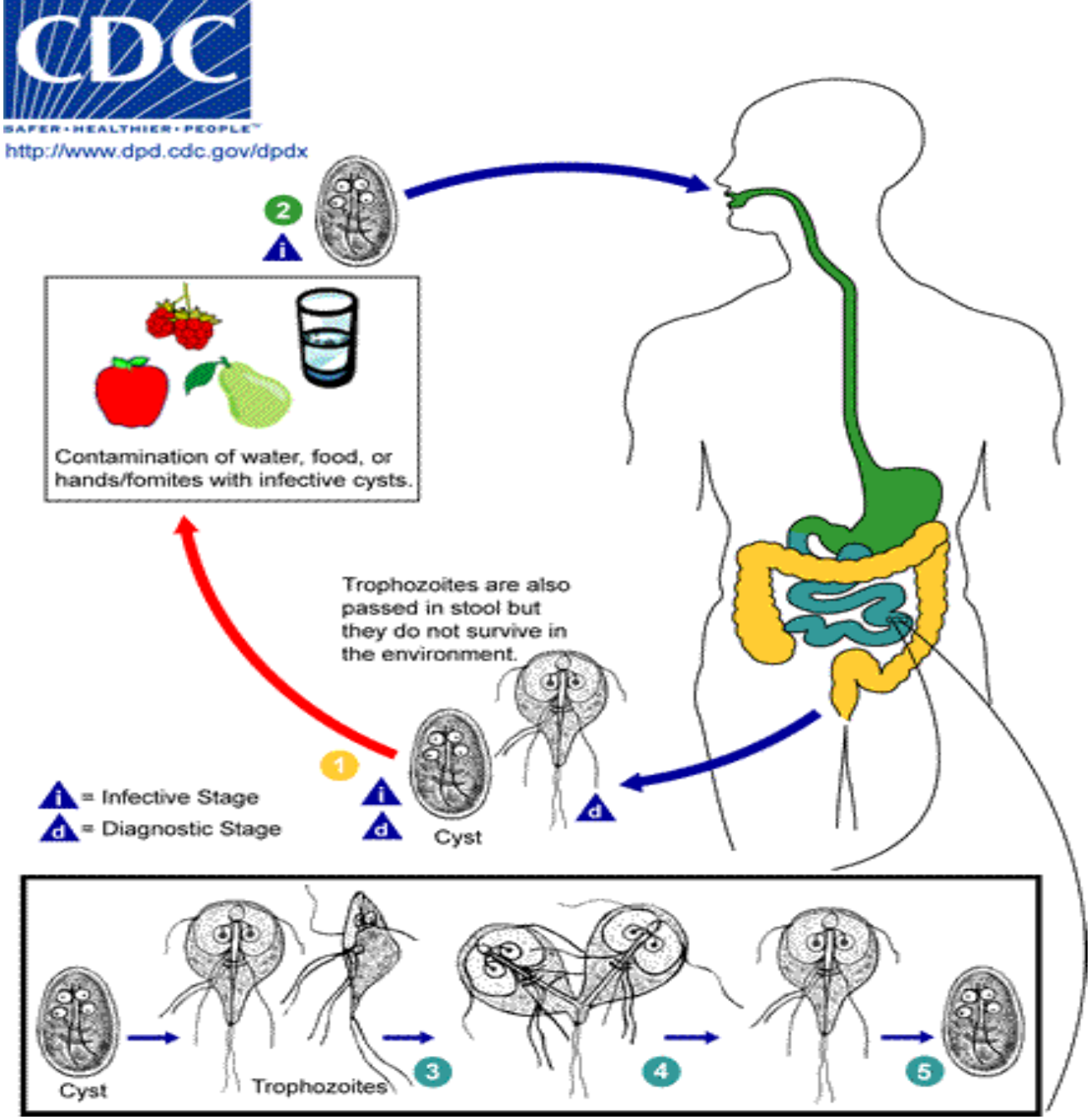
Entamoeba gingivalis



There is no known cyst stage for *Entamoeba gingivalis*; trophozoites live in the oral cavity of humans, residing in the gingival pockets near the base of the teeth. They are not considered pathogenic, and feed on bacteria and other debris. Trophozoites are transmitted person-to-person orally by kissing or fomites (such as eating utensils). The trophozoite stage of *E. gingivalis* is morphologically similar to that of *E. histolytica*, and the two should be differentiated, as both can be coughed up in sputum specimens (for the latter, when present in pulmonary abscesses).

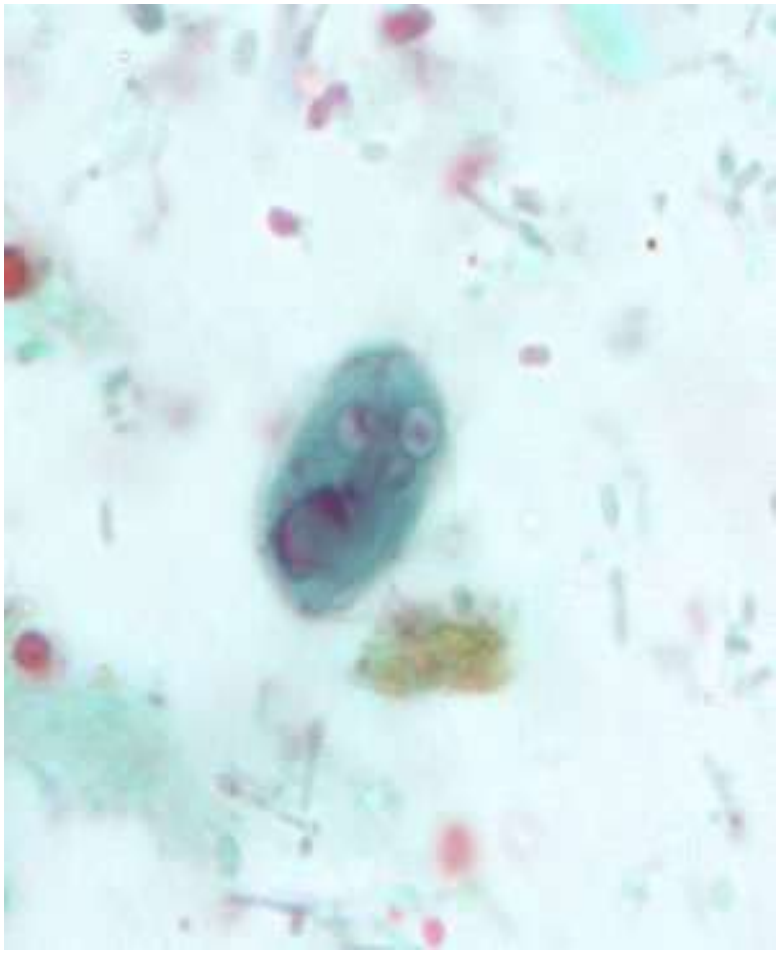


Trophozoite of *Entamoeba gingivalis* stained with trichrome



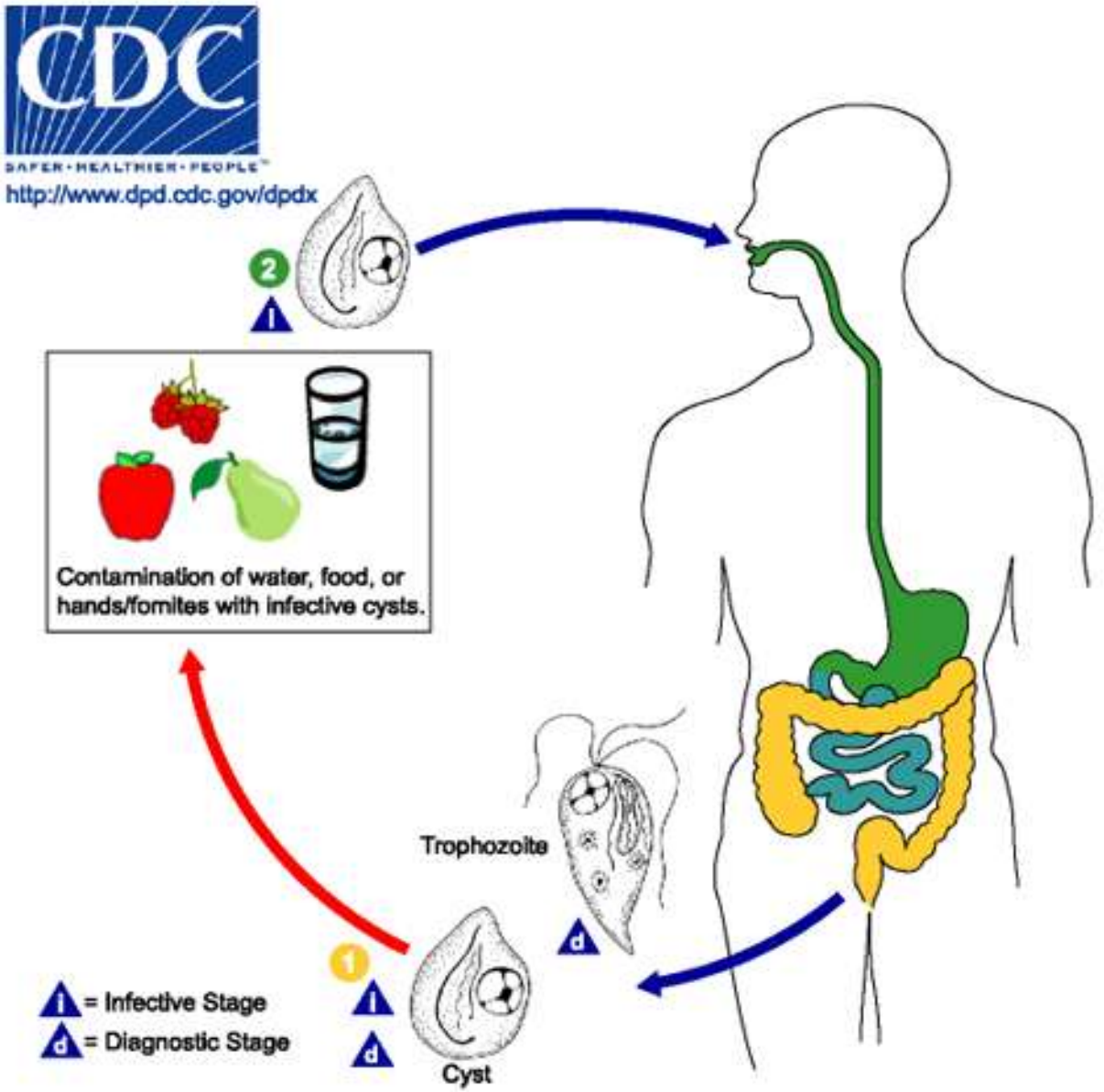
Life Cycle Of *Giardia lambilia(G. duodenalis)*

1

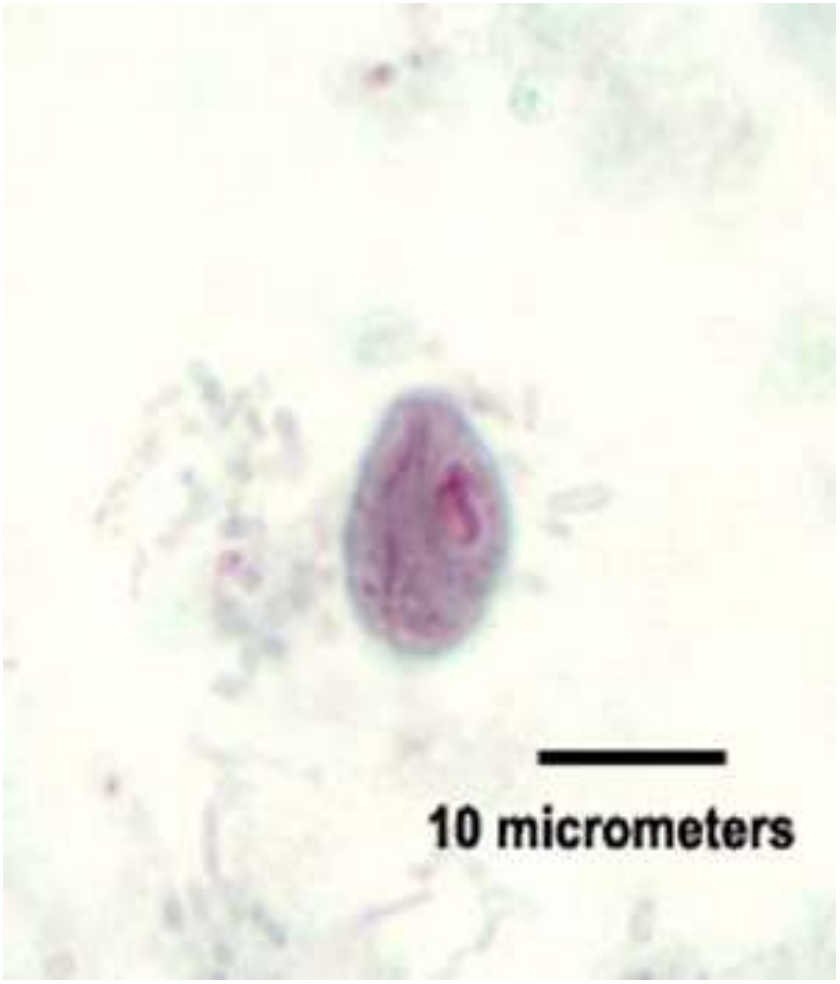


2

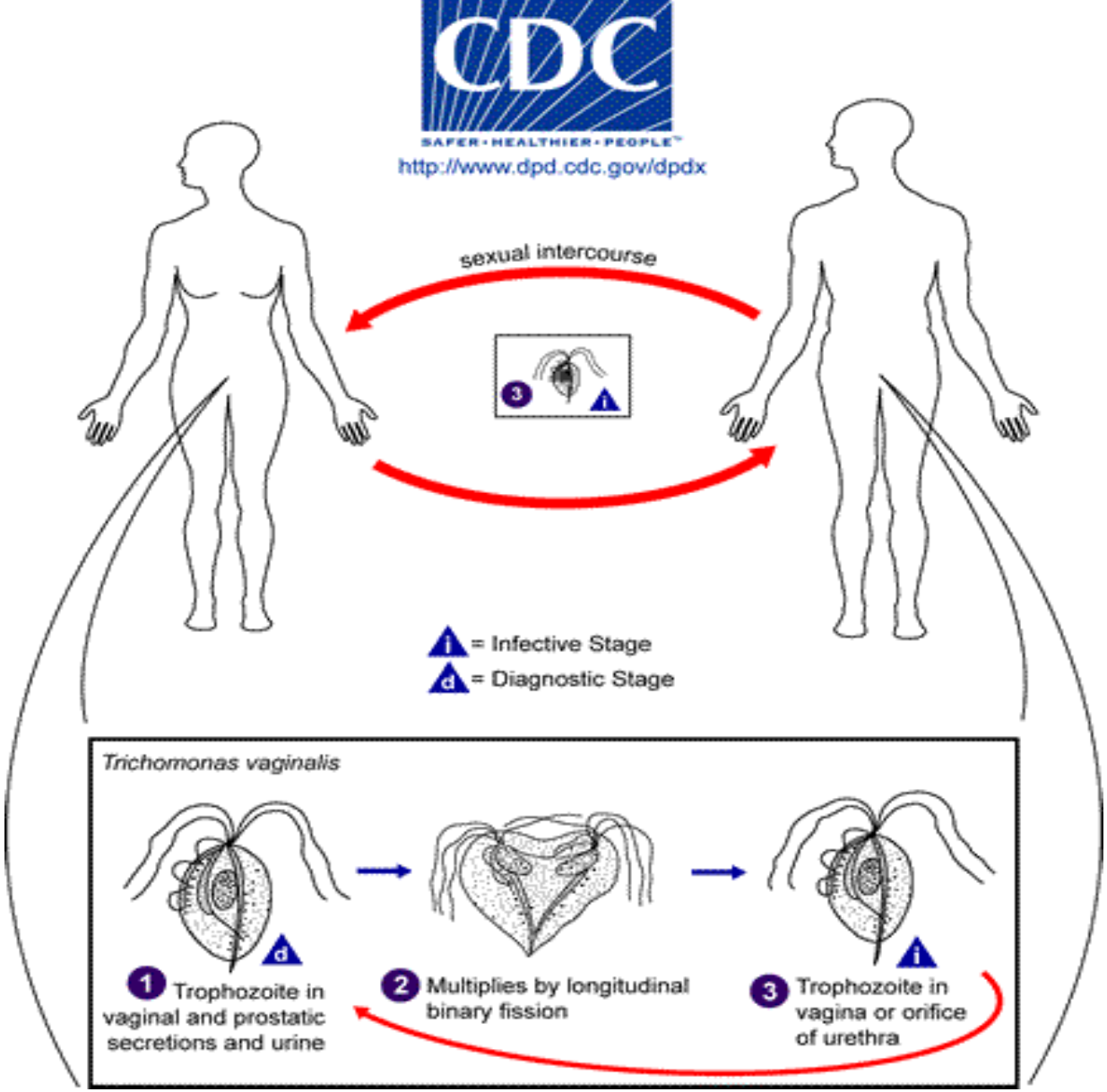
Giardia lambilia : 1-trophozoite 2- cyst



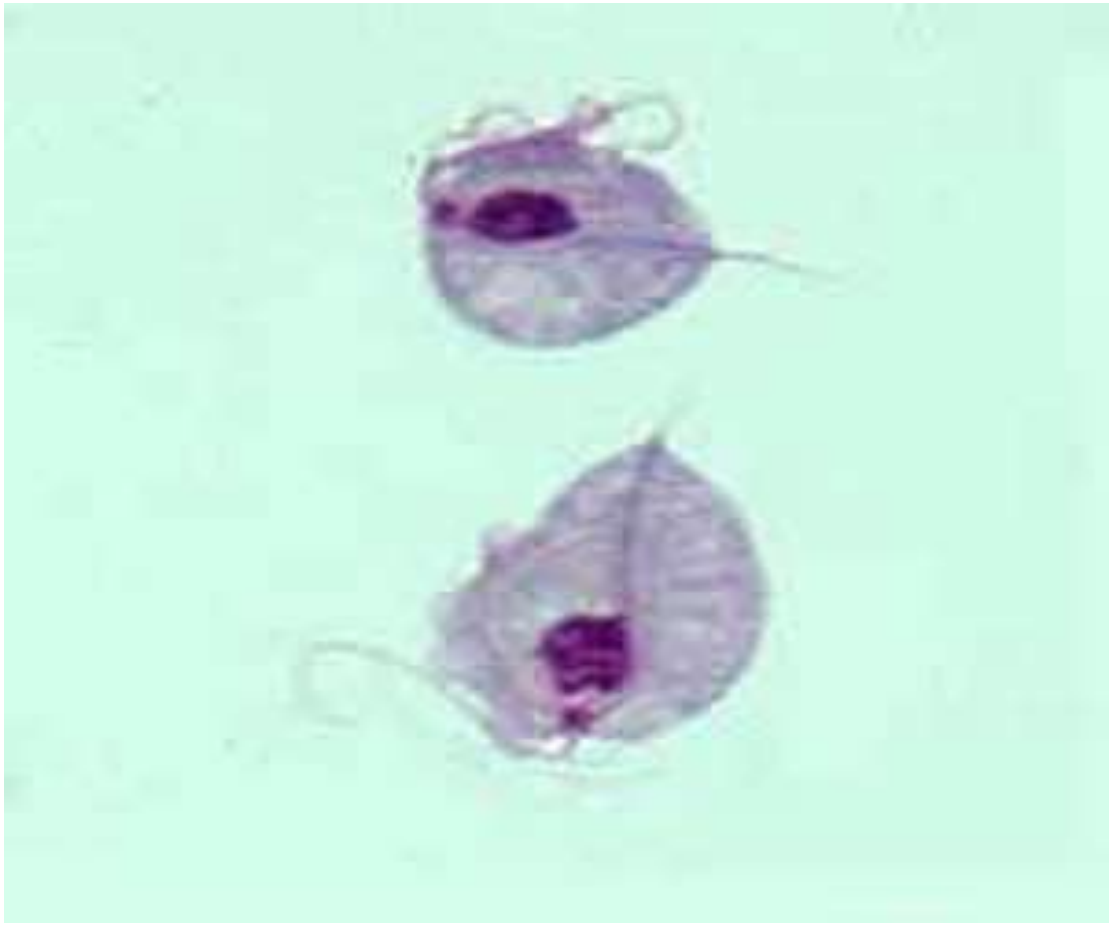
Life Cycle of *Chilomastix mesnili*



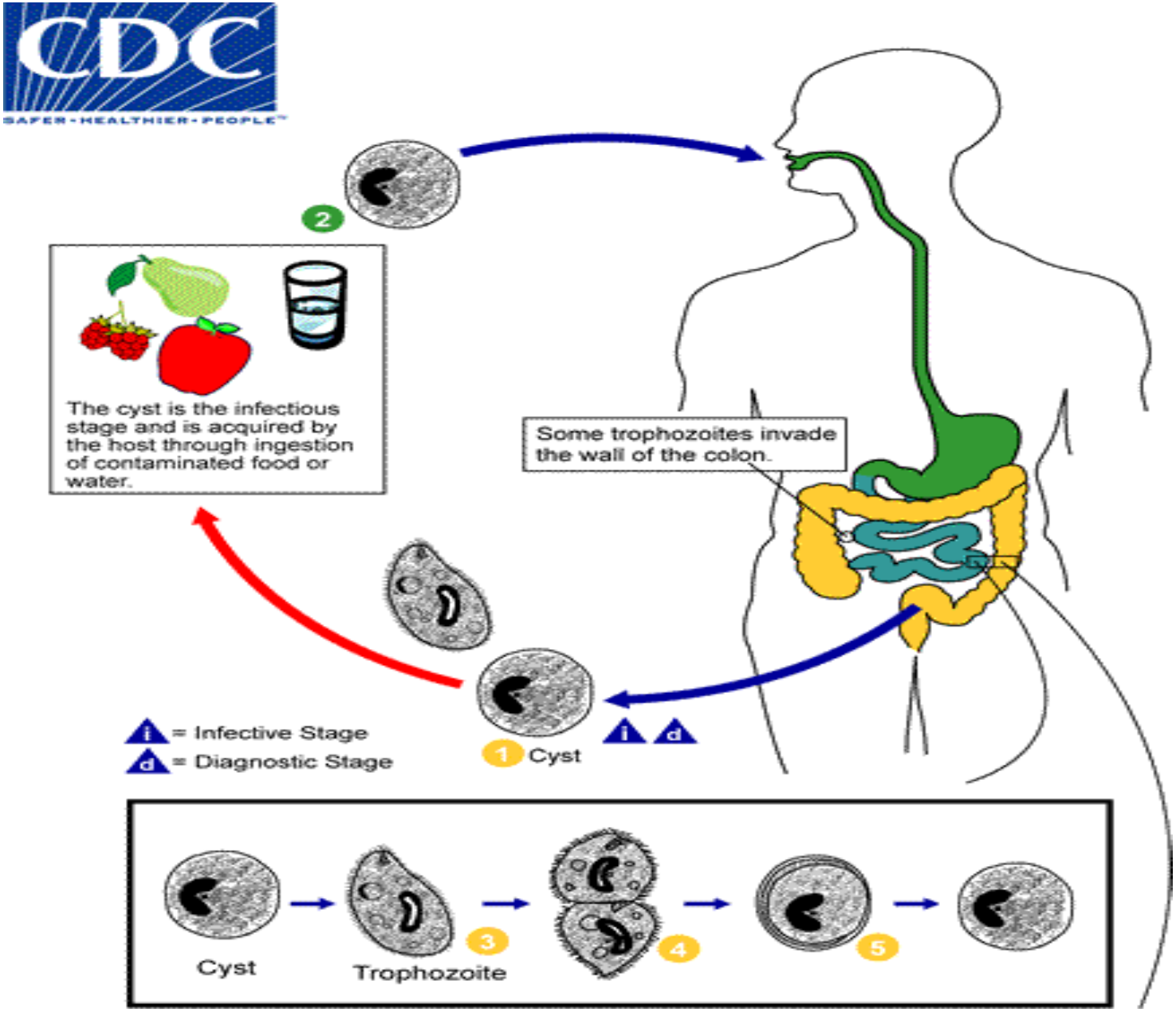
Left, *Chilomastix mesnili* trophozoite, silver stain**.**
Right*, C. mesnili* cyst*.*



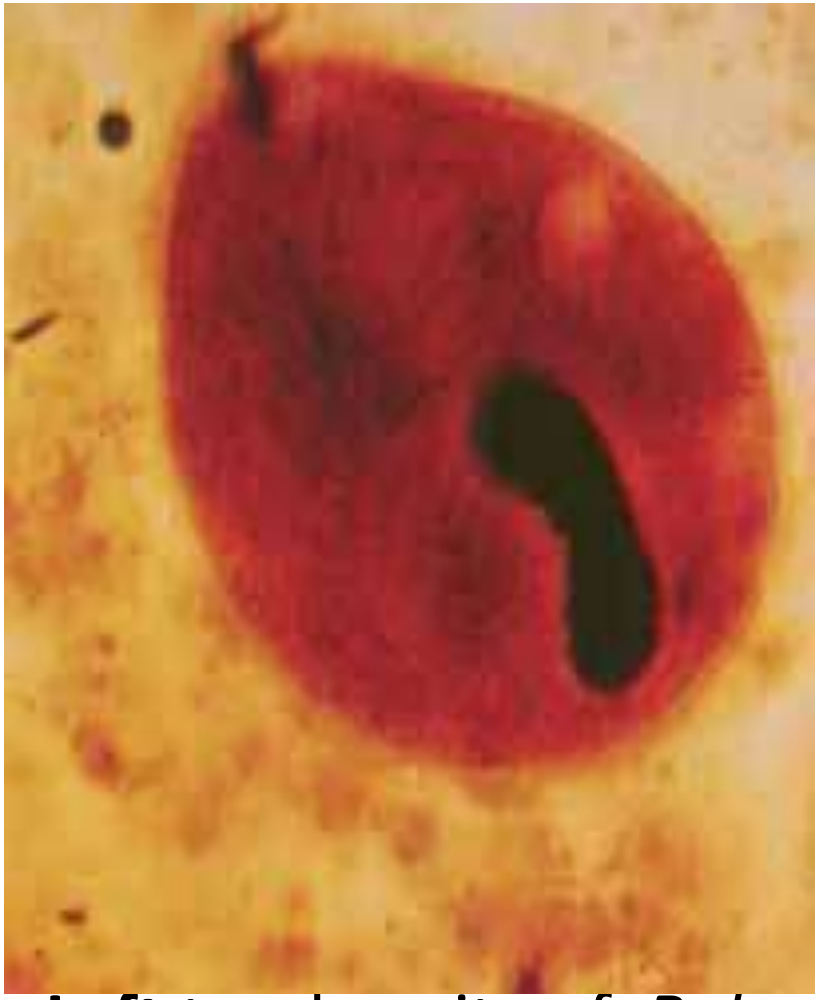
Life Cycle of *Trichomonas vaginalis*

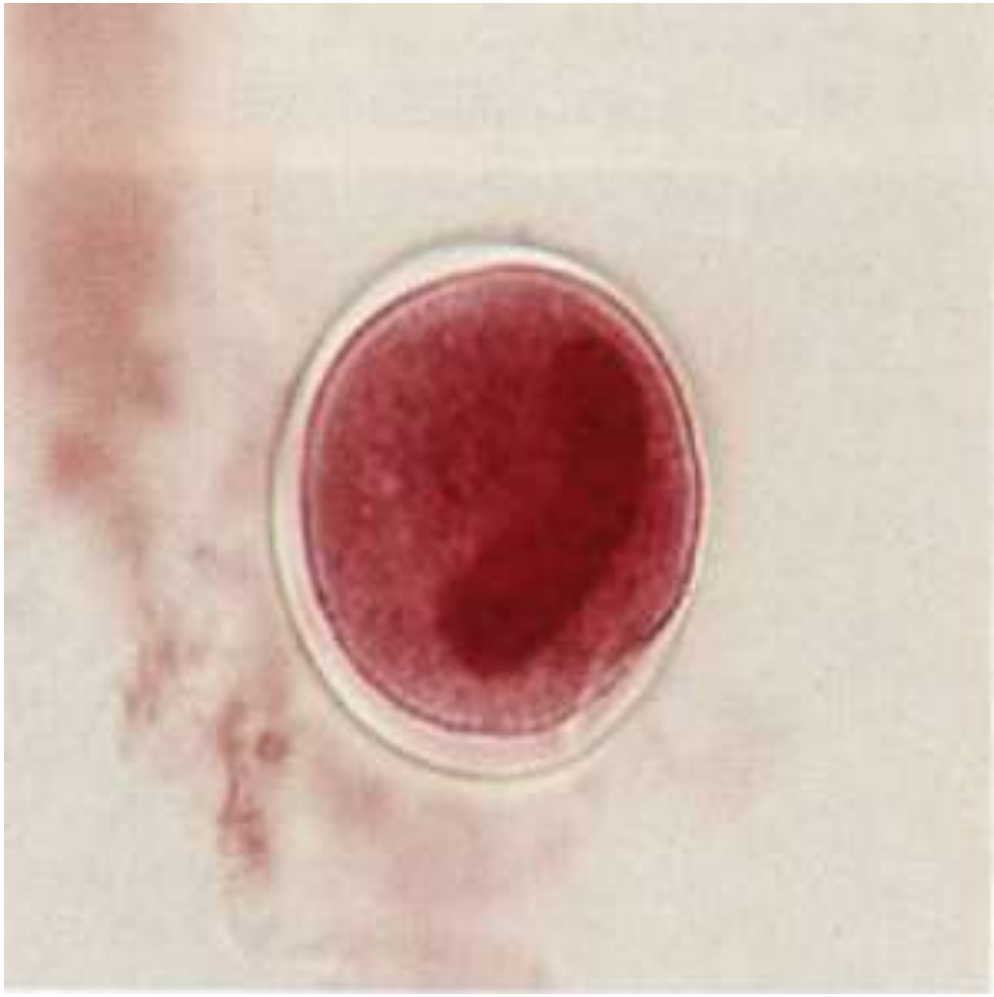


Trophozoite of *Trichomonas vaginalis*



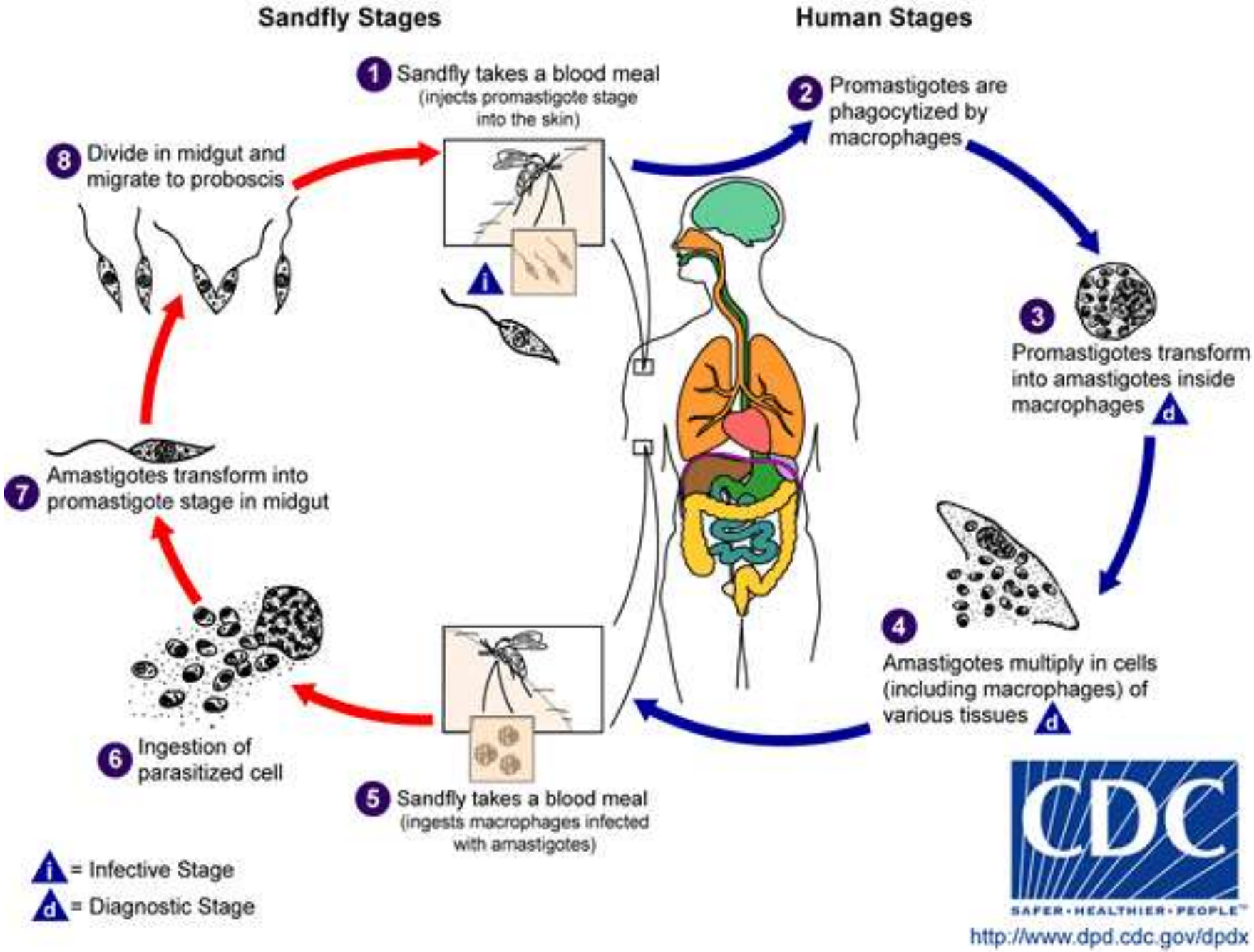
Life Cycle of *Balantidium coli*



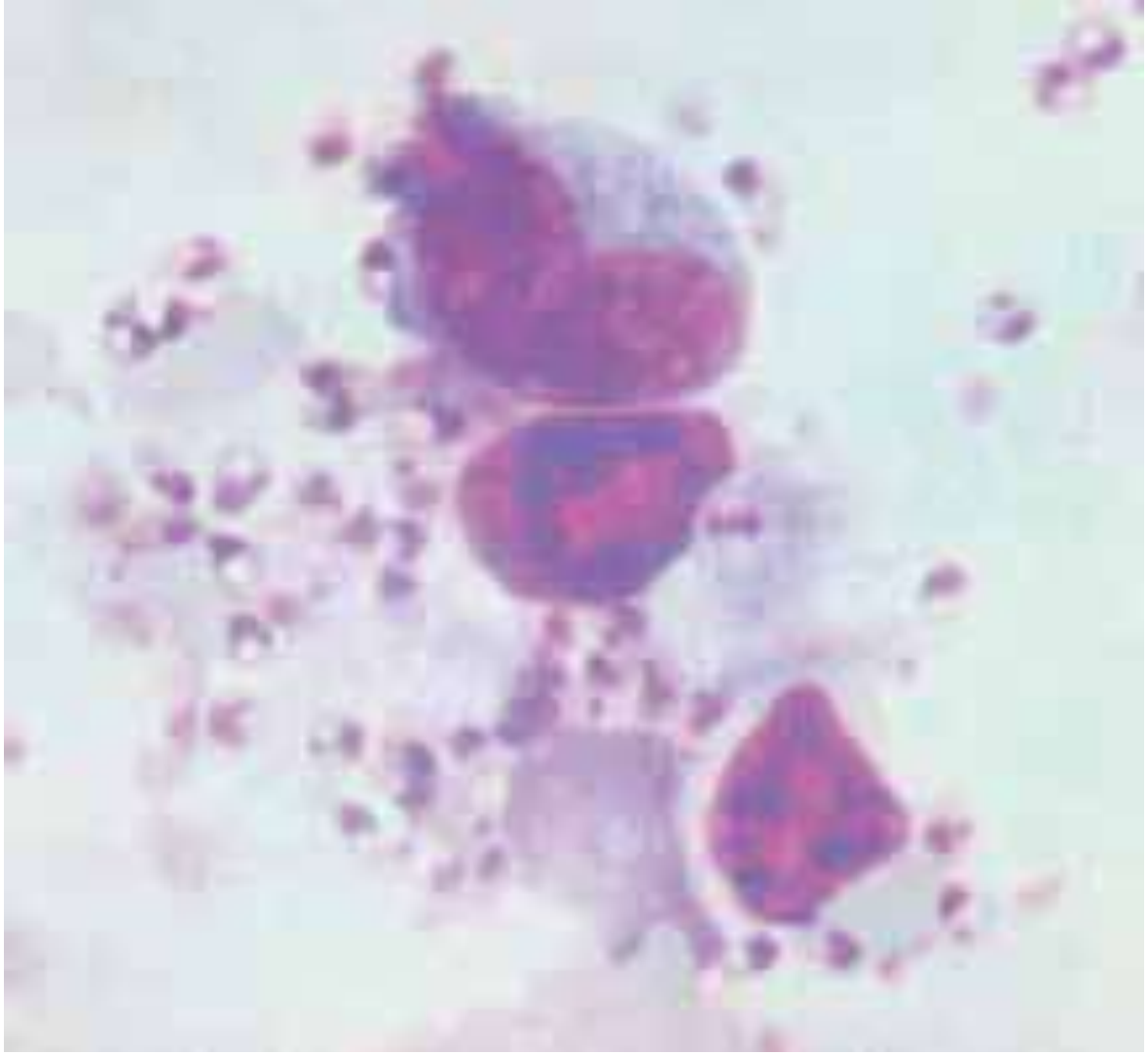


Left trophozoite of *Balantidium coli.*

Right cyst of *Balantidium coli*

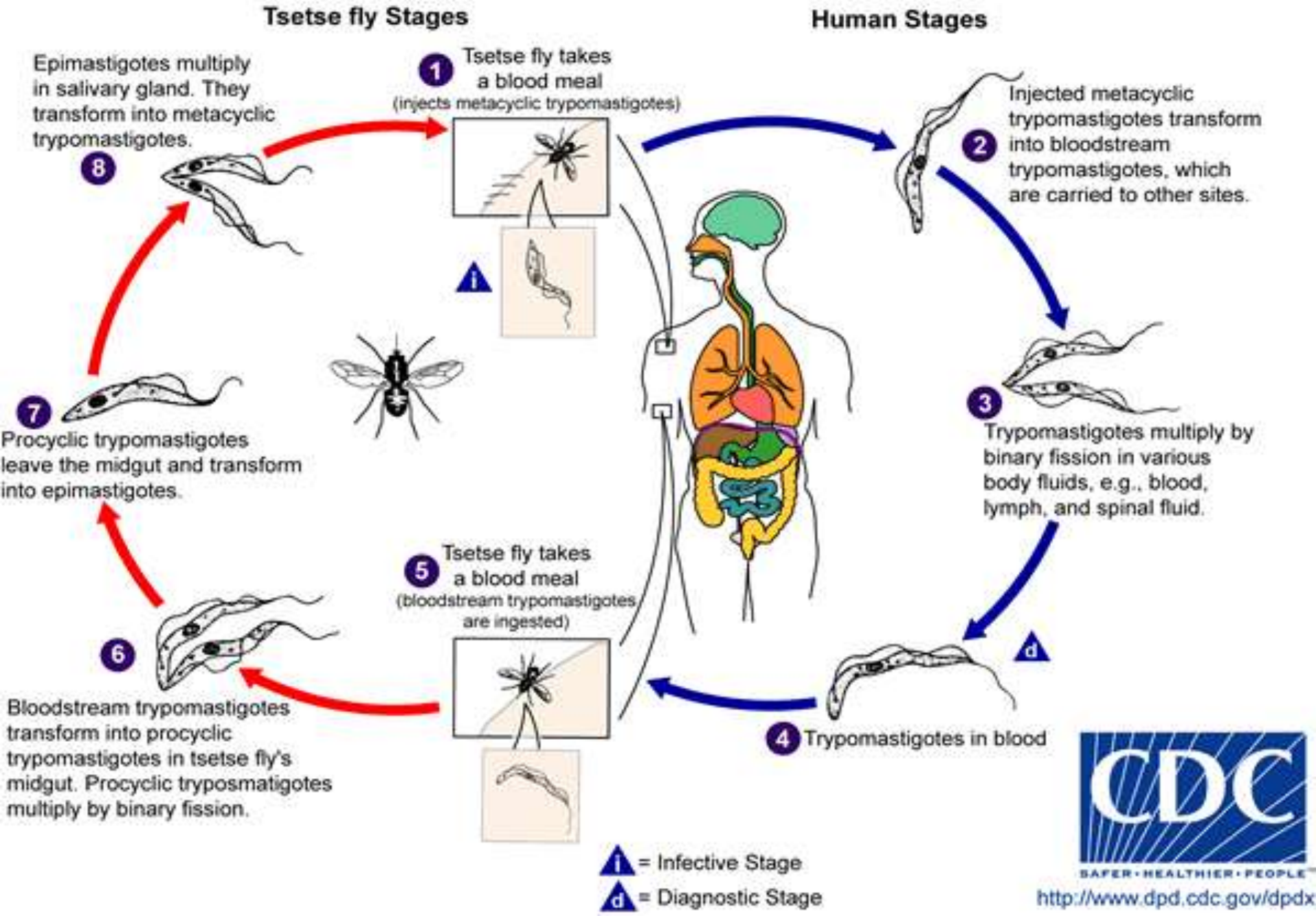


Life cycle of *Leishmania*

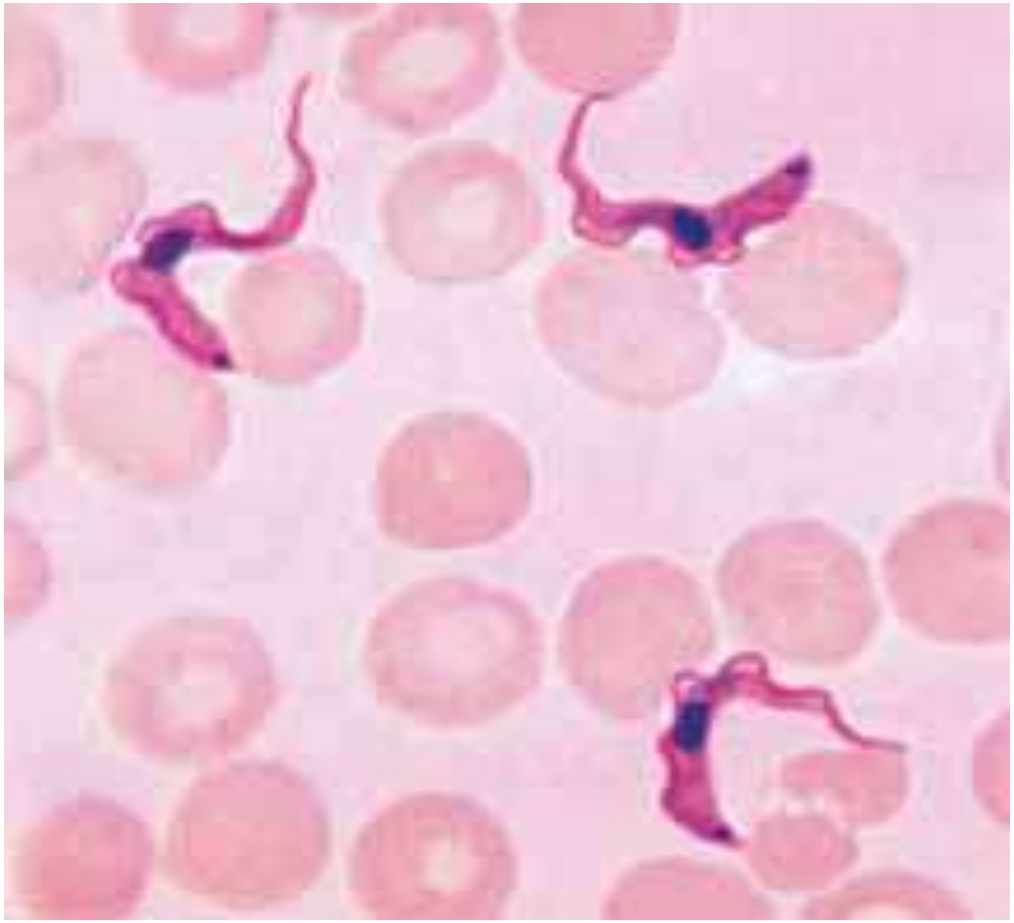


Leishmania donovani amastigotes.

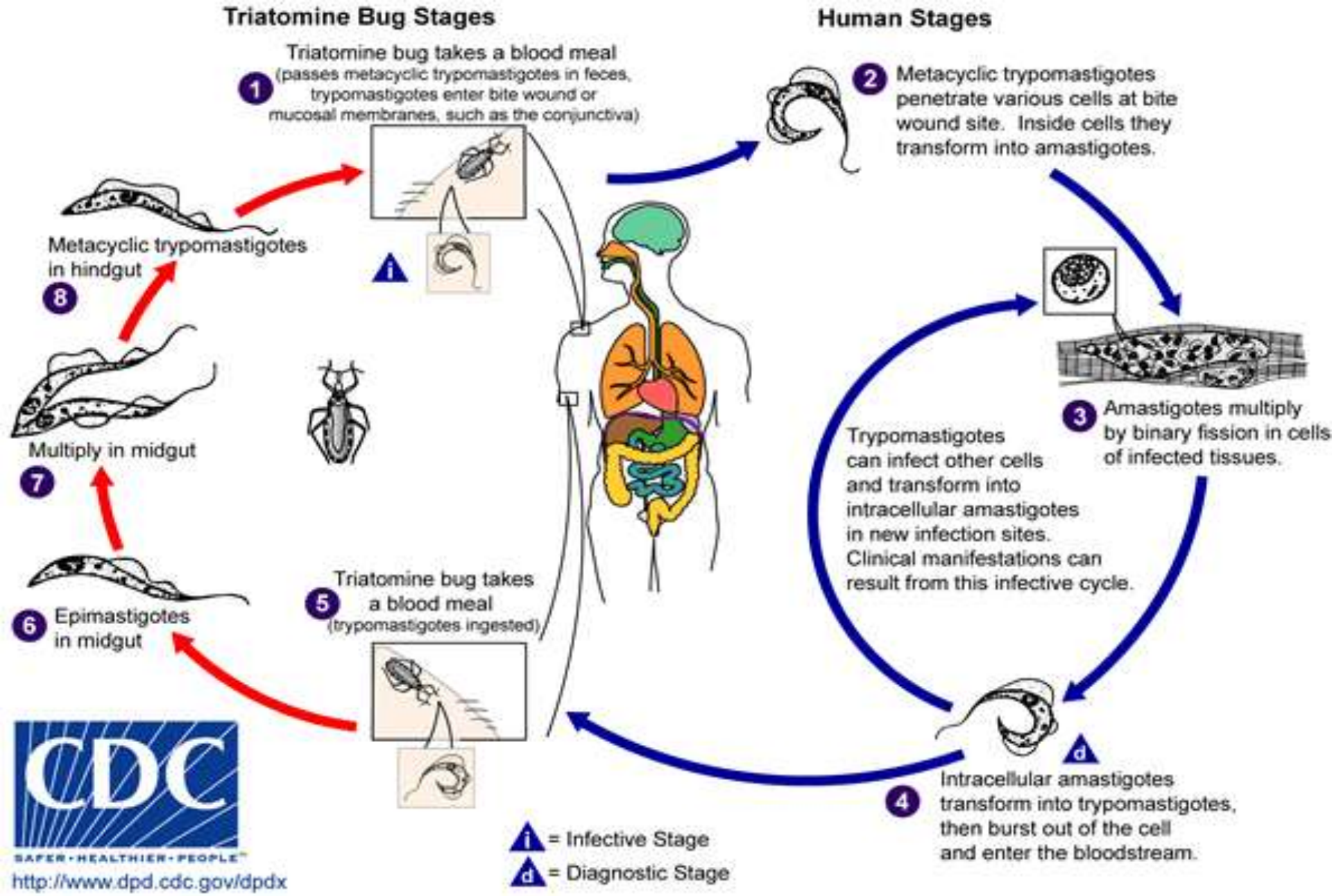
Lieshmania spp. Promastigote in culture stained with Giemsa stain



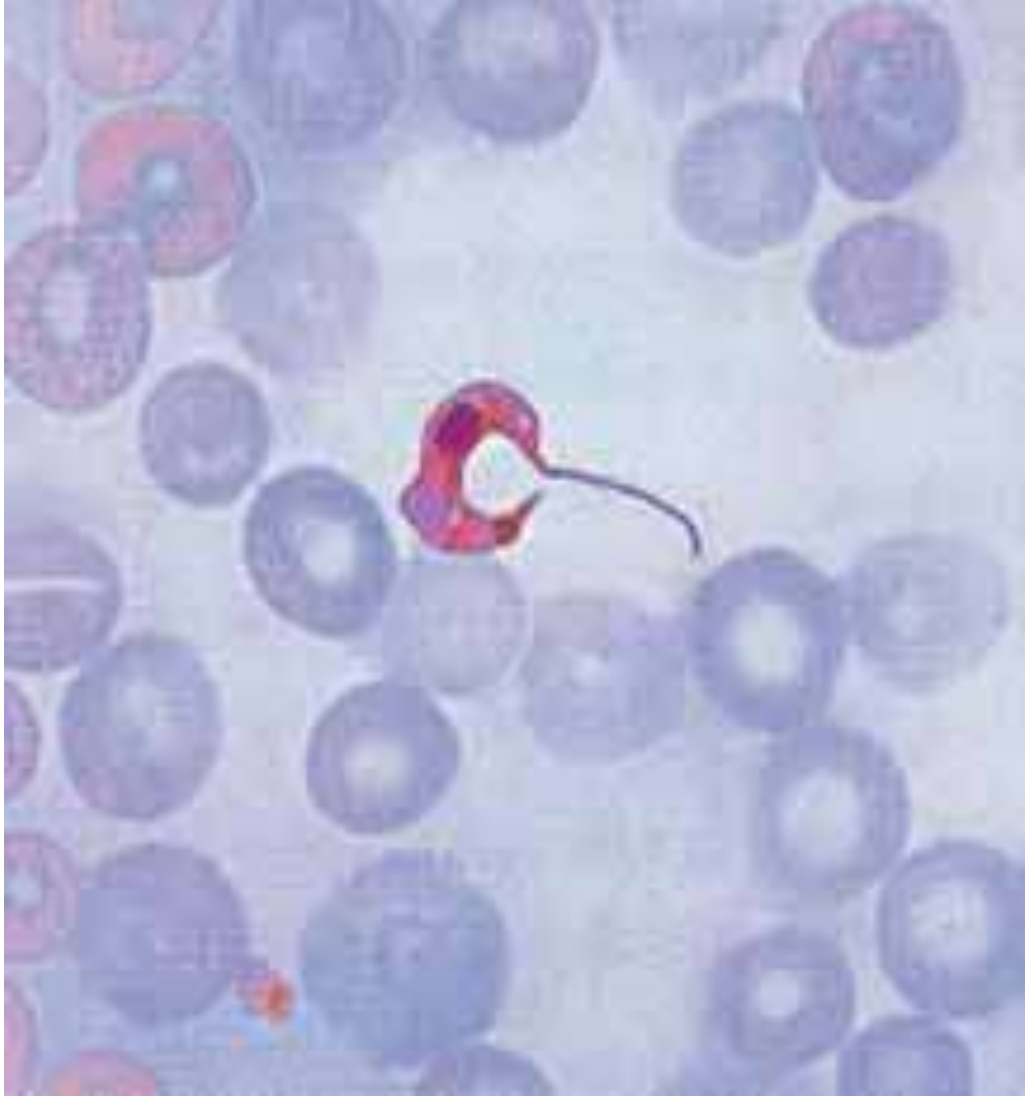
Life cycle of African Trypanosomiasis



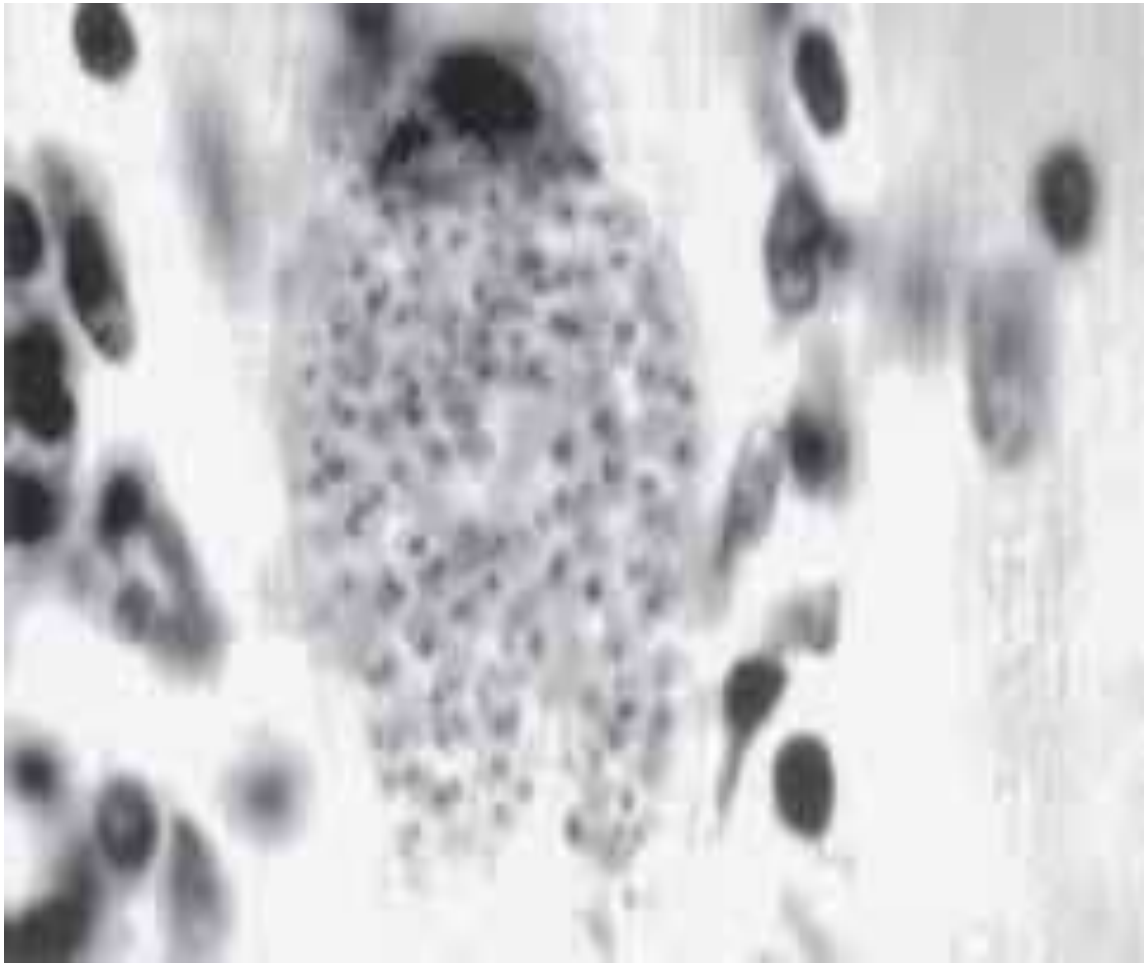
Trypanosoma gambiense trypomastigote in blood film.



