# Diagnostic Parasitology

## Medically Important Amebae

**Kaitlin Landfried, MLS(ASCP)$^{CM}$**

# Disclaimer

- This presentation was meant to provide students with both didactic and laboratory skills as they apply to clinical parasitology. It is meant for educational purposes only and does not represent Cleveland Clinic views or practices.
- The presentation contains images and other references copyrighted by another entity or person and credits shall be given to the rightful owners of the materials and I claim no copyright to the said content.
- Most of the information was adopted from the Textbook of Diagnostic Microbiology by Mahon & Lehman (see citation) but condensed for bite sized learning.

# Protozoa

- Unicellular parasites
- Classified according to their motility organelles
  - Amebae move by pseudopodia
  - Ciliates move by cilia
  - Flagellates move by flagella
  - Sporozoa are nonmotile
- All reproduce asexually except for the sporozoa

**Cyst:** nonmotile form, resistance to environmental factors
**Trophozoite:** feeding, motile form, replicates in the host and responsible for causing damage

# The Amebae

## Intestinal

- Six medically important members
  - *Entamoeba histolytica/dispar/moshkovkii*
  - *Entamoeba hartmanni*
  - *Entamoeba coli*
  - *Endolimax nana*
  - *Iodamoeba butschlii*
  - *Blastocystis* species
- Includes pathogens and nonpathogens
- Humans are the only known host except for *Blastocystis* species
- Cyst is the infective form
- Fecal oral transmission

## Tissue

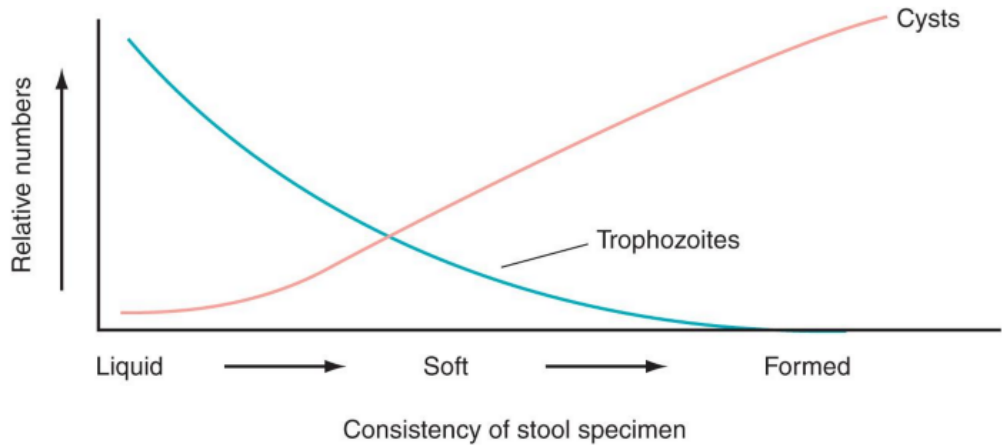
- Three medically important members
  - *Naegleria fowleri*
  - *Acanthamoeba species*
  - *Balamuthia mandrillaris*
- Free-living in soil and water
- Trophozoite is the infective form

Species identification is determined based on size, number of nuclei, nuclear structure, and presence of specific internal structures

# Amebae Microscopic Identification

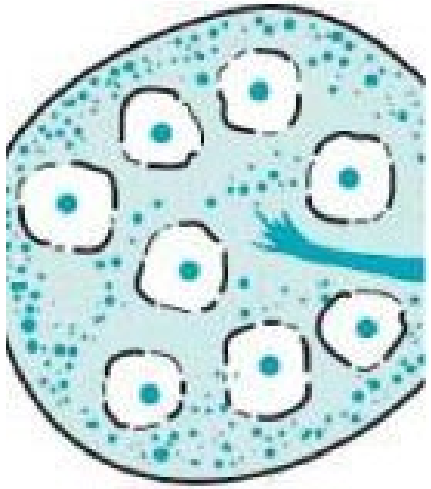
## STEP ONE

- Cyst vs Trophozoite
  - Amebic cysts can have more than one nucleus, depending on maturity, and lack motility organelles
  - Amebic trophozoites have one nucleus and motility organelles

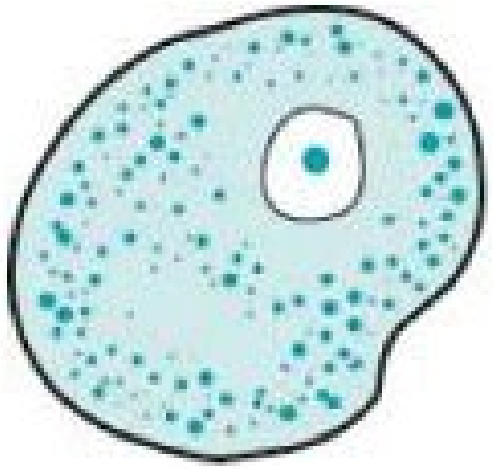


# Amebae Microscopic Identification

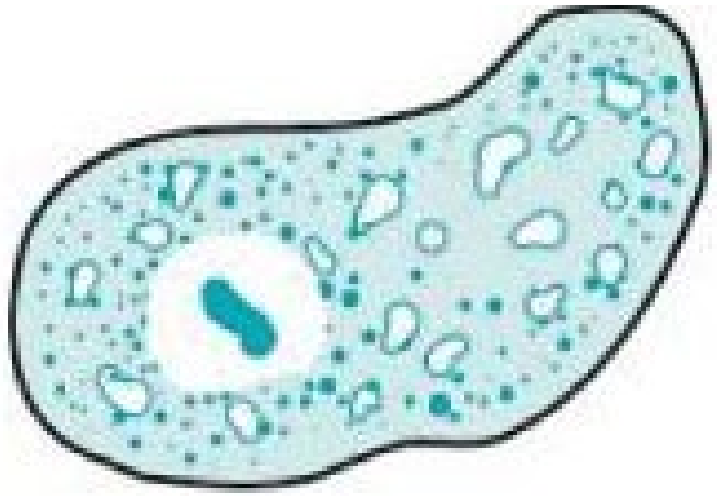
- Cyst or trophozoite?



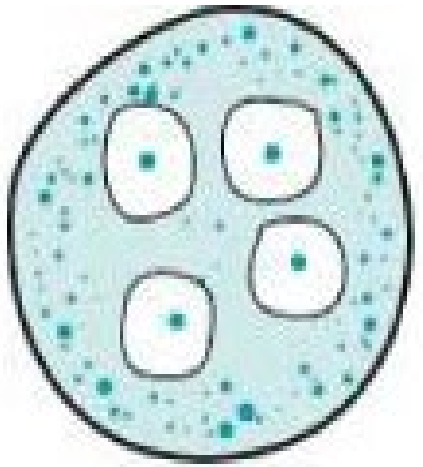
Cyst



Trophozoite



Trophozoite

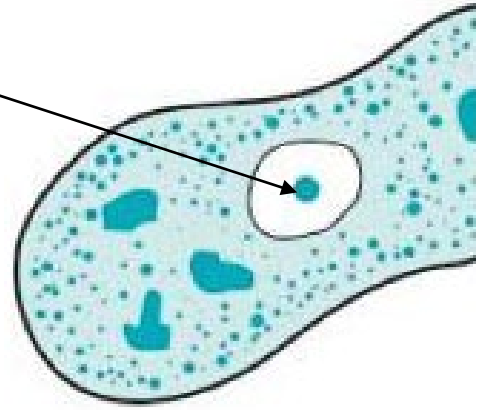


Cyst

# Amebae Microscopic Identification

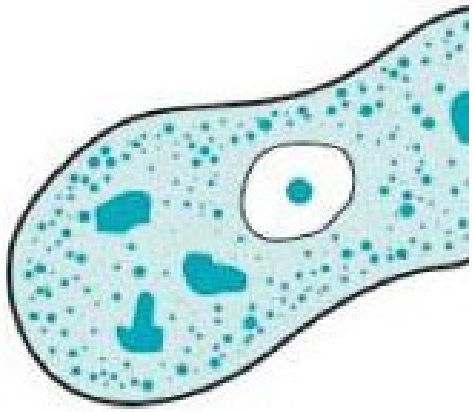
## STEP TWO

- Describe the **<u>karyosome</u>: a chromatin mass within the nucleus of protozoa**
  - Shape: regular(circular) vs irregular
  - Size: dot-like (small) or blot-like (large)
  - Location: central or eccentric

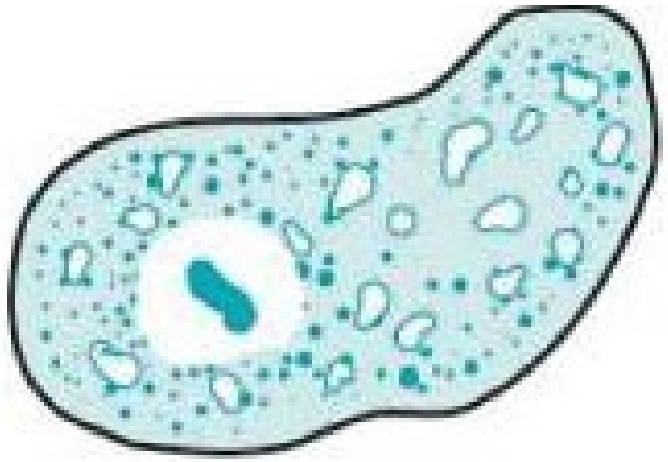


# Amebae Microscopic Identification

- Describe the karyosome.