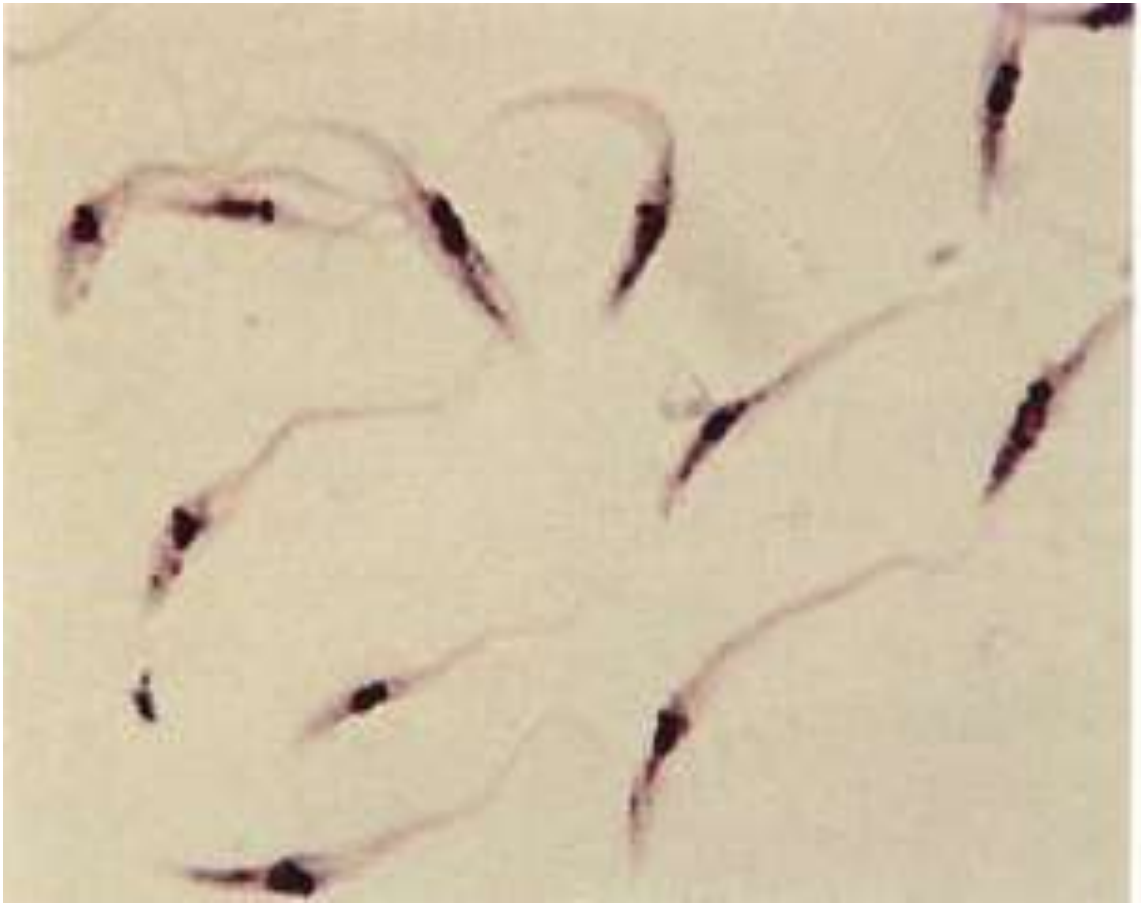
Life cycle of American Trypanosomiasis



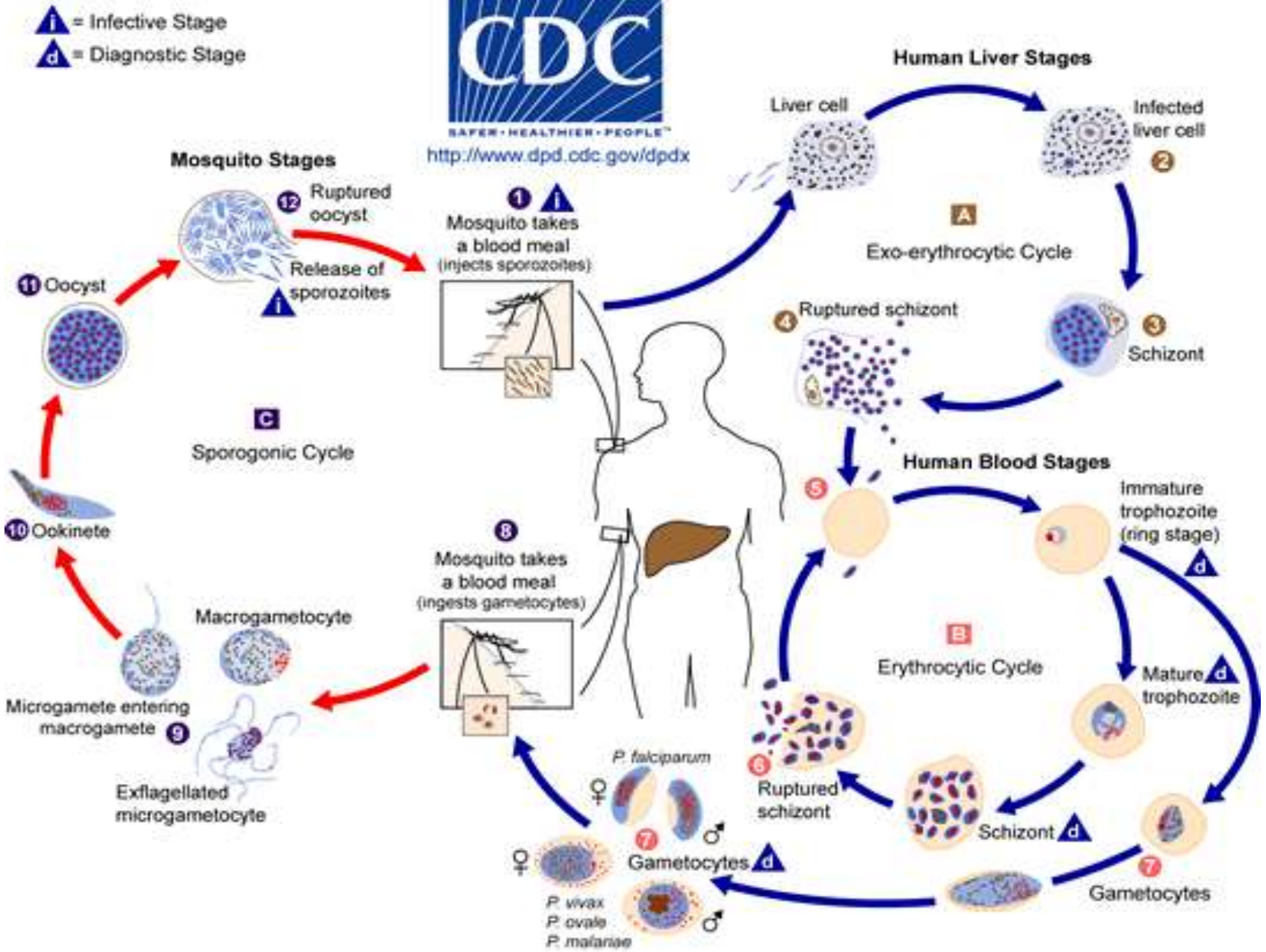
Trypanosoma cruzi trypomastigote.



Trypanosoma cruzi amastigote parasites in cardiac muscle (2500×



Promastigote of *Trypanosoma cruzi* grown in NNN media



Life Cycle of *Plasmodium* parasite

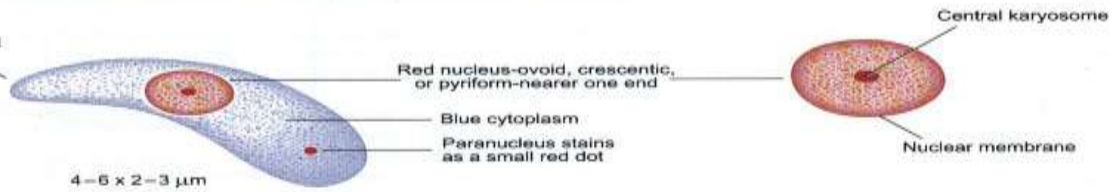
Tissue protozoa

Toxoplasma gondii

Toxoplasma has a very wide mammalian host range.

Morphology

Tachyzoite



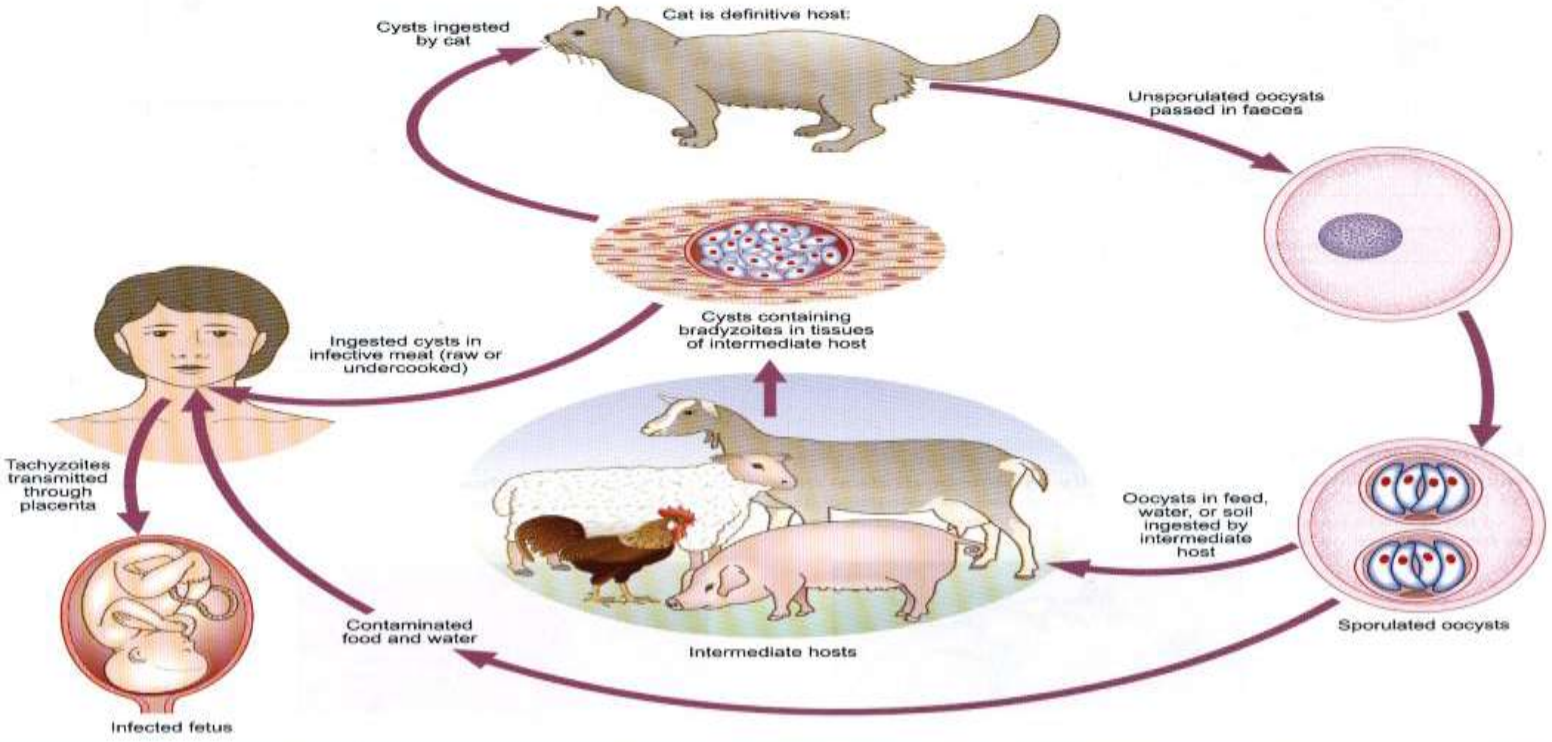
Habitat

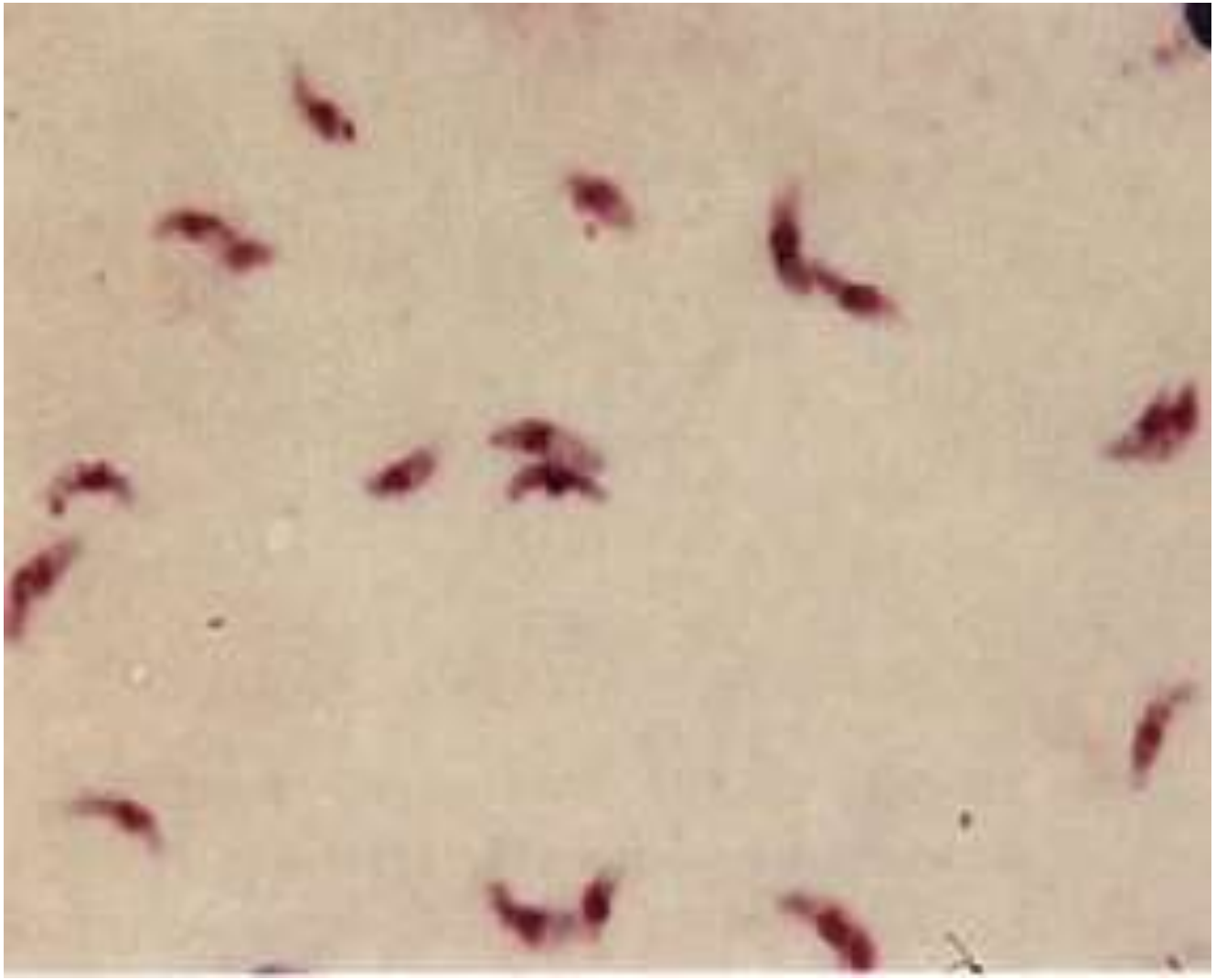
Tachyzoites: single (free or intracellular) or in masses (pseudocysts)

In nucleated cells, especially macrophages

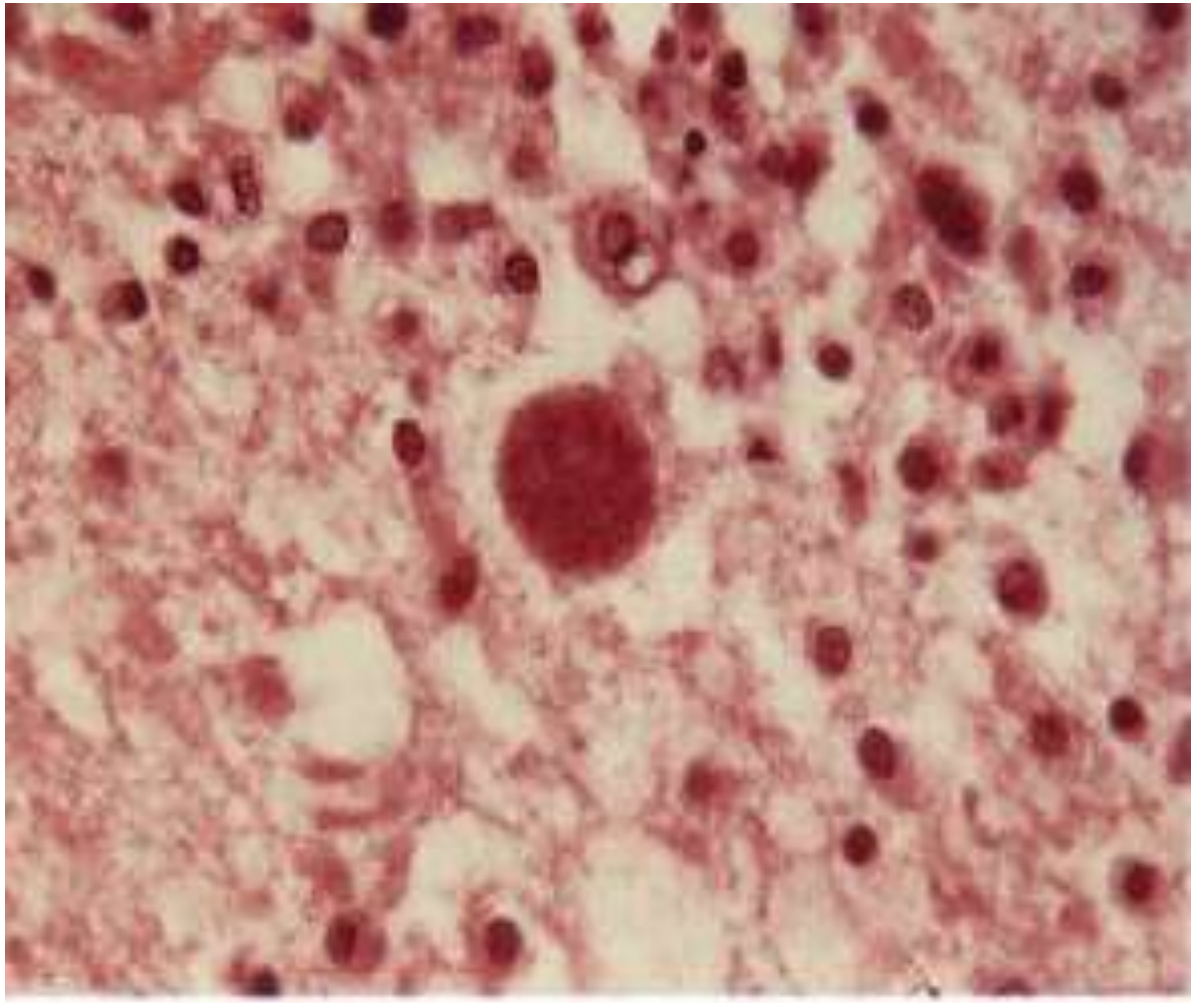
Bradyzoites (similar to tachyzoites but less active metabolically) in tissue cysts

Life cycle





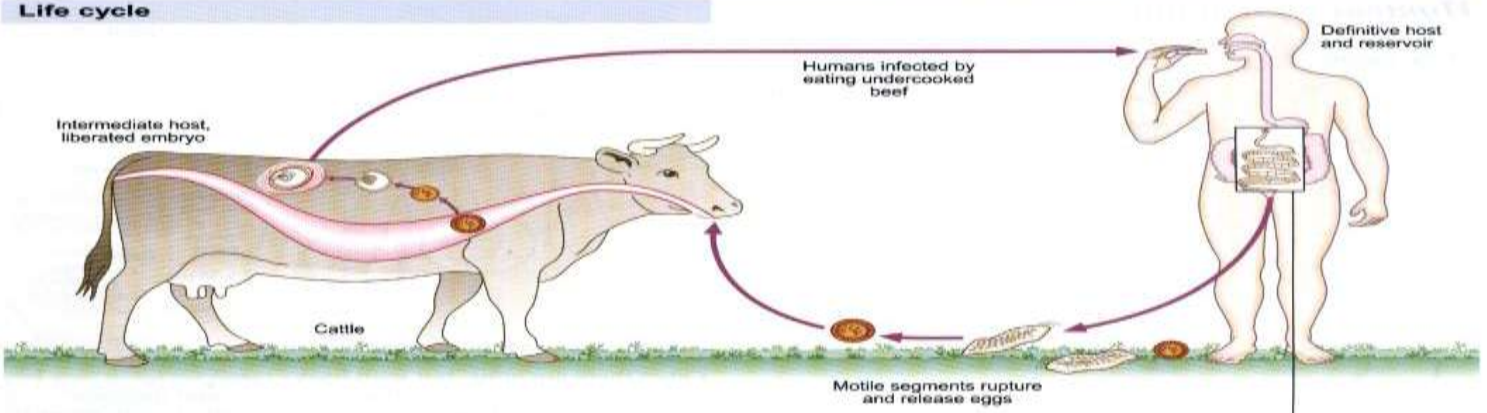
15-33 *Toxoplasma gondii*. Trophozoites. Cul-
ture. Giemsa stain (×1250). Trophozoites of T. gondii

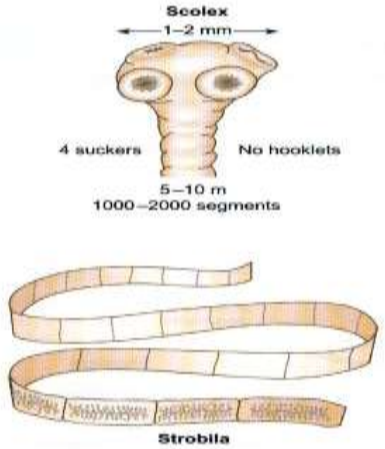


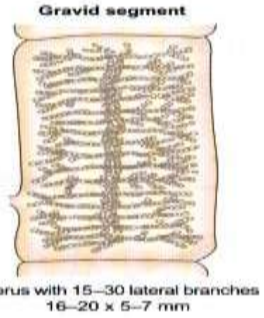
Toxoplasma gondii. Cyst. Brain.

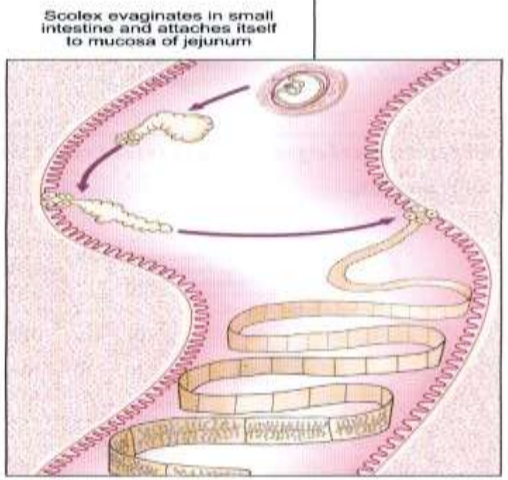
Taenia saginata (beef tape worm)

Life cycle









Pathology and Clinical features

Usually there is no pathology as *Cysticercus bovis* is unknown in humans. Occasionally there is vague alimentary upset.

Laboratory diagnosis

Gravid segments, ova and scolex can be found in faeces. Uterine branches of the mature segments may be seen in a crush preparation between two glass slides, or by Indian ink preparation, as in *T. solium*. Ova are also found on the perianal skin (on clear adhesive tape slides).

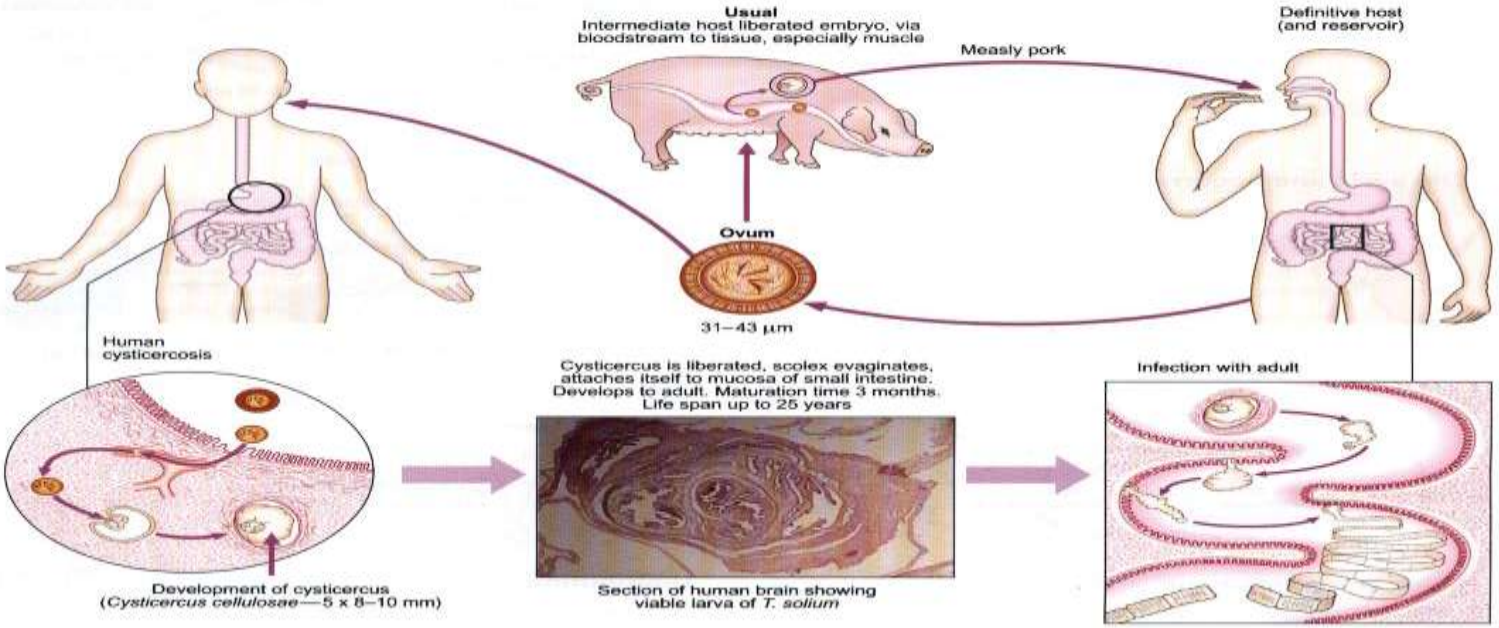
Distribution

Taenia saginata is found in beef-eating areas, especially in the tropics.

Cestode (tape) worms

Taenia solium (pork tape worm)

Life cycle



Pathology and Clinical features

Infection by larvae (cysticercosis). Cysticerci, generally multiple, may occur in any site but are more frequent in the brain and muscle. They excite reaction in the area, especially when they die, which manifests as inflammation, fibrosis and later some calcification. This leads to focal CNS syndromes, especially epilepsy.

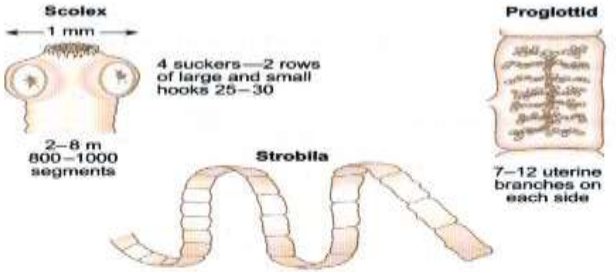
Infection with adults. Often there can be no pathology, but there might be mild irritation of intestinal mucosa.

Laboratory diagnosis

Eosinophilia.

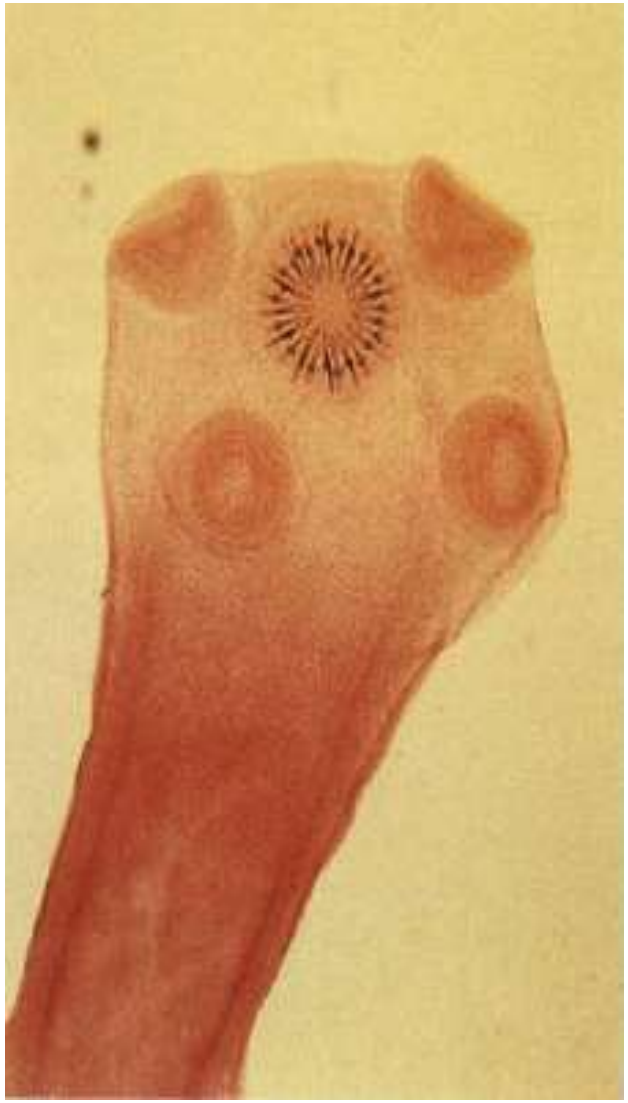
Larval infections. There are several methods, including histological examination of biopsy material, serology (IFAT, ELISA, EITB) and radiology (CT or MRI scan of the brain, X-ray of the thigh muscles).

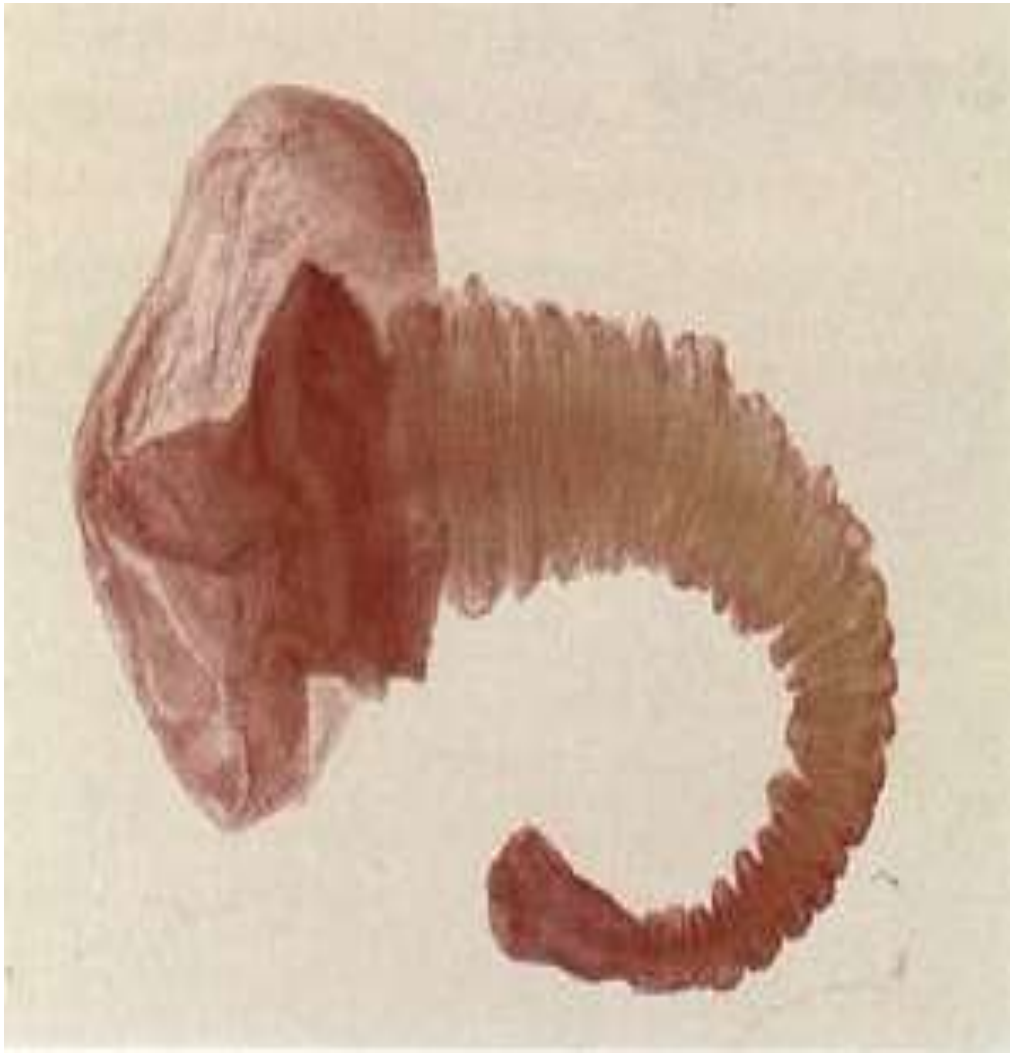
Pure infection with the adult. Gravid segments, ova and scolex can be found in faeces. The uterine branches of the mature segments can be demonstrated by injection of Indian ink through the uterine pore.



Distribution

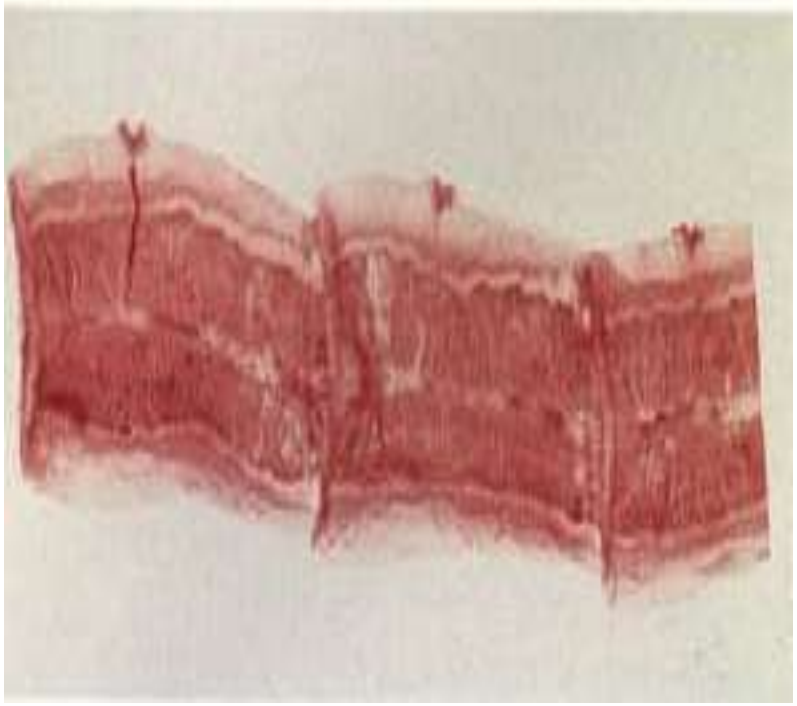
5 million people infected worldwide. *Taenia solium* is endemic in pig-rearing areas of the world where hygiene and animal husbandry are poor.

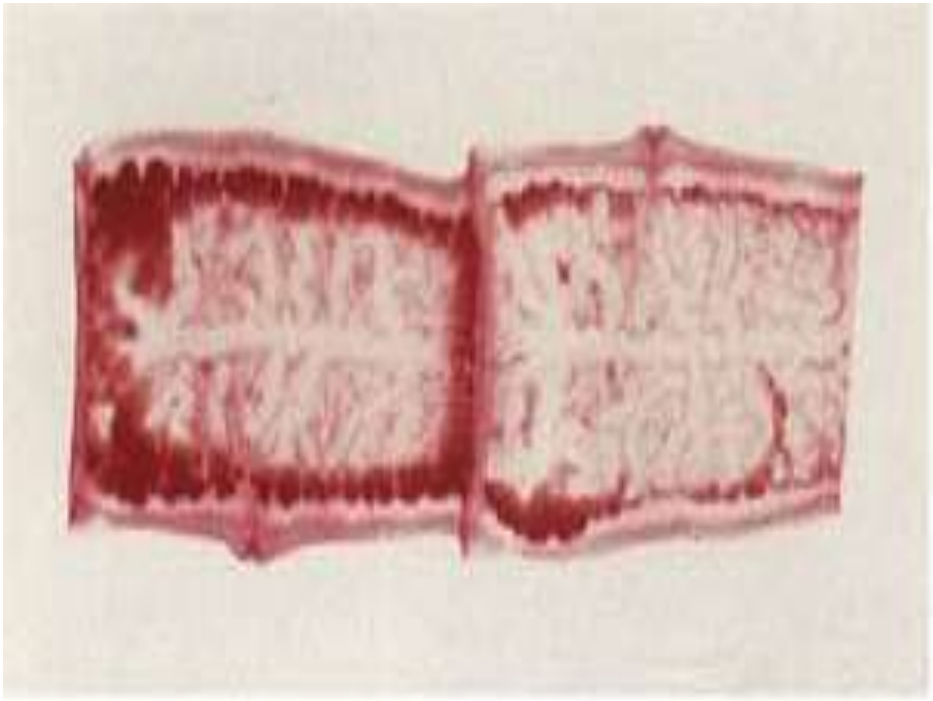




A B

Taenia Solium: A- Scolex B- *Cystisercus cellulosae*

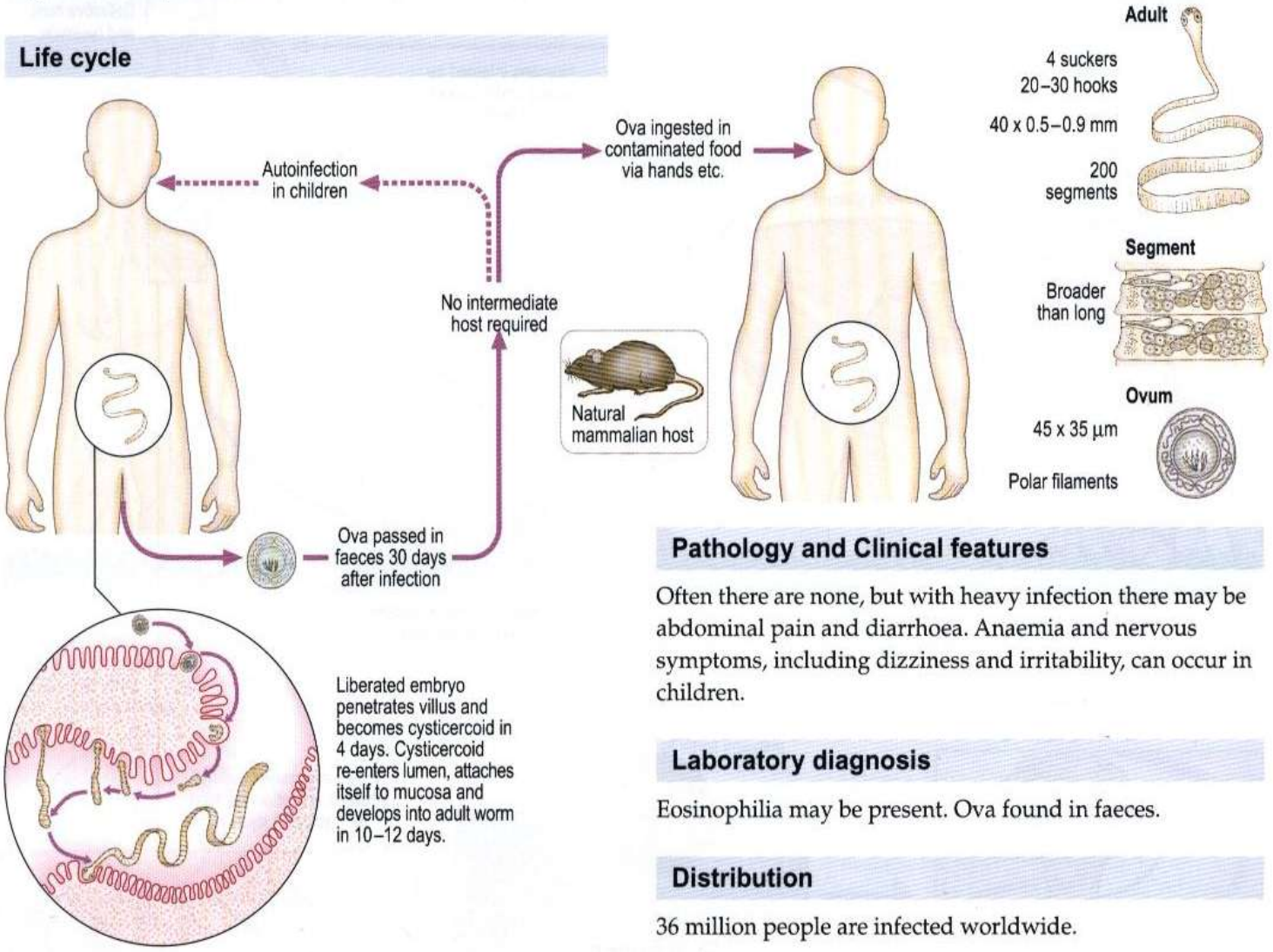




Gravid Proglottid (segment) of *Taenia solium* (right) & *Taenia saginata*(left)

Hymenolepis nana

Life cycle



Pathology and Clinical features

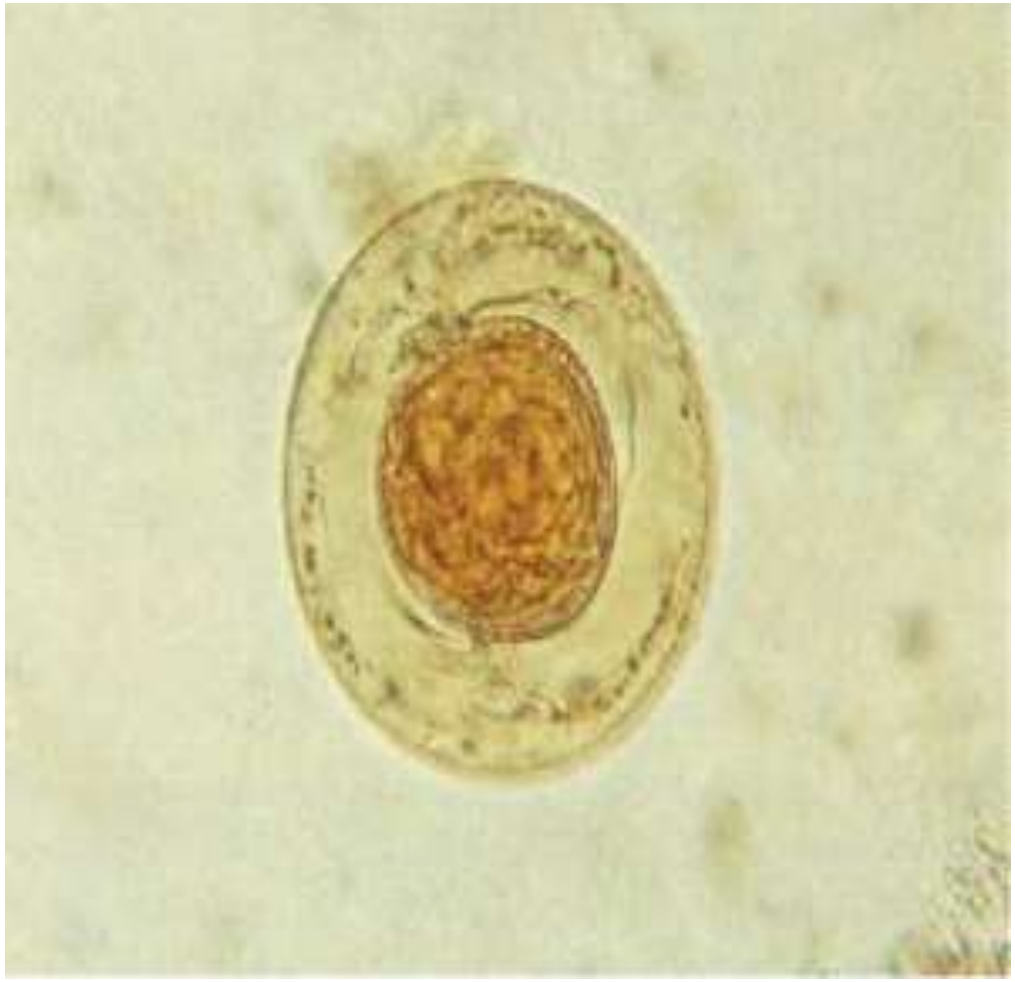
Often there are none, but with heavy infection there may be abdominal pain and diarrhoea. Anaemia and nervous symptoms, including dizziness and irritability, can occur in children.

Laboratory diagnosis

Eosinophilia may be present. Ova found in faeces.

Distribution

36 million people are infected worldwide.