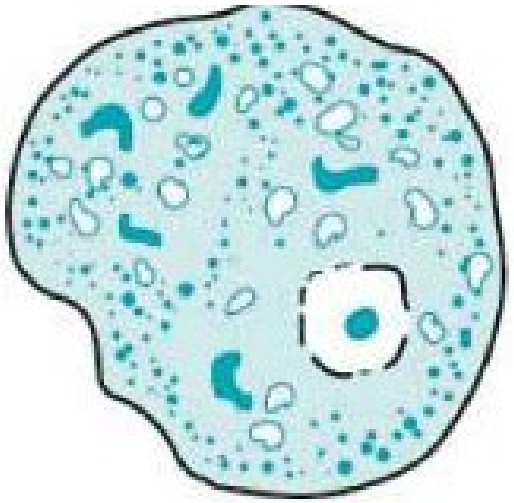


regular
dot-like
centrally located



irregular
blot-like
centrally located

regular
blot-like
centrally located

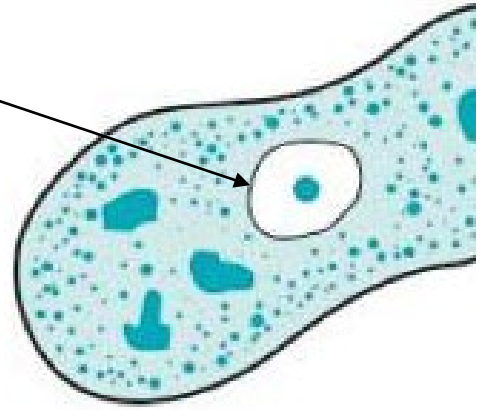


regular
dot-like
eccentrically located

# Amebae Microscopic Identification

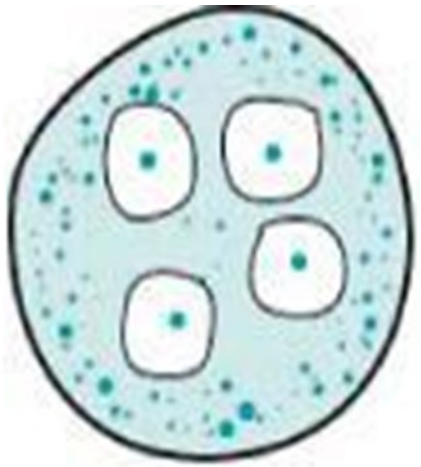
## STEP THREE

- Describe the **peripheral chromatin: DNA present on the nuclear membrane of some protozoa.**
  - Present/absent
  - Distribution: even vs uneven

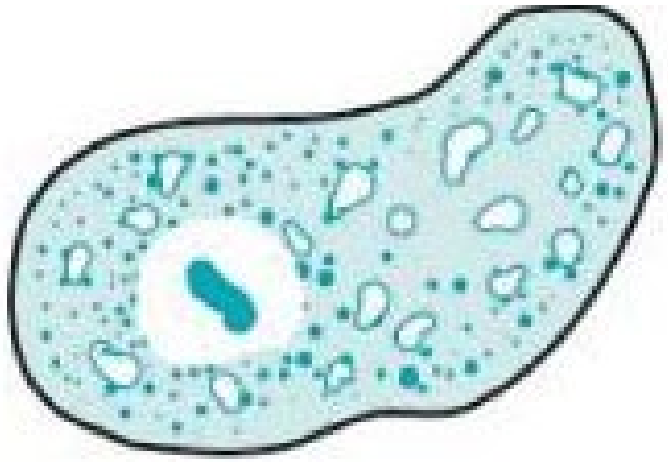


# Amebae Microscopic Identification

- Describe the peripheral chromatin

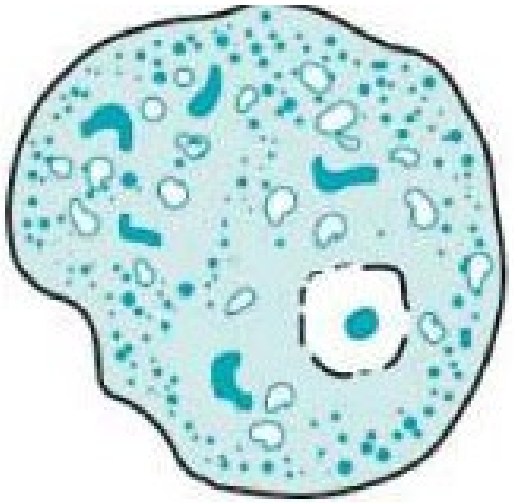


present
evenly distributed



absent

absent

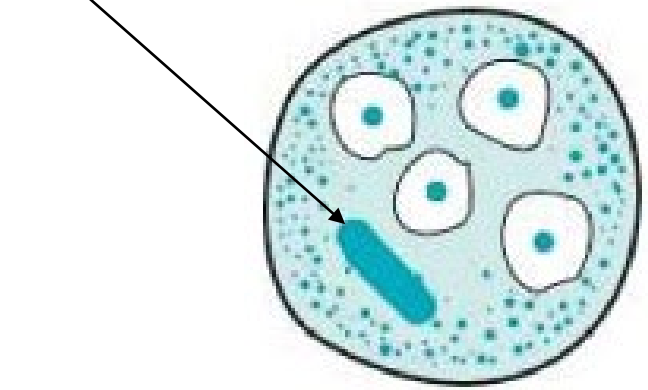


present
unevenly distributed

# Amebae Microscopic Identification

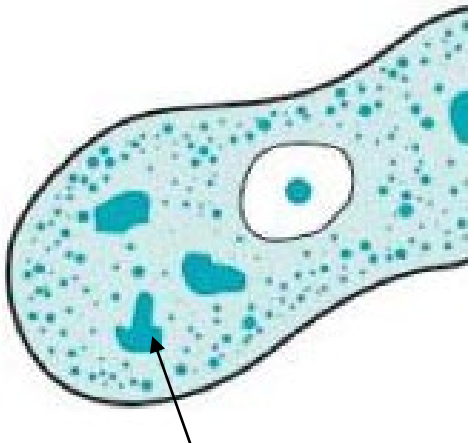
## STEP FOUR

- Describe unique features:
  - Chromatoidal bars: condensed RNA found in the cytoplasm of some amebic cysts
    - a bar-like structure rounded or splintered
  - Vacuoles
  - Ingested red blood cells

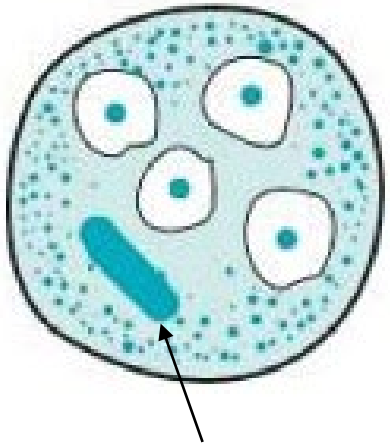


# Amebae Microscopic Identification

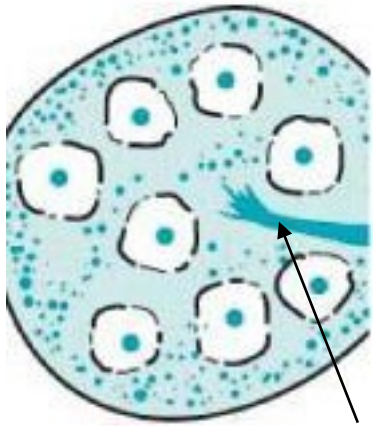
- Describe the unique features



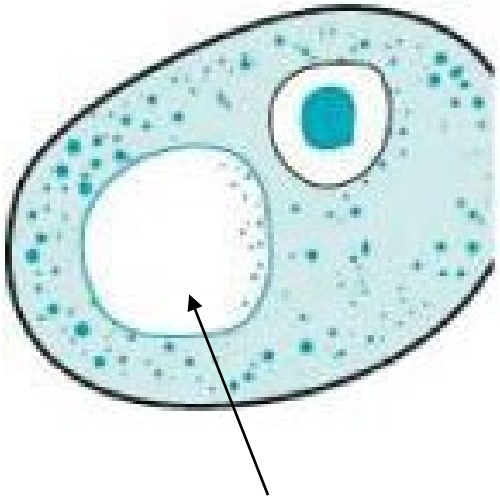
ingested RBCs



rounded chromatoidal bar



splintered chromatoidal bar



large vacuole

# Amebae Microscopic Identification

## STEP FIVE

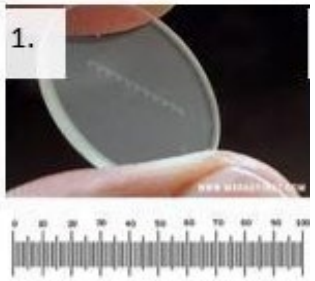
- Measure the size
  - Use the ocular micrometer to measure the size of the organism in µm
  - Some ameba look so similar the only way to differentiate is size
  - Always double check the size with the expected size for the parasite of suspicion

# Amebae Microscopic Identification

## Calibration of the Ocular Micrometer

### Instructions

1. Insert the ocular micrometer into a 10X eyepiece. The ocular micrometer is divided into ocular divisions (OD).
2. Place the calibrated stage micrometer slide on the stage and focus on the scale. The stage micrometer has a calibrated scale which is divided into 0.1 millimeters (mm) and 0.01 mm units.
3. Adjust the field so the 0 line of the ocular micrometer (OM) scale is exactly superimposed upon the 0.0 line of the stage micrometer (SM) scale.
4. Without moving the stage micrometer, locate the point as far to the extreme right as possible where any two lines are exactly superimposed upon each other.
5. Count the number of divisions (mm) on the stage micrometer between the 0.0 line and the superimposed line to the far right.
6. Count the number of ocular divisions on the ocular micrometer between the 0 line and the superimposed line to the far right.
7. Divide the distance determined in step 5 by the number of ocular divisions in step 6 and multiply by 1000 to give the ocular micrometer units in µm.
8. Repeat steps 3 through 7 for each objective on the microscope.
9. If at any time the ocular micrometer is moved to a different microscope or a new objective is added to the microscope, the calibration procedure must be completed again.