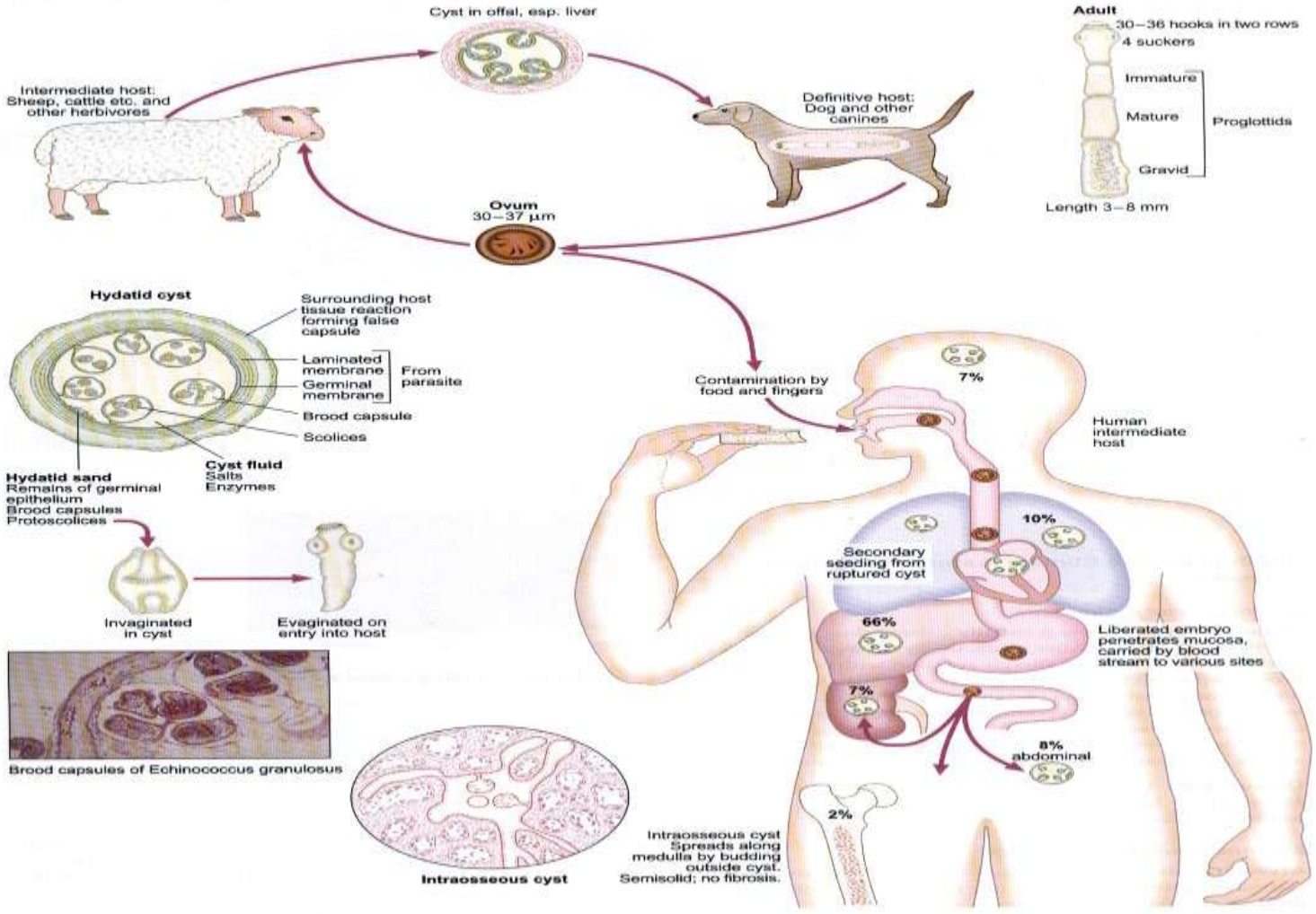


Hymenolepis nana. Egg. Feces.

Echinococcus granulosus (dog tape worm)

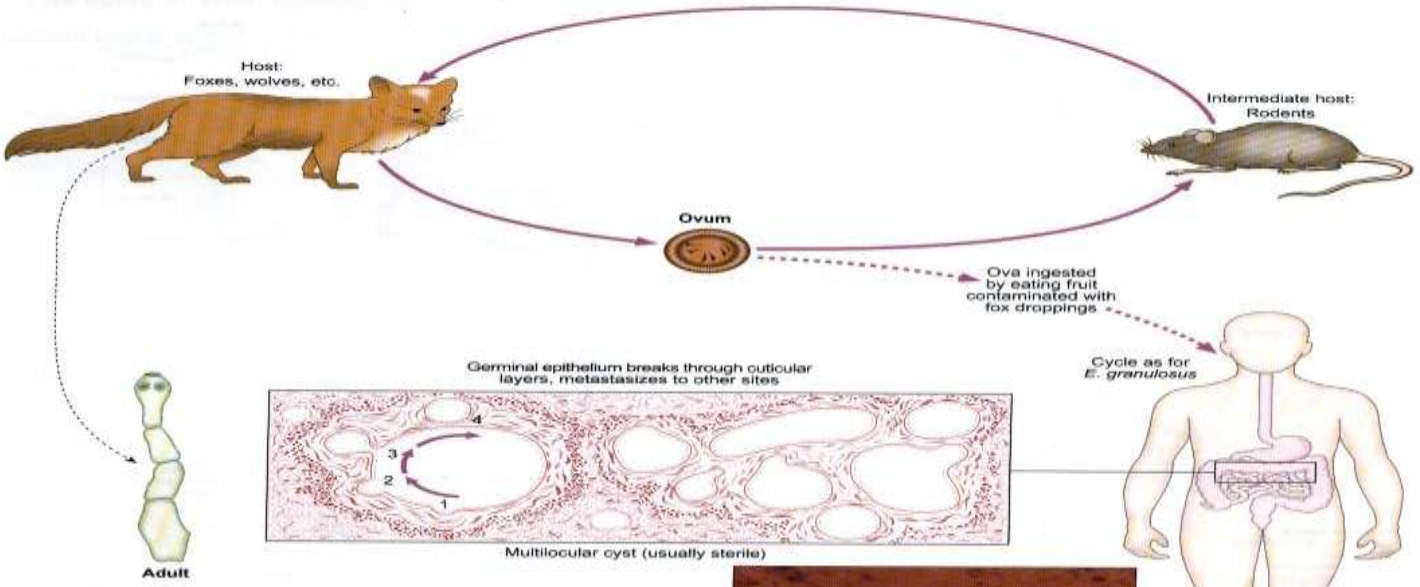
Life cycle

Echinococcus granulosus causes hydatid disease.



Echinococcus multilocularis

Life cycle



Pathology and Clinical features of hydatid disease

Echinococcus granulosus

Unilocular cysts. There is usually surrounding inflammatory reaction and fibrosis. After years, the cyst may die, shrink and calcify. There is general allergic reaction with eosinophilia, bronchospasm, etc. Pressure effects can cause local tissue damage and obstruction of natural channels. Rupture or leakage of the cyst can accentuate the allergic reaction. There can be anaphylactic shock and sometimes secondary implantation, for example in the peritoneal region. There can also be secondary infection with formation of abscess.

Osseus cysts. Usually there is no fibrosis although there is some cellular infiltration. Destruction of the bone can sometimes lead to spontaneous fracture.

Echinococcus multilocularis

Alveolar cysts. There are local pressure effects and allergy. Germinal epithelium can act like a neoplasm with local infiltration or distant metastases.

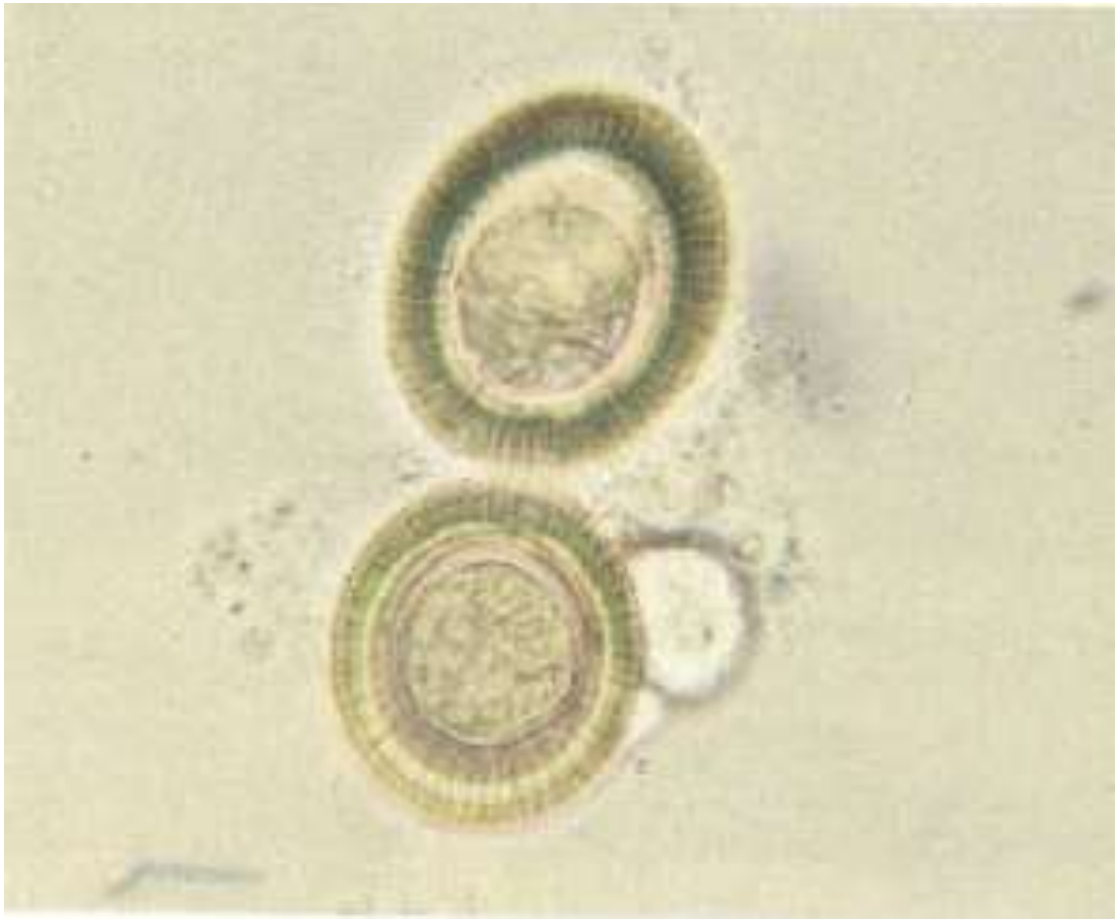
Eosin penetration of dead protoscolices

Laboratory diagnosis of hydatid disease

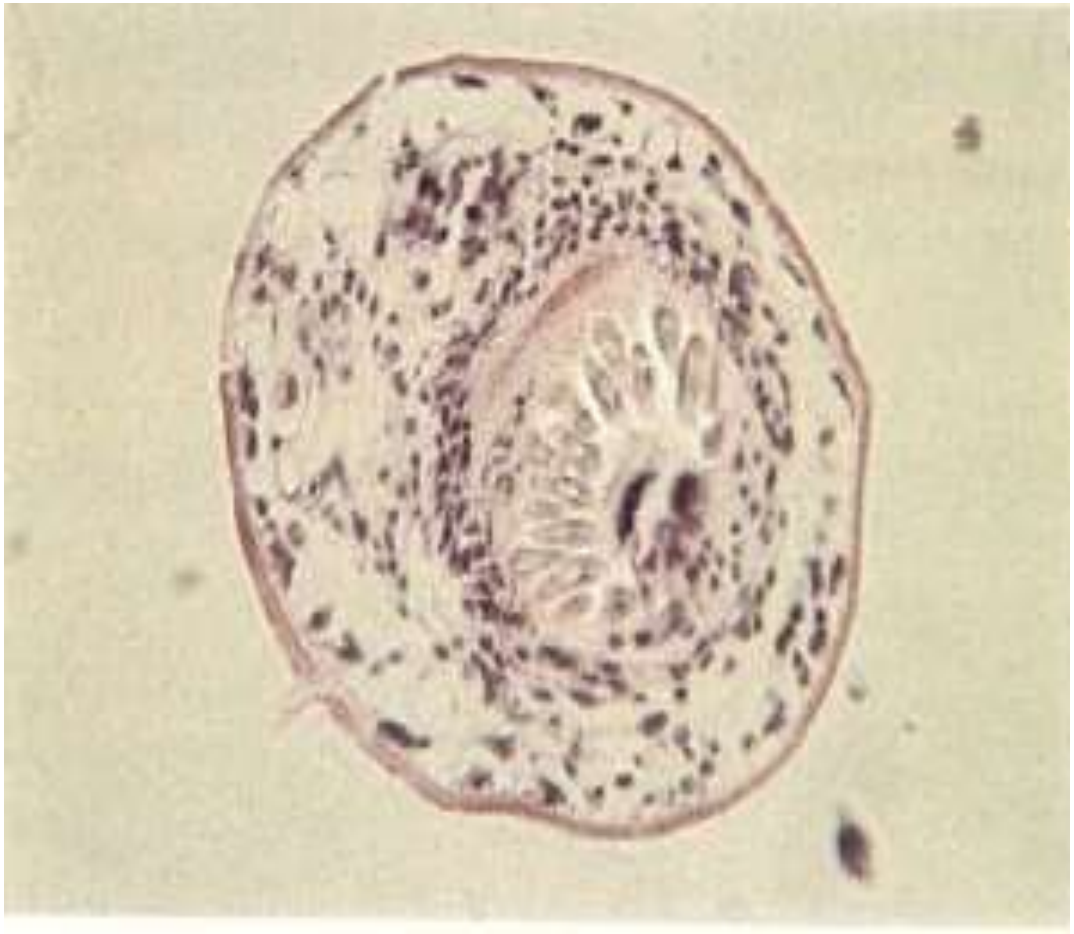
Use serological tests on serum (e.g. ELISA, complement fixation, counter current immunoelectrophoresis for Arc 5 or immunoblot). Microscopy of cyst fluid from operative specimens can be used to assess viability of protoscolices. Histological examination of a removed specimen is another possibility.

Distribution

1 million infected worldwide. *E. multilocularis* is rare in humans, but occurs in Northern Europe, Asia, North America and Arctic regions. *E. granulosus* is widespread in sheep-rearing areas of the world. Eradication is well advanced in Australia and New Zealand.



15-118 *Echinococcus granulosus*. Eggs. Iodine stain (×800). The eggs of *E. granulosus* are identical to the eggs of the *Taenia* spp. They are spherical with a thick, radially striated shell and measure 30 to 40 μm in diameter.



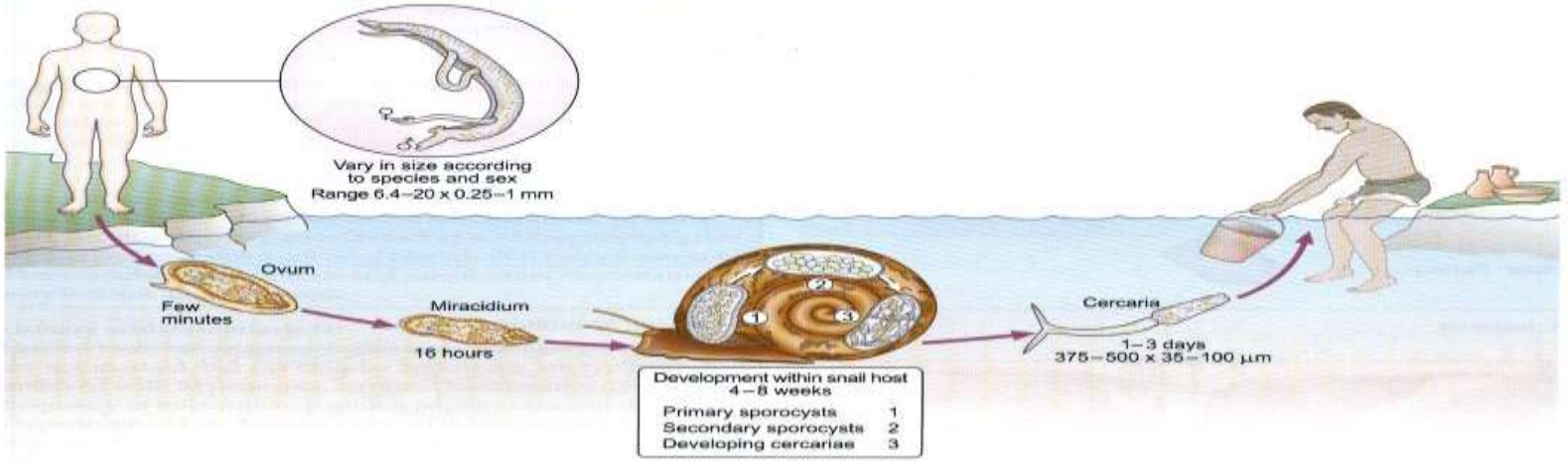
A **B**

Echinococcus granulosus A- adult worm B-hydatid cyst in liver

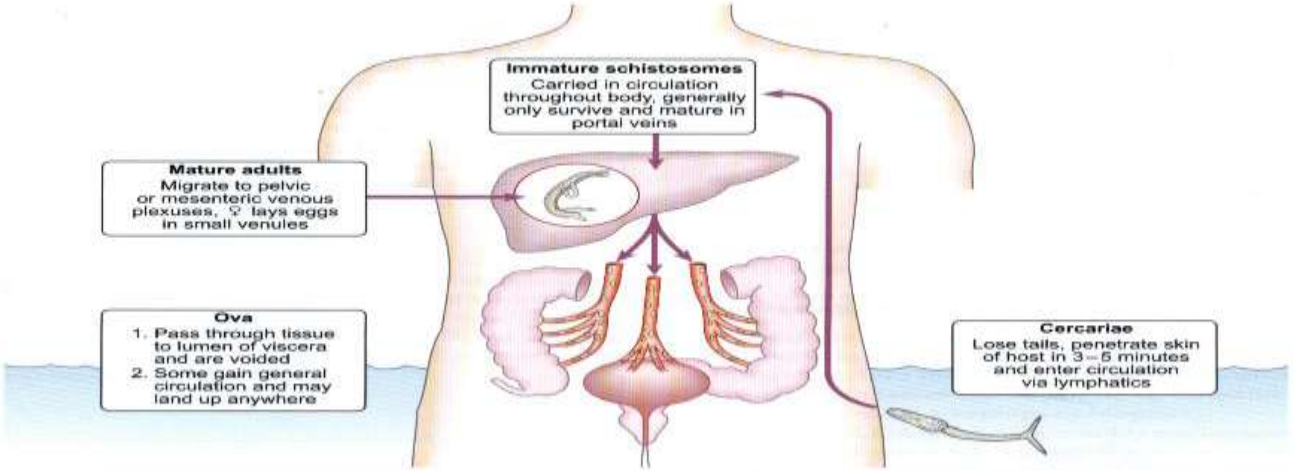
Trematode (flat) worms

Schistosoma species (blood flukes)

Life cycle for all species



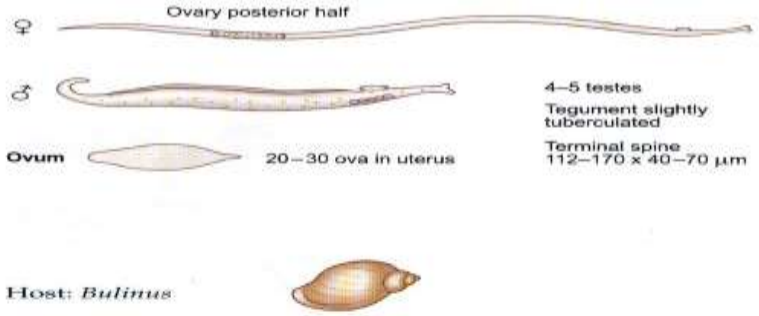
Life cycle in humans



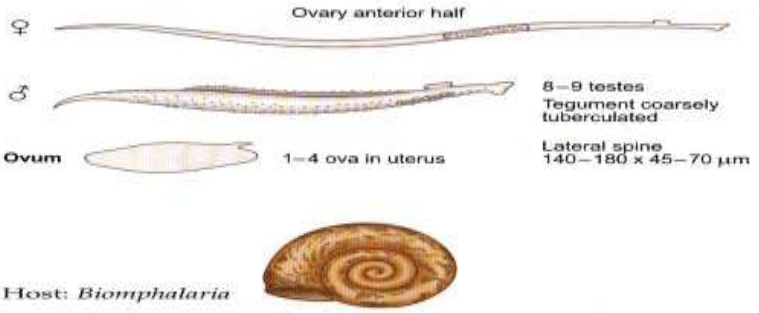
Schistosoma species (blood flukes) (Continued)

Morphology

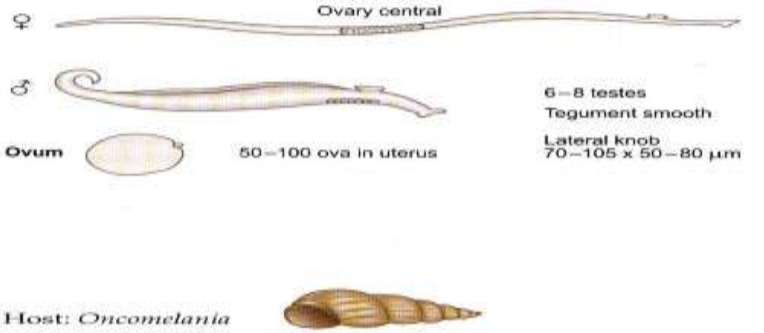
S. haematobium



S. mansoni



S. japonicum

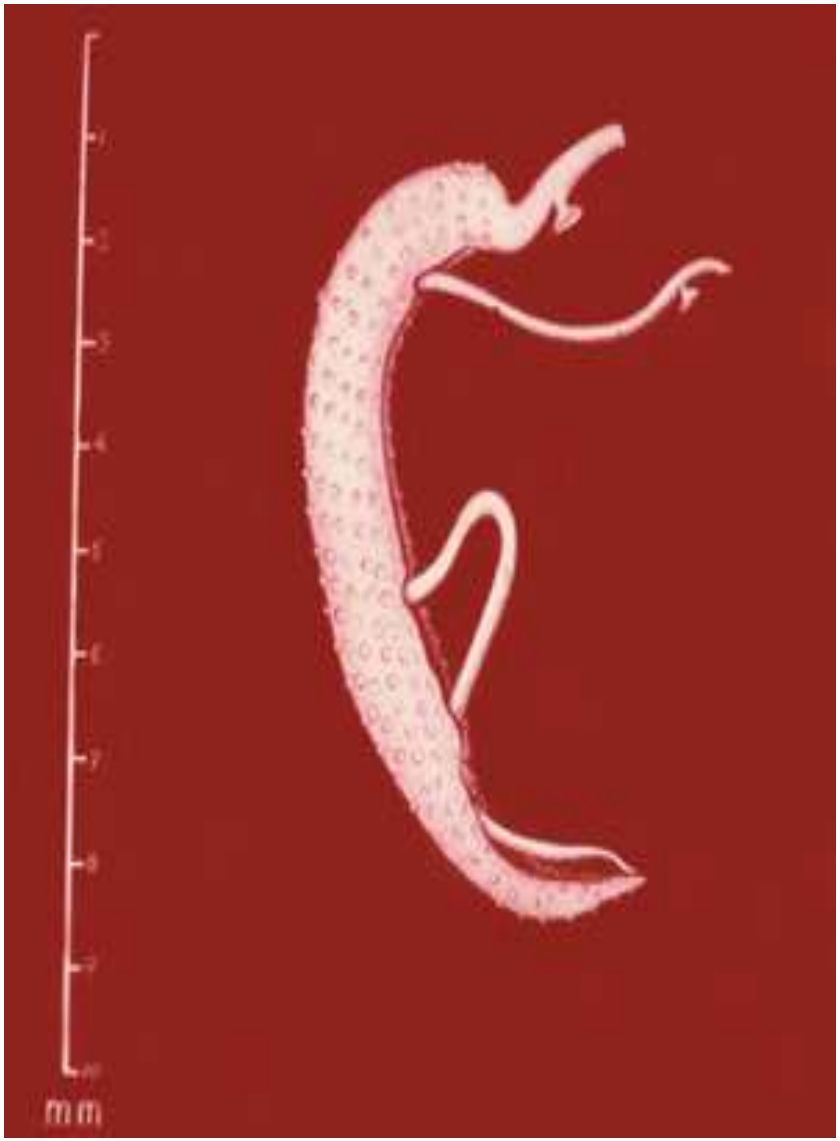


Distribution

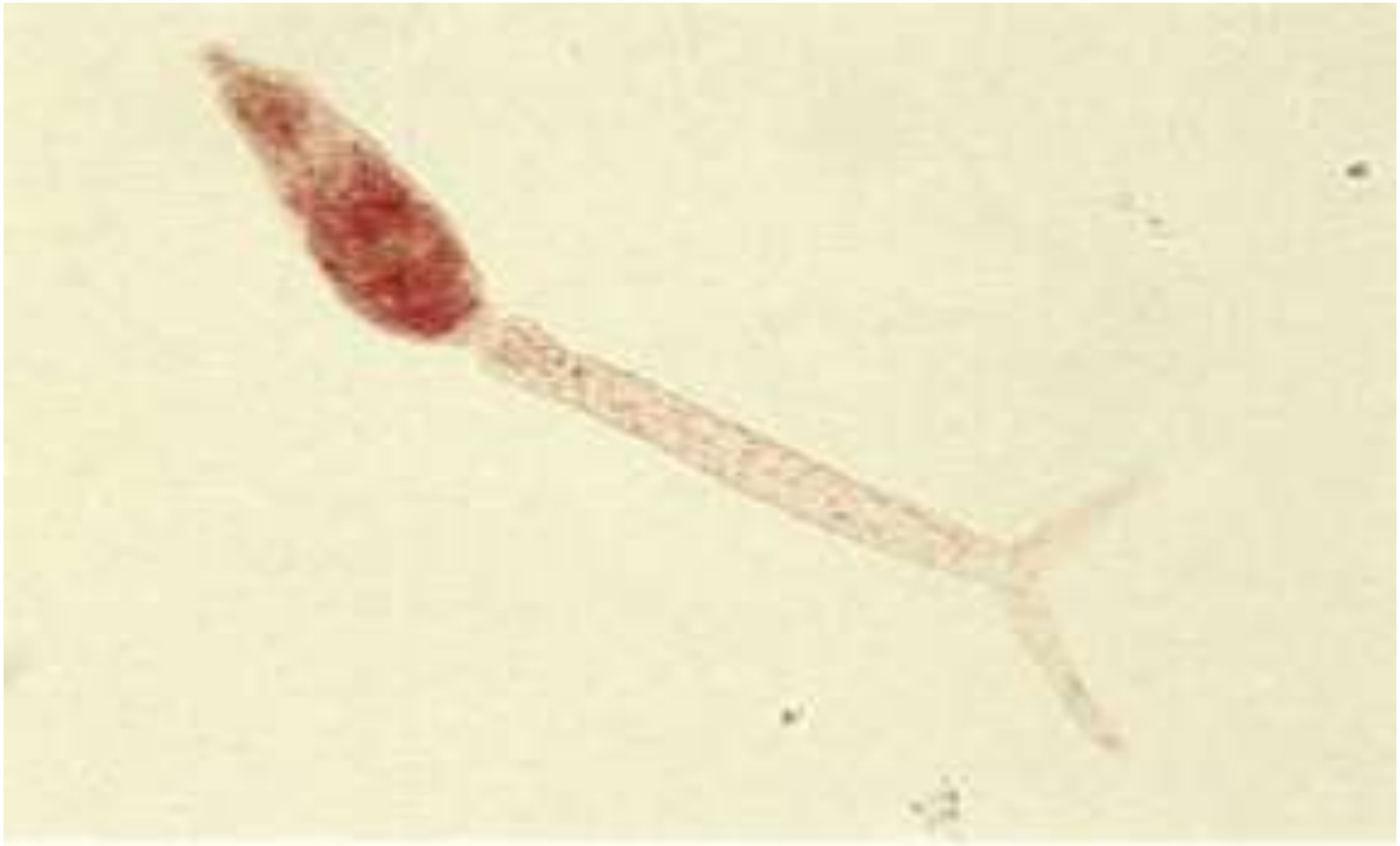
S. haematobium: 78 million

S. mansoni: 57 million

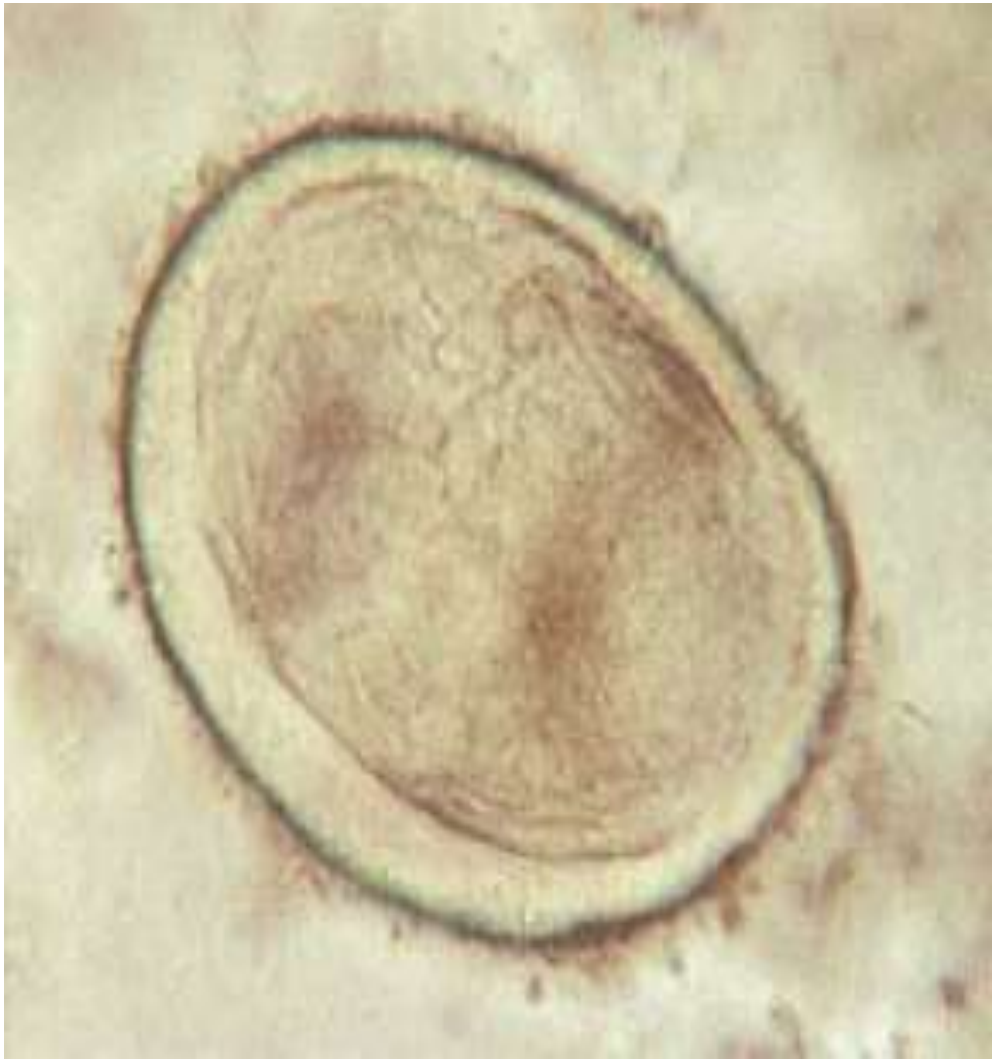
S. japonicum: 69 million



Adults of *S. mansoni*. The thin female resides in the gynecophoral canal of the thicker male.

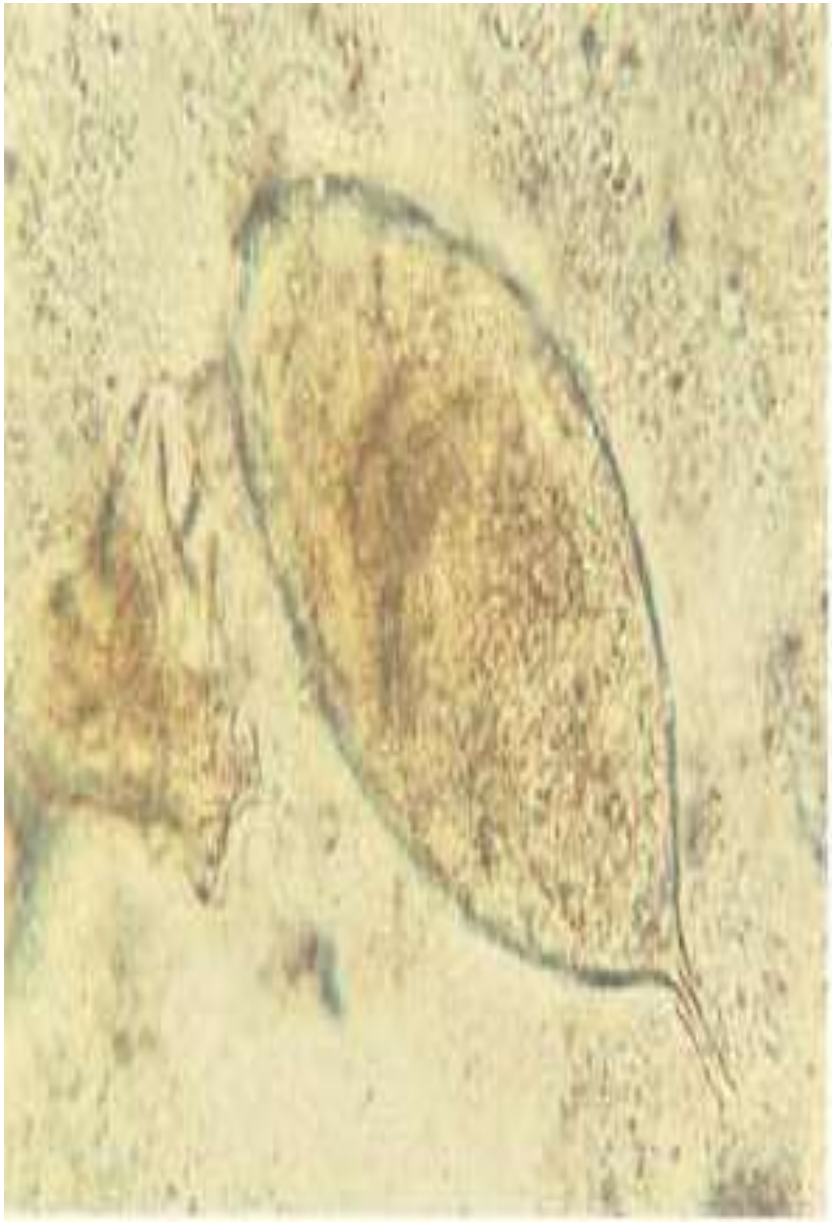


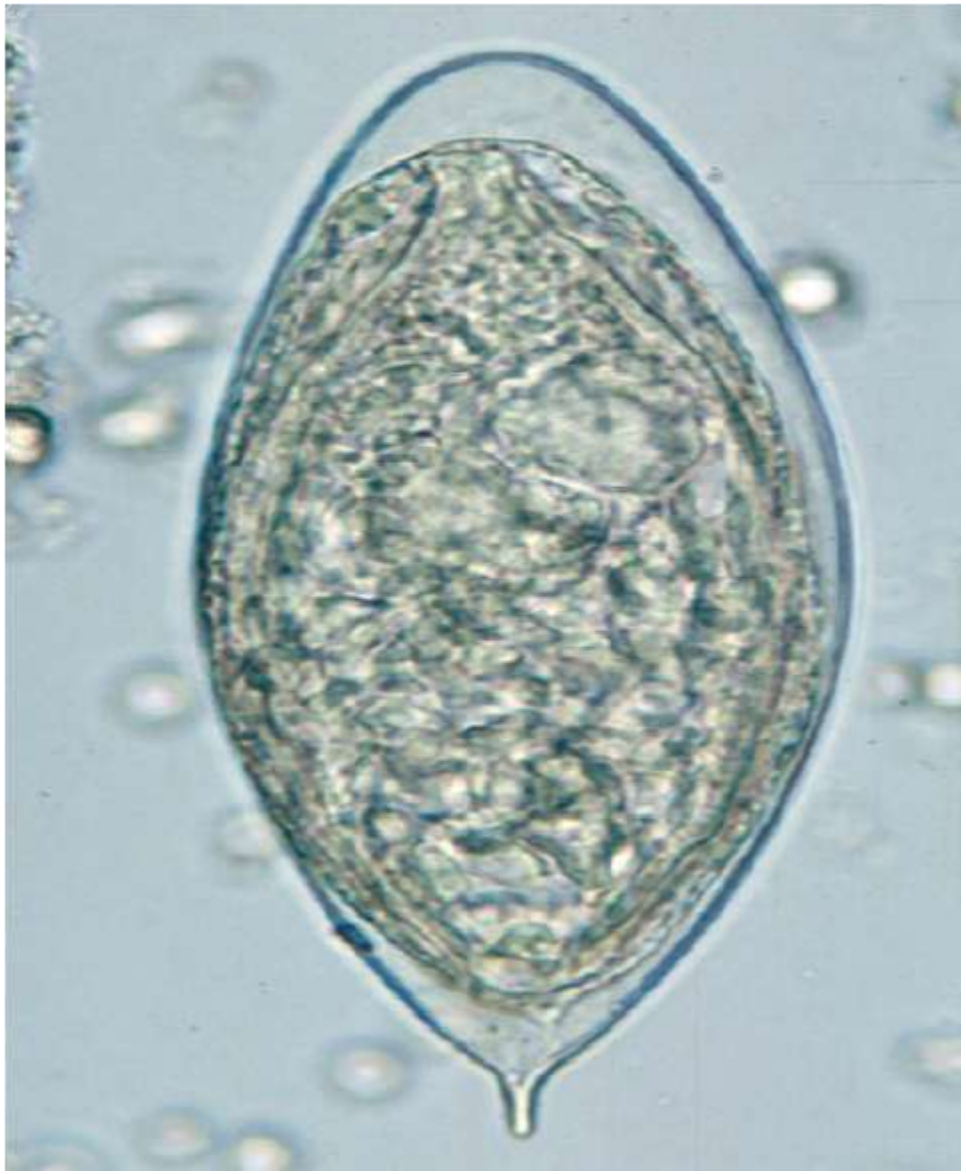
Cercaria of *Schistosoma spp.*



Schistosoma japonicum. Egg. Feces.

Schistosoma mansoni. Egg. Feces.

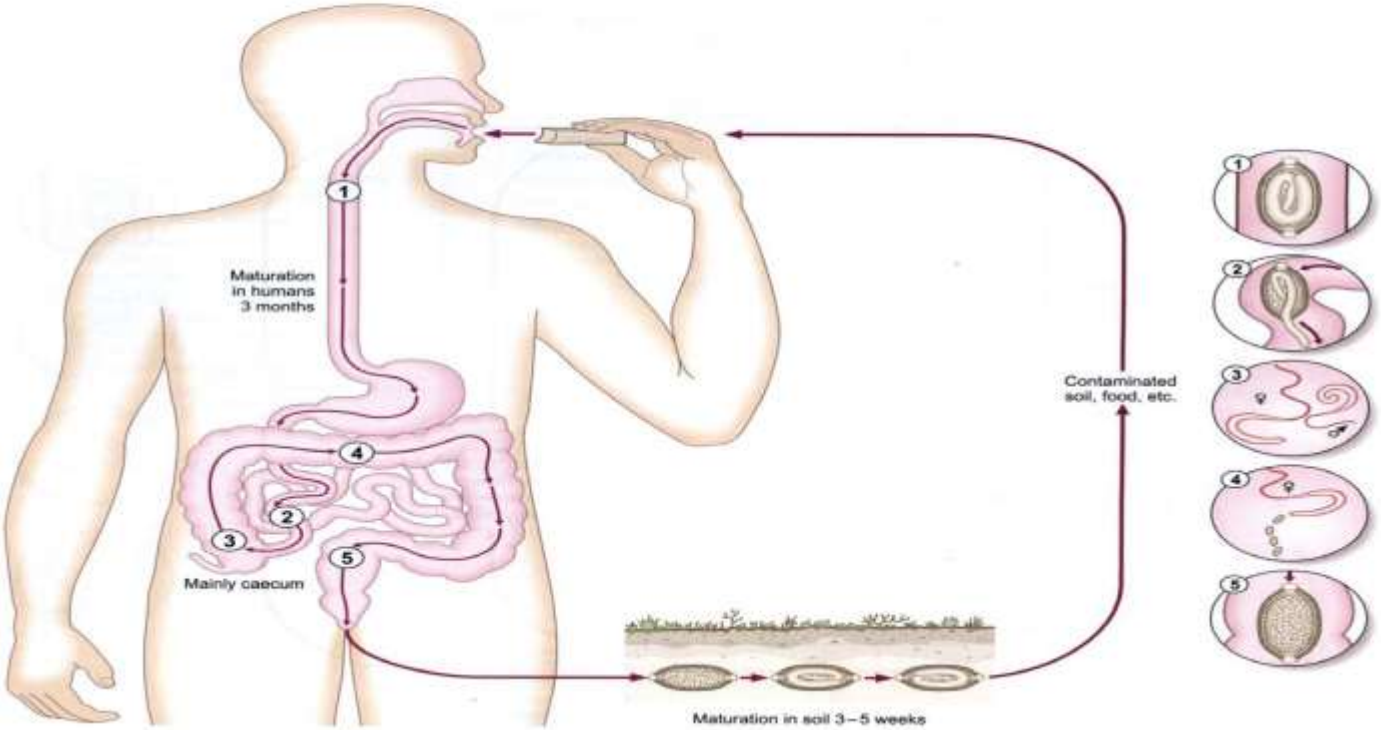




***Schistosoma haematobium*. Egg.**

Trichuris trichiura (whip worm)

Life cycle



Pathology and Clinical features

Light infections may be asymptomatic. Heavy infections can result in the trichuris dysentery syndrome, rectal prolapse, rectal bleeding, anaemia, growth stunting and growth retardation in children.

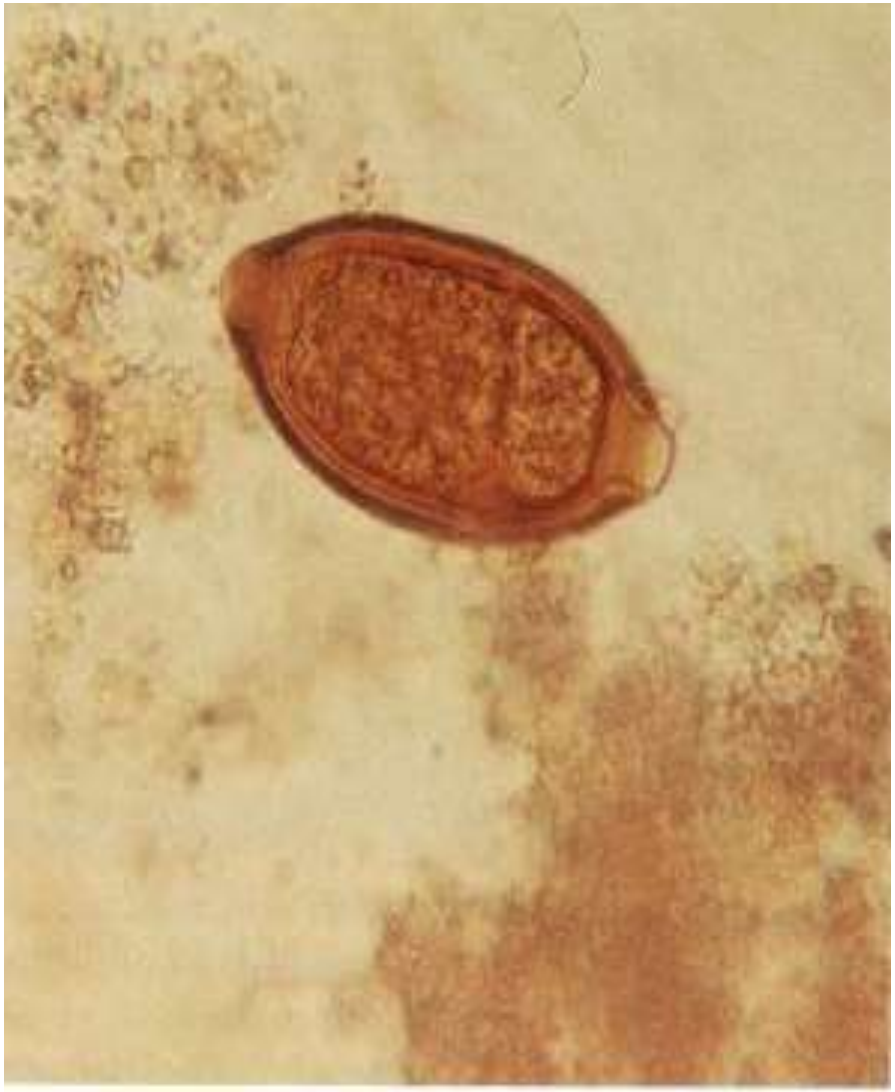
Laboratory diagnosis

Eosinophilia may occur.

Ova may be recovered in faeces by concentration methods.

Distribution

1.3 billion infected worldwide.

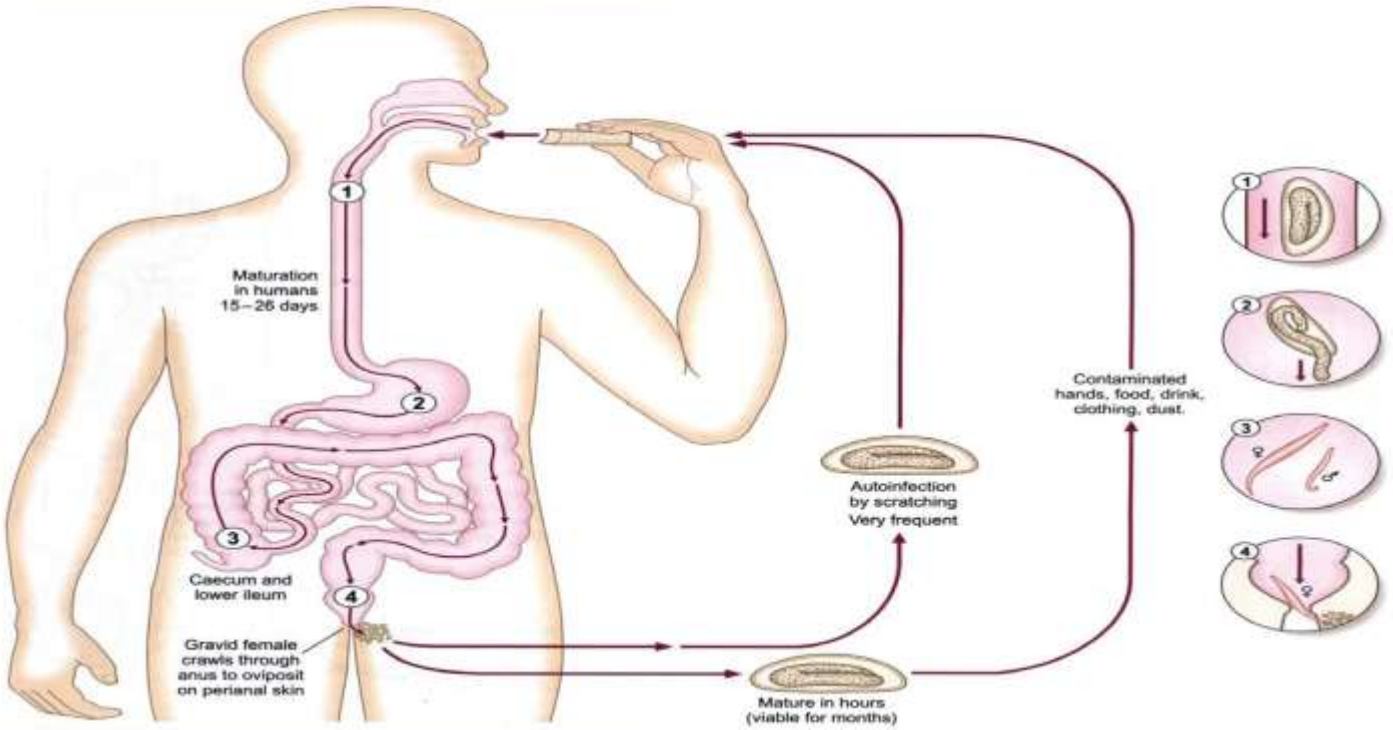


Trichuris trichiura. Egg. Feces

Nematode (round) worms

Enterobius vermicularis (thread or pin worm)

Life cycle



Distribution

350 million infected worldwide, often group or institutional infection.

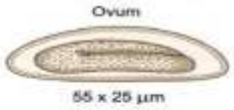
Pathology and Clinical features

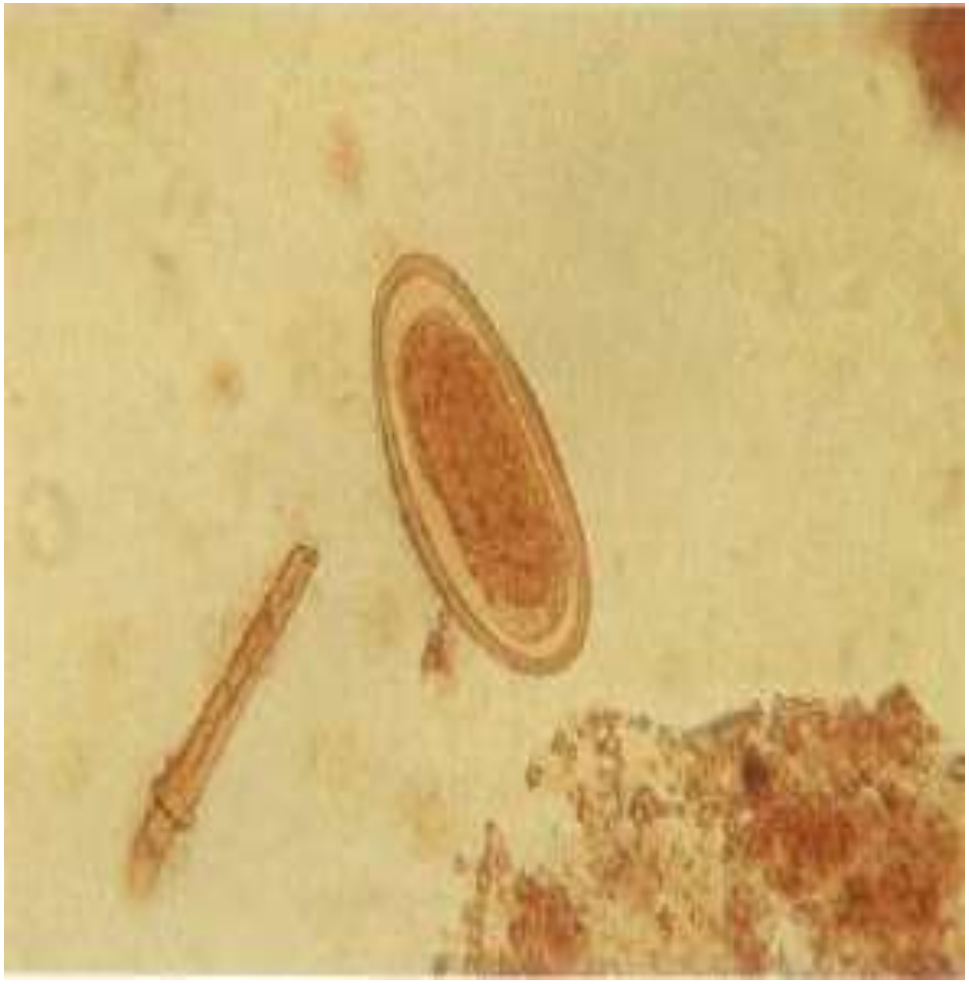
Most infections are asymptomatic. Perianal itching may be troublesome. In females, migrating worms may cause pruritis vulvae or vaginitis. Rarely, urinary tract infection or appendicitis can occur. Migration into the peritoneal cavity has been recorded.