ocular micrometer

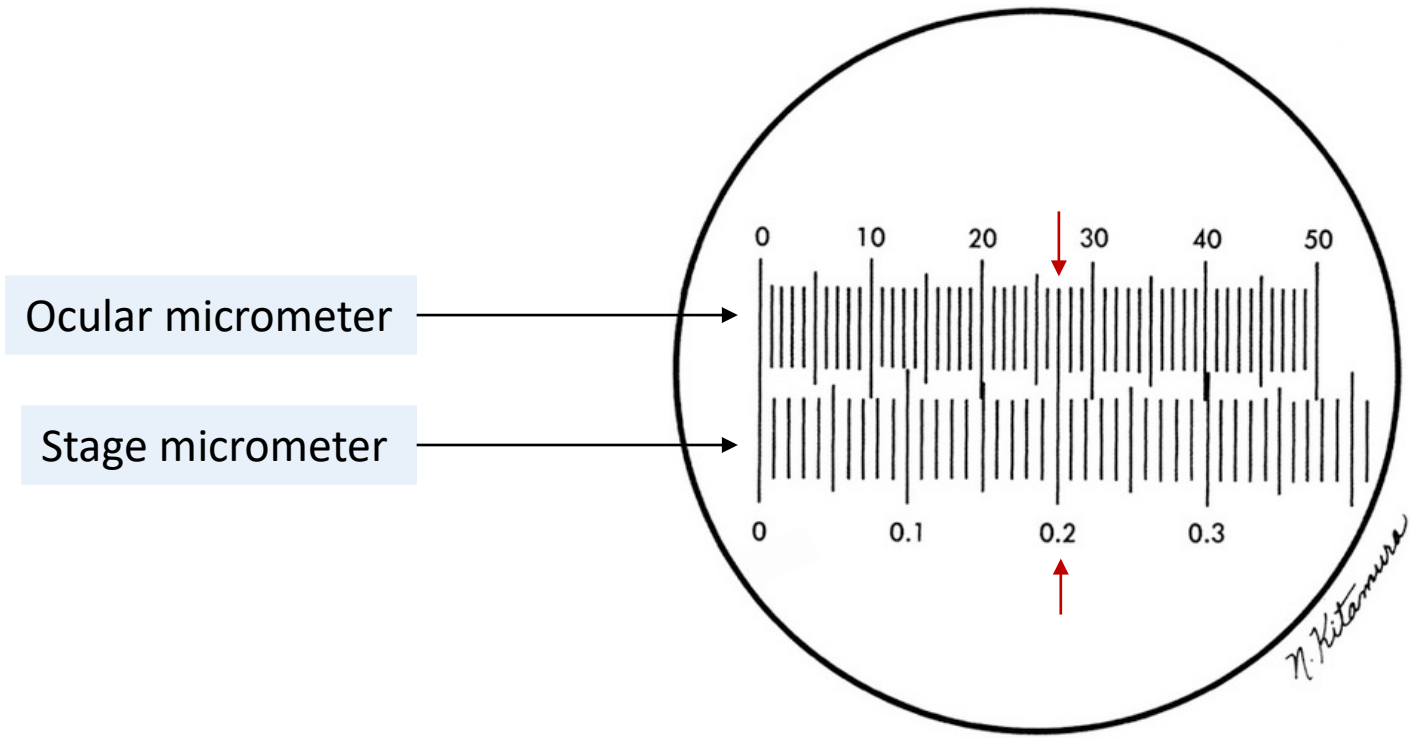

stage micrometer

Use me!

7. $$\frac{\text{Length seen on stage micrometer}}{\text{\# of divisions counted on ocular micrometer}} \times 1000 = \_\_\_\_\ \mu m$$

# Amebae Microscopic Identification

- Calibrate the ocular micrometer on 100x

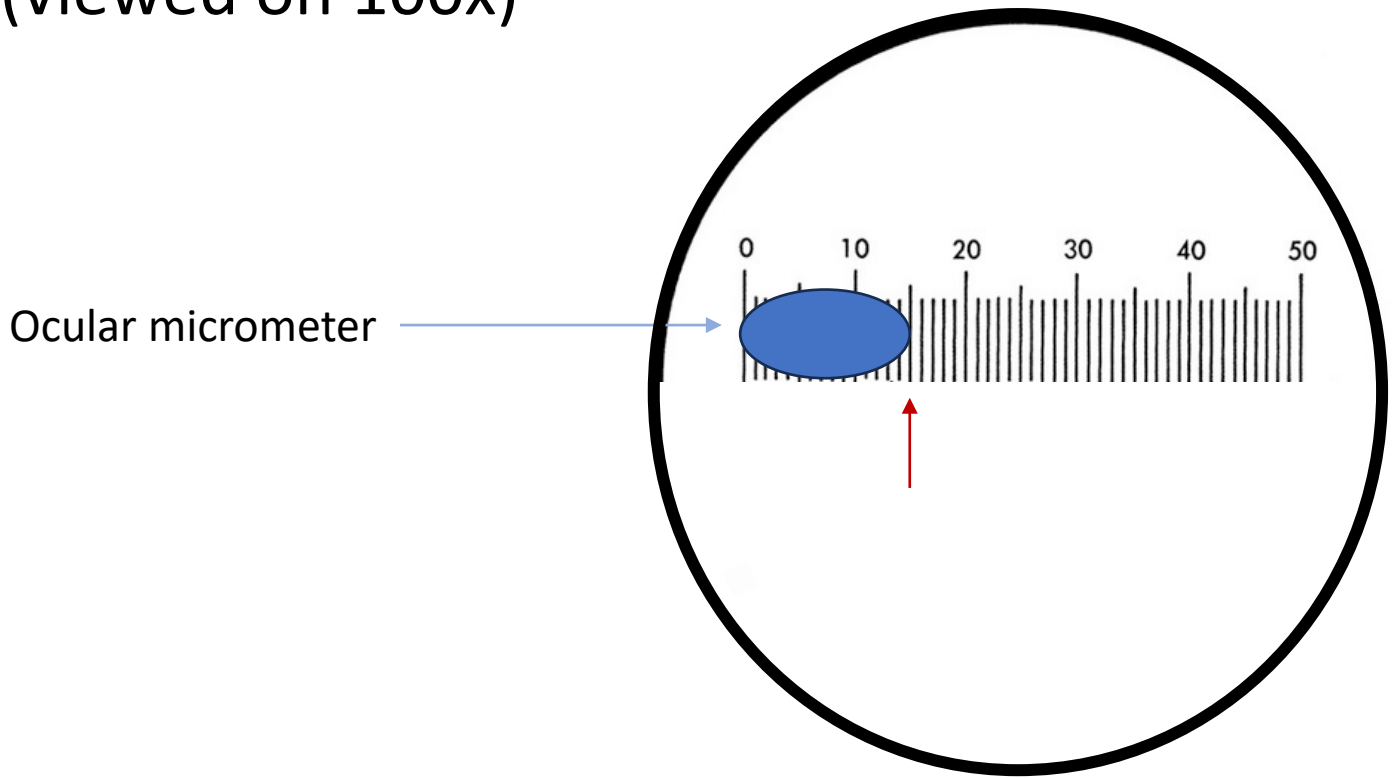



7. $\frac{\text{Length seen on stage micrometer}}{\text{\# of divisions counted on ocular micrometer}} \times 1000 = \_\_\_\_\ \mu m$

$\frac{0.2mm}{27} \times 1{,}000 = 7.4\ \mu m$

# Amebae Microscopic Identification

- Determine the size of the object in micrometers (viewed on 100x)




Ocular distance x calibration = size of object in micrometers

15 x 7.4µm = 111µm

# Amebae Microscopic Identification

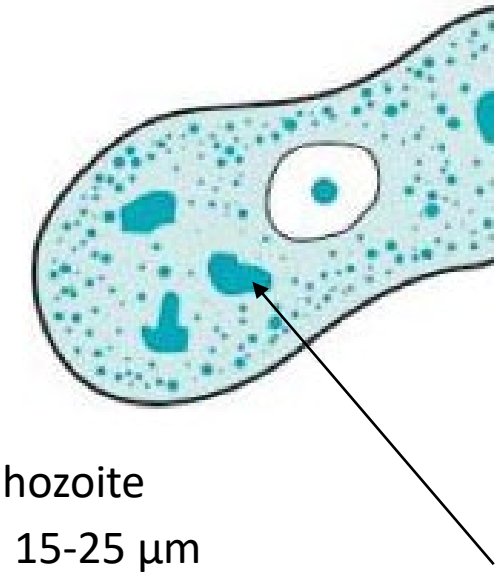
| | |
|---|---|
| STEP ONE | Form (Fluffy) |
| STEP TWO | Karyosome (Kittens) |
| STEP THREE | Peripheral chromatin (Purr) |
| STEP FOUR | Unique characteristic (Uncontrollably) |
| STEP FIVE | Size (Sunday) EVERY |

# Intestinal Amebae: *Entamoeba histolytica/dispar/moshkovkii*

- Three species are morphologically identical organisms
  - *Entamoeba histolytica* is considered a pathogen
  - *Entamoeba dispar* and *Entamoeba moshkovkii* are consider nonpathogenic
- *Entamoeba histolytica* is found worldwide
  - Asymptomatic colonization
  - Amebic dysentery
    - Acute: vague abdominal symptoms
    - Chronic: formation of ameboma
  - Extraintestinal amebiasis
    - Organism erodes the intestines and enters the circulation

# Intestinal Amebae: *Entamoeba histolytica/dispar/moshkovkii*

Nucleus: **regular dot-like centrally** located **karyosome** with **evenly distributed peripheral chromatin**

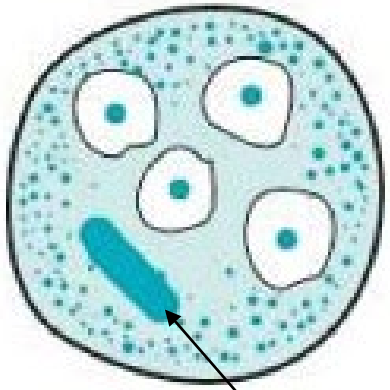


Trophozoite

Size: 15-25 µm

Unique: presence of ingested RBCs

***ingested RBCs definitively identify the organism as *Entamoeba histolytica* ***

Cyst

Size: 10-20 µm

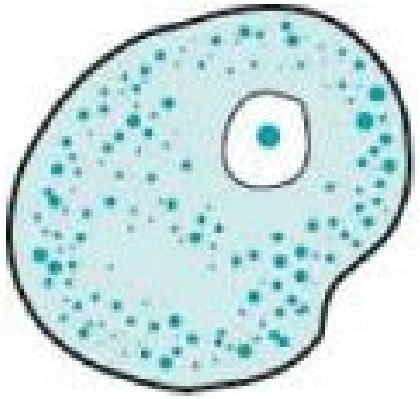
Unique: may contain chromatoidal bars with rounded ends