Laboratory diagnosis

Mild eosinophilia.

Ova can be recovered from the perianal area using clear adhesive tape or a cotton swab moistened with saline. Early morning collection before washing gives best recovery. In females, ova may occasionally be recovered from urine.

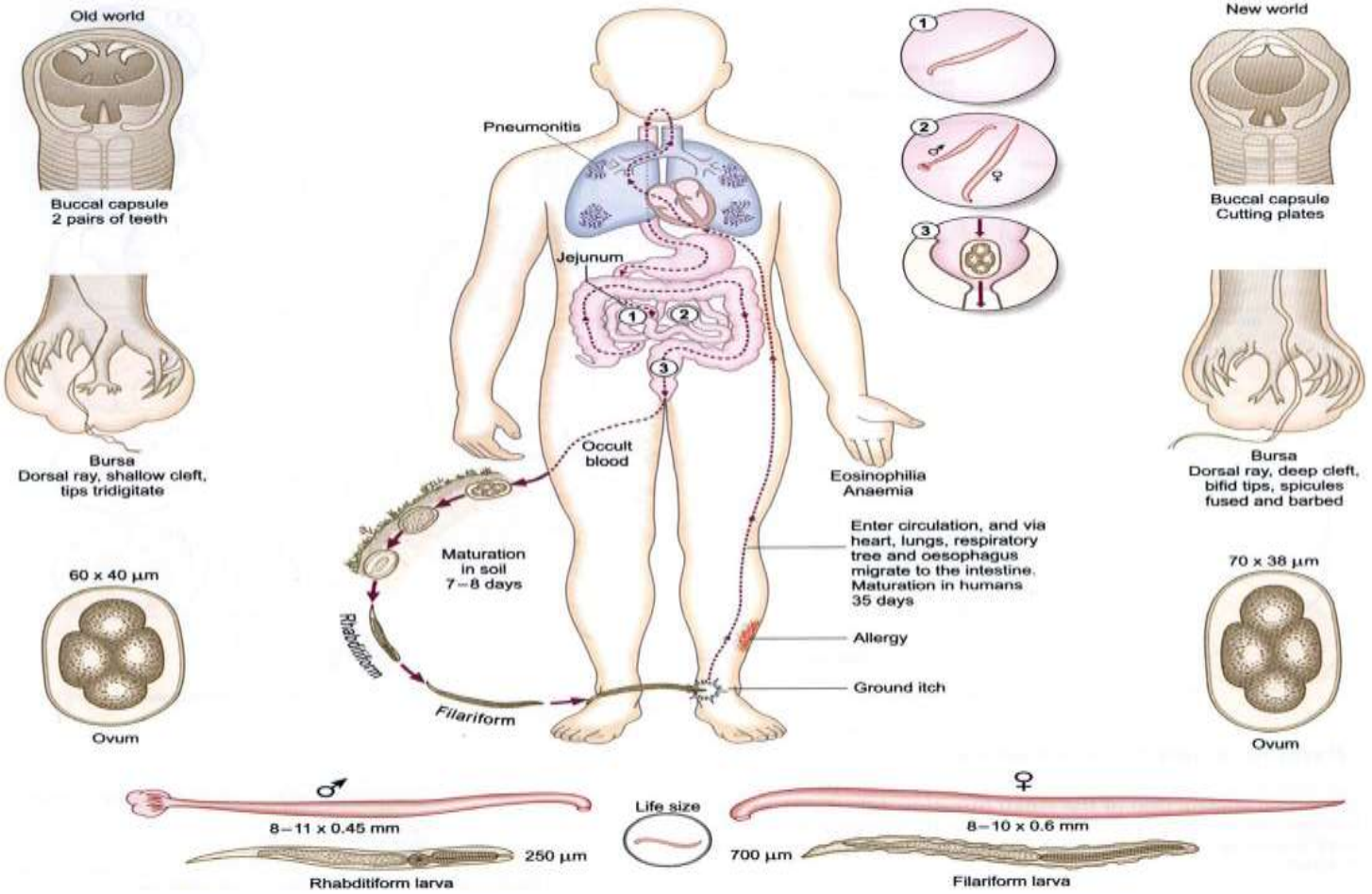




Enterobius vermicularis

Hookworms

Ancylostoma duodenale — *Necator americanus*



Pathology and Clinical features

Ground itch may follow skin penetration by filariform larvae. Pneumonitis can result from larval migration through the lungs. Adult worms in the jejunum ingest blood. Occult gastrointestinal bleeding occurs. Iron deficiency anaemia and its sequelae in heavy infections.

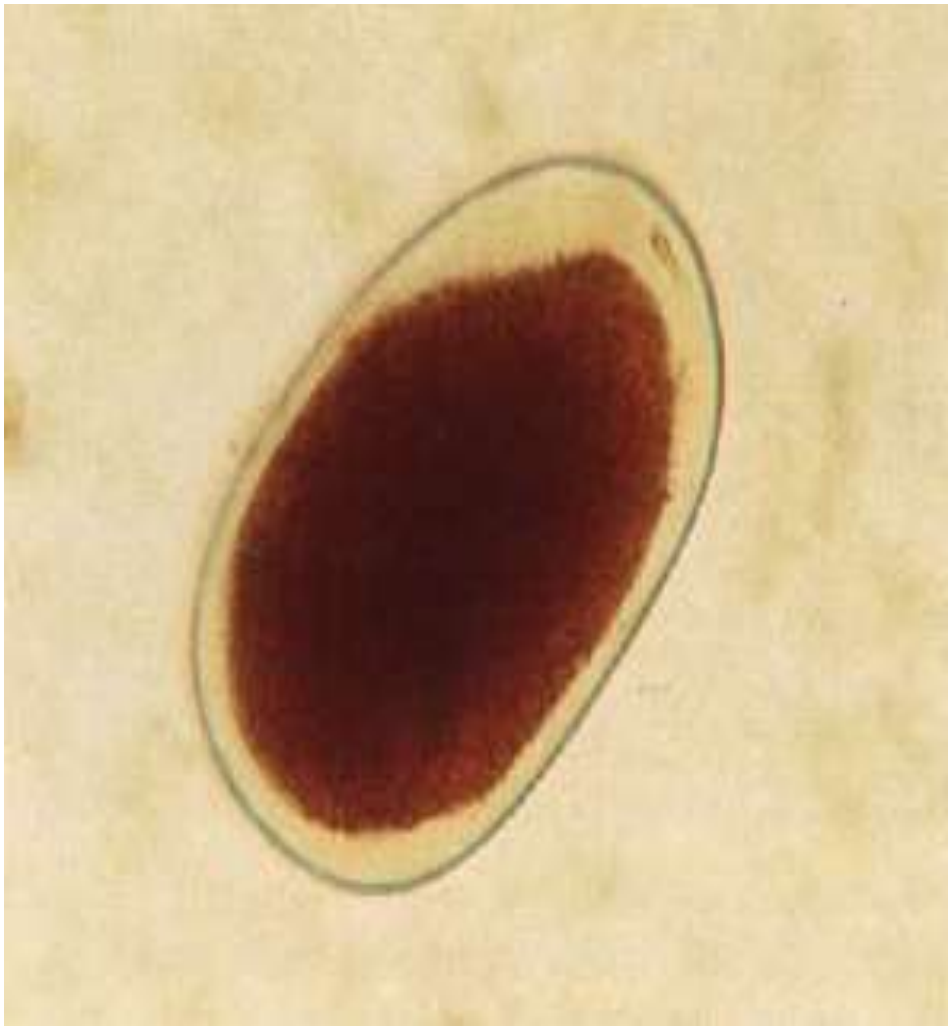
Distribution

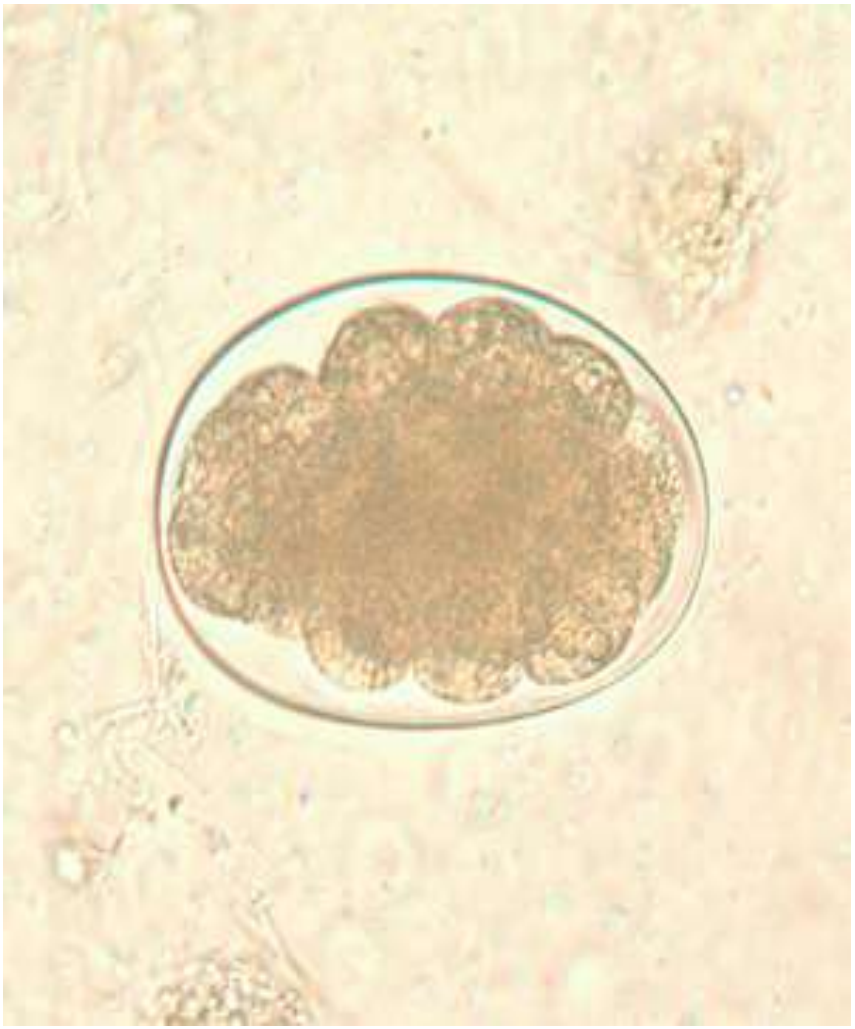
900 million infected worldwide.

Laboratory diagnosis

Eosinophilia.

Ova may be recovered from faeces by concentration methods. Rhabditiform larvae may be seen in old faecal specimens and must be distinguished from *Strongyloides* by the appearance of the buccal cavity.

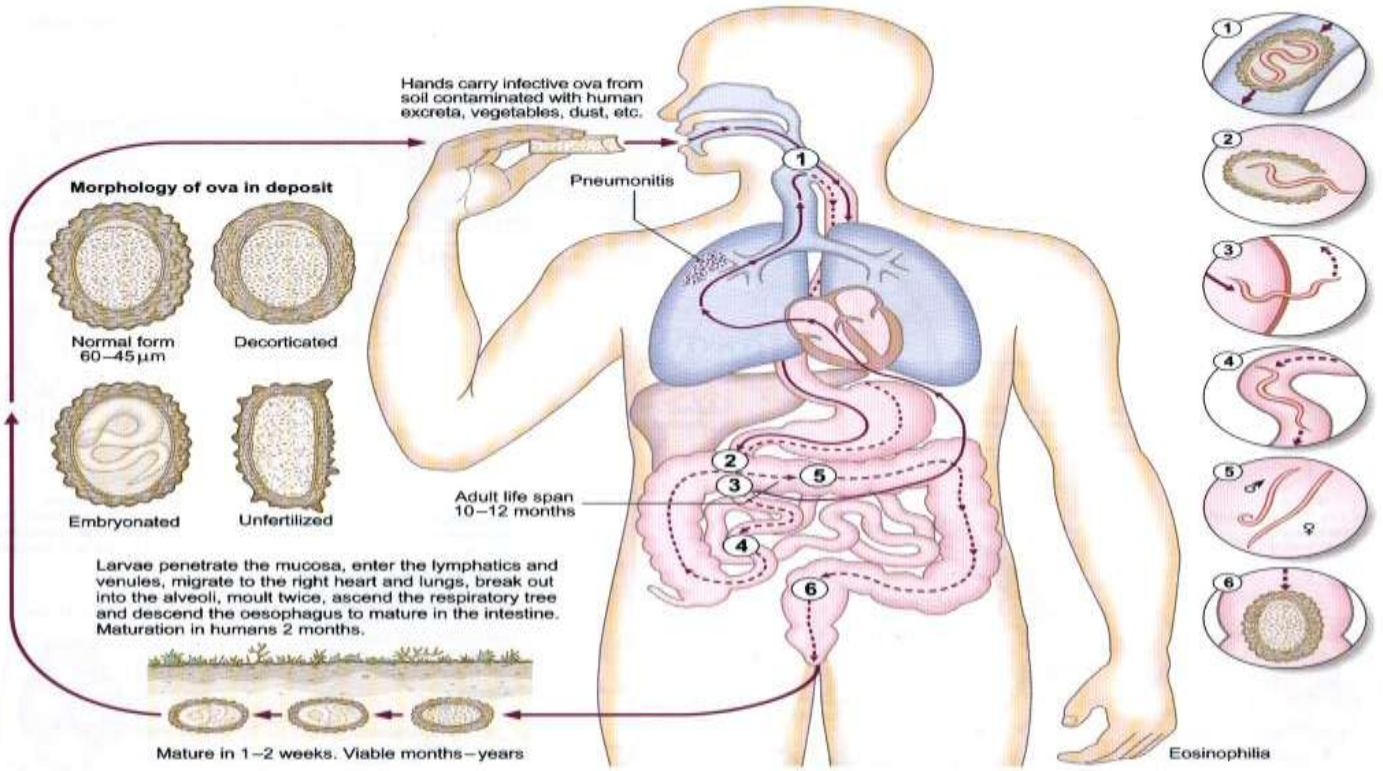




Egg of *Ancylostoma duodenale*

Ascaris lumbricoides (round worm)

Life cycle



Pathology and Clinical features

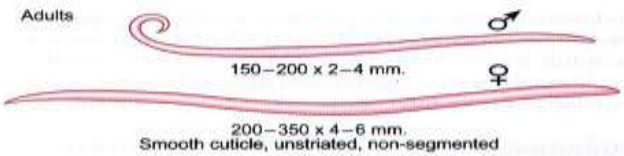
Larvae can cause pneumonitis with eosinophilia. Adult worms can cause obstruction of the small intestine, bile ducts and trachea; also appendicitis, pancreatitis and peritonitis. Children may vomit up a bolus of adult worms, or cough up immature worms.

Head of adult to show arrangement of the three lips

Laboratory diagnosis

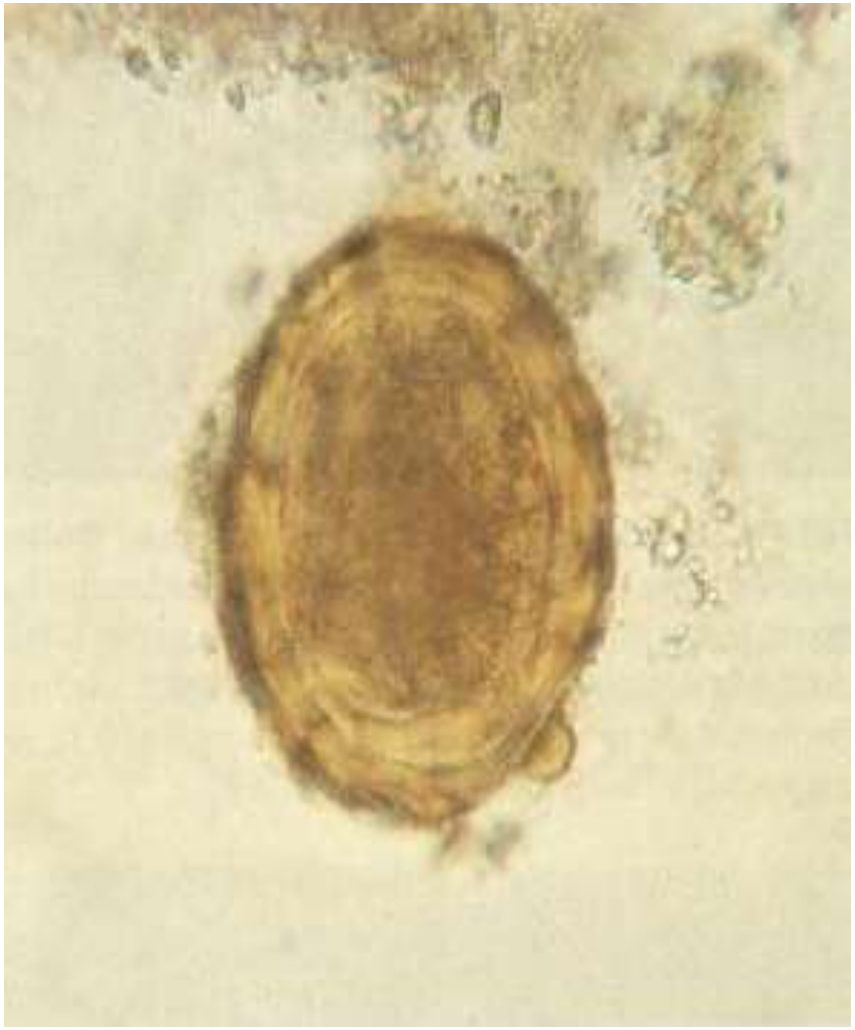
Ova may be recovered from faeces by concentration methods. Rarely larvae can be found in sputum, and must be distinguished from those of *Strongyloides*. Eosinophilia is present in the larval invasion stage.

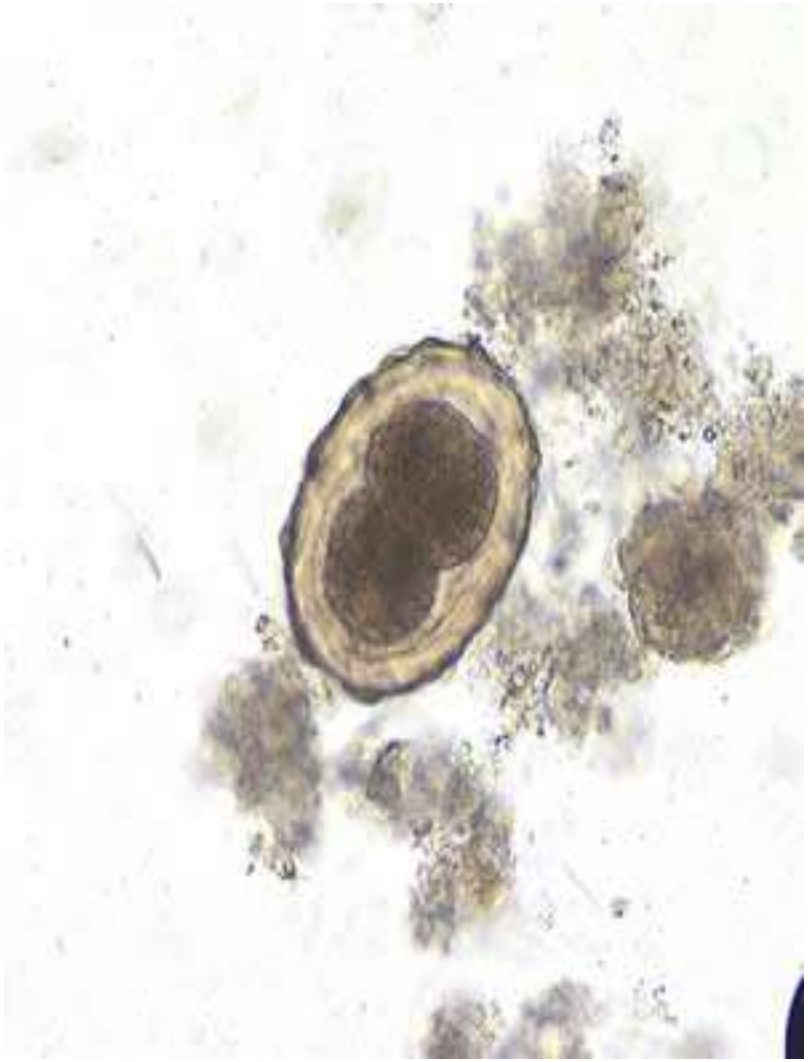
No specific serology is currently available.



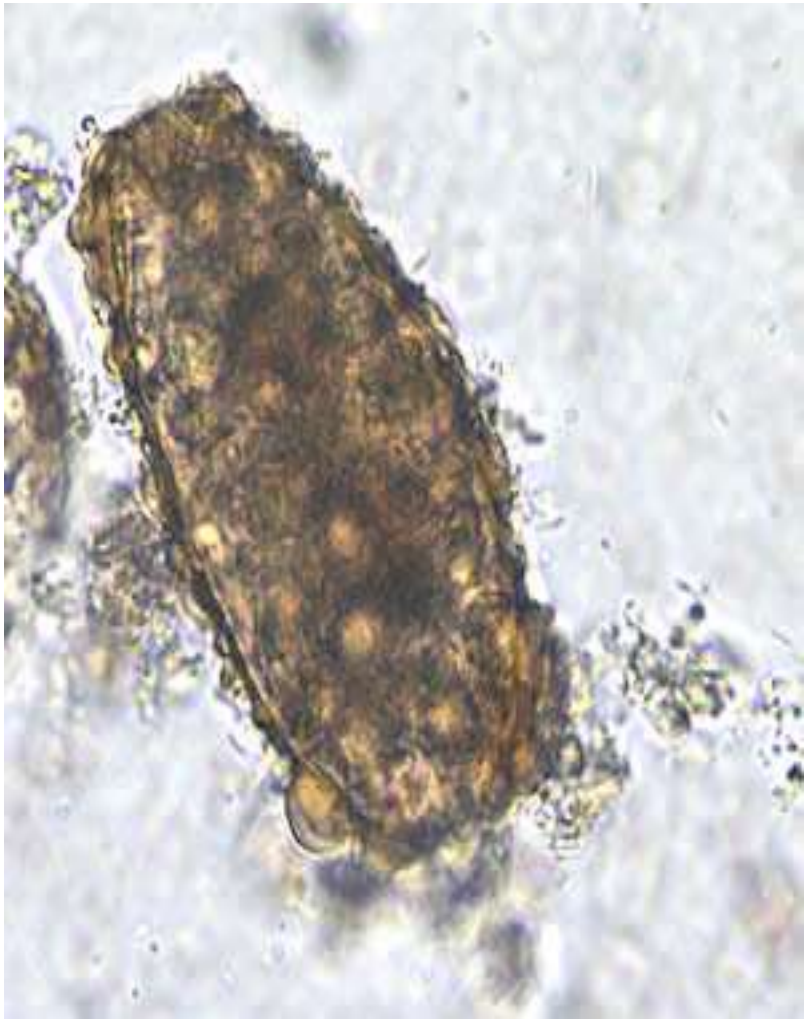
Distribution

1.47 billion infected worldwide.





Fertilized egg of *Ascaris lumbricoides*



Unfertilized egg of *Ascaris lumbricoides*

Syllabus of Human Parasitology

Phylum: Protozoa

Class: **Amoeba**

Intestinal and oral amoeba

Entamoeba hystolytica, *E. coli, Endolimux nana, Iodomoeba butschilli, Entamoeba gingivalis*

Class: **Flagellates**

Intestinal flagellates

Giardia lambilia, Chilomastics mesnelli

Genital tract flagellates

Trichomonas vaginalis

Blood and tissues flagellates

Leishamnia tropica, L. donovani, L. mexicana brasielensis

Trypanosoma gambiense, T. rhodesiense, T. cruzi

Class: **Ciliates**

Balantidium coli

Class: **Sporozoa**

Plasmodium vivax, P. falciparum, P. ovale, P. malariae

Class: **Coccidia**

Toxoplasma gondii

Phyllum: Platyhelminthes (FLAT WORMS)

Class: **Cestoda(es) TAPE WORMS**

Taenia saginata, T. solium, Hymenolepis nana,

Ecchinococcus granulosus, E. multilocularis

Class: **Trematoda(es)**

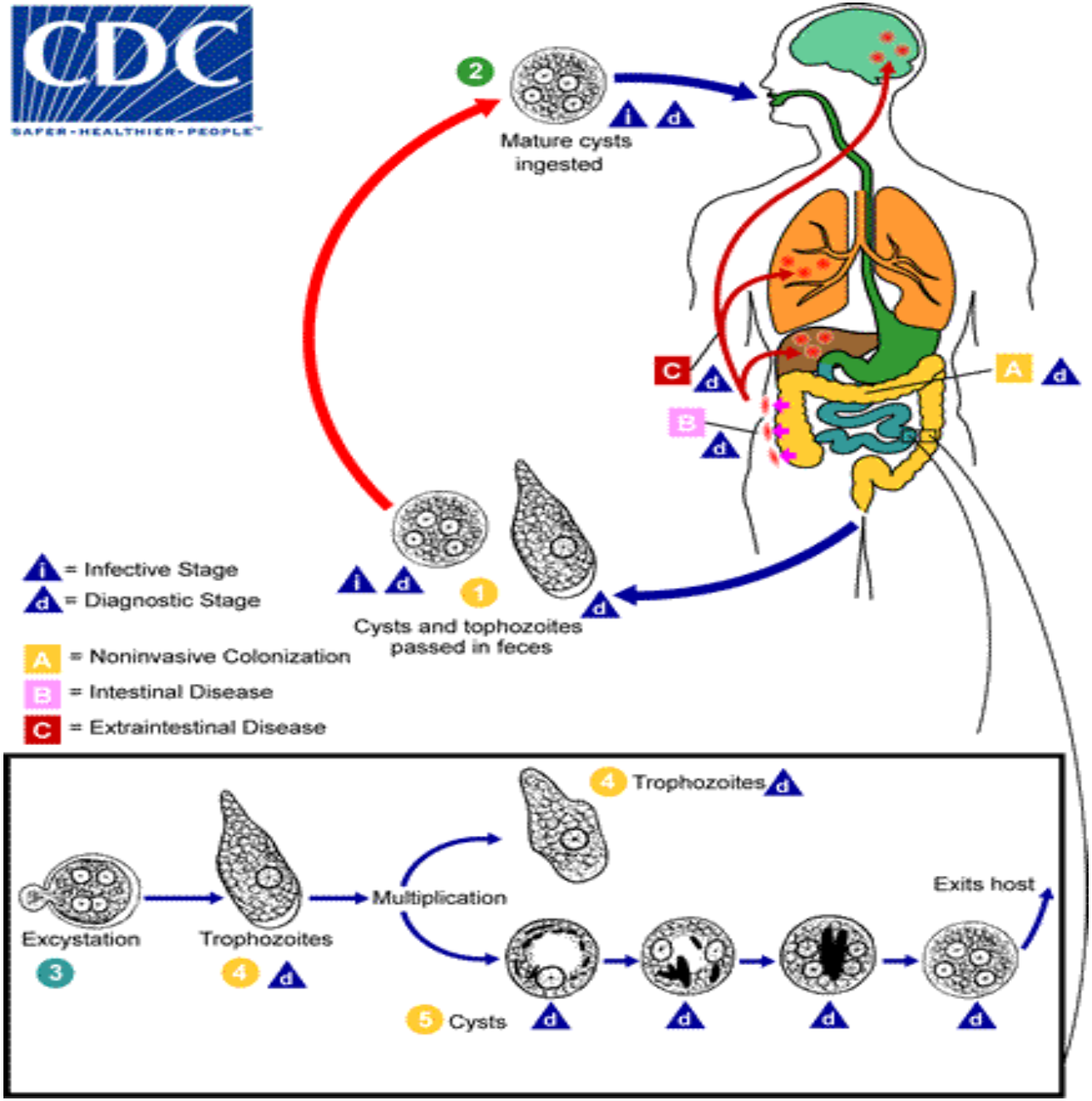
Schistosoma mansoni, S. japonicum, S. haematobium

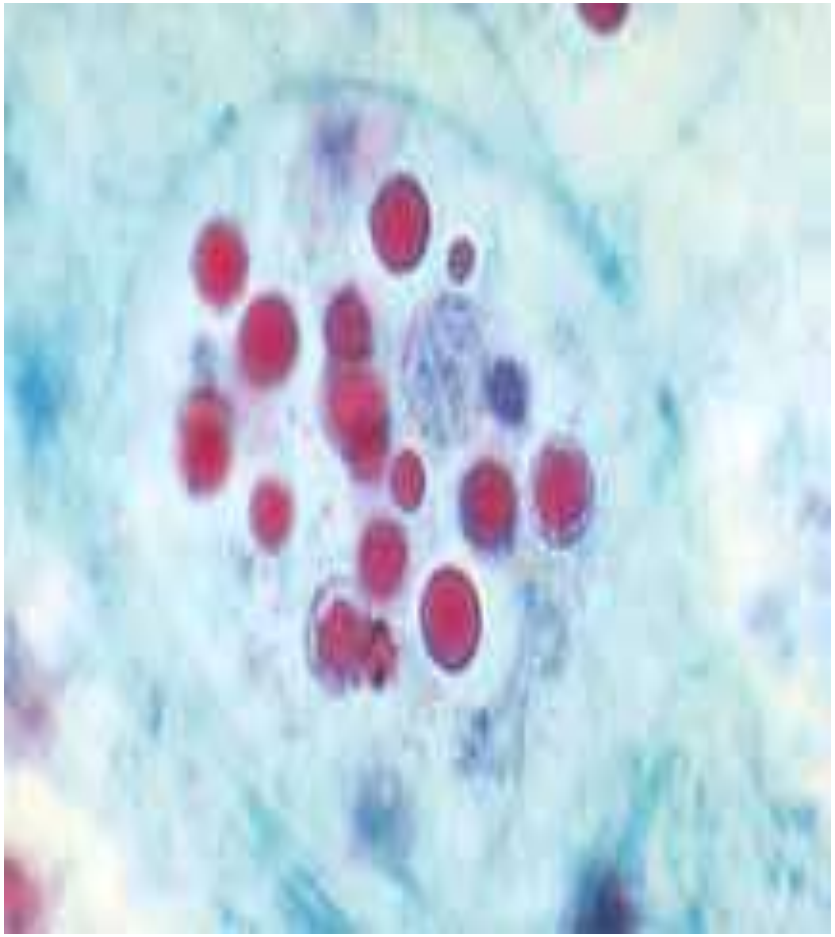
Phyllum: Nematohelminthes(Nematoda) ROUND WORMS

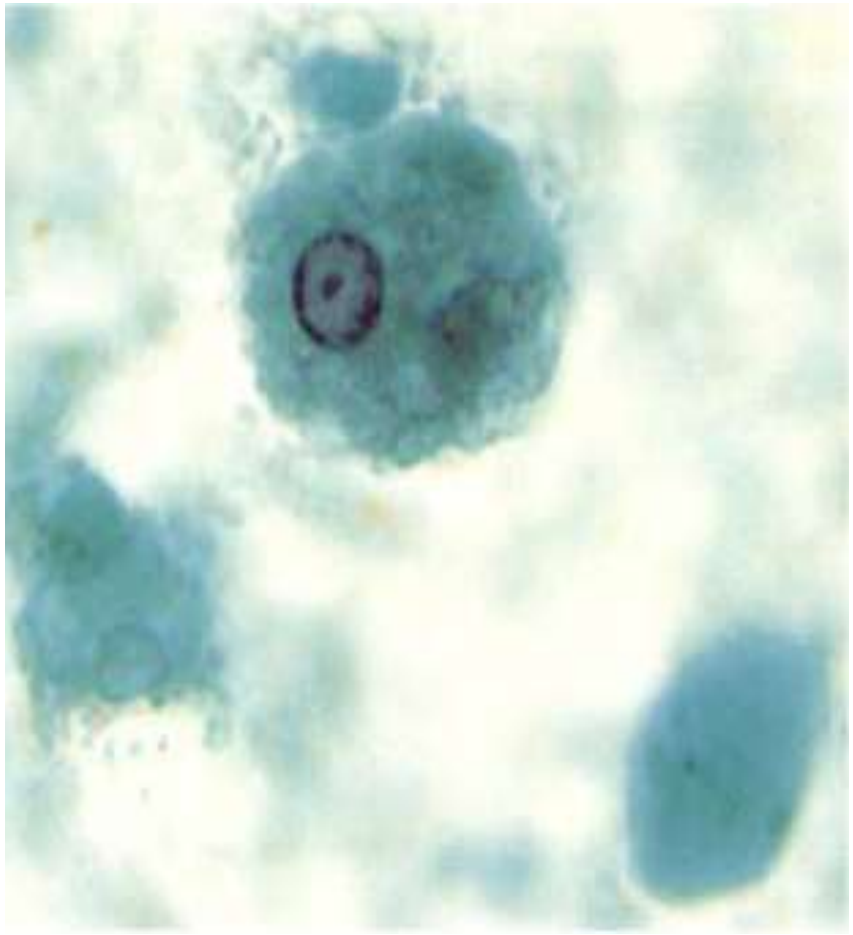
Trichuris trichuira, Enterobius vermicularis, Ancylostoma duodenale, Ascaris lumbricoides

Phyllum : Acanthocephala

Phyllum: Arthropoda(es)

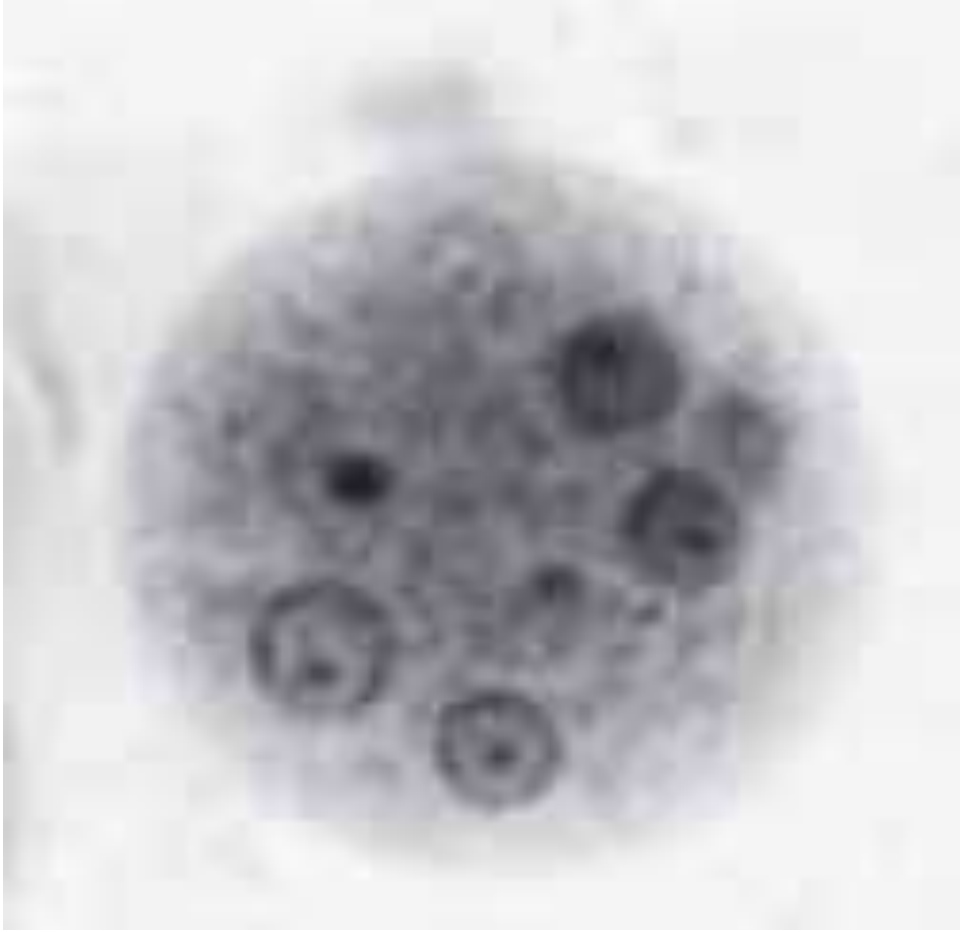

CDC
SAFER·HEALTHIER·PEOPLE™
2
Mature cysts ingested
i
d
C
d
A
d
B
d
i
d
1
Cysts and tophozoites passed in feces
d
i = Infective Stage
d = Diagnostic Stage
A = Noninvasive Colonization
B = Intestinal Disease
C = Extraintestinal Disease
4 Trophozoites d
Multiplication
Exits host
Excystation
3
Trophozoites
4 d
5 Cysts
d
d
d
d



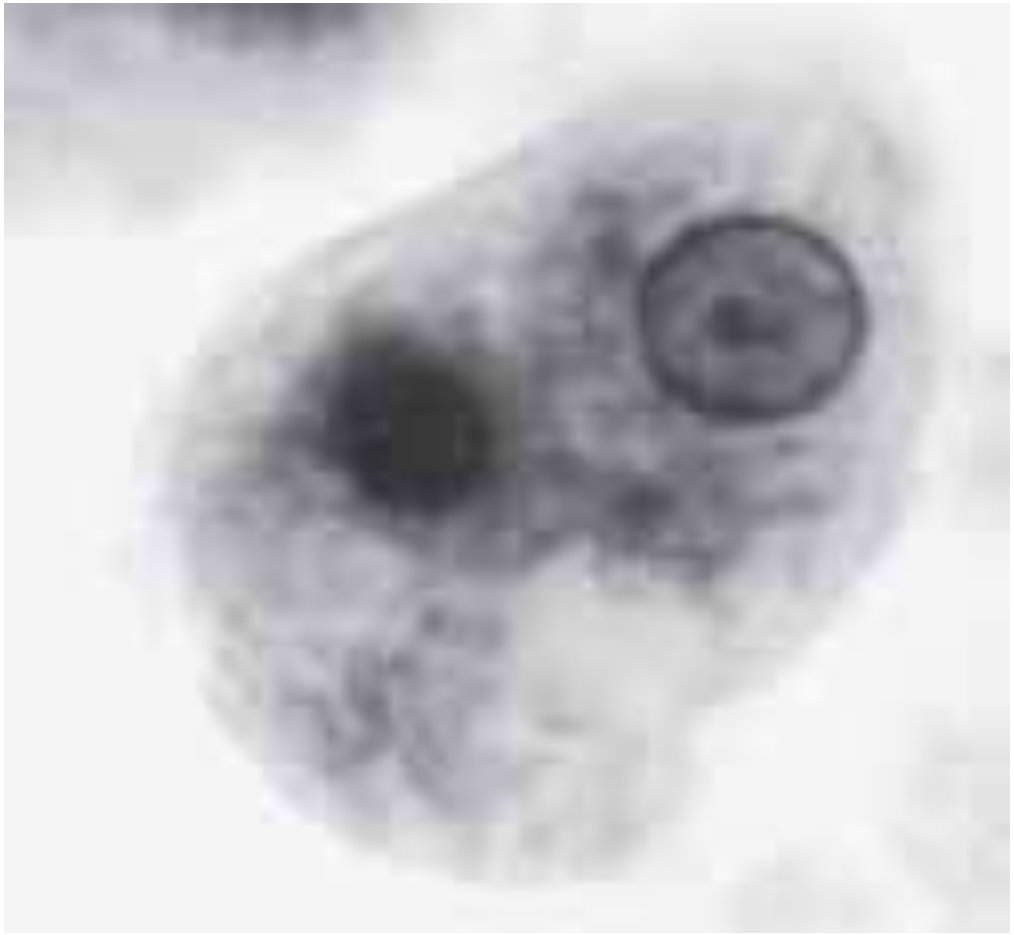


1 2

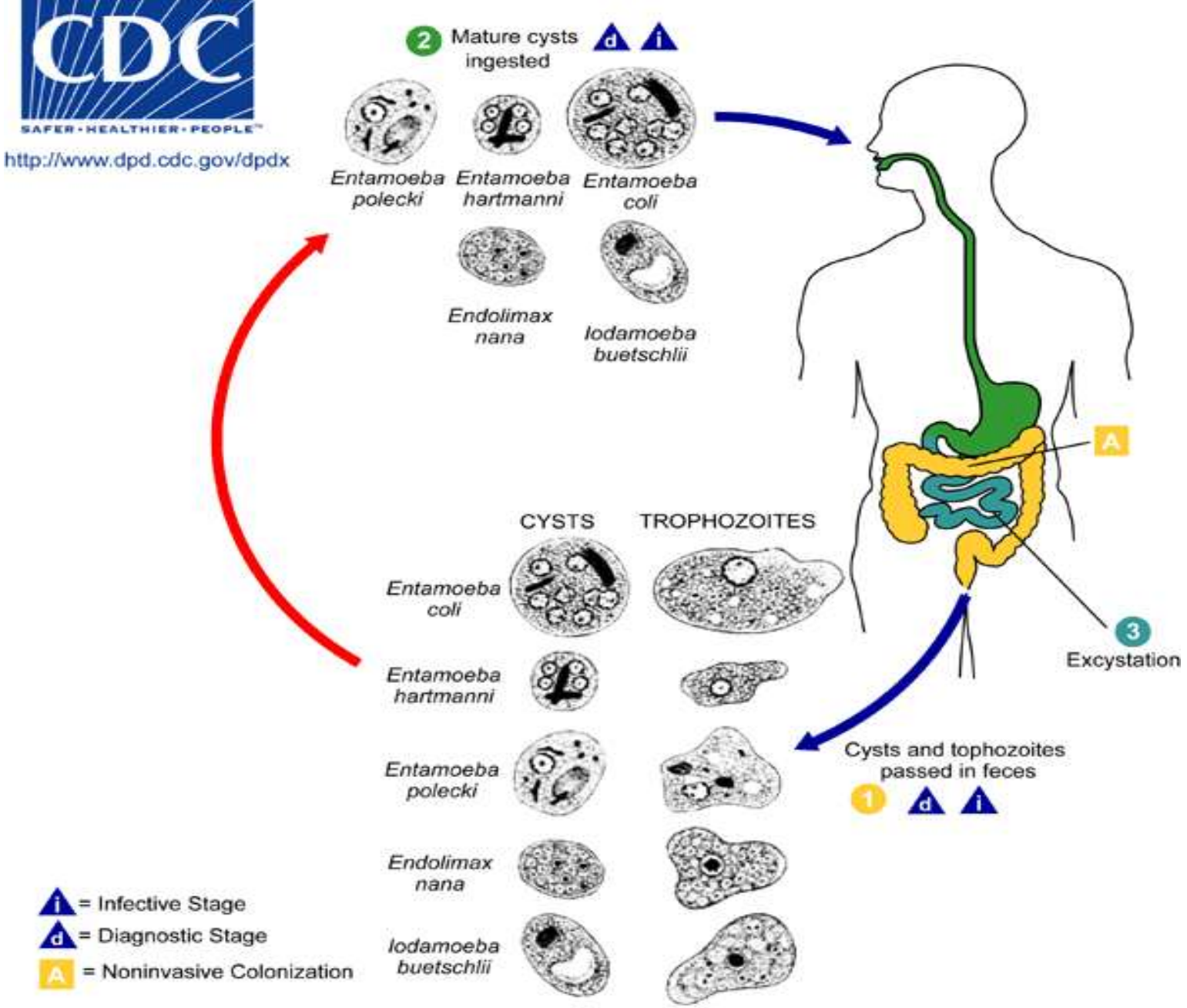
Entamoeba histolytica trophozoite 1-containing ingested red blood cells 2-stained with trichrome stain



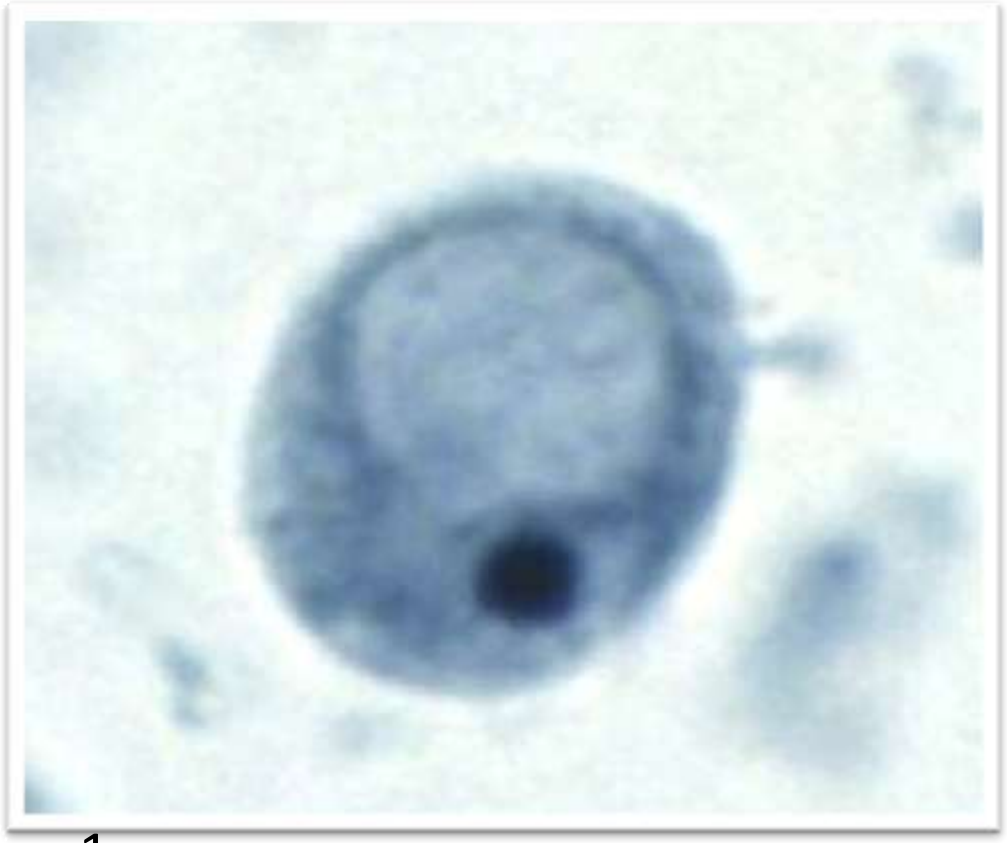
Mature Cyst of *Entamoeba histolytica*



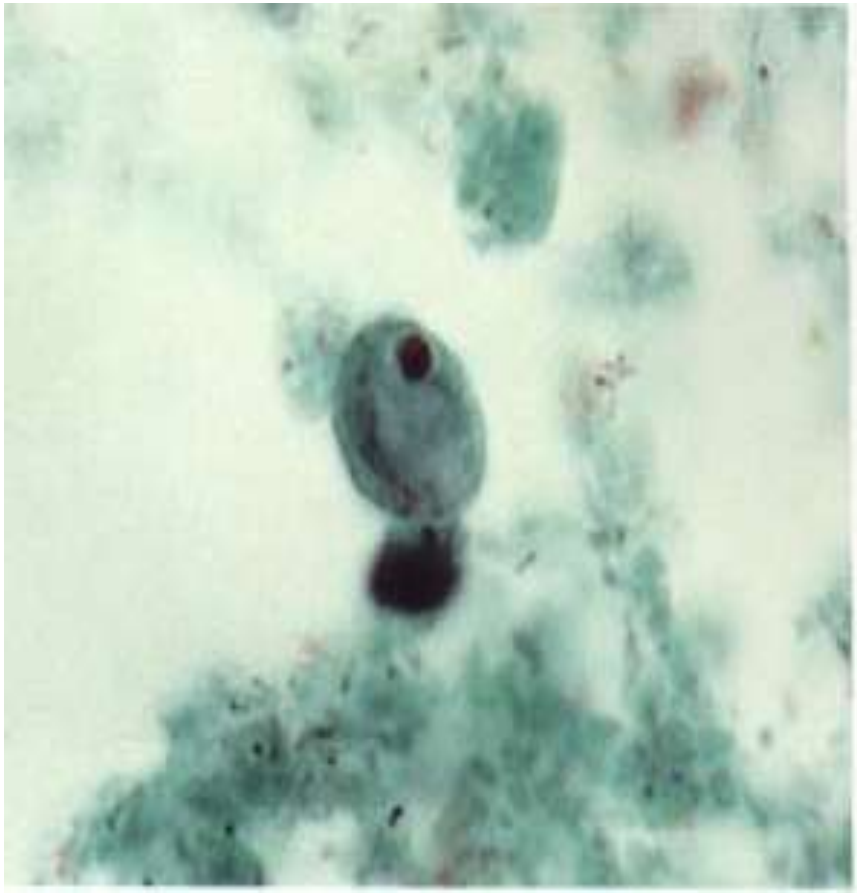
Trophozoite of *Entamoeba hystolytica*



Life Cycle of Commensal Intestinal Amoebas



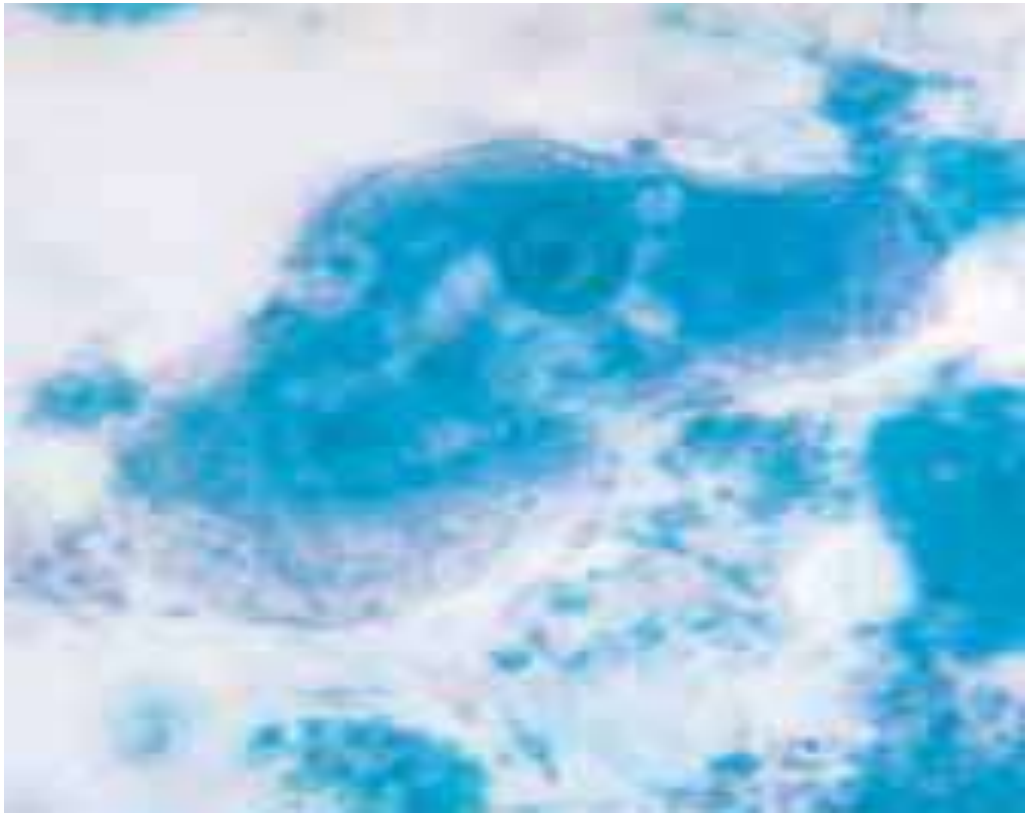
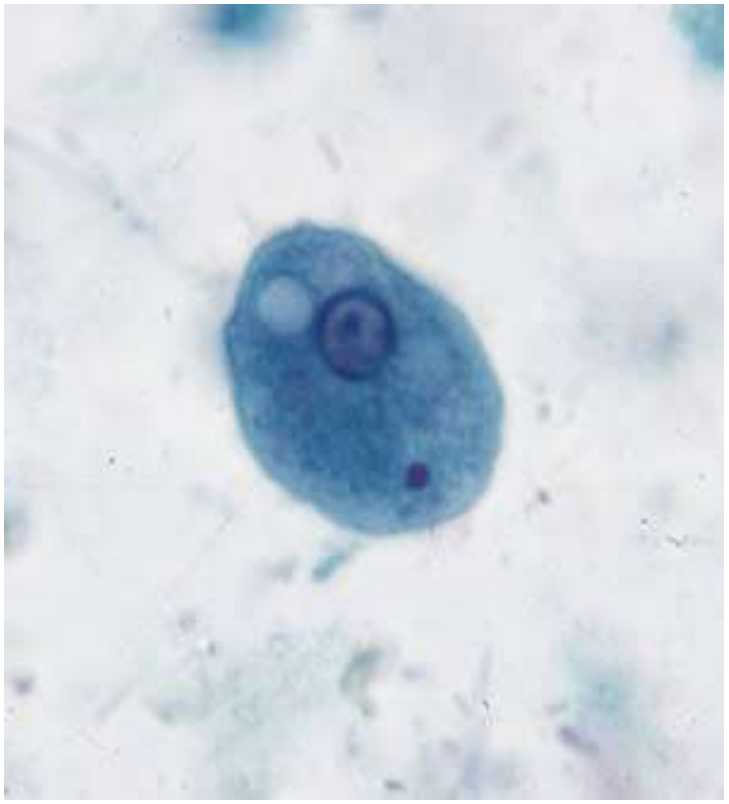
1



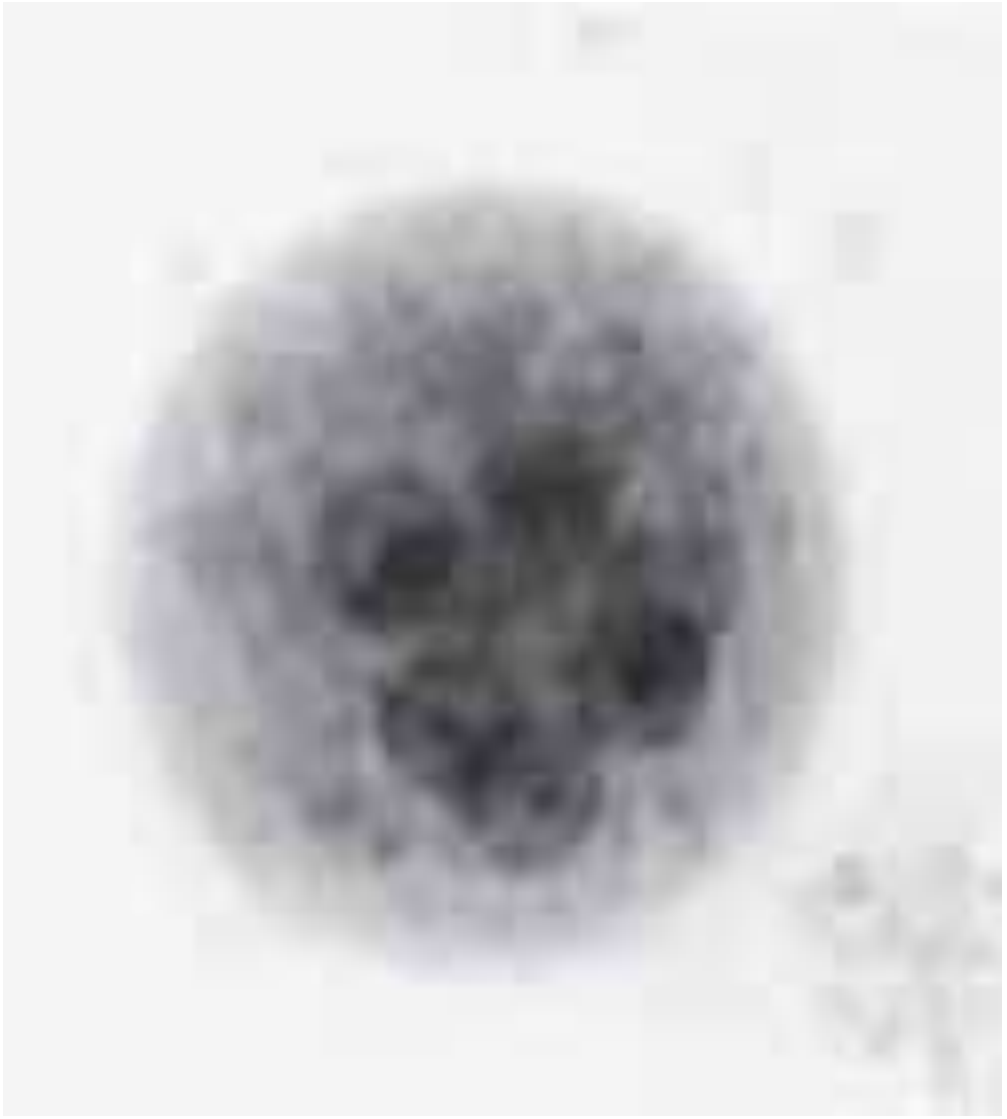
2

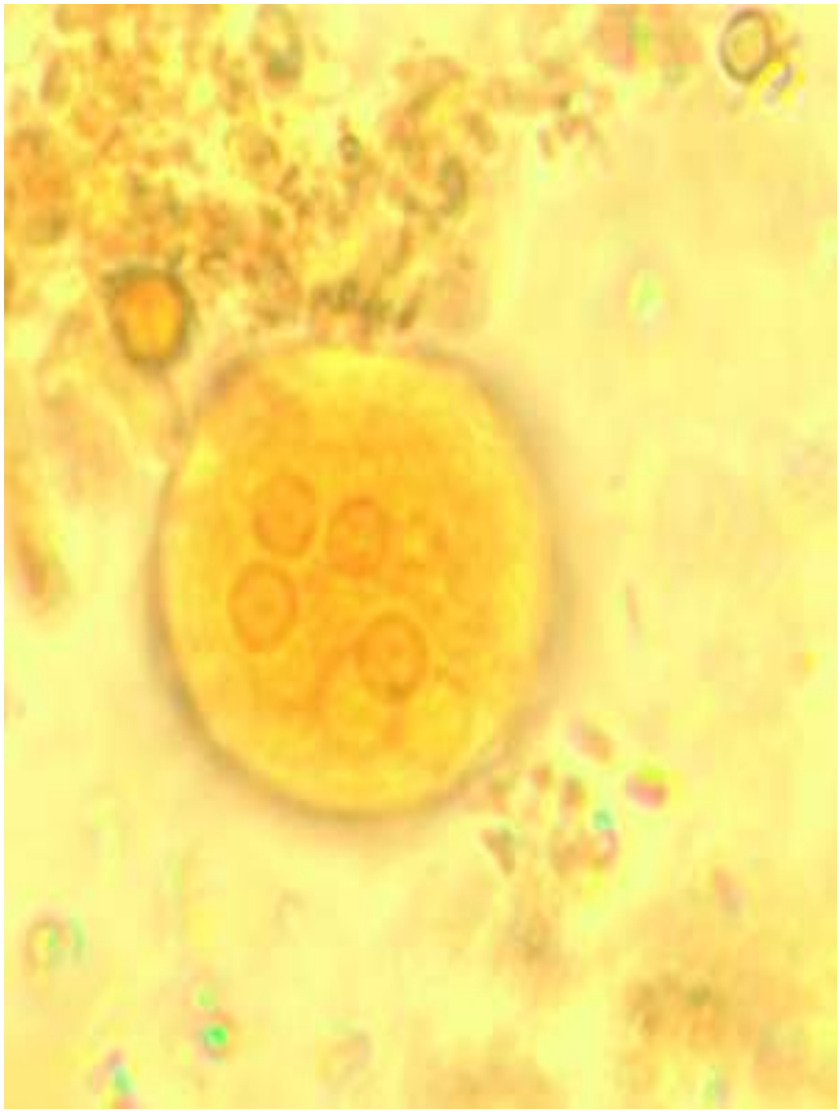
Iodomoeba butschlii 1- Cyst

2- Trophozoite

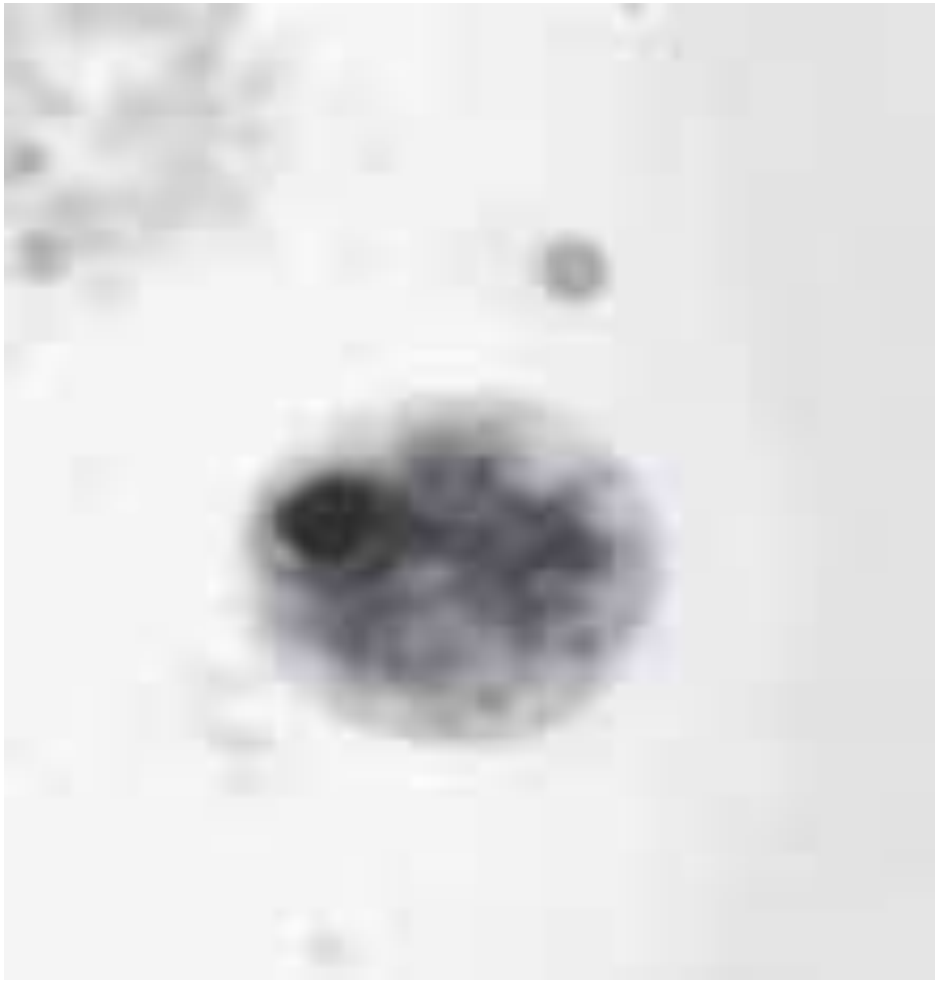



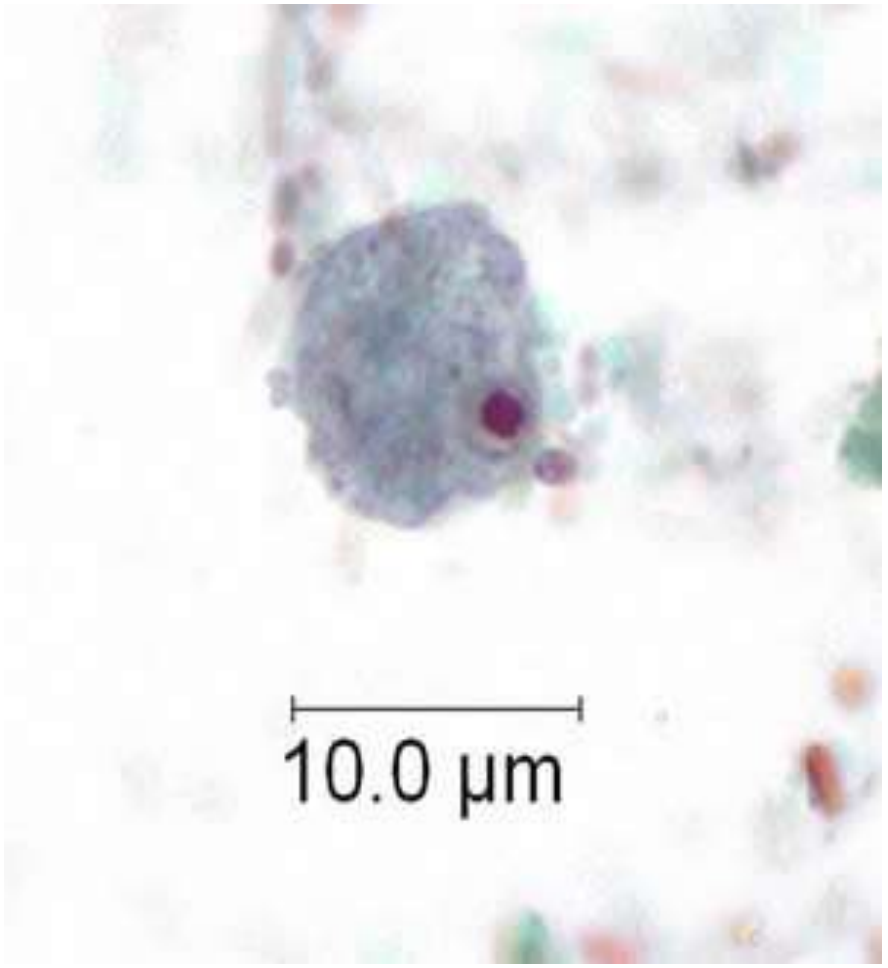
Entamoeba coli trophozoite



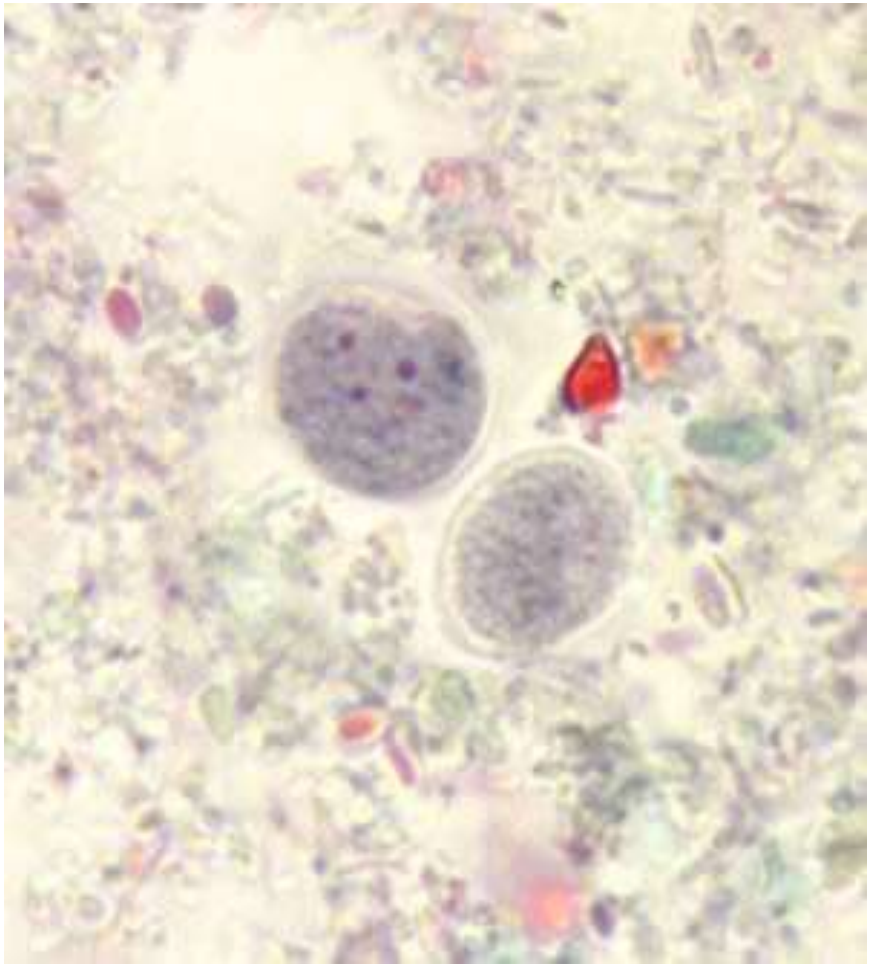


Cyst of *Entamoeba coli*



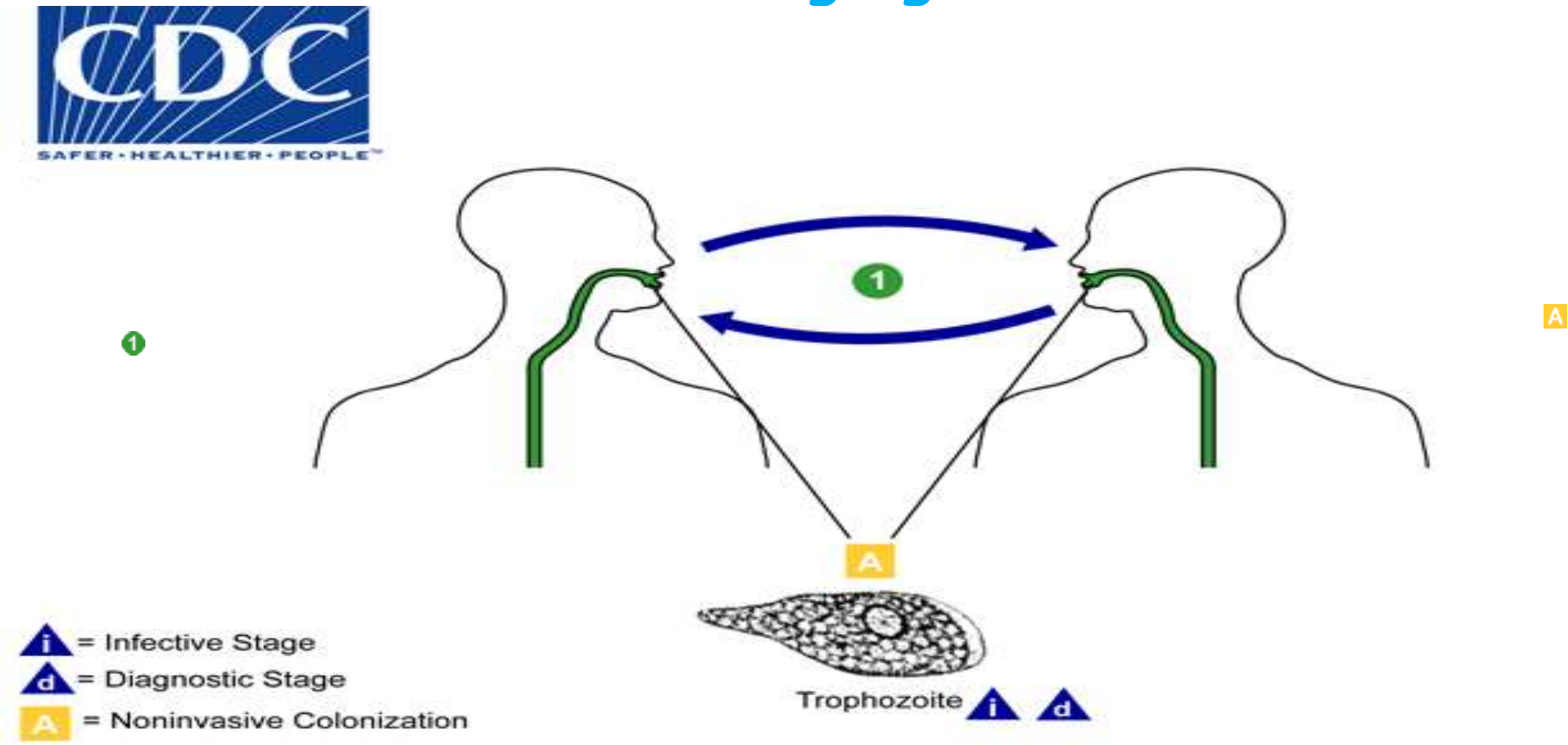


Trophozoite of *Endolimax nana*

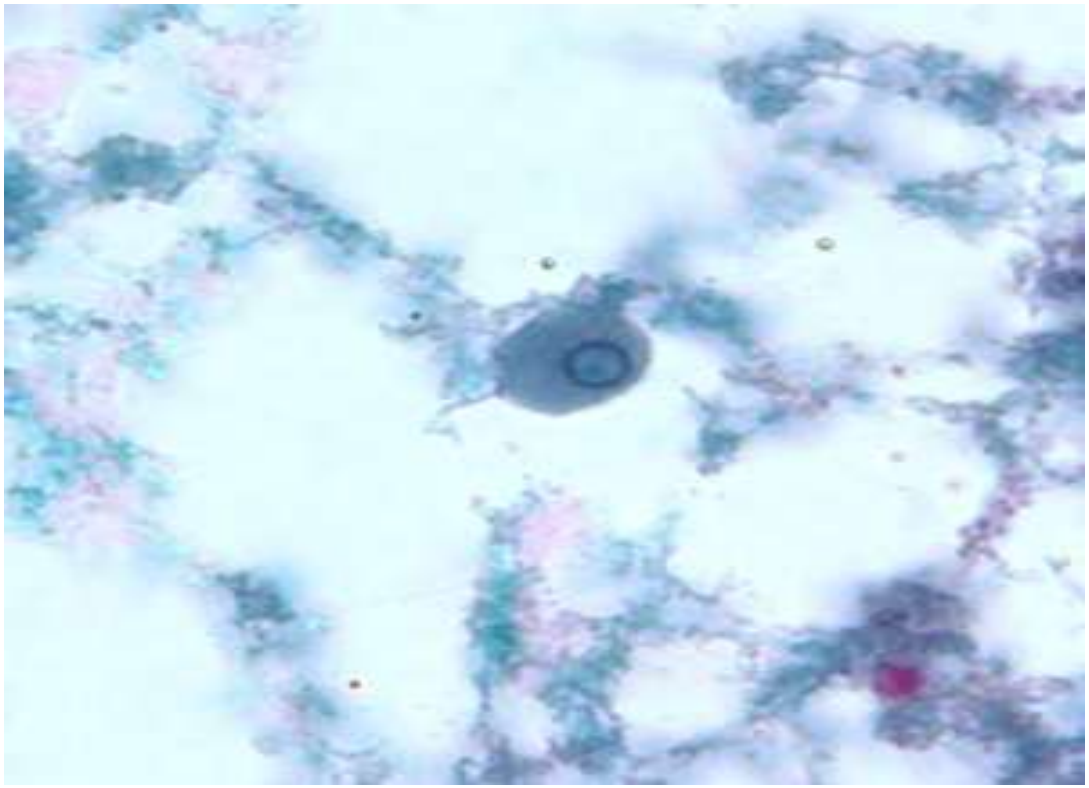


Cyst of *Endolimax nana*

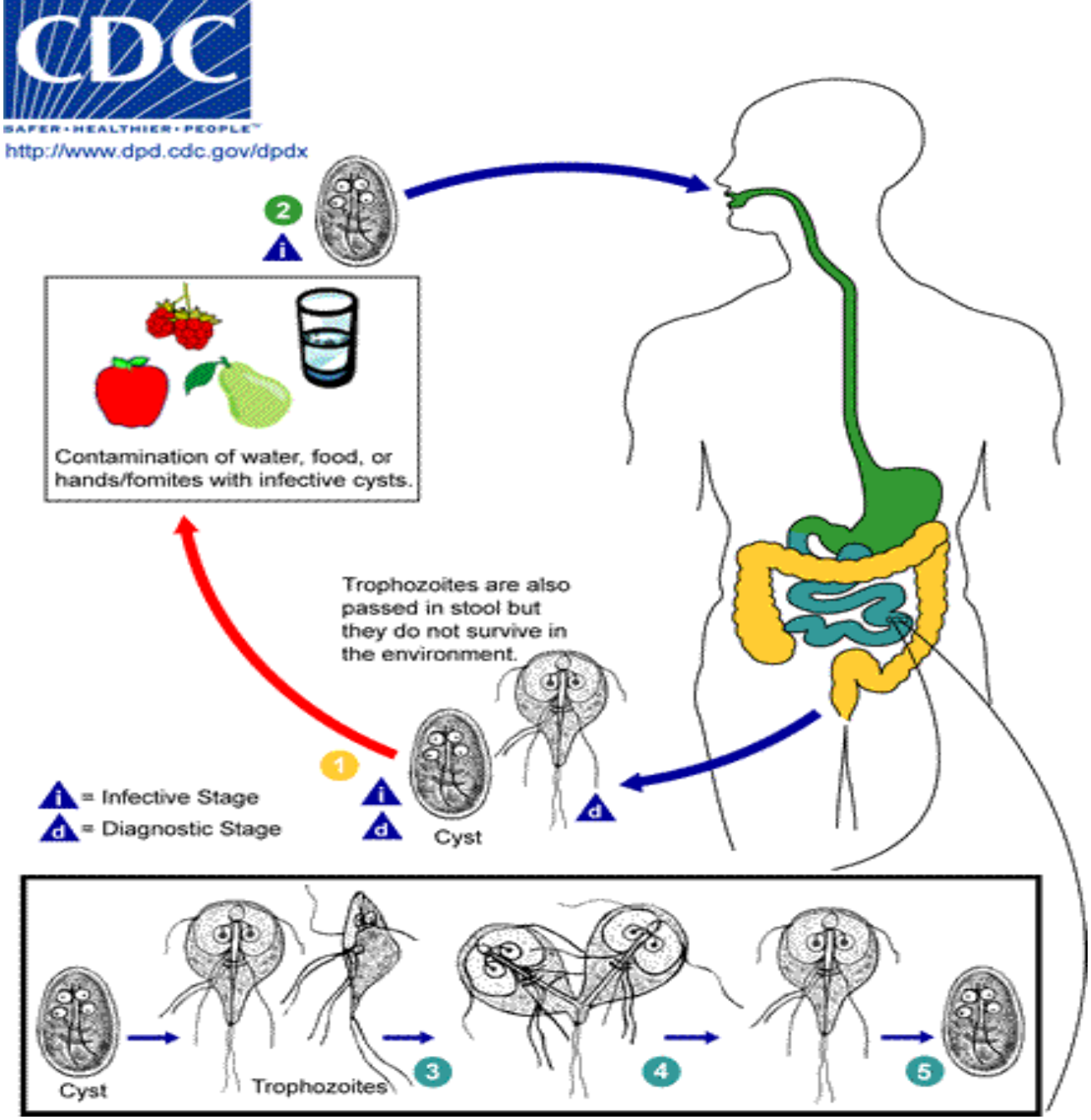
Entamoeba gingivalis



There is no known cyst stage for *Entamoeba gingivalis*; trophozoites live in the oral cavity of humans, residing in the gingival pockets near the base of the teeth. They are not considered pathogenic, and feed on bacteria and other debris. Trophozoites are transmitted person-to-person orally by kissing or fomites (such as eating utensils). The trophozoite stage of *E. gingivalis* is morphologically similar to that of *E. histolytica*, and the two should be differentiated, as both can be coughed up in sputum specimens (for the latter, when present in pulmonary abscesses).

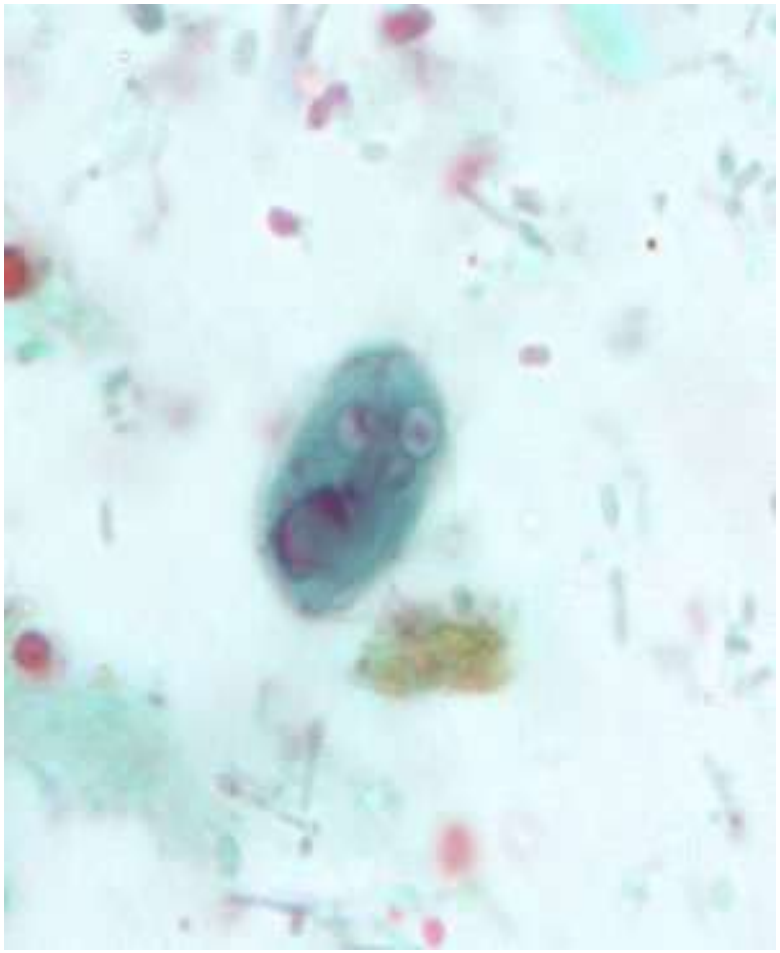


Trophozoite of *Entamoeba gingivalis* stained with trichrome



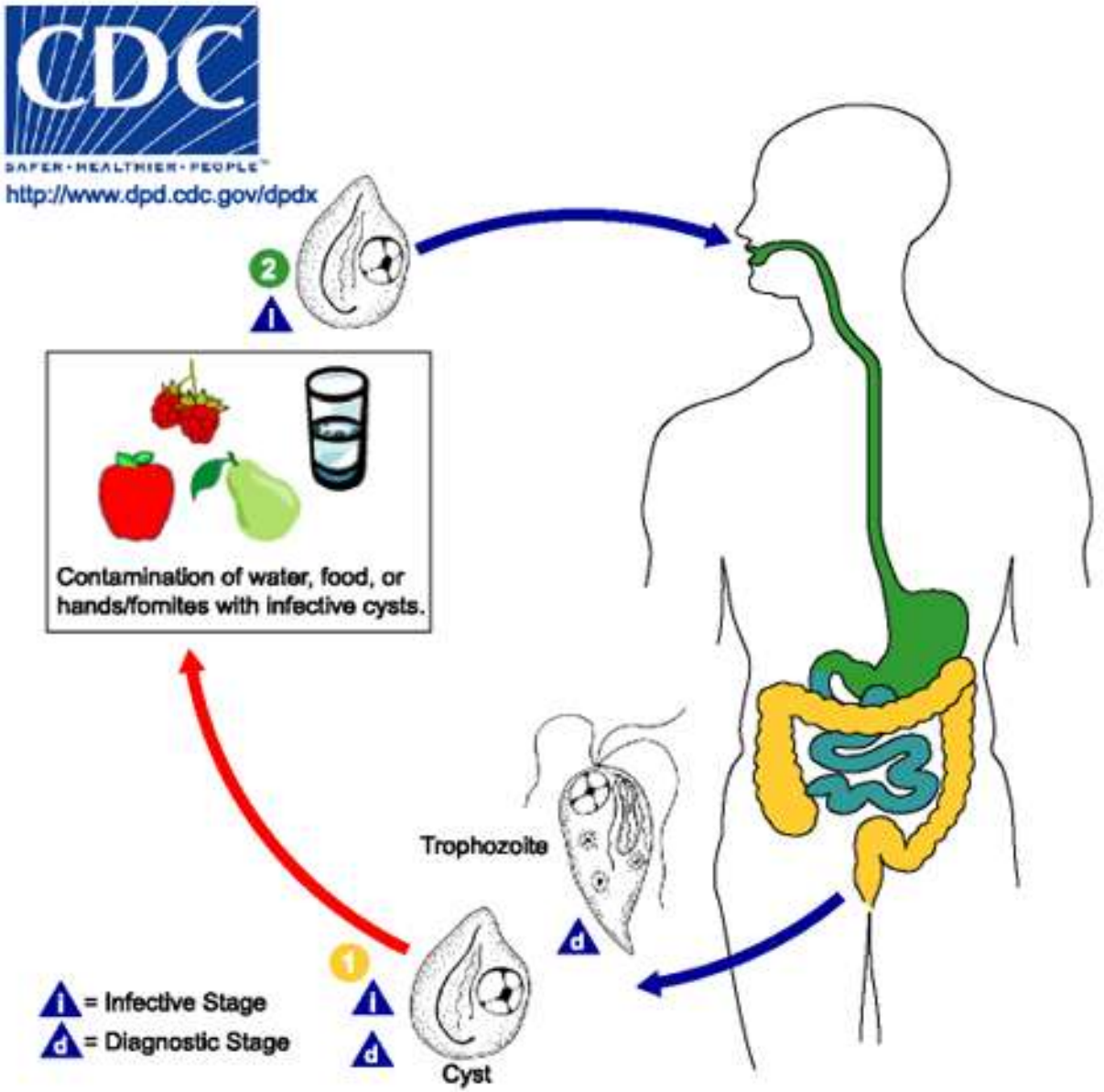
Life Cycle Of *Giardia lambilia(G. duodenalis)*

1

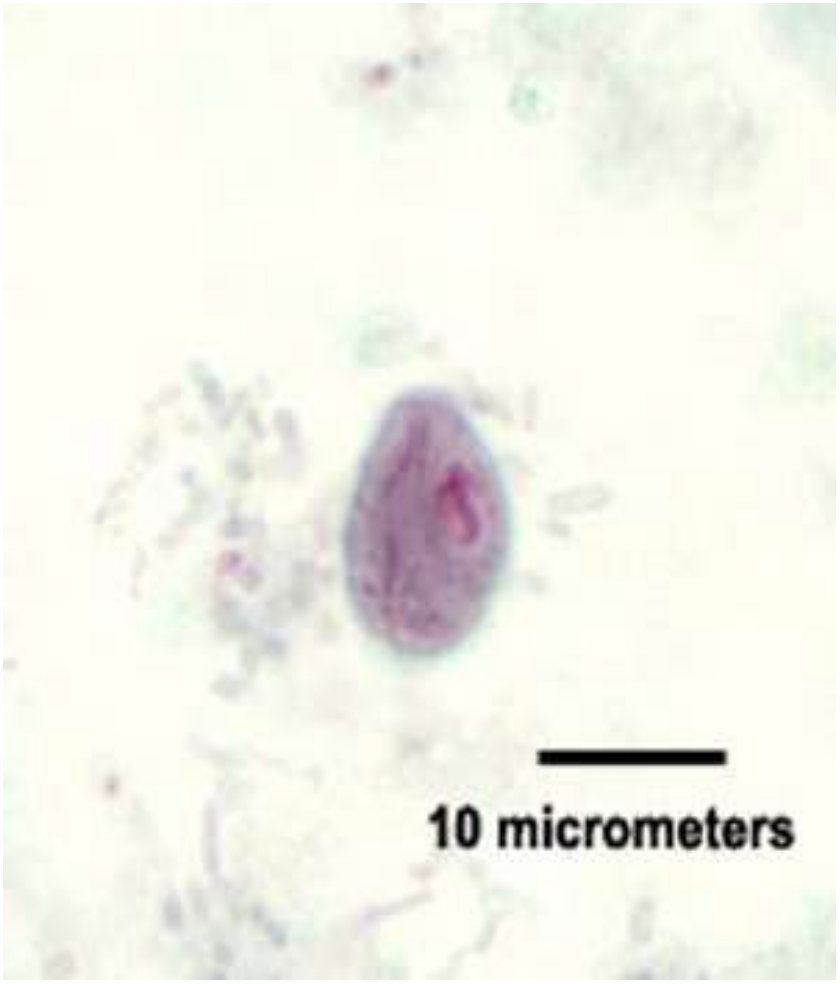


2

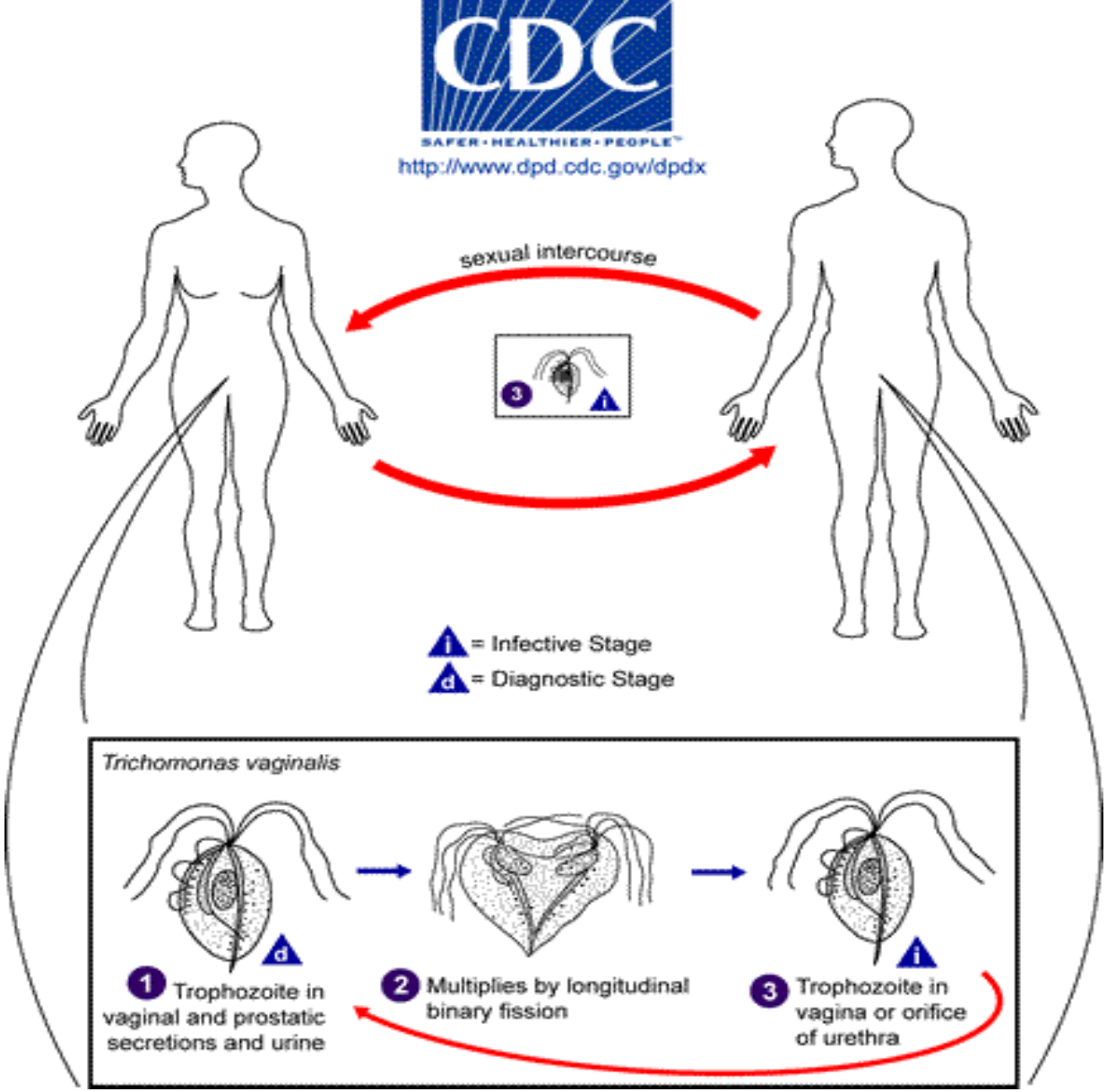
Giardia lambilia : 1-trophozoite 2- cyst



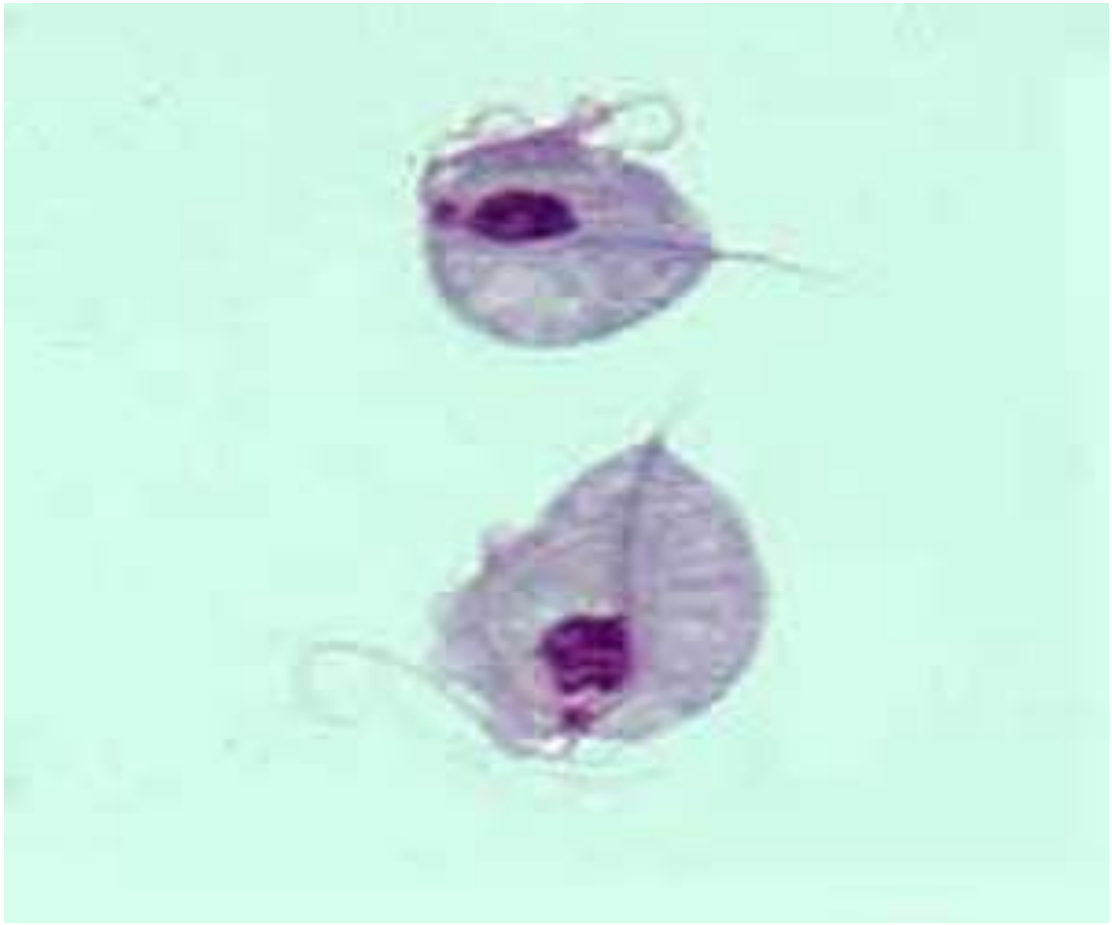
Life Cycle of *Chilomastix mesnili*



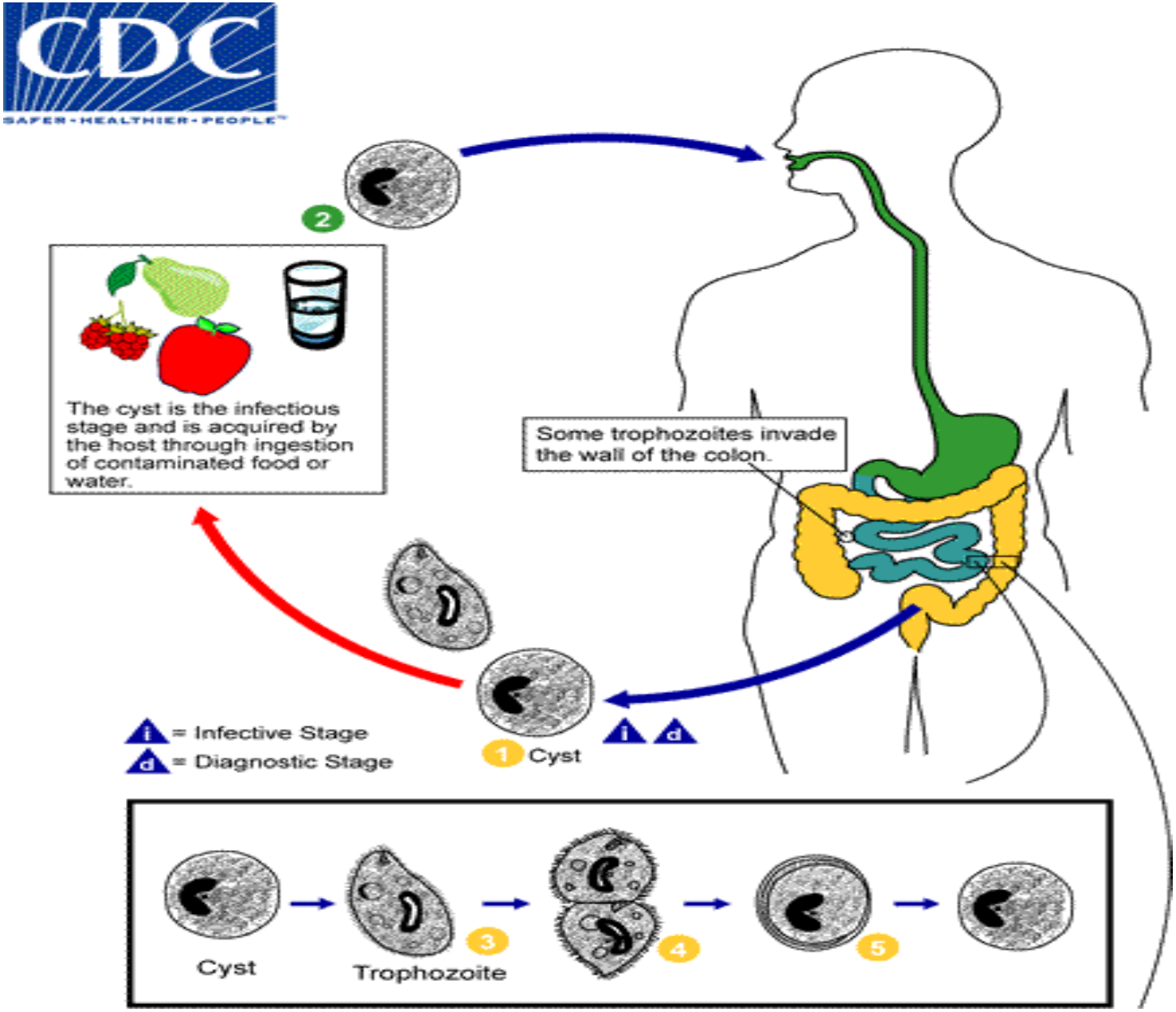
Left, *Chilomastix mesnili* trophozoite, silver stain**.**
Right*, C. mesnili* cyst*.*