# Intestinal Amebae: *Entamoeba histolytica*

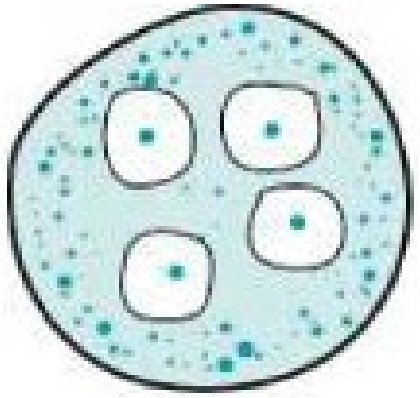
- Other tests for identification
  - Tests that detect *E. histolytica* antigen in stool provide evidence of current infection
    - More sensitive than a microscopic examination of feces
    - These tests use the EIA method with monoclonal antibodies to proteins (e.g. serine-rich antigen or the galactose/ N-acetylgalactose adhesion lectins)
  - Multiplex molecular assays for GI syndromic testing are also available, and *E. histolytica* is often included

# Intestinal Amebae: *Entamoeba hartmanni*

Nucleus: **regular dot-like centrally** located **karyosome** with **evenly distributed peripheral chromatin**



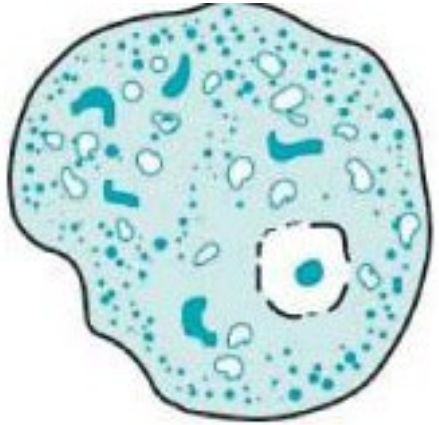
Trophozoite
Size: ≤12 µm



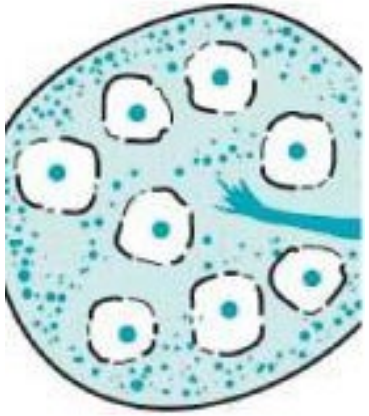
Cyst
Size: ≤10 µm
Unique: may contain chromatoidal bars with rounded ends

# Intestinal Amebae: *Entamoeba coli*

Nucleus: **regular dot-like eccentrically** located **karyosome** with **uneven peripheral chromatin**



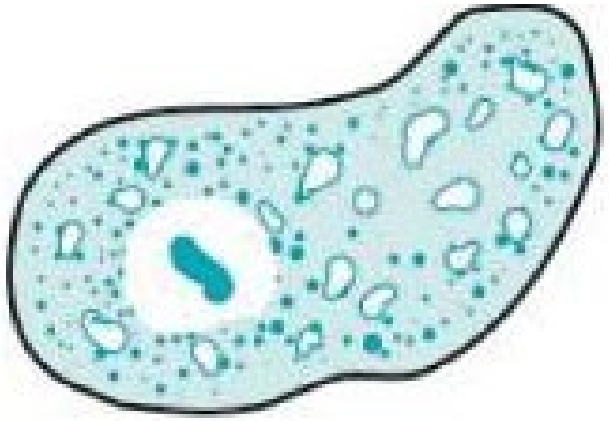
Trophozoite
Size: 15-50µm



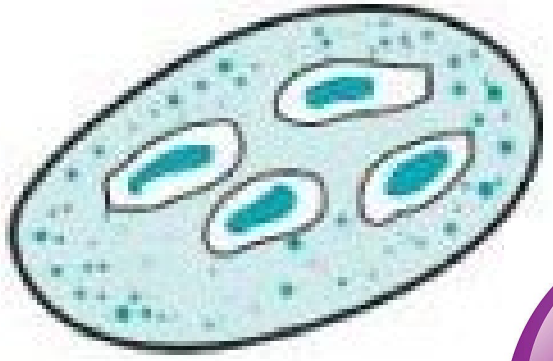
Cyst
Size: 15-25µm
Unique: may contain chromatoidal bars with splintered ends.
Up to eight nuclei.