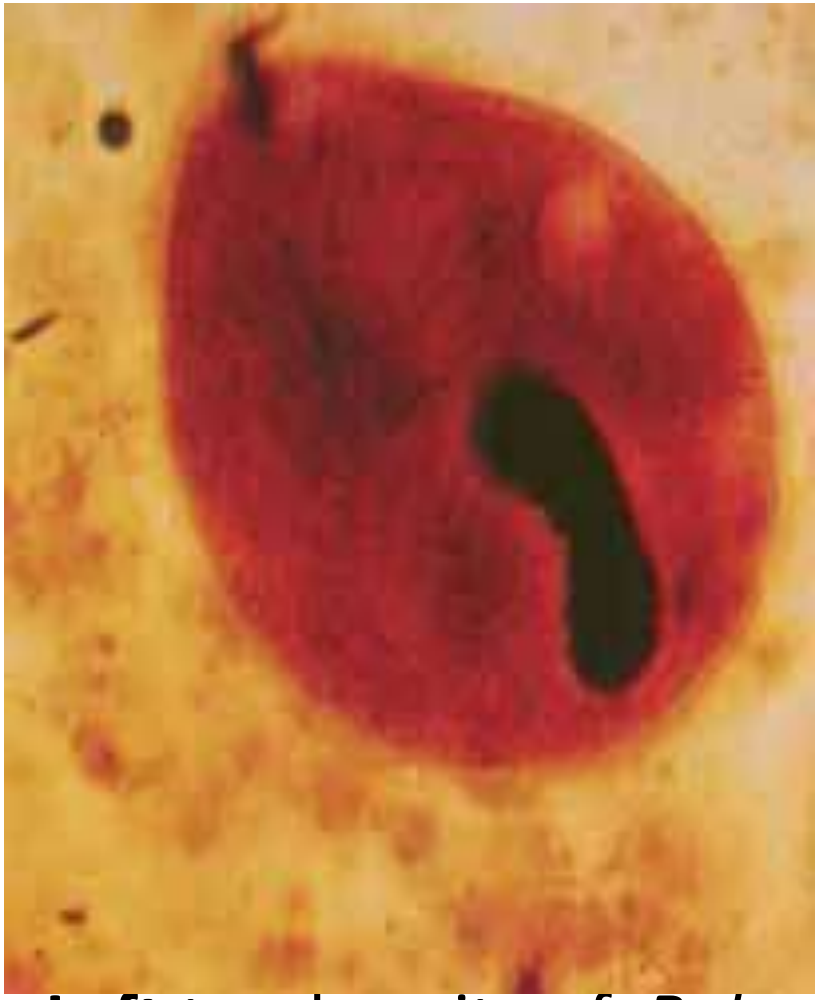
Life Cycle of *Trichomonas vaginalis*

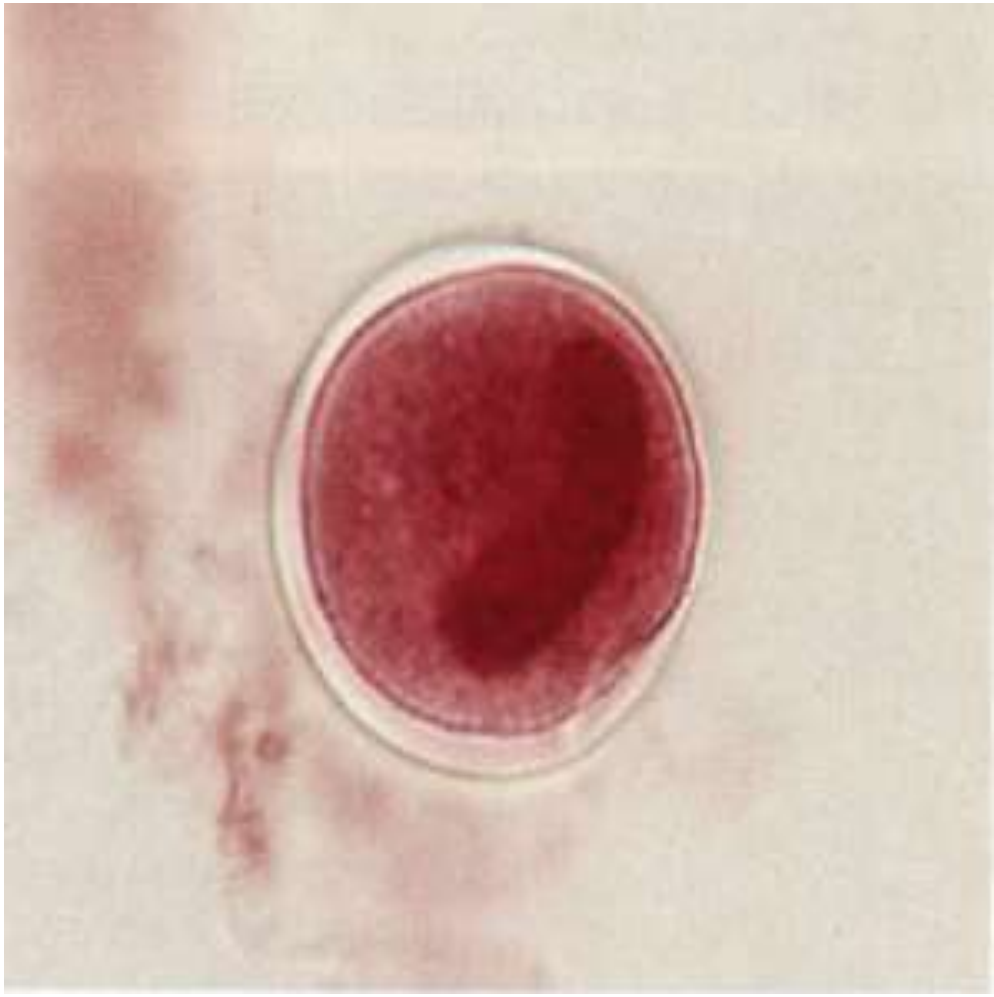


Trophozoite of *Trichomonas vaginalis*



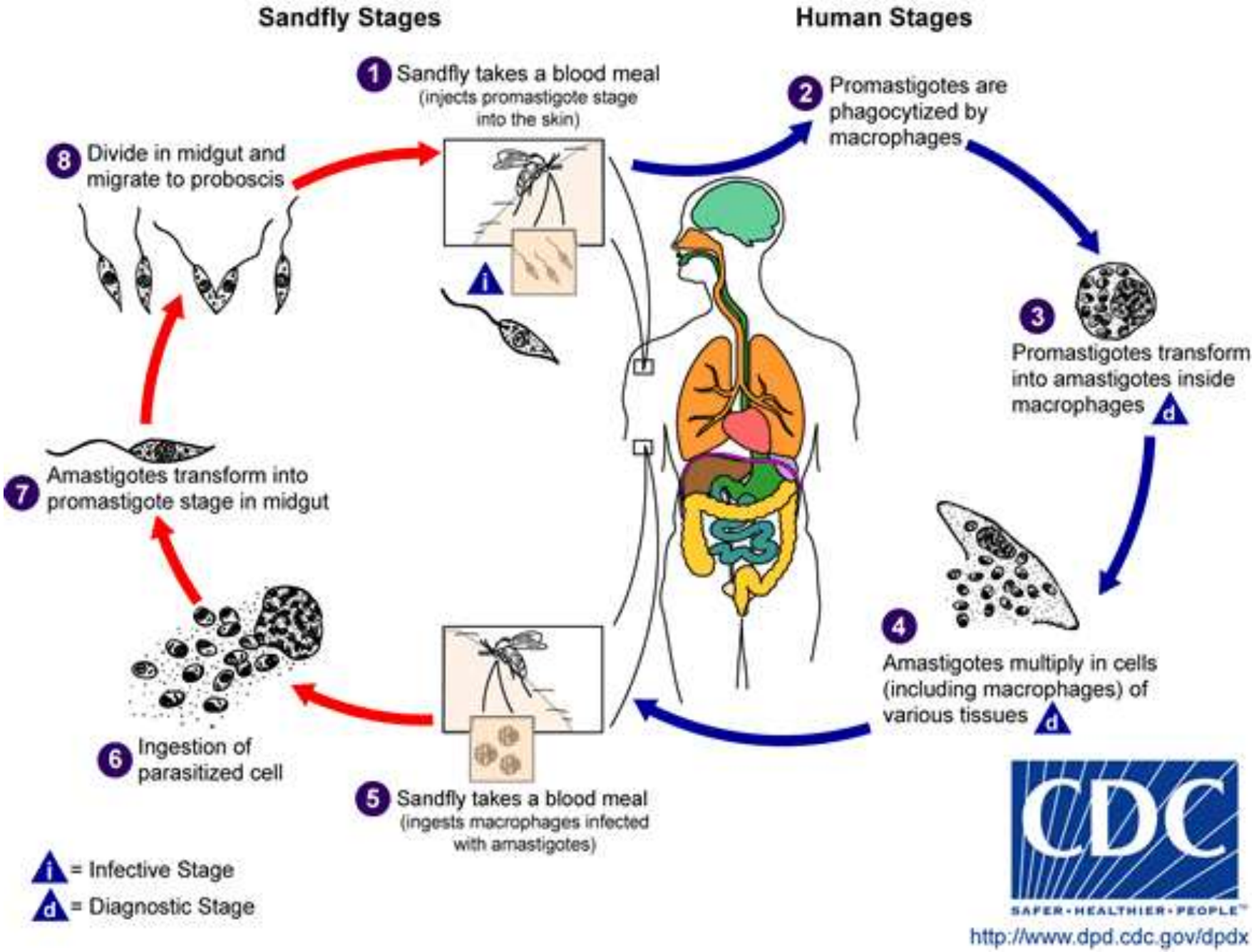
Life Cycle of *Balantidium coli*



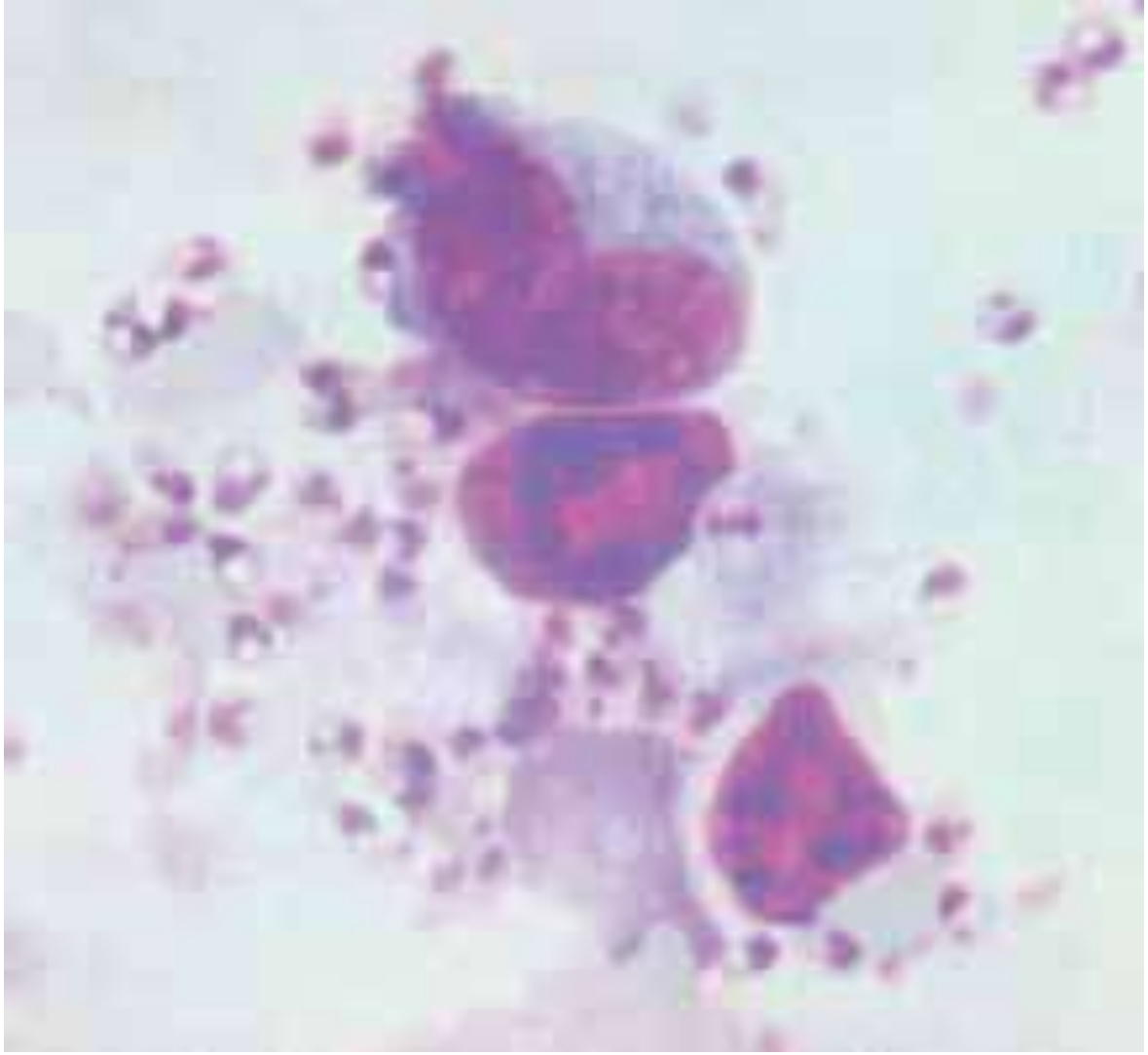


Left trophozoite of *Balantidium coli.*

Right cyst of *Balantidium coli*

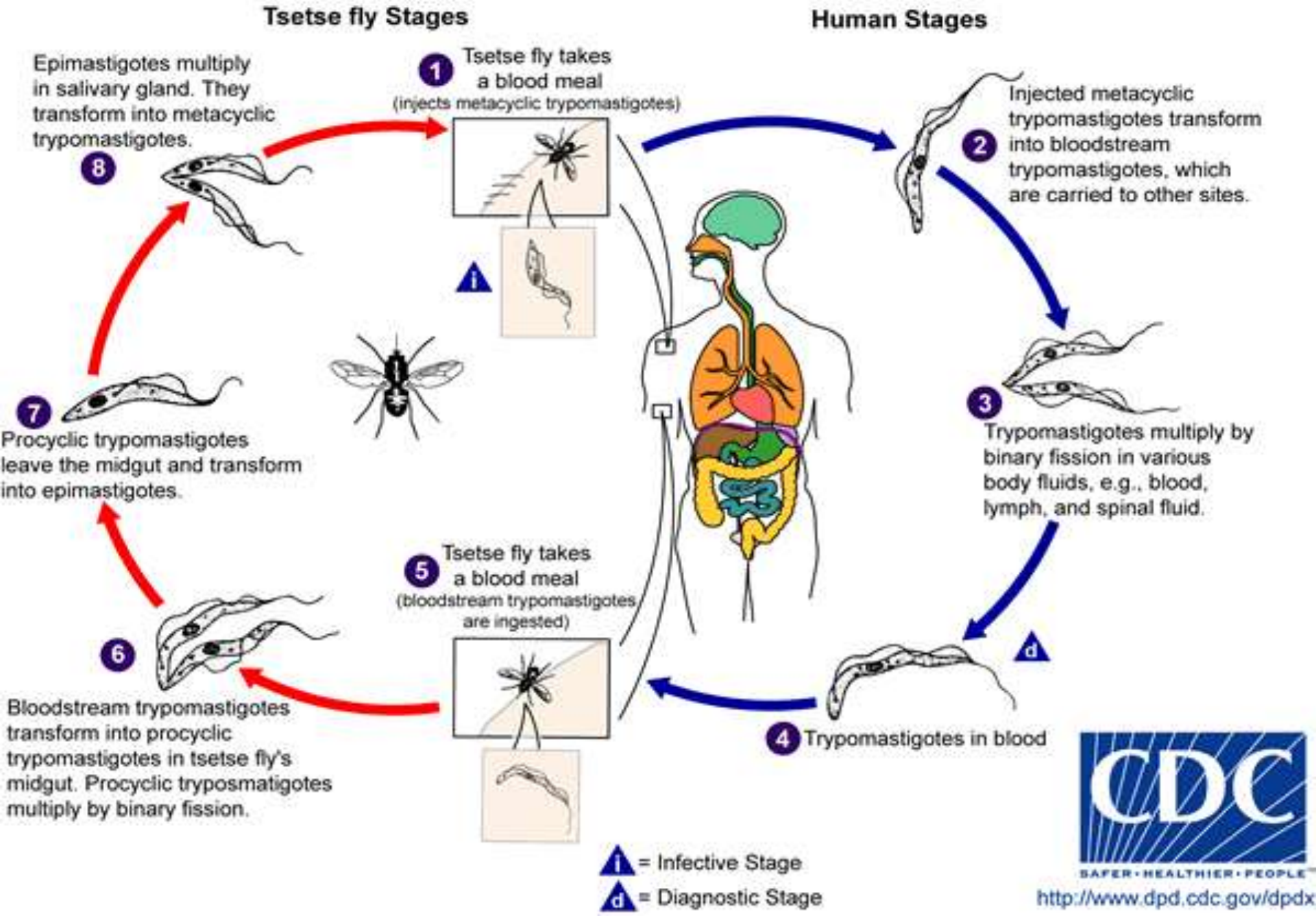


Life cycle of *Leishmania*

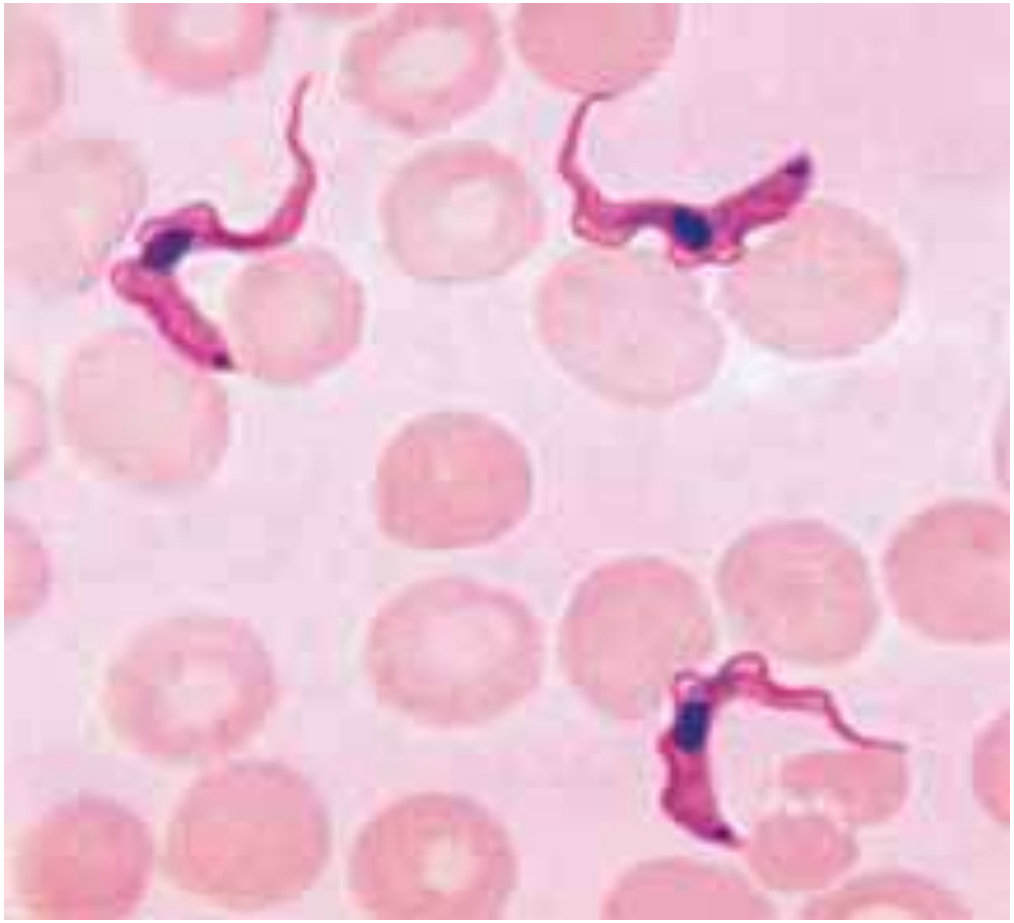


Leishmania donovani amastigotes.

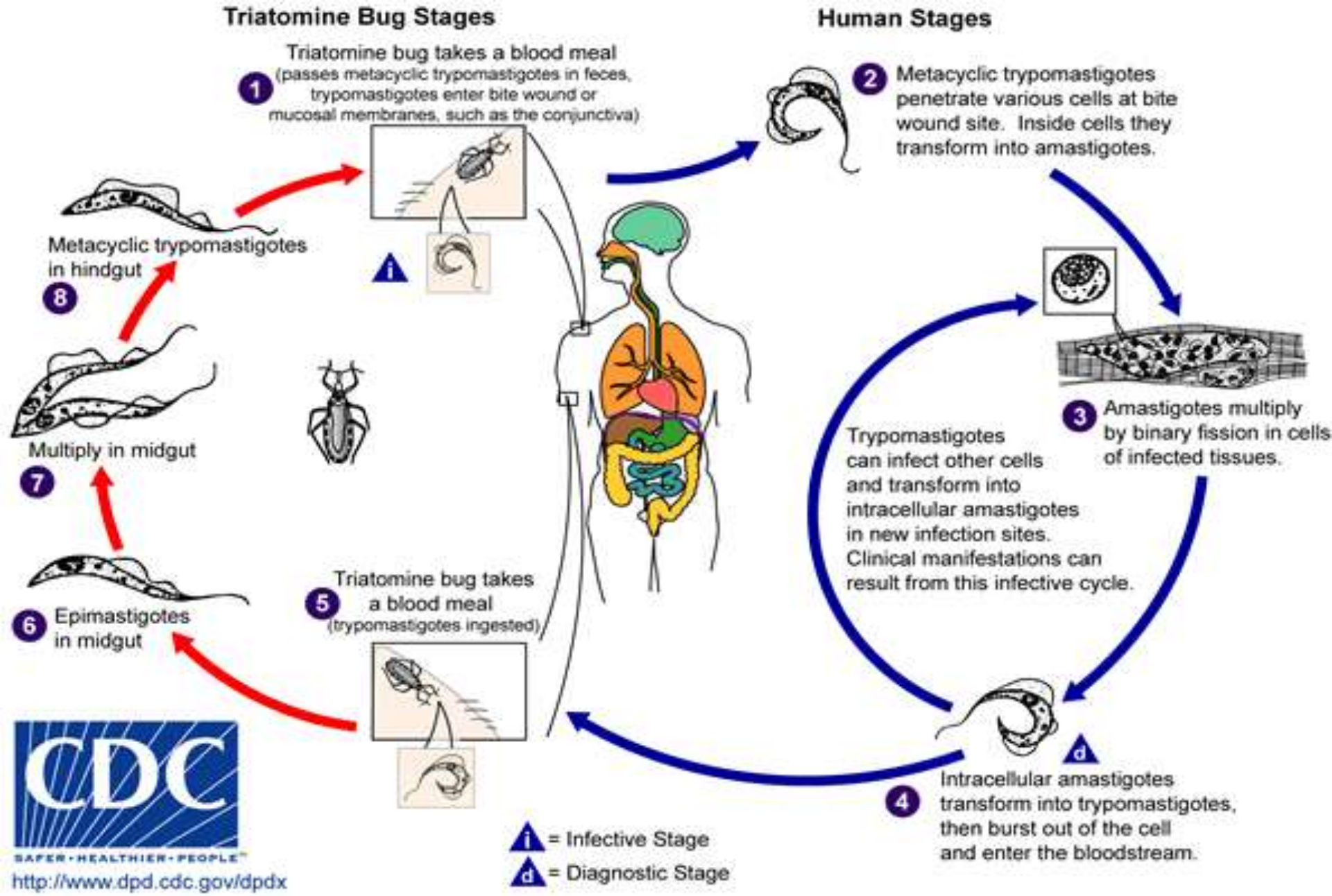
Lieshmania spp. Promastigote in culture stained with Giemsa stain



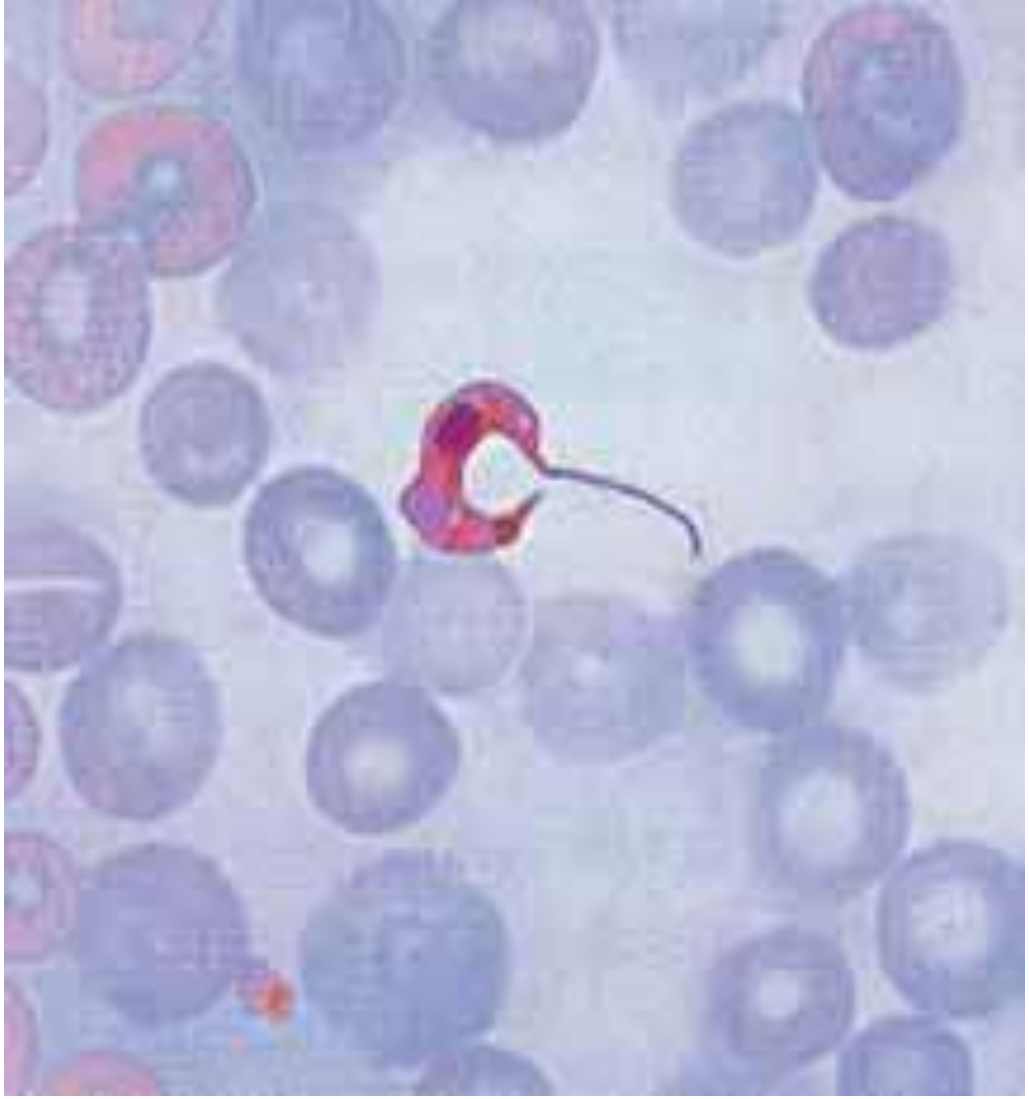
Life cycle of African Trypanosomiasis



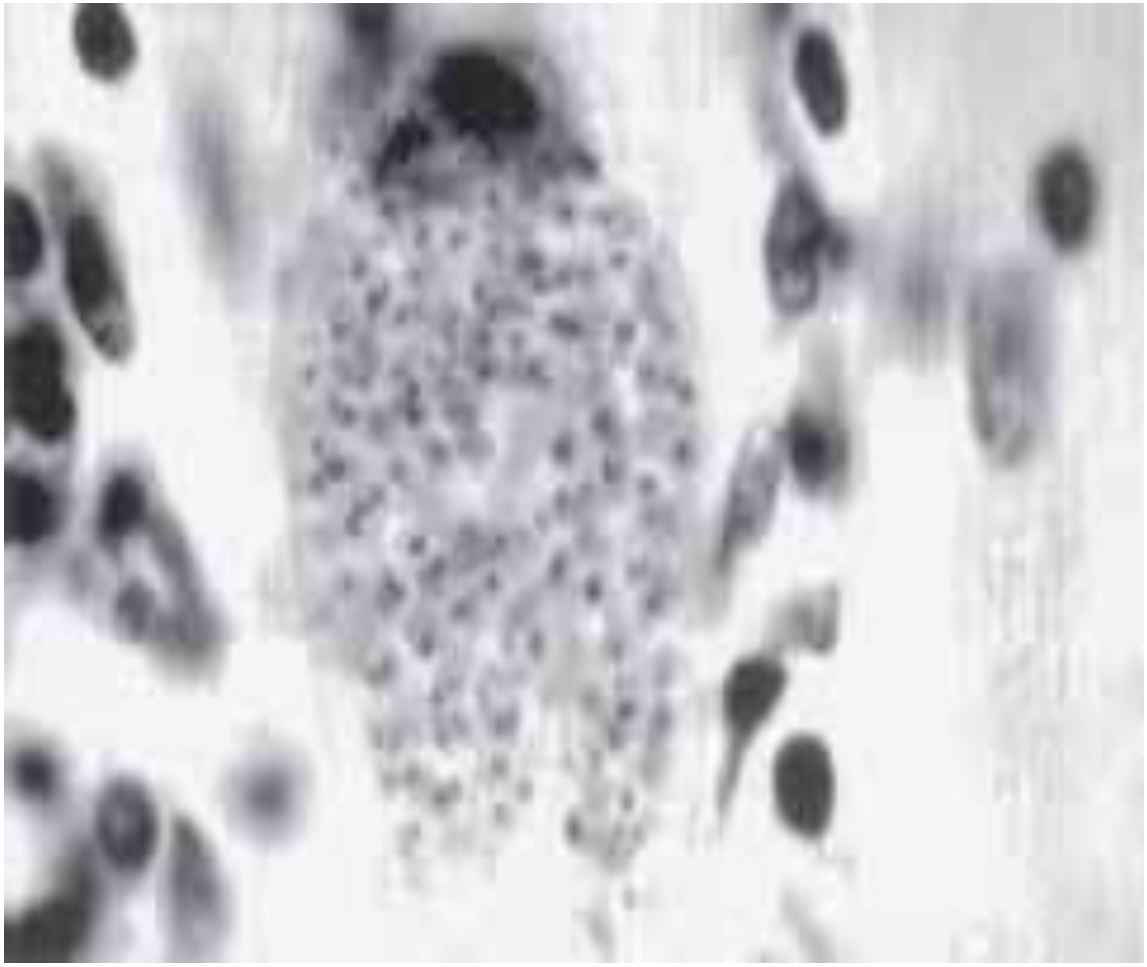
Trypanosoma gambiense trypomastigote in blood film.



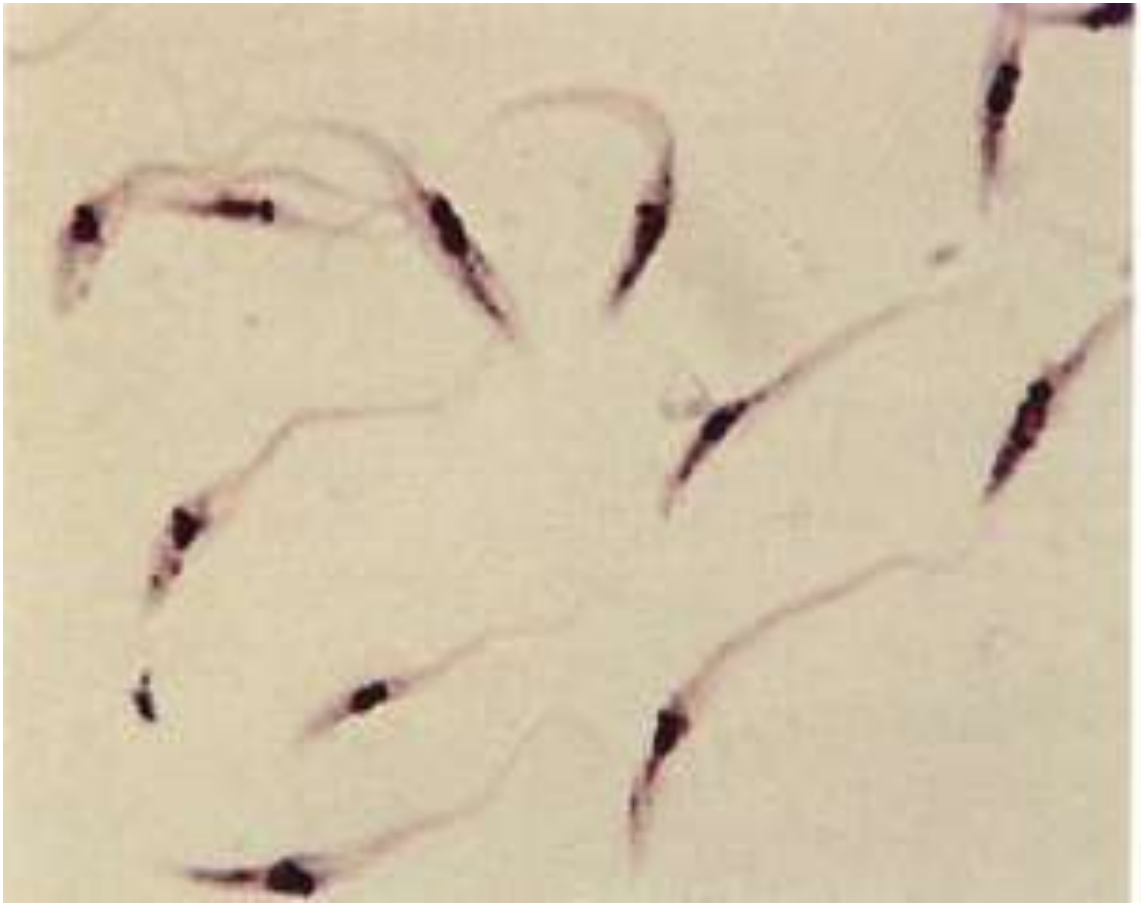
Life cycle of American Trypanosomiasis



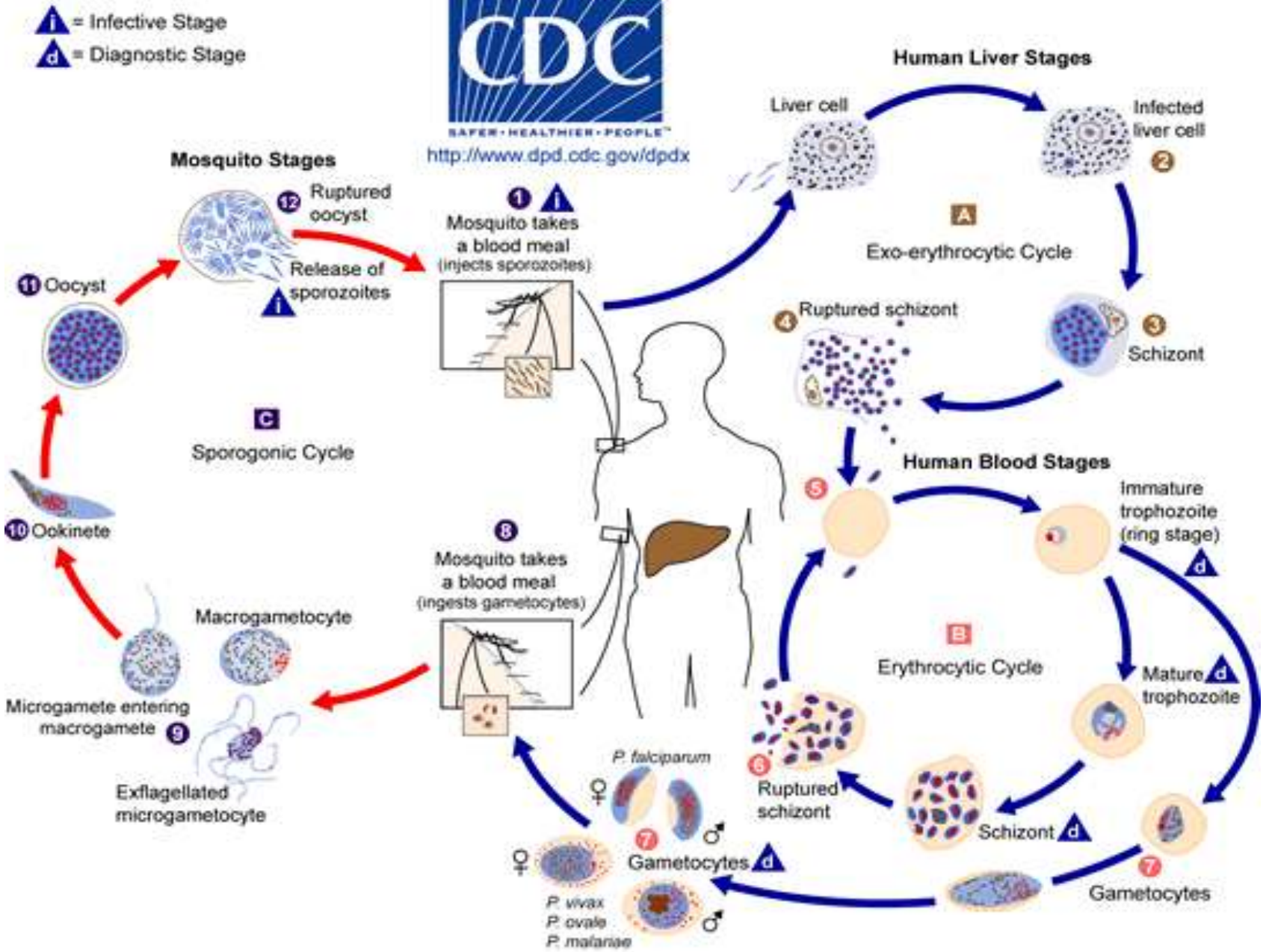
Trypanosoma cruzi trypomastigote.



Trypanosoma cruzi amastigote parasites in cardiac muscle (2500×



Promastigote of *Trypanosoma cruzi* grown in NNN media



Life Cycle of *Plasmodium* parasite

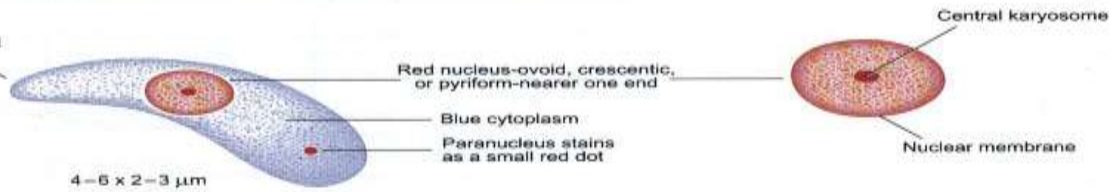
Tissue protozoa

Toxoplasma gondii

Toxoplasma has a very wide mammalian host range.

Morphology

Tachyzoite



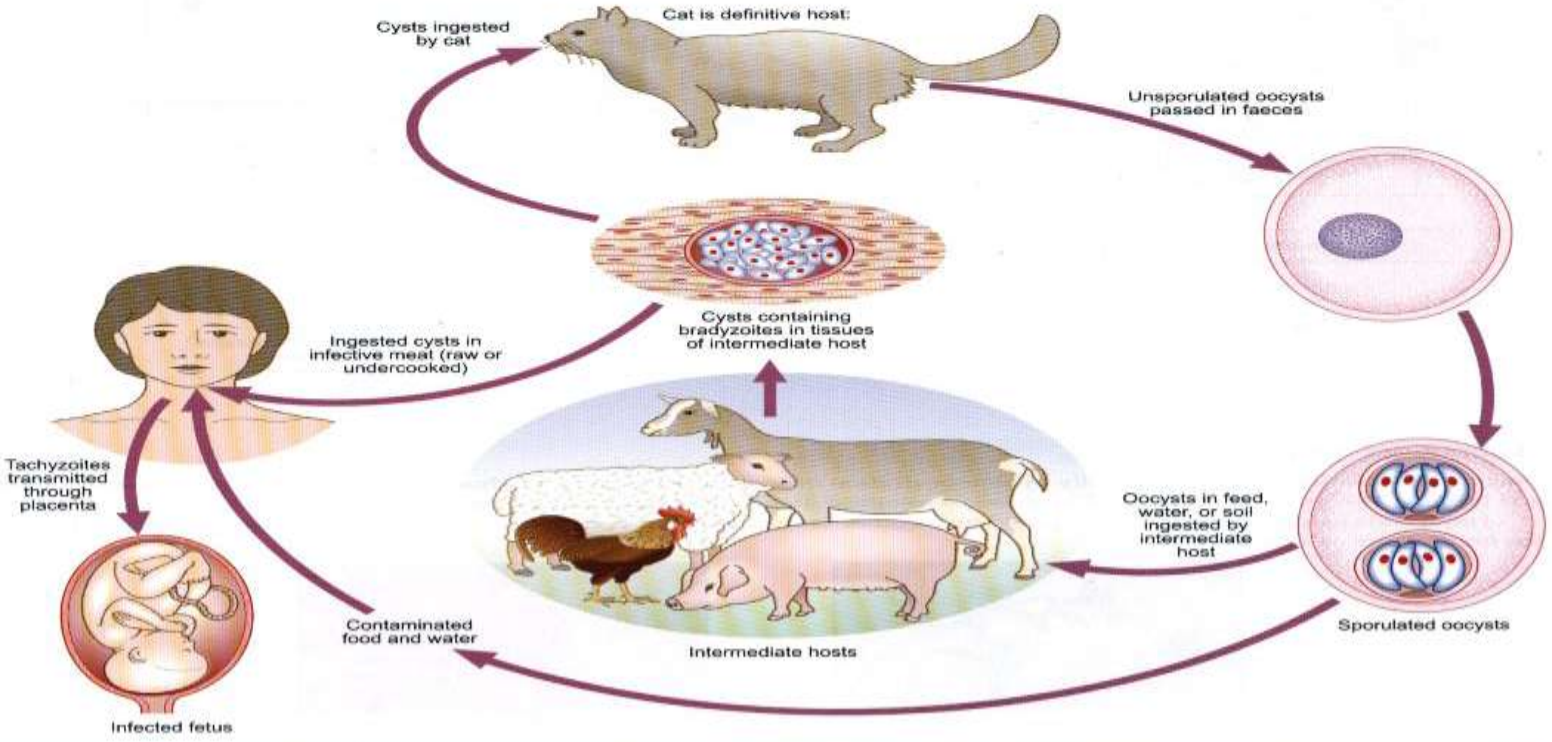
Habitat

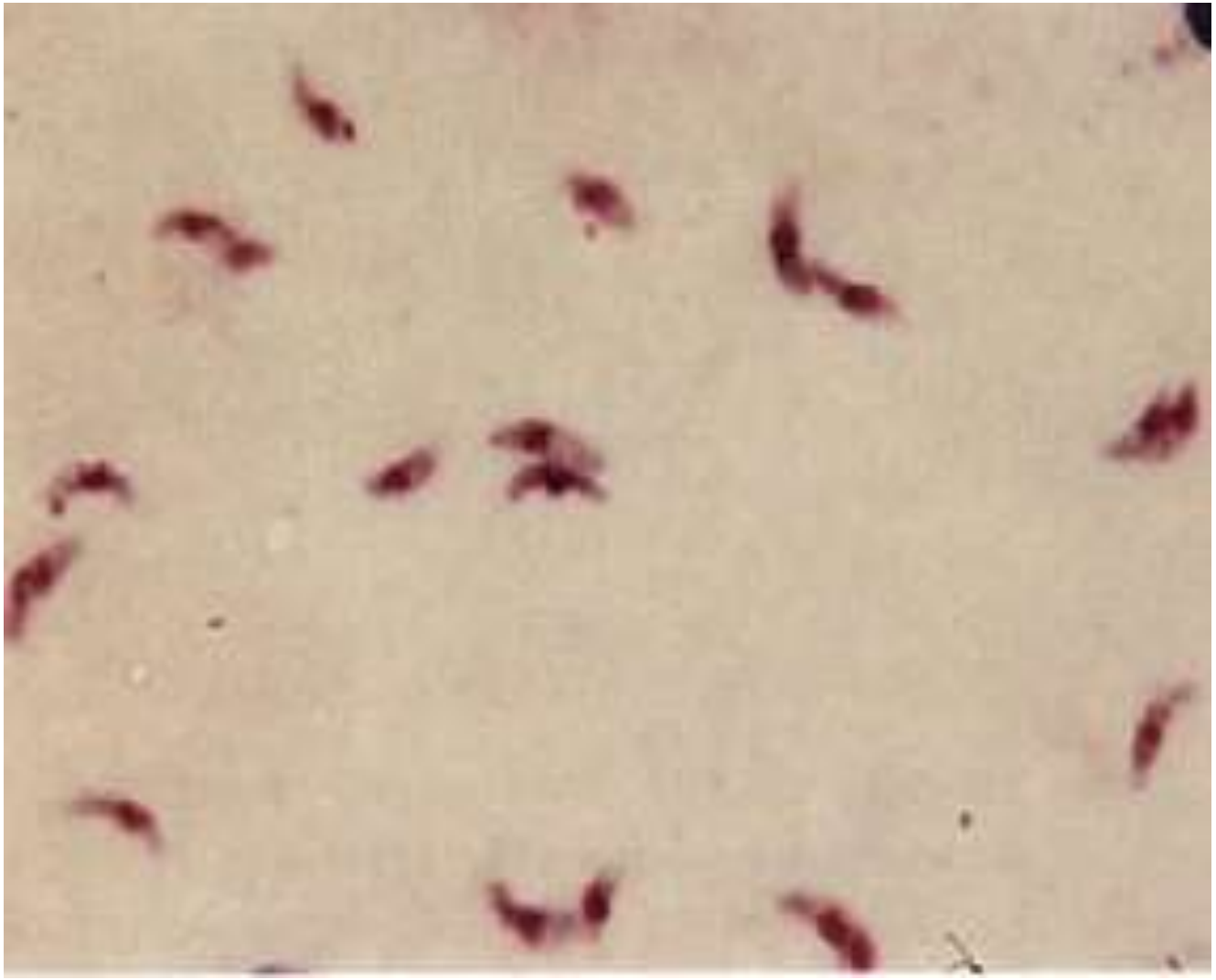
Tachyzoites: single (free or intracellular) or in masses (pseudocysts)

In nucleated cells, especially macrophages

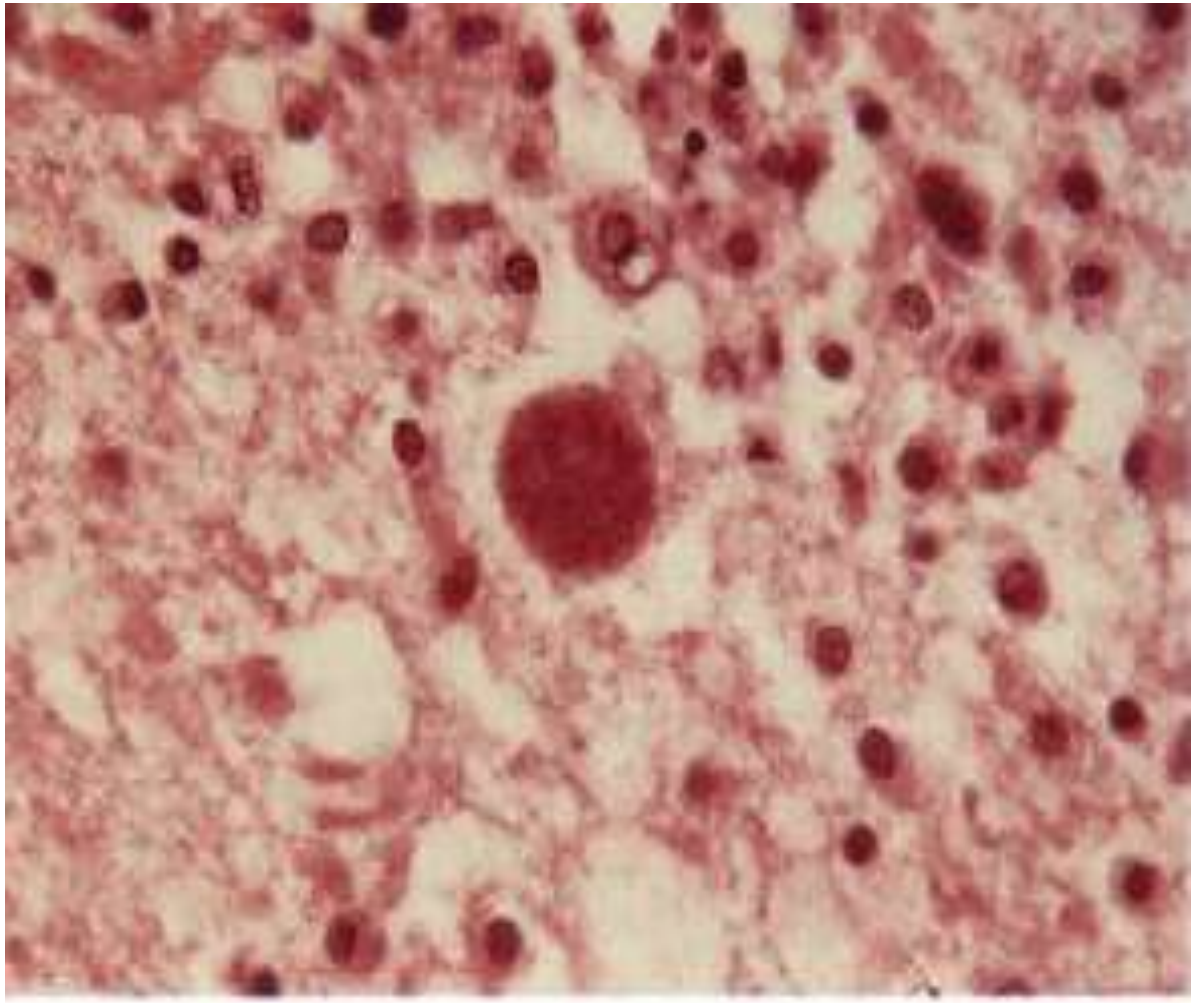
Bradyzoites (similar to tachyzoites but less active metabolically) in tissue cysts

Life cycle





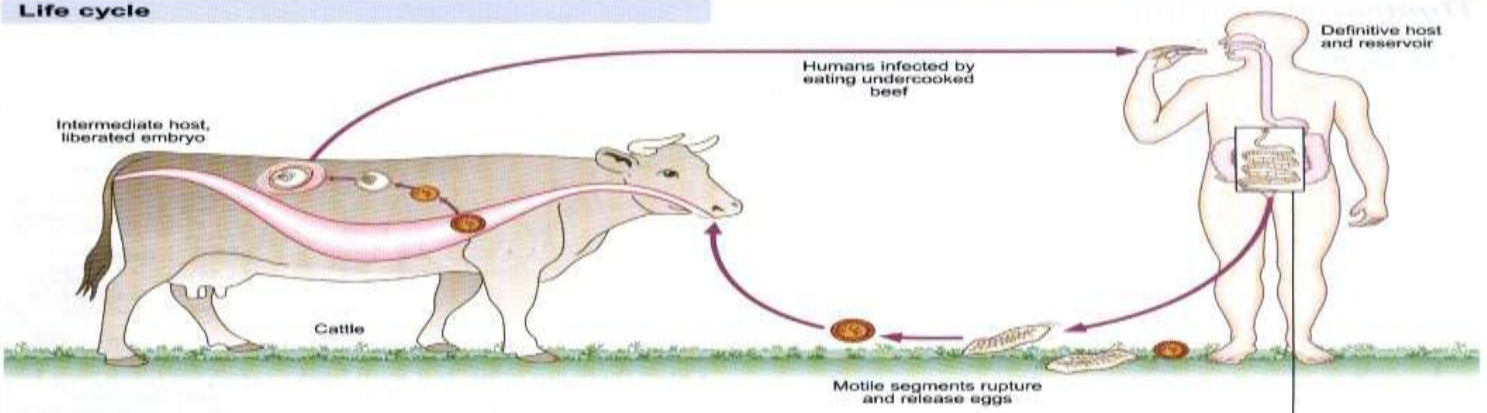
15-33 *Toxoplasma gondii*. Trophozoites. Cul-
ture. Giemsa stain (×1250). Trophozoites of T. gondii

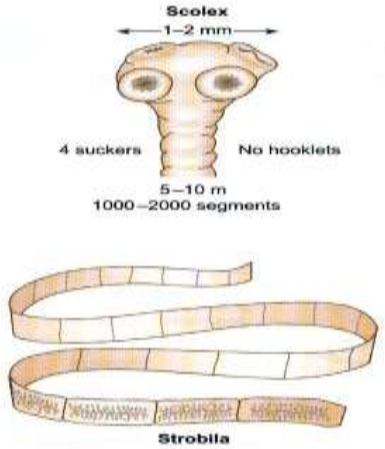


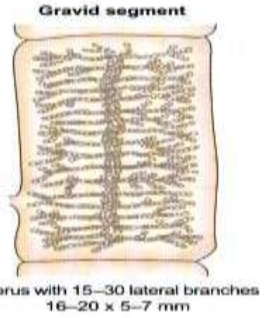
Toxoplasma gondii. Cyst. Brain.

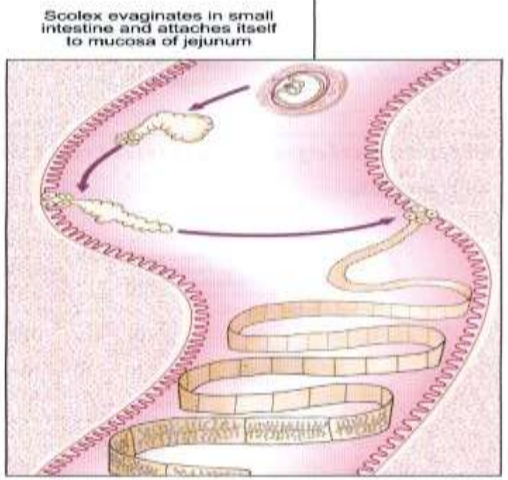
Taenia saginata (beef tape worm)

Life cycle









Pathology and Clinical features

Usually there is no pathology as *Cysticercus bovis* is unknown in humans. Occasionally there is vague alimentary upset.

Laboratory diagnosis

Gravid segments, ova and scolex can be found in faeces. Uterine branches of the mature segments may be seen in a crush preparation between two glass slides, or by Indian ink preparation, as in *T. solium*. Ova are also found on the perianal skin (on clear adhesive tape slides).

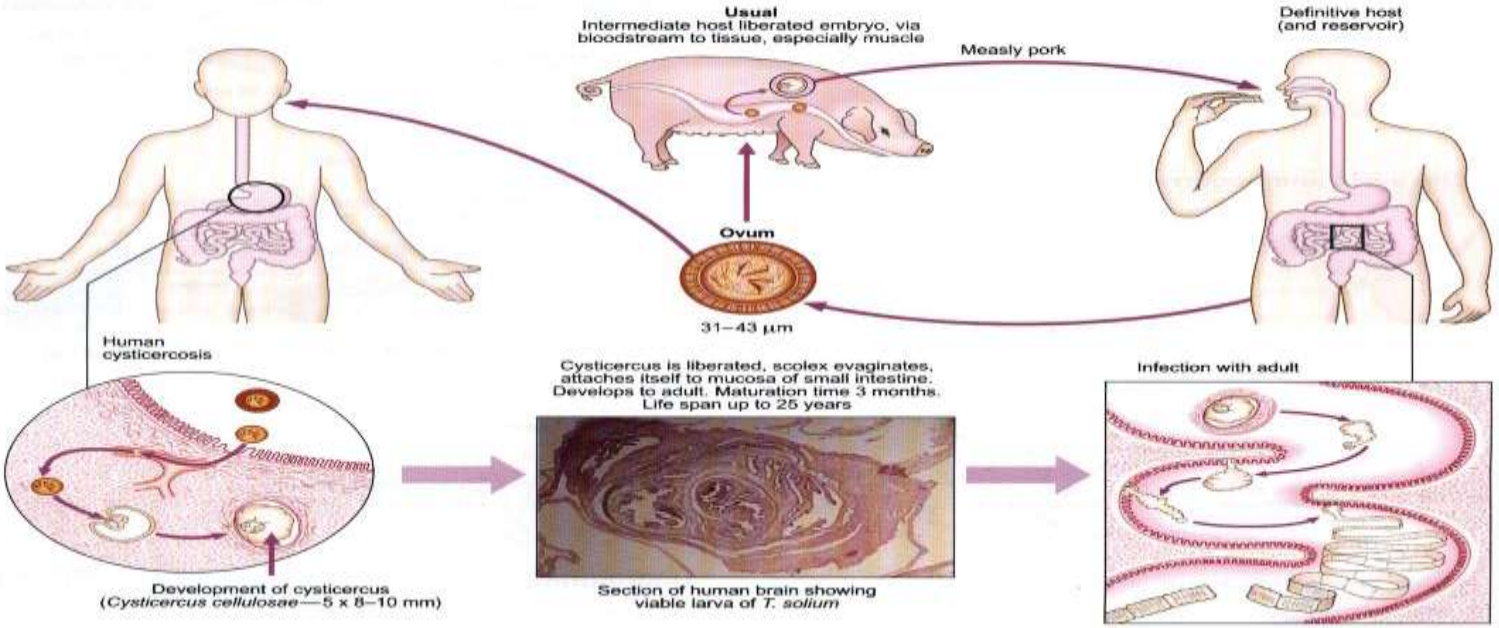
Distribution

Taenia saginata is found in beef-eating areas, especially in the tropics.

Cestode (tape) worms

Taenia solium (pork tape worm)

Life cycle



Pathology and Clinical features

Infection by larvae (cysticercosis). Cysticerci, generally multiple, may occur in any site but are more frequent in the brain and muscle. They excite reaction in the area, especially when they die, which manifests as inflammation, fibrosis and later some calcification. This leads to focal CNS syndromes, especially epilepsy.

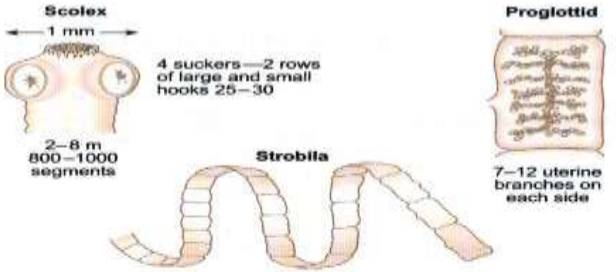
Infection with adults. Often there can be no pathology, but there might be mild irritation of intestinal mucosa.

Laboratory diagnosis

Eosinophilia.

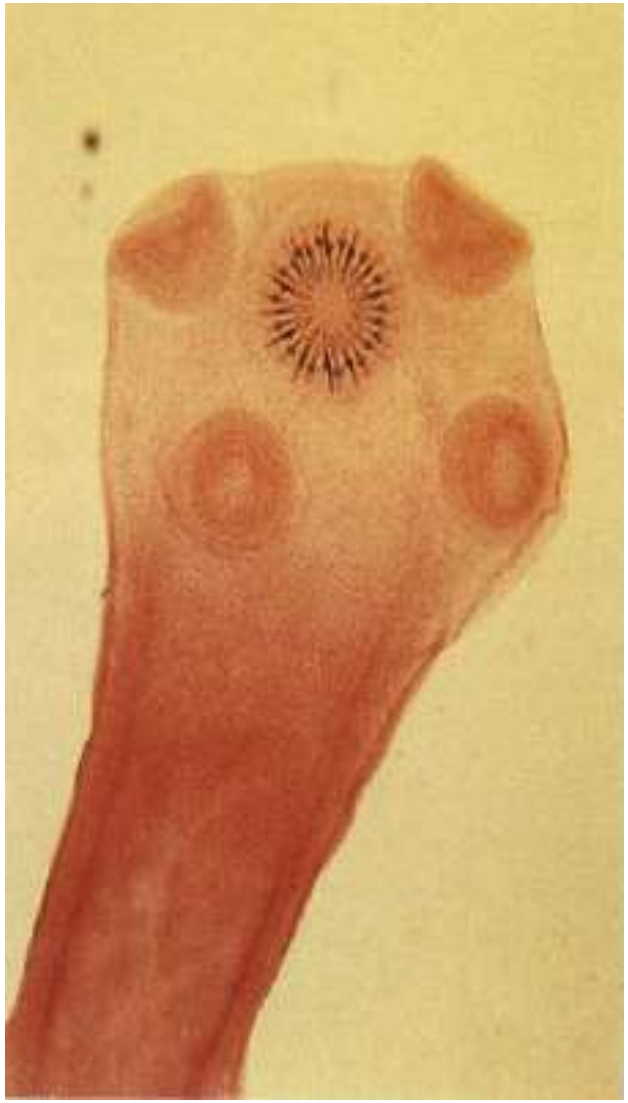
Larval infections. There are several methods, including histological examination of biopsy material, serology (IFAT, ELISA, EITB) and radiology (CT or MRI scan of the brain, X-ray of the thigh muscles).

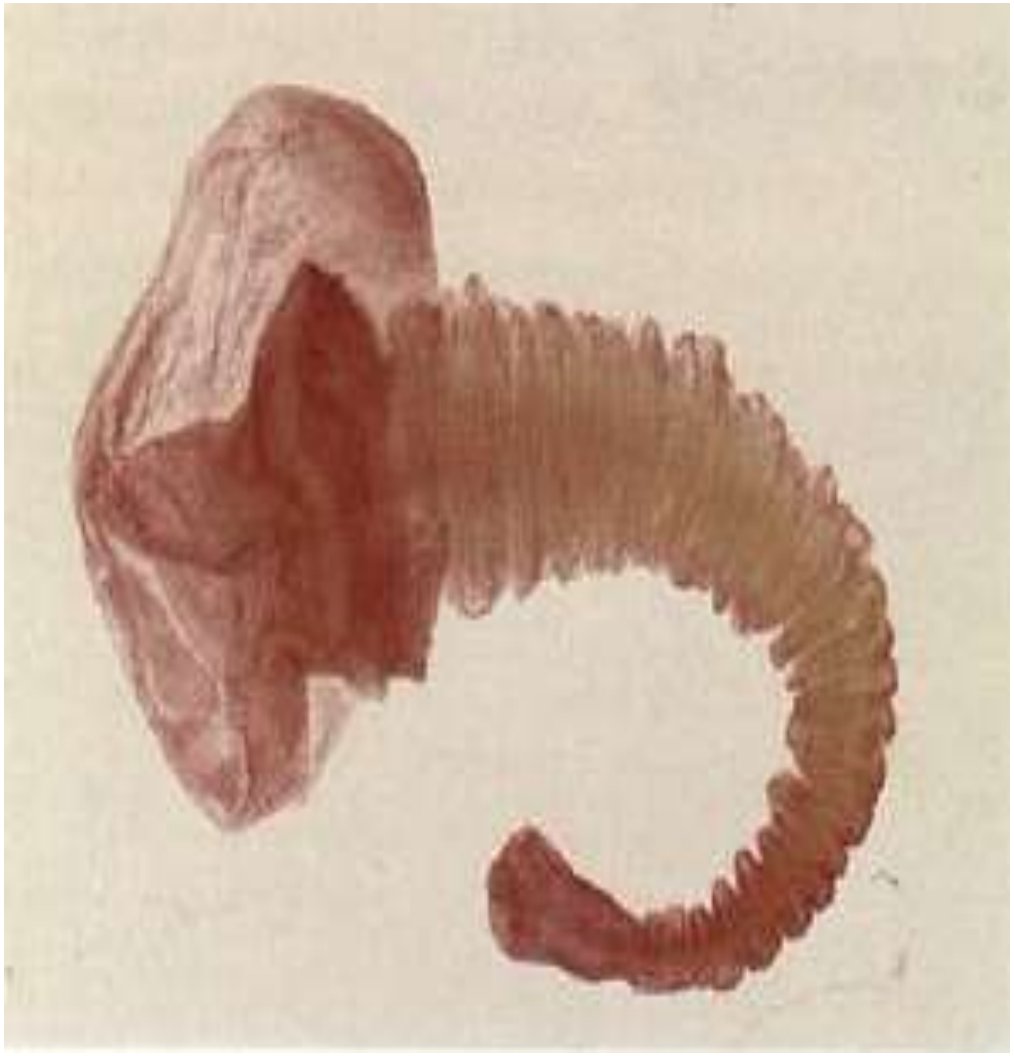
Pure infection with the adult. Gravid segments, ova and scolex can be found in faeces. The uterine branches of the mature segments can be demonstrated by injection of Indian ink through the uterine pore.



Distribution

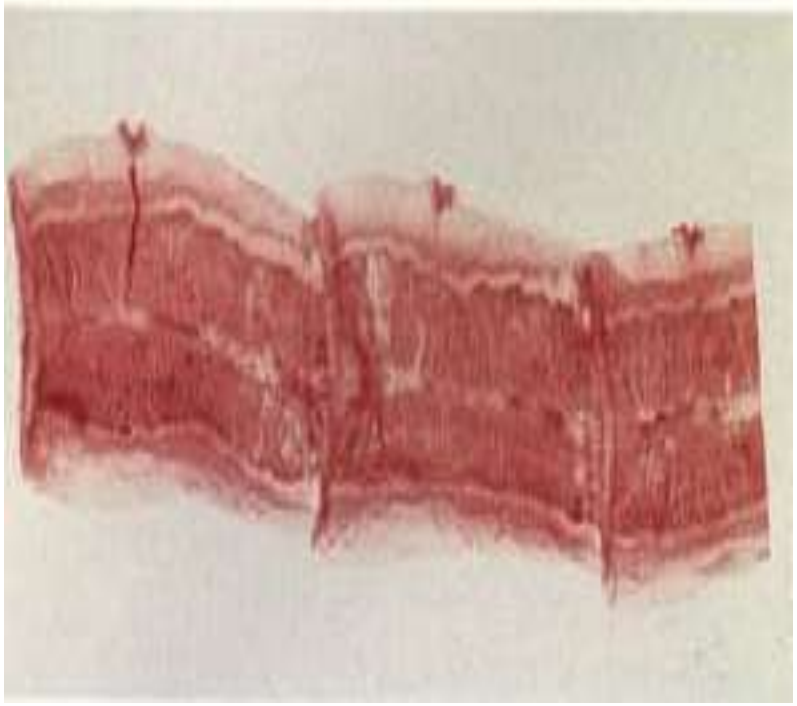
5 million people infected worldwide. *Taenia solium* is endemic in pig-rearing areas of the world where hygiene and animal husbandry are poor.

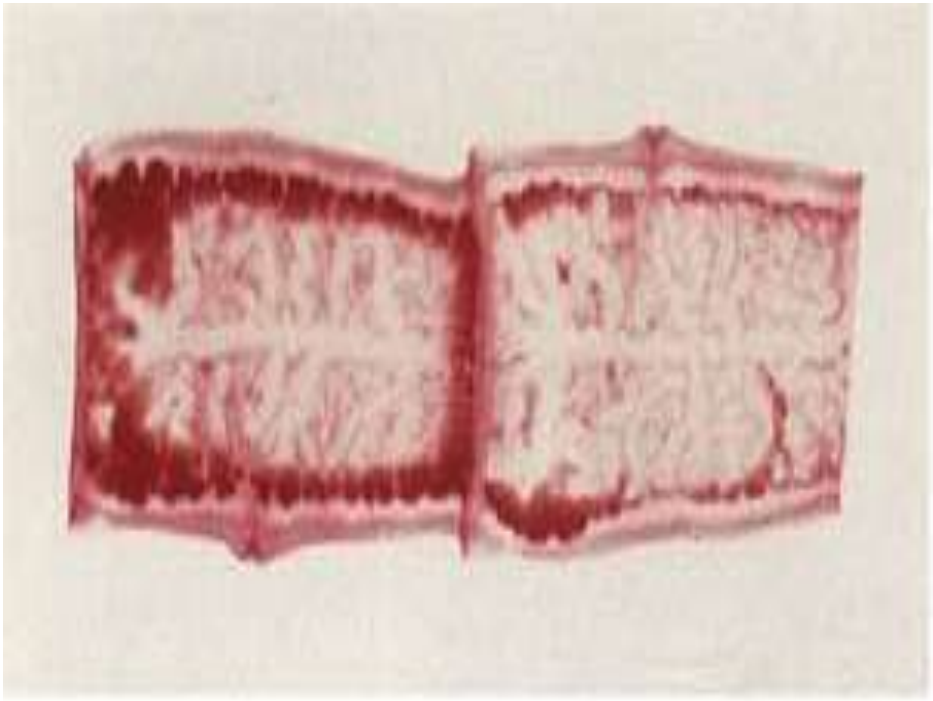




A B

Taenia Solium: A- Scolex B- *Cystisercus cellulosae*

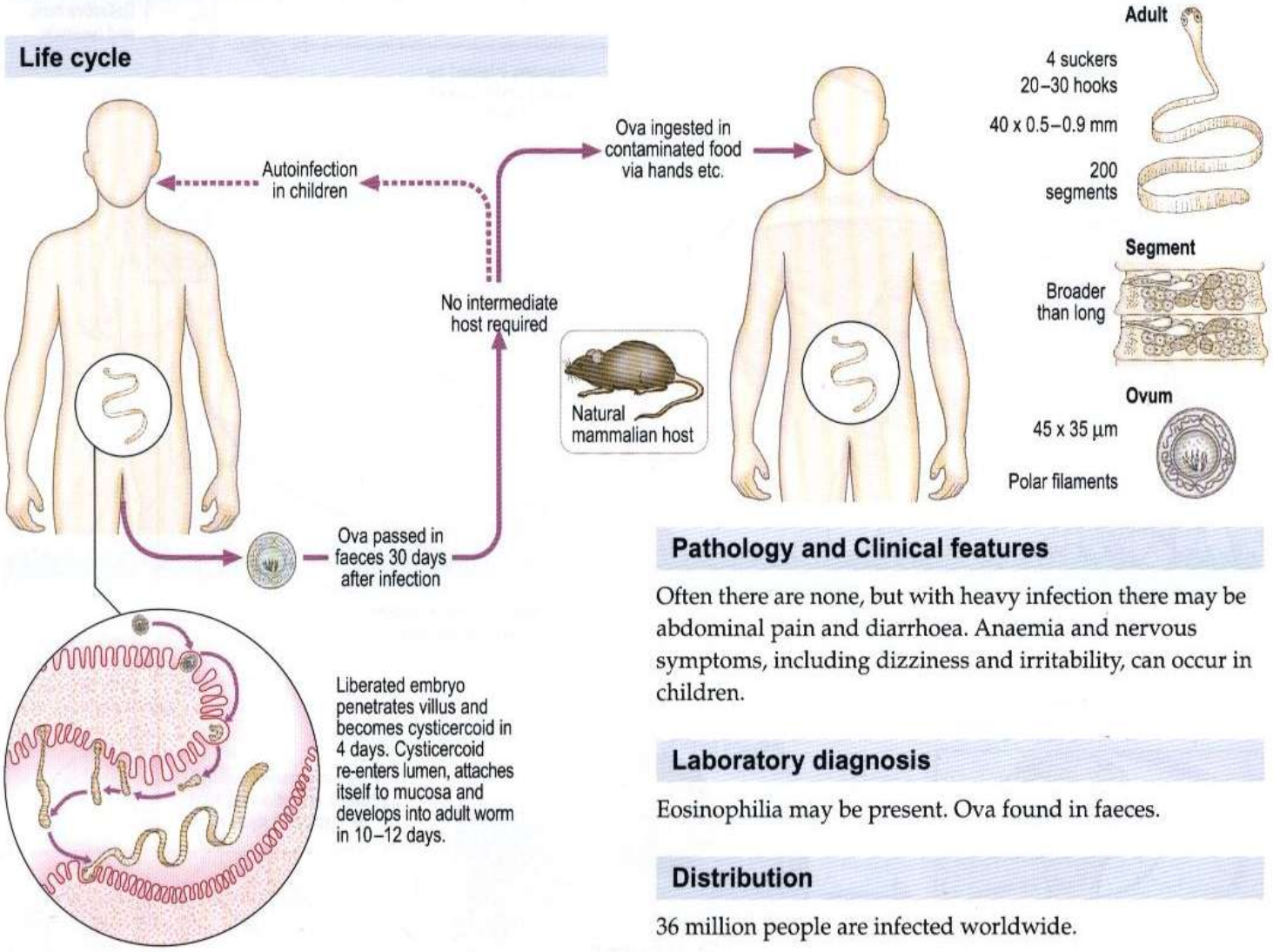




Gravid Proglottid (segment) of *Taenia solium* (right) & *Taenia saginata*(left)

Hymenolepis nana

Life cycle



Pathology and Clinical features

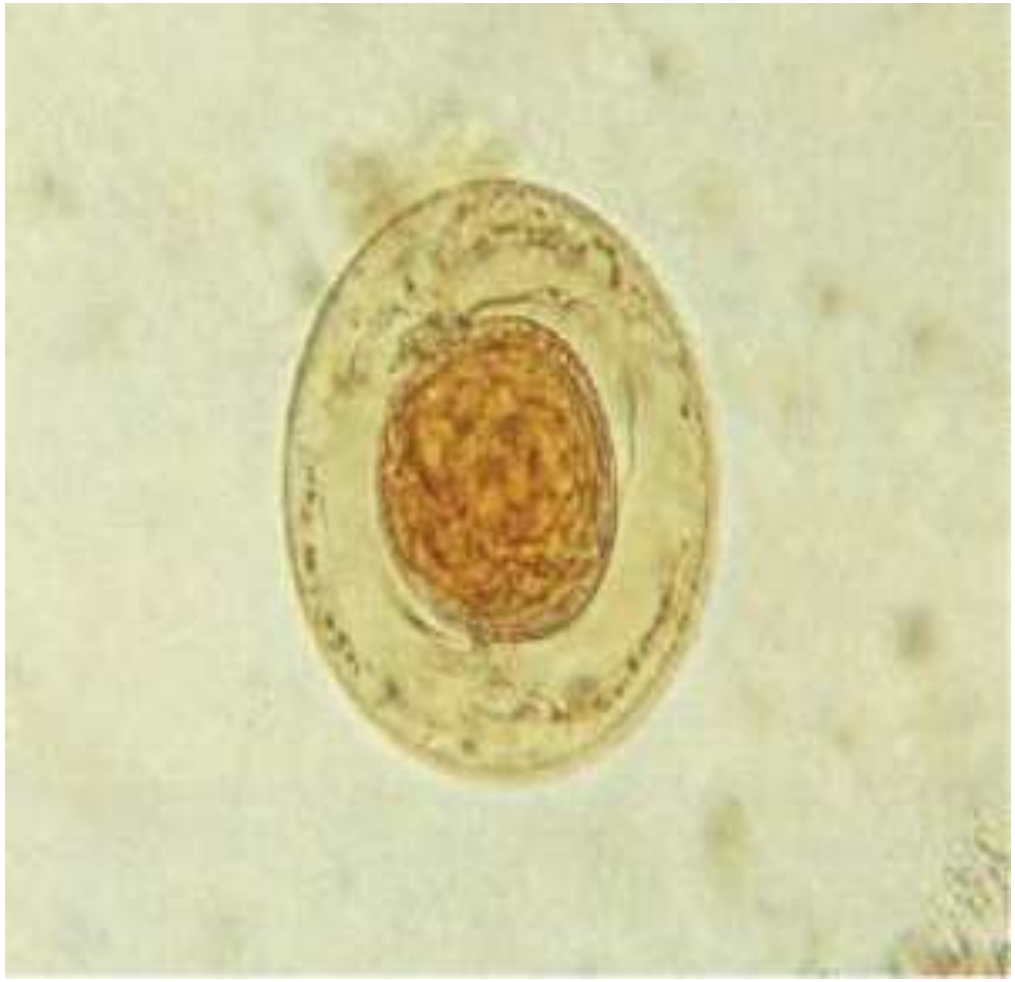
Often there are none, but with heavy infection there may be abdominal pain and diarrhoea. Anaemia and nervous symptoms, including dizziness and irritability, can occur in children.

Laboratory diagnosis

Eosinophilia may be present. Ova found in faeces.

Distribution

36 million people are infected worldwide.

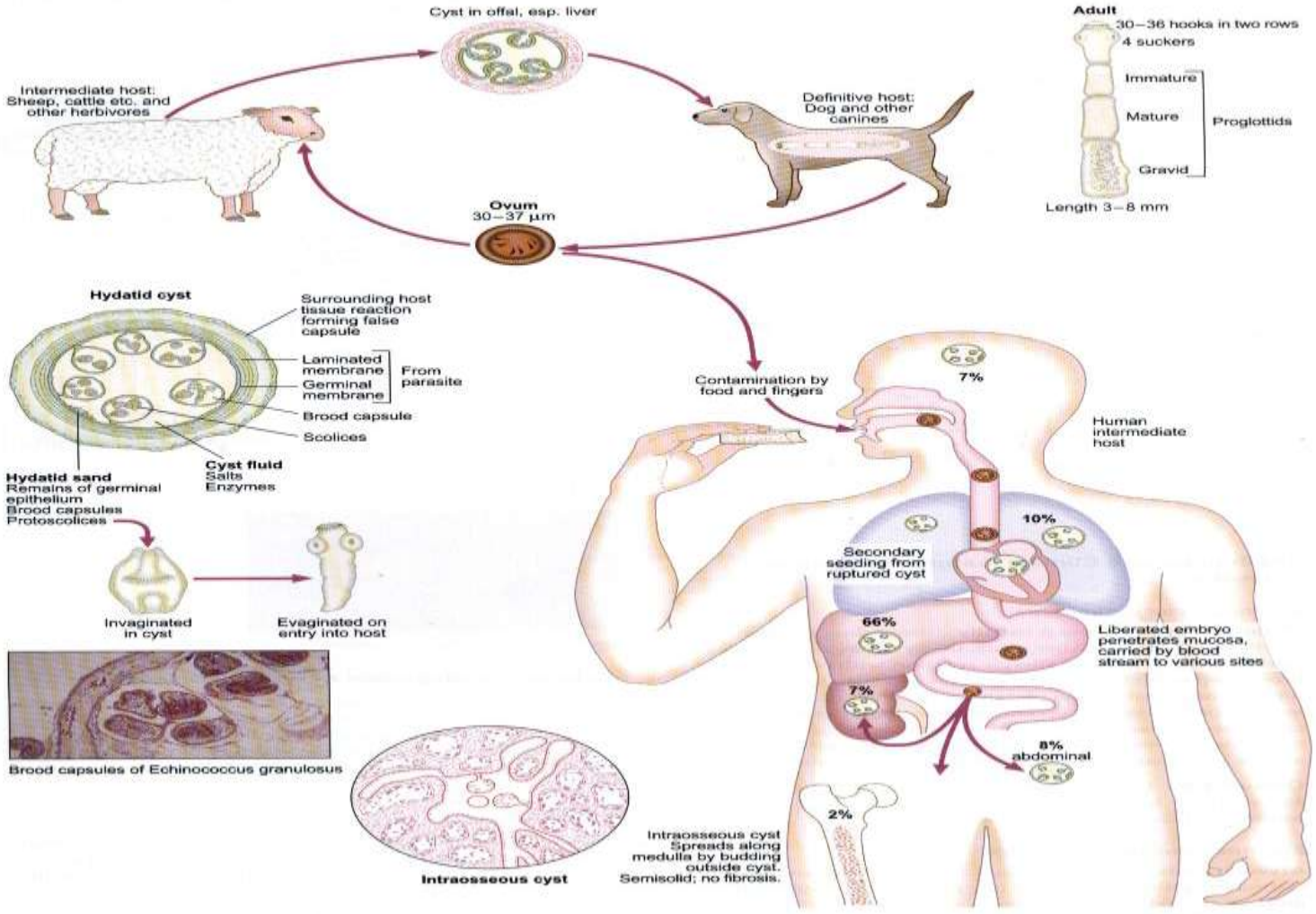


Hymenolepis nana. Egg. Feces.

Echinococcus granulosus (dog tape worm)

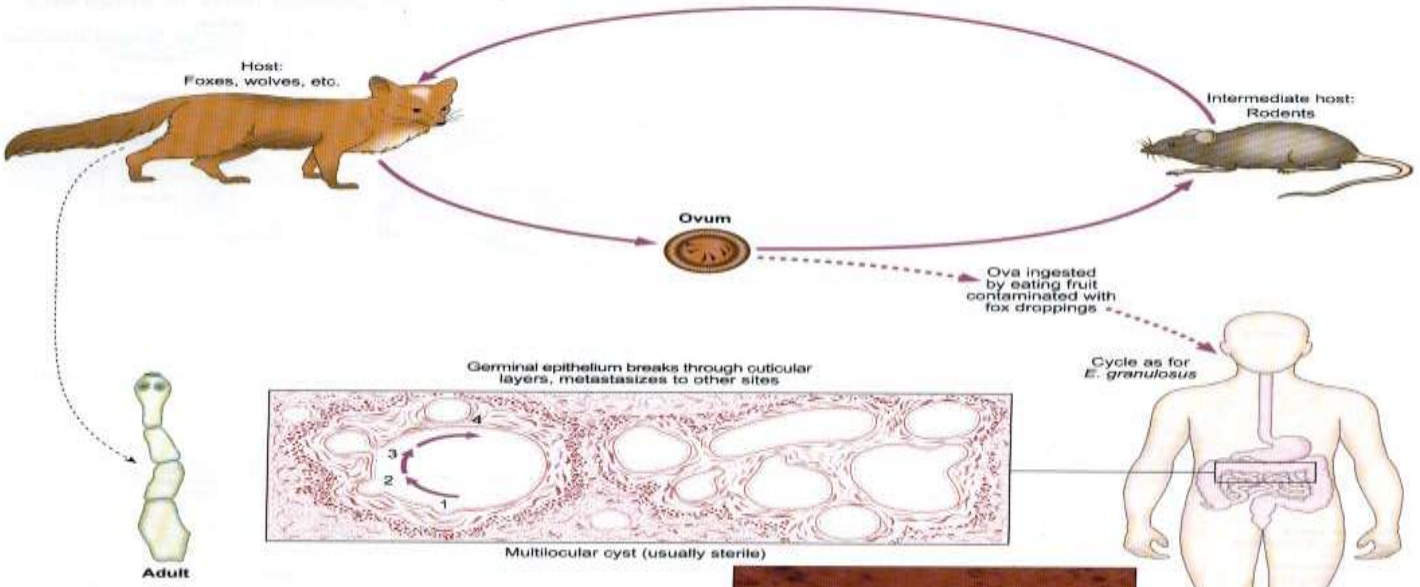
Life cycle

Echinococcus granulosus causes hydatid disease.



Echinococcus multilocularis

Life cycle



Pathology and Clinical features of hydatid disease

Echinococcus granulosus

Unilocular cysts. There is usually surrounding inflammatory reaction and fibrosis. After years, the cyst may die, shrink and calcify. There is general allergic reaction with eosinophilia, bronchospasm, etc. Pressure effects can cause local tissue damage and obstruction of natural channels. Rupture or leakage of the cyst can accentuate the allergic reaction. There can be anaphylactic shock and sometimes secondary implantation, for example in the peritoneal region. There can also be secondary infection with formation of abscess.

Osseus cysts. Usually there is no fibrosis although there is some cellular infiltration. Destruction of the bone can sometimes lead to spontaneous fracture.

Echinococcus multilocularis

Alveolar cysts. There are local pressure effects and allergy. Germinal epithelium can act like a neoplasm with local infiltration or distant metastases.

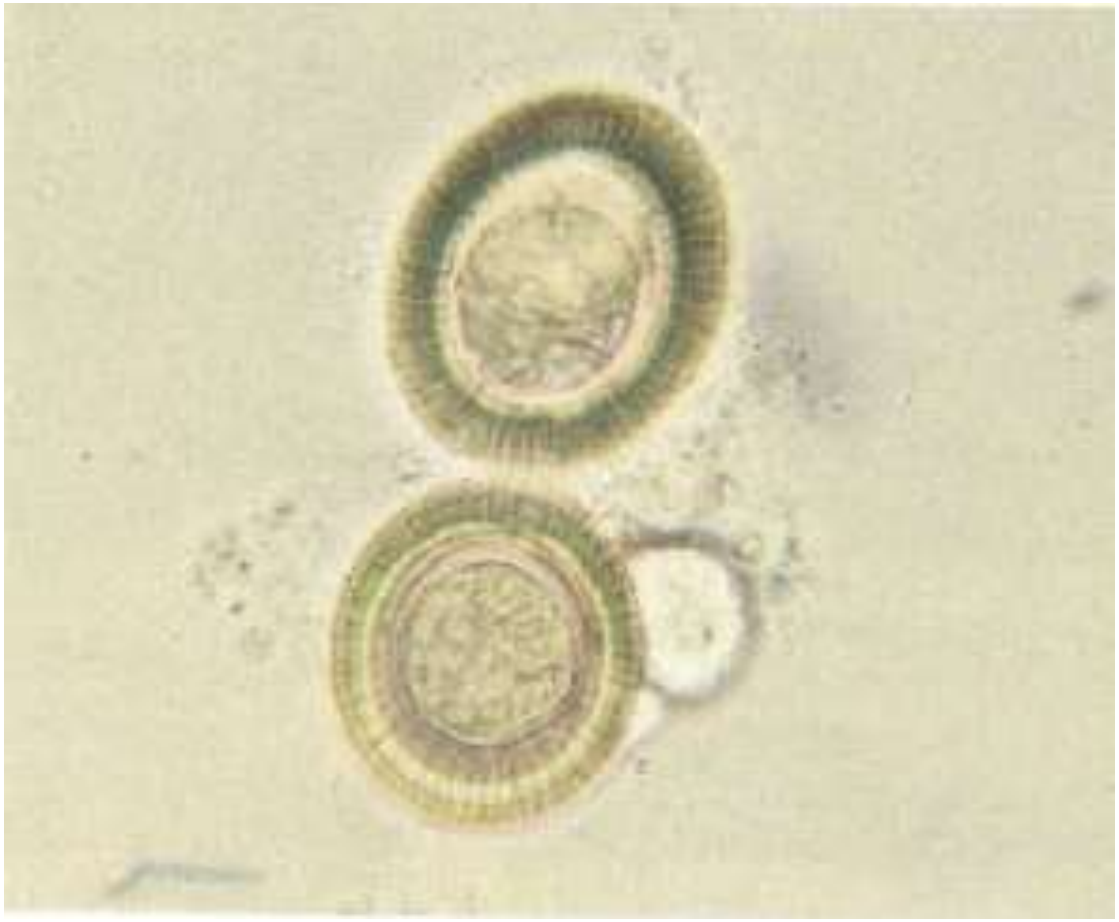
Eosin penetration of dead protoscolices

Laboratory diagnosis of hydatid disease

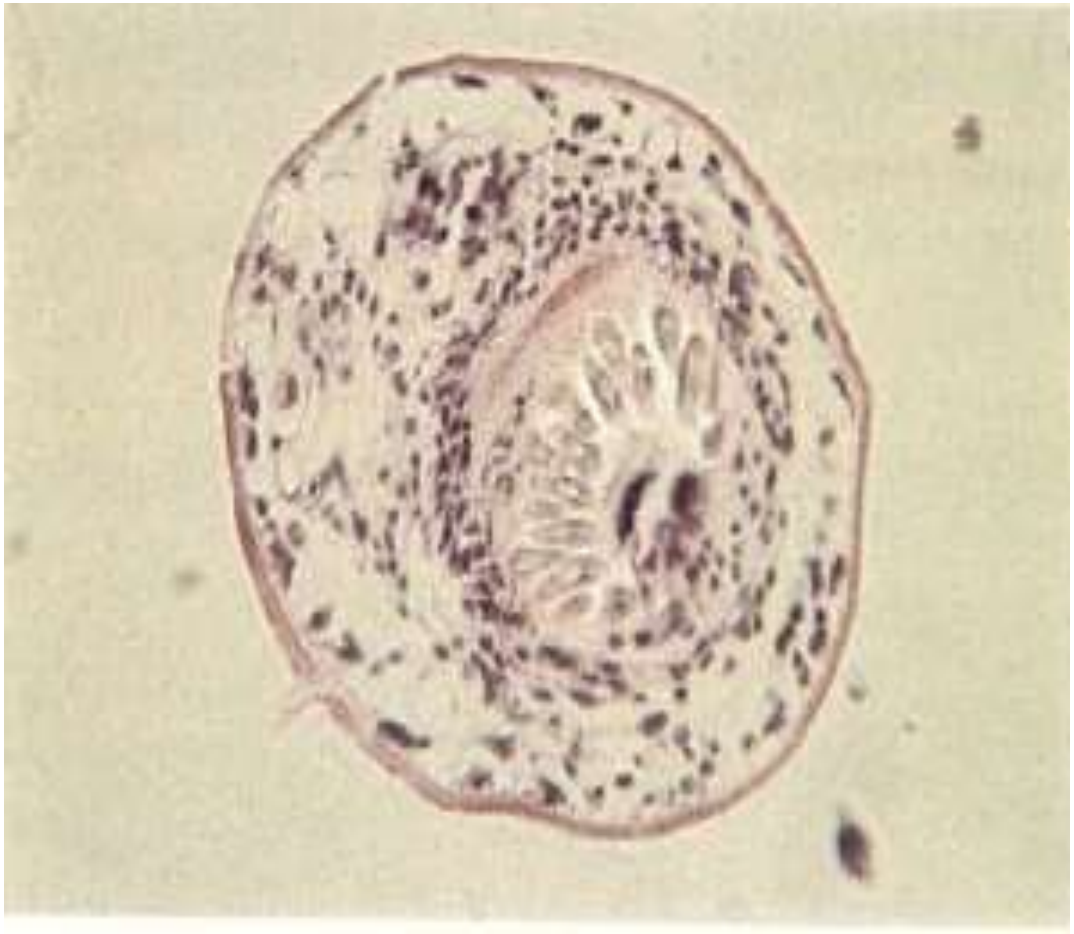
Use serological tests on serum (e.g. ELISA, complement fixation, counter current immunoelectrophoresis for Arc 5 or immunoblot). Microscopy of cyst fluid from operative specimens can be used to assess viability of protoscolices. Histological examination of a removed specimen is another possibility.

Distribution

1 million infected worldwide. *E. multilocularis* is rare in humans, but occurs in Northern Europe, Asia, North America and Arctic regions. *E. granulosus* is widespread in sheep-rearing areas of the world. Eradication is well advanced in Australia and New Zealand.



15-118 *Echinococcus granulosus*. Eggs. Iodine stain (×800). The eggs of *E. granulosus* are identical to the eggs of the *Taenia* spp. They are spherical with a thick, radially striated shell and measure 30 to 40 μm in diameter.



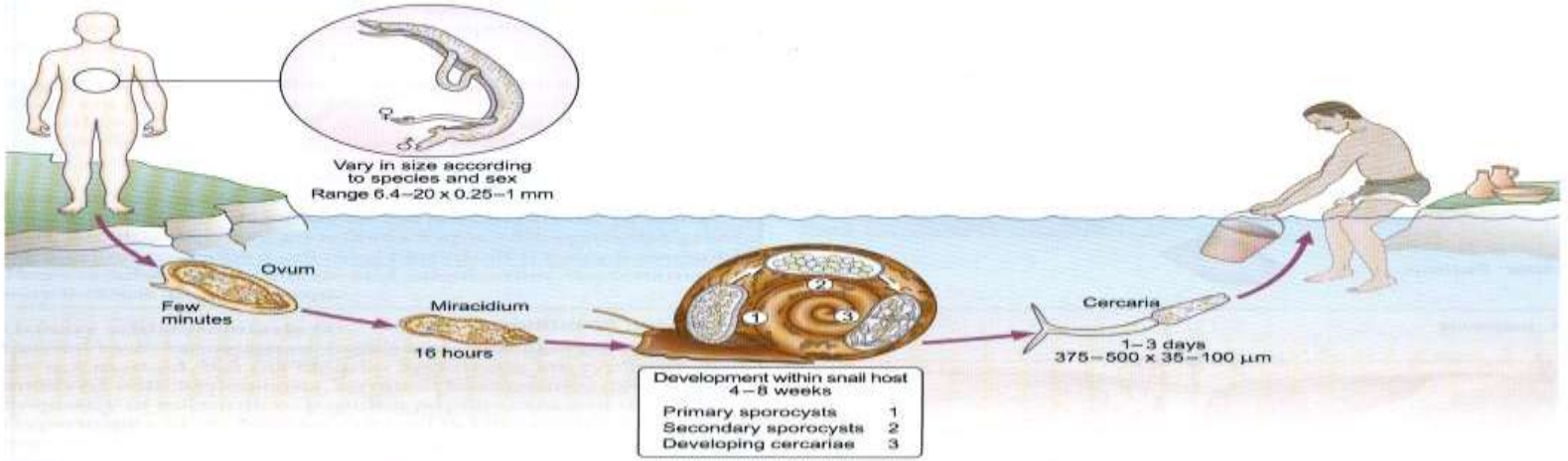
A **B**

Echinococcus granulosus A- adult worm B-hydatid cyst in liver

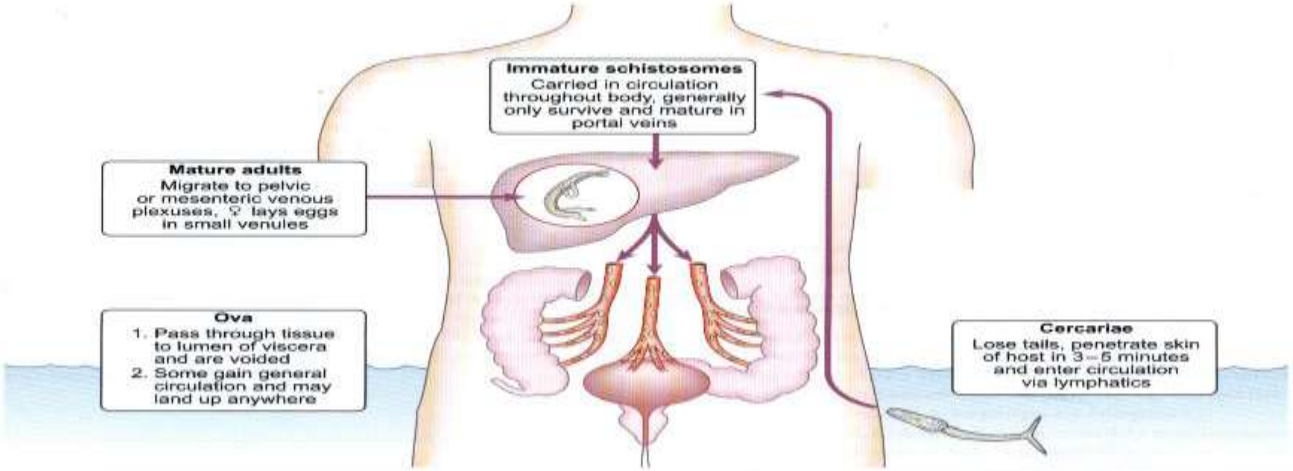
Trematode (flat) worms

Schistosoma species (blood flukes)

Life cycle for all species



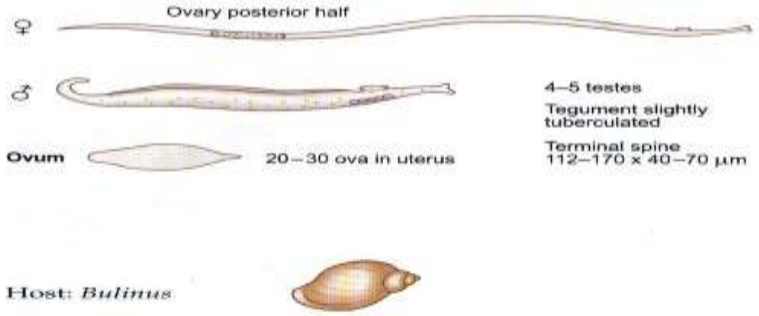
Life cycle in humans



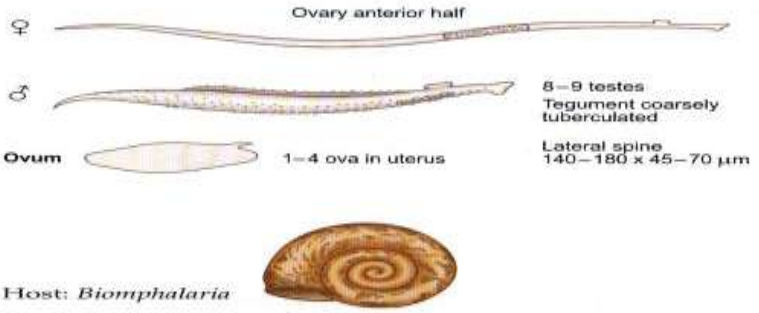
Schistosoma species (blood flukes) (Continued)

Morphology

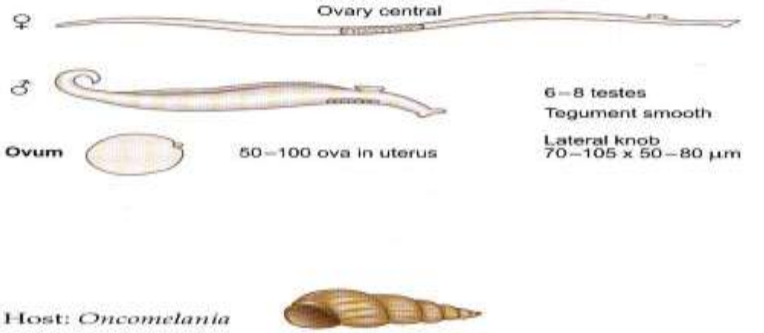
S. haematobium



S. mansoni



S. japonicum

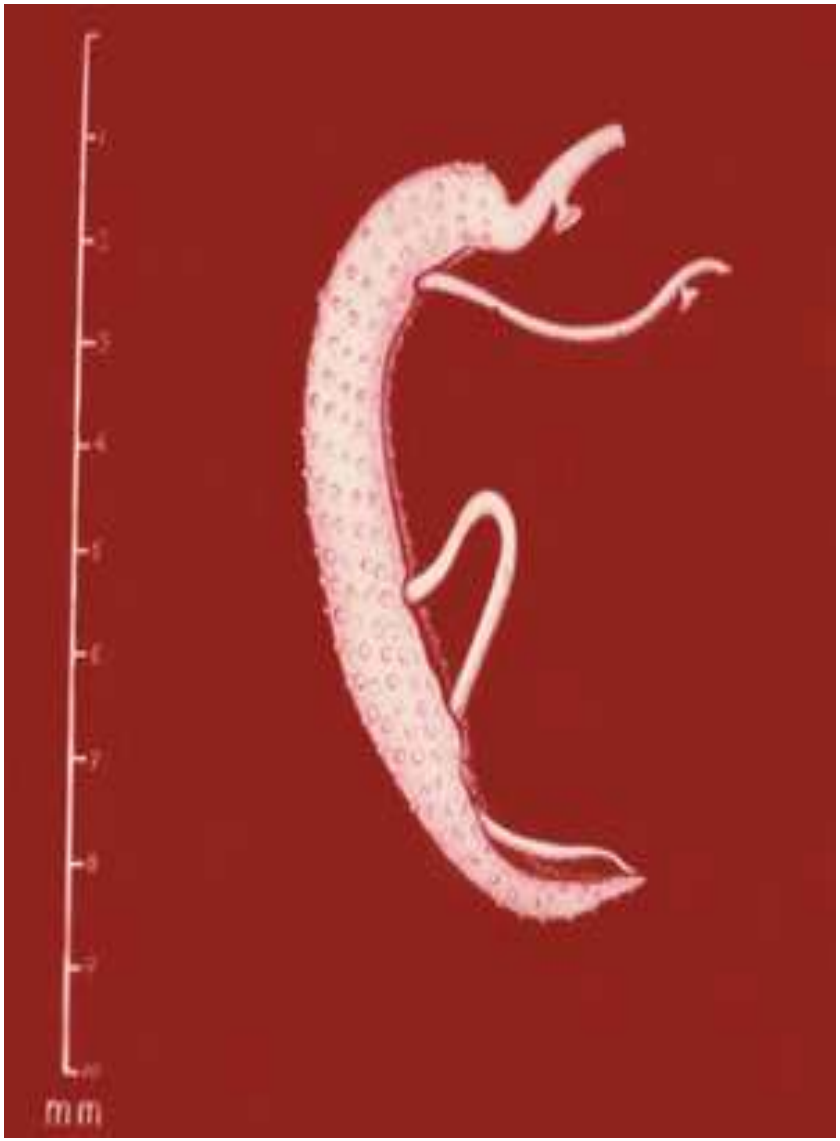


Distribution

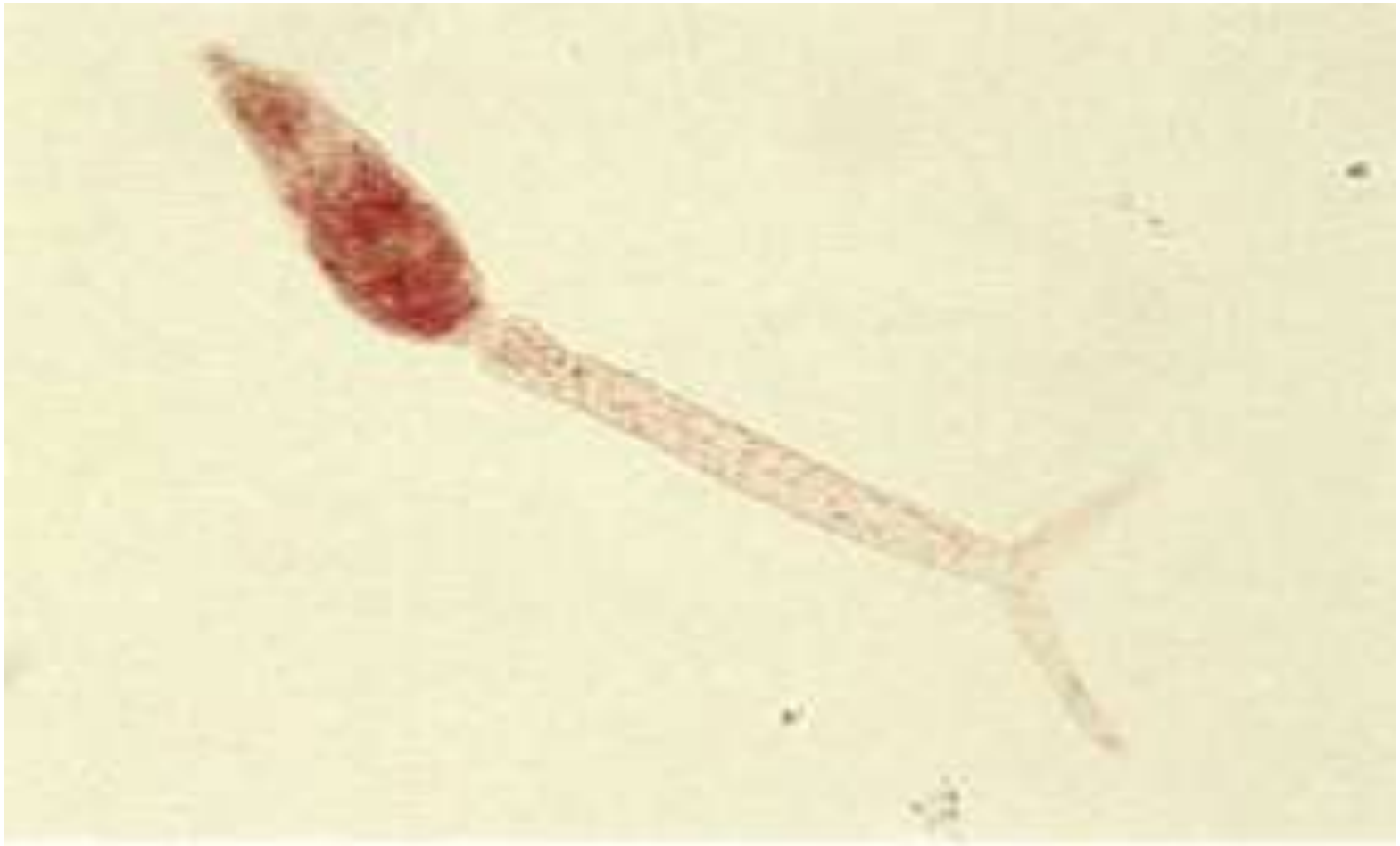
S. haematobium: 78 million

S. mansoni: 57 million

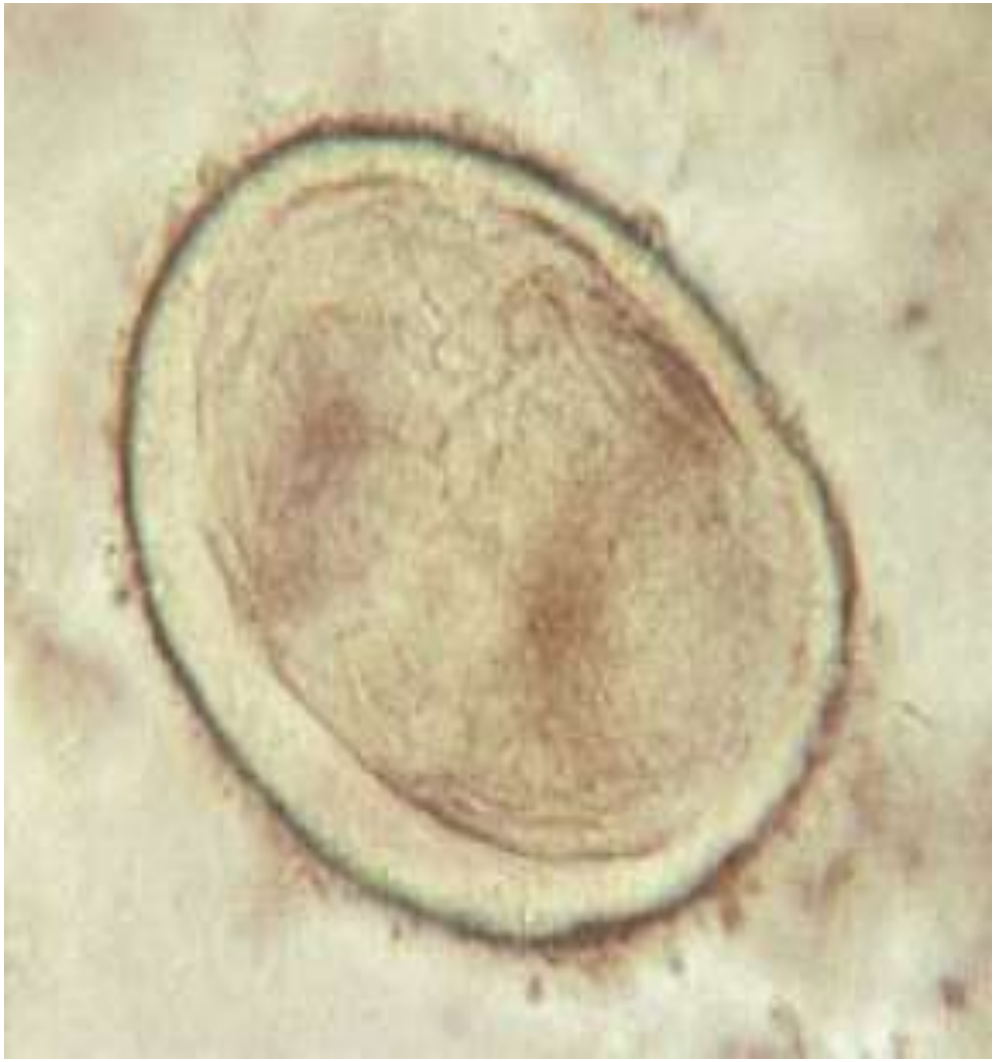
S. japonicum: 69 million



Adults of *S. mansoni*. The thin female resides in the gynecophoral canal of the thicker male.