# Intestinal Amebae: *Endolimax nana*

Nucleus: **irregular blot-like centrally** located **karyosome** without **peripheral chromatin**



Trophozoite
Size: 5-12µm

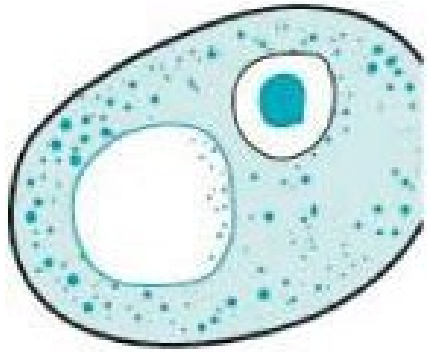


Cyst
Size: 5-12µm

# Intestinal Amebae: *Iodamoeba butschlii*

Nucleus: **regular blot-like centrally** located **karyosome** surrounded by achromatic granules <u>without</u> **peripheral chromatin**

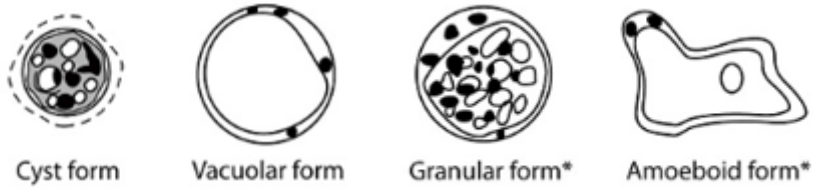
Trophozoite
Size: 6-20µm



Cyst
Size: 6-15µm
Unique: large vacuole.
one nucleus.

# Intestinal Amebae: *Blastocystis* species

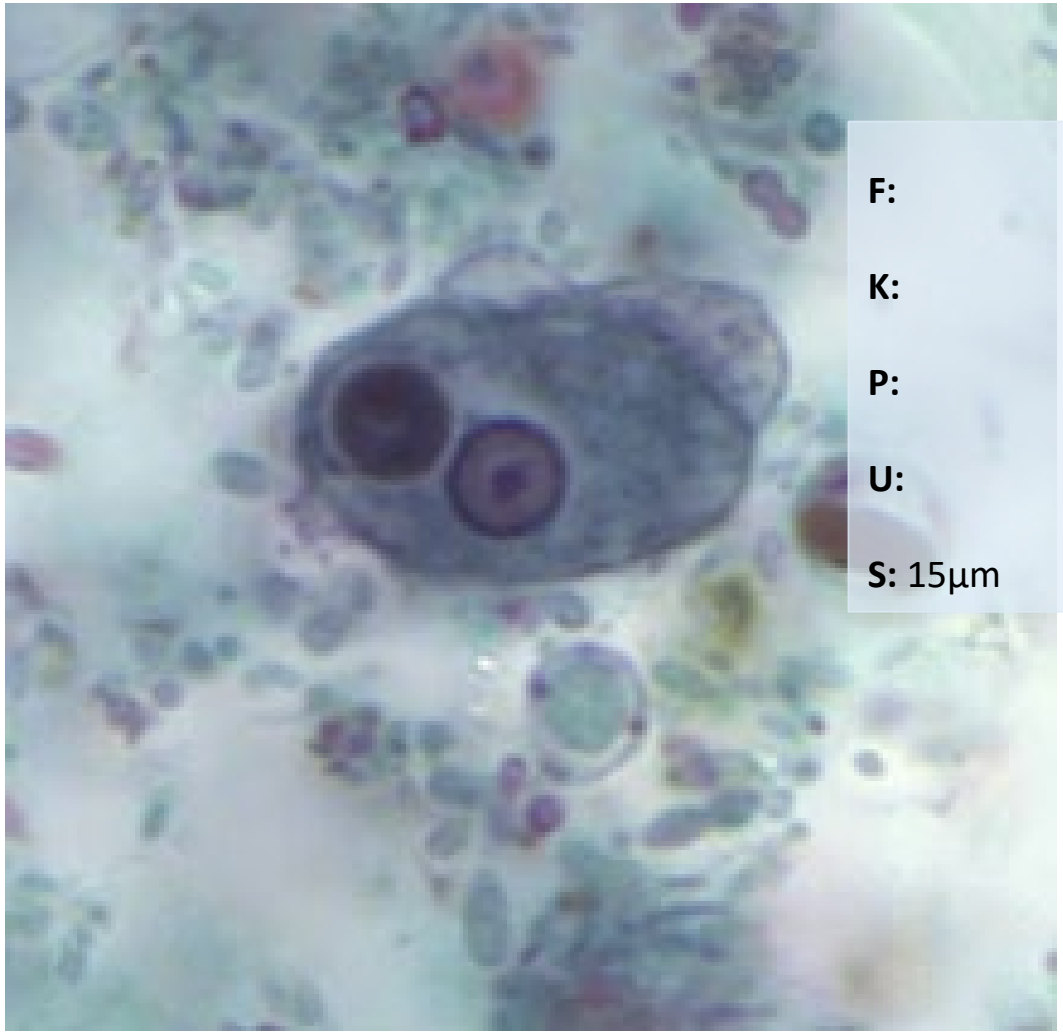
- Not exactly an ameba, but instead closely related to brown algae
- Controversy over pathogenicity
  - Considered a pathogen when patient is symptomatic