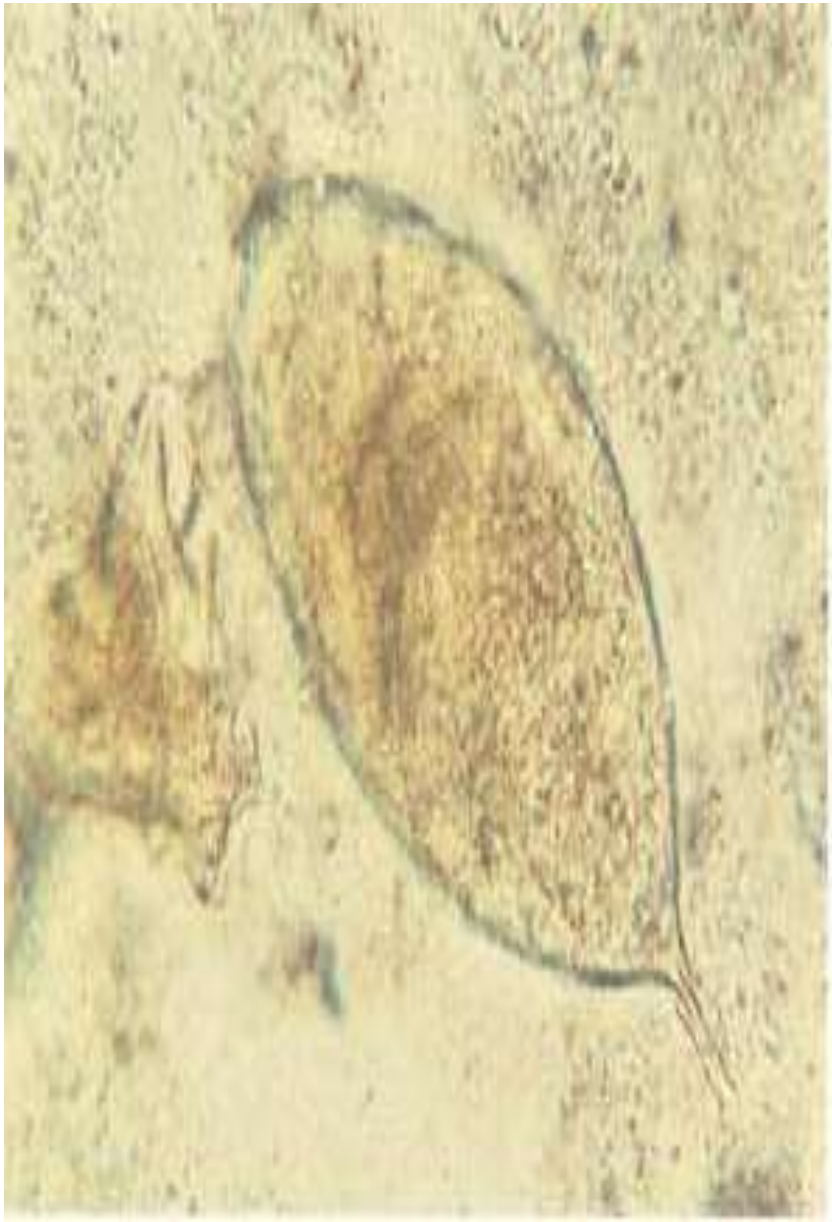


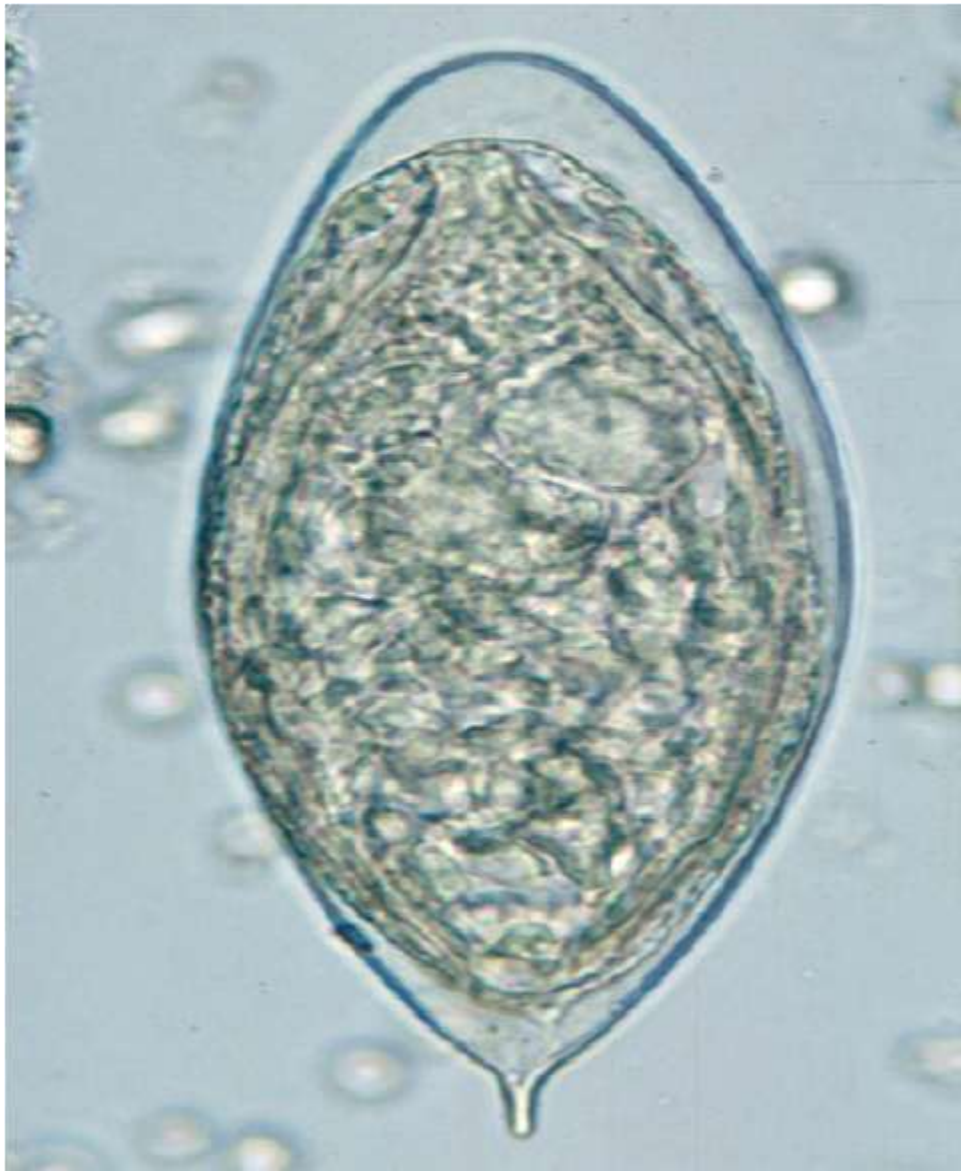
Cercaria of *Schistosoma spp.*



Schistosoma japonicum. Egg. Feces.

Schistosoma mansoni. Egg. Feces.

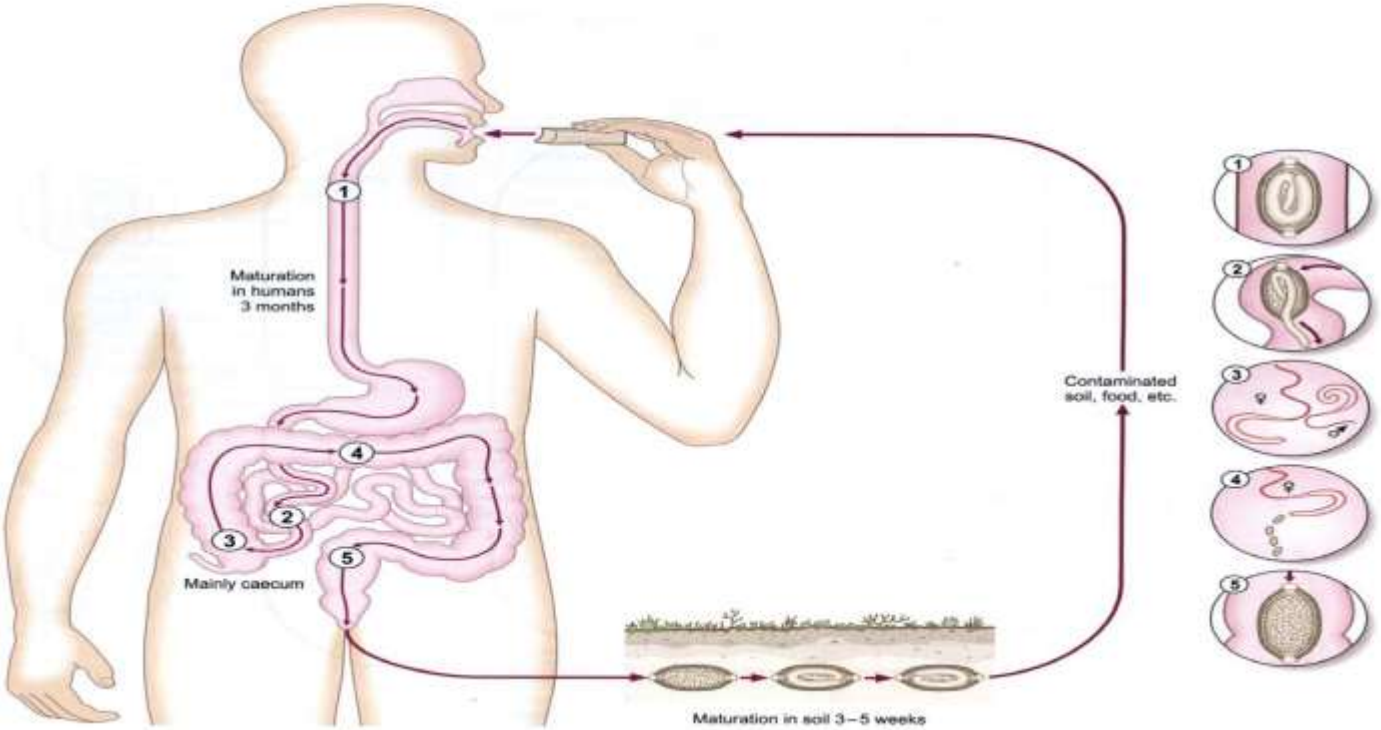




***Schistosoma haematobium*. Egg.**

Trichuris trichiura (whip worm)

Life cycle



Pathology and Clinical features

Light infections may be asymptomatic. Heavy infections can result in the trichuris dysentery syndrome, rectal prolapse, rectal bleeding, anaemia, growth stunting and growth retardation in children.

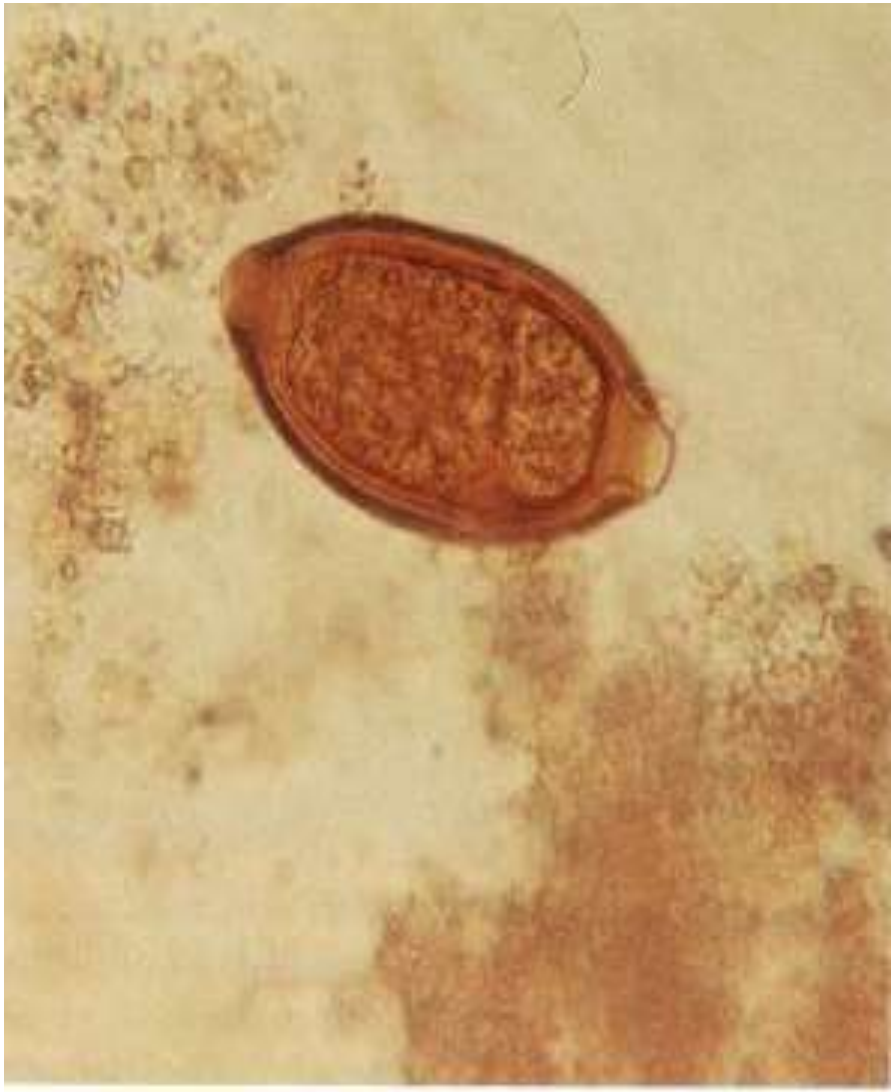
Laboratory diagnosis

Eosinophilia may occur.

Ova may be recovered in faeces by concentration methods.

Distribution

1.3 billion infected worldwide.

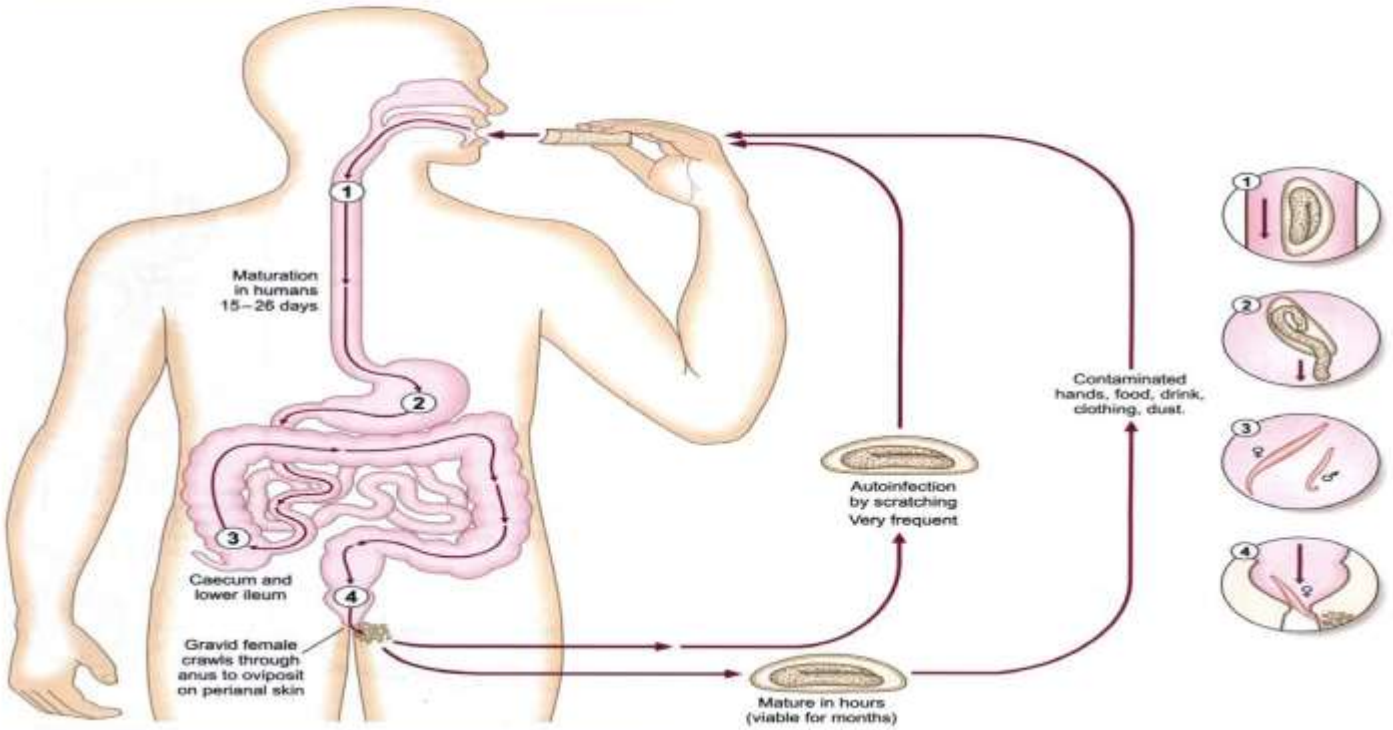


Trichuris trichiura. Egg. Feces

Nematode (round) worms

Enterobius vermicularis (thread or pin worm)

Life cycle



Distribution

350 million infected worldwide, often group or institutional infection.

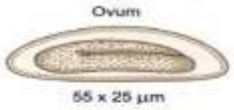
Pathology and Clinical features

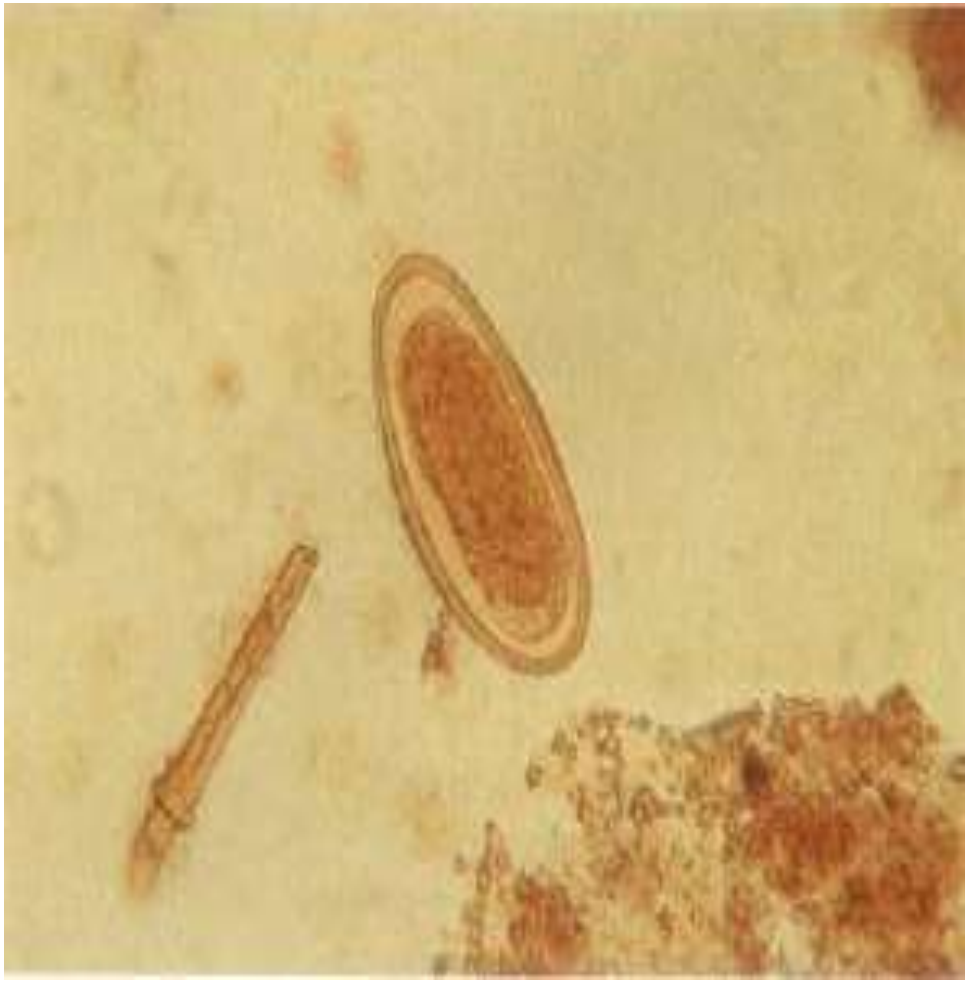
Most infections are asymptomatic. Perianal itching may be troublesome. In females, migrating worms may cause pruritis vulvae or vaginitis. Rarely, urinary tract infection or appendicitis can occur. Migration into the peritoneal cavity has been recorded.

Laboratory diagnosis

Mild eosinophilia.

Ova can be recovered from the perianal area using clear adhesive tape or a cotton swab moistened with saline. Early morning collection before washing gives best recovery. In females, ova may occasionally be recovered from urine.

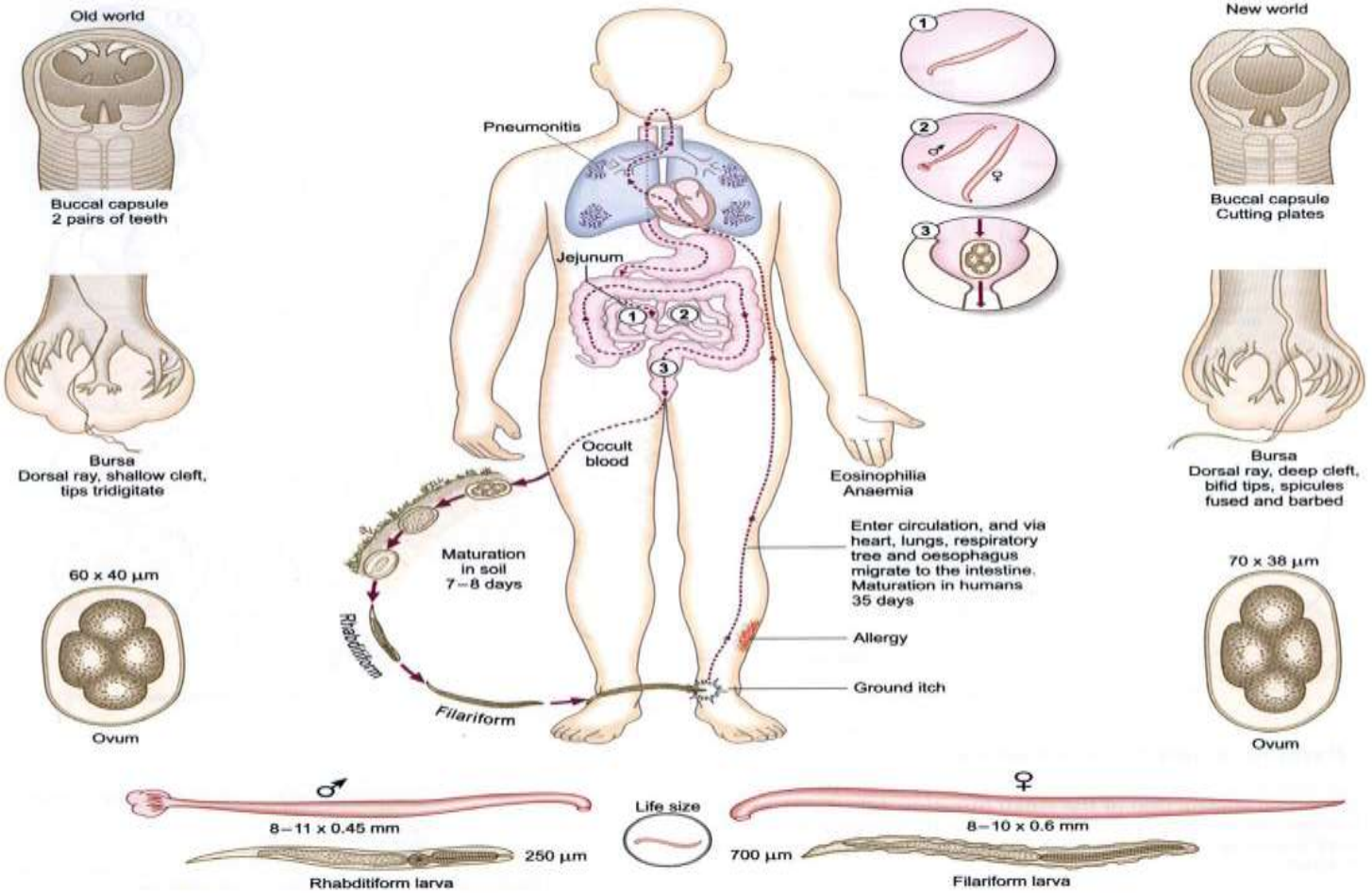




Enterobius vermicularis

Hookworms

Ancylostoma duodenale — *Necator americanus*



Pathology and Clinical features

Ground itch may follow skin penetration by filariform larvae. Pneumonitis can result from larval migration through the lungs. Adult worms in the jejunum ingest blood. Occult gastrointestinal bleeding occurs. Iron deficiency anaemia and its sequelae in heavy infections.

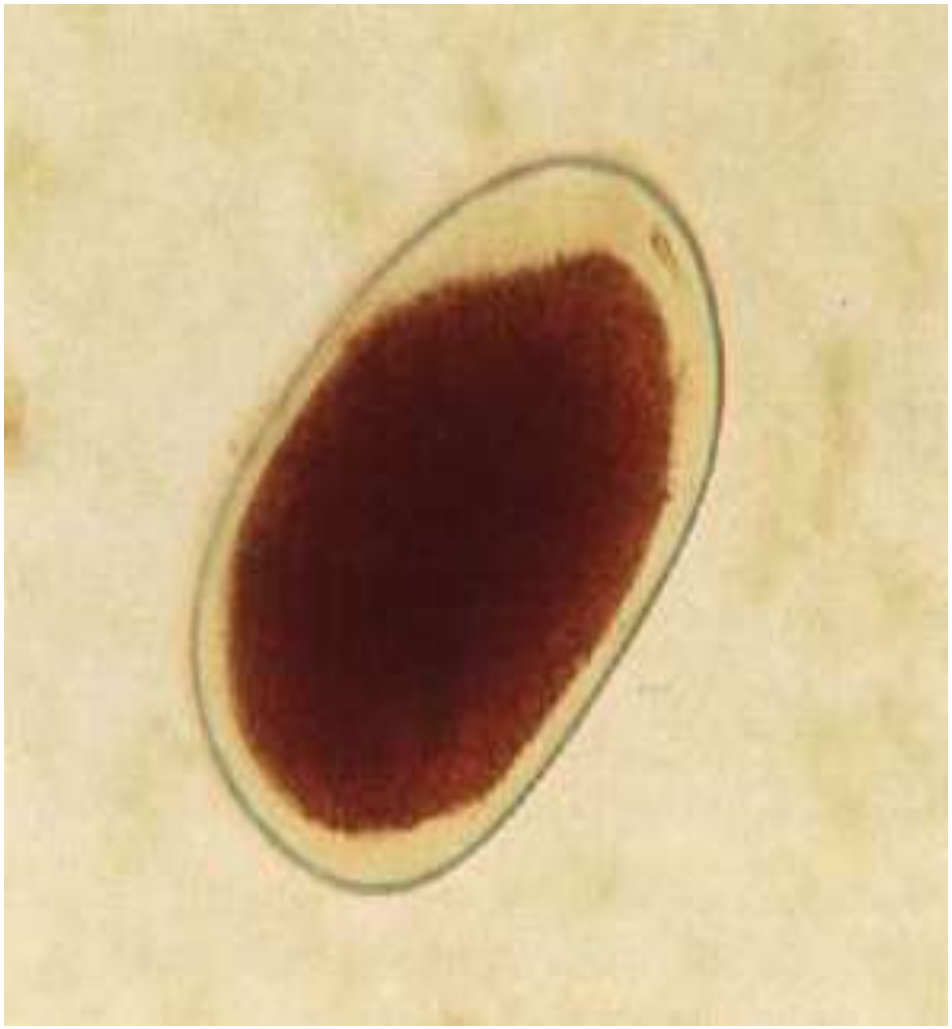
Distribution

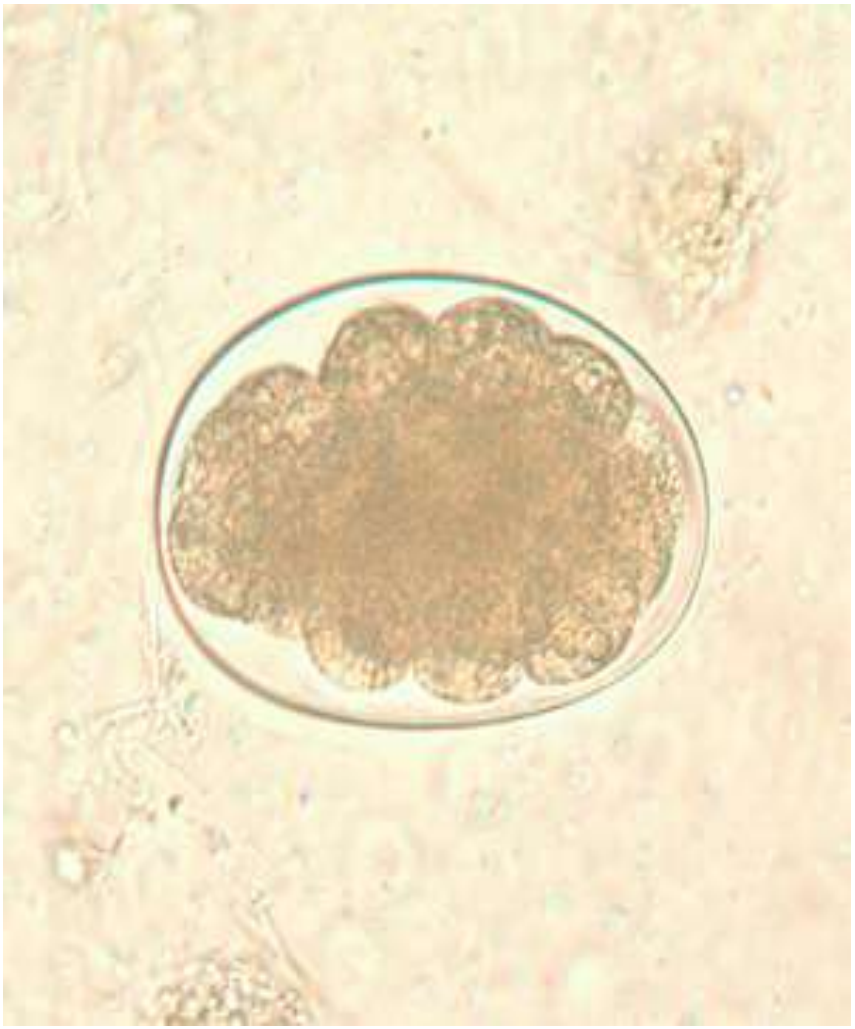
900 million infected worldwide.

Laboratory diagnosis

Eosinophilia.

Ova may be recovered from faeces by concentration methods. Rhabditiform larvae may be seen in old faecal specimens and must be distinguished from *Strongyloides* by the appearance of the buccal cavity.

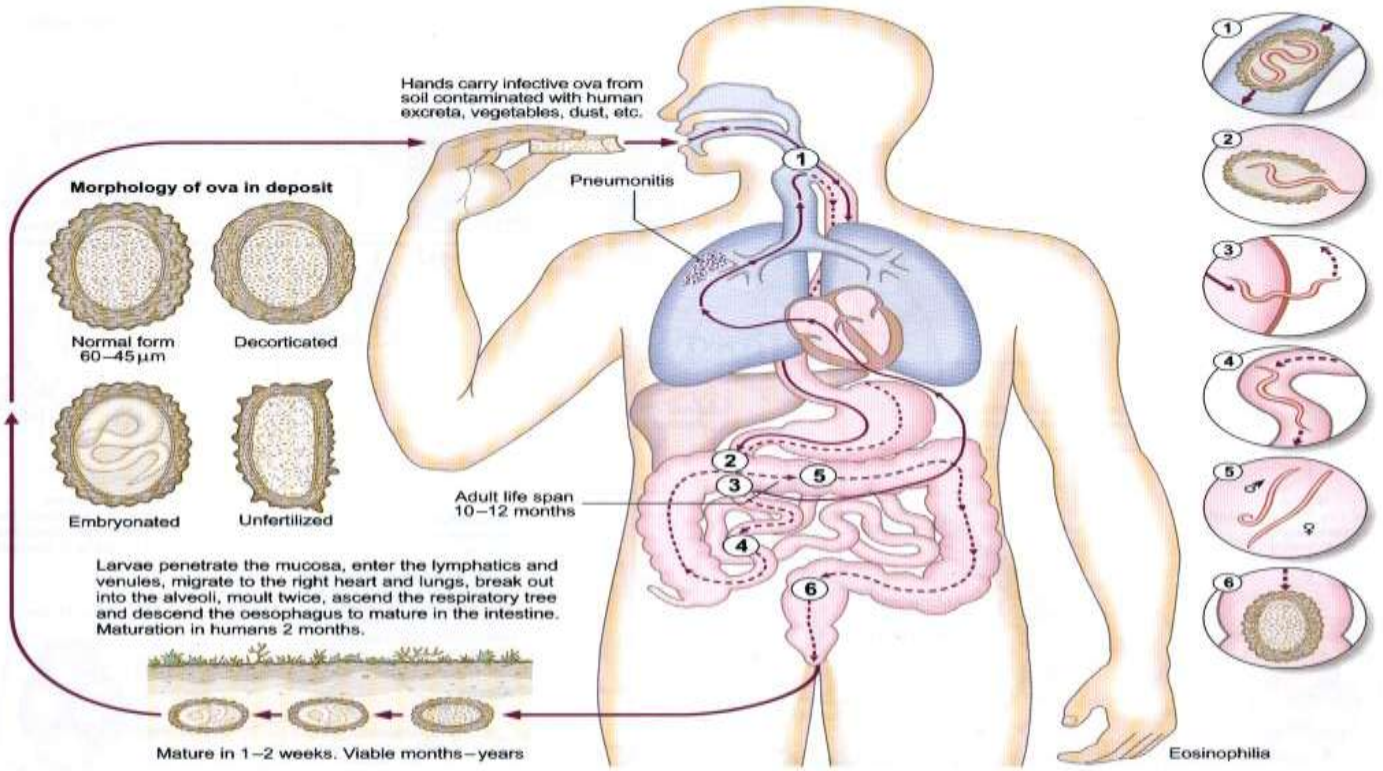




Egg of *Ancylostoma duodenale*

Ascaris lumbricoides (round worm)

Life cycle



Pathology and Clinical features

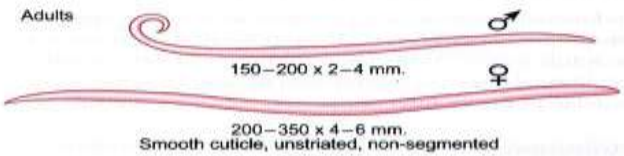
Larvae can cause pneumonitis with eosinophilia. Adult worms can cause obstruction of the small intestine, bile ducts and trachea; also appendicitis, pancreatitis and peritonitis. Children may vomit up a bolus of adult worms, or cough up immature worms.

Head of adult to show arrangement of the three lips

Laboratory diagnosis

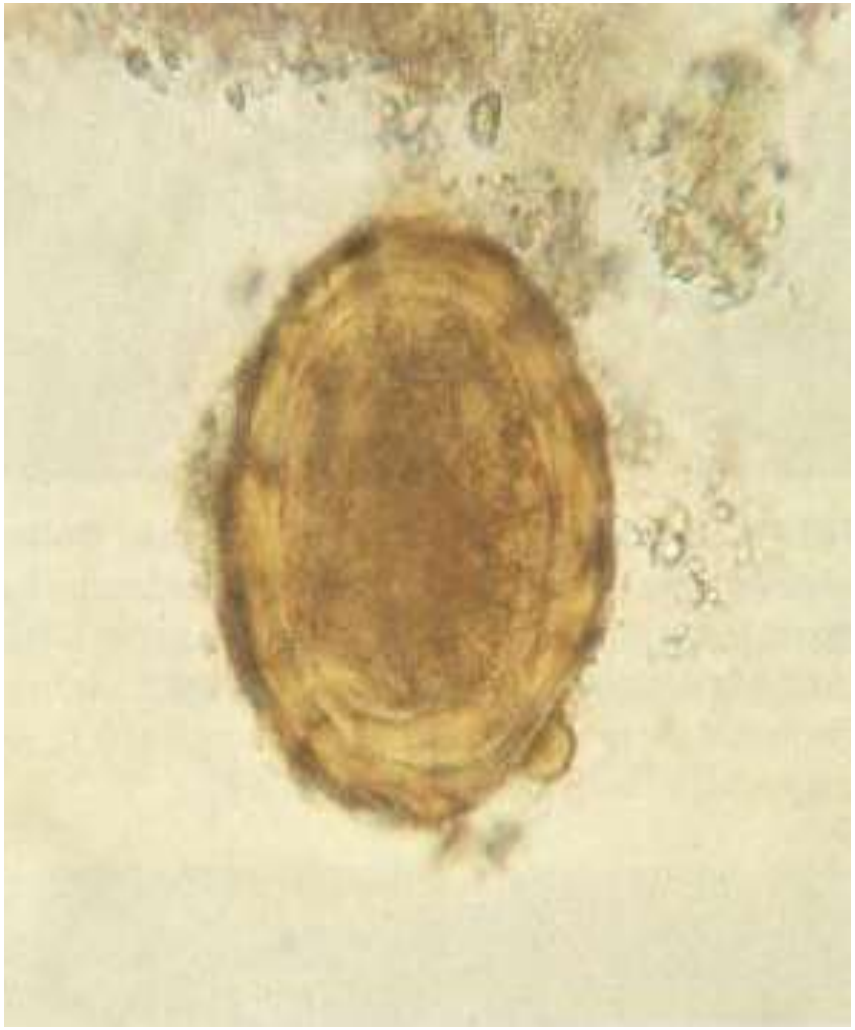
Ova may be recovered from faeces by concentration methods. Rarely larvae can be found in sputum, and must be distinguished from those of *Strongyloides*. Eosinophilia is present in the larval invasion stage.

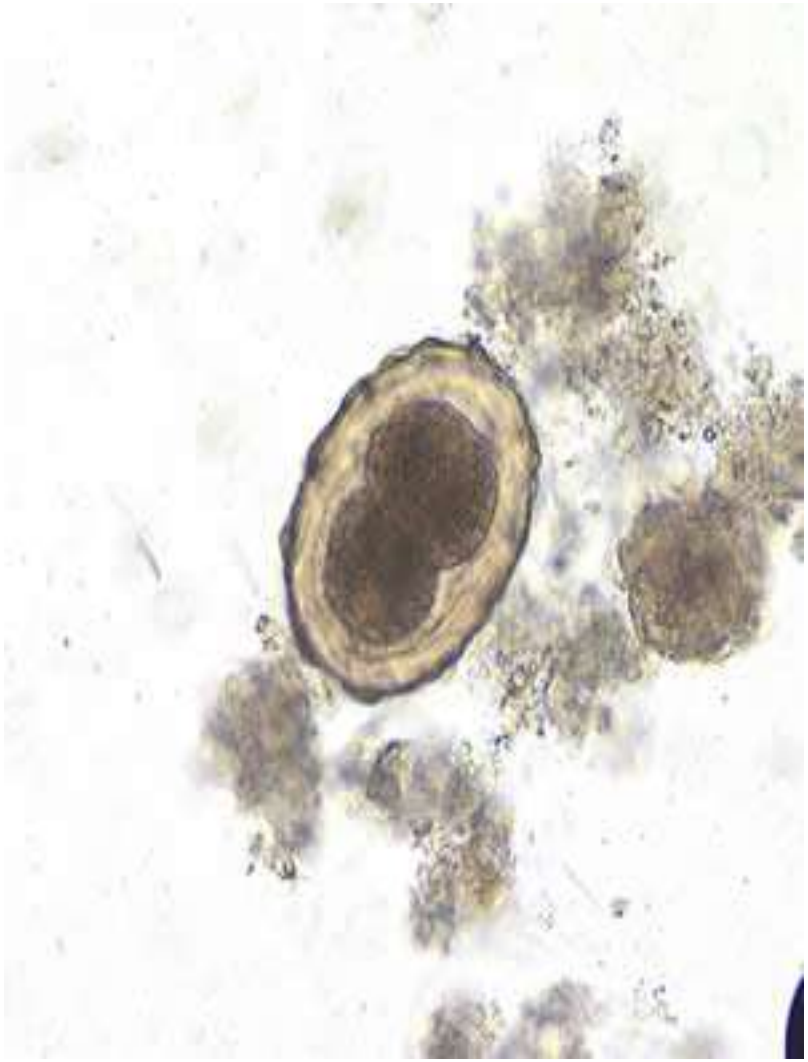
No specific serology is currently available.



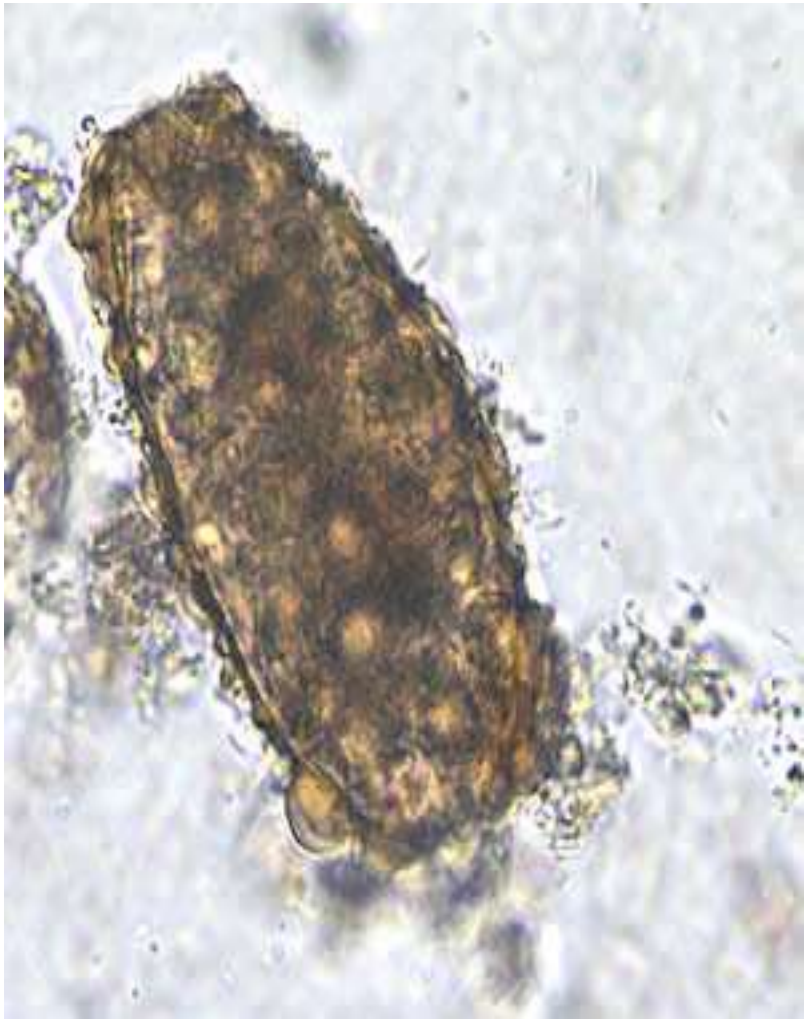
Distribution

1.47 billion infected worldwide.





Fertilized egg of *Ascaris lumbricoides*



Unfertilized egg of *Ascaris lumbricoides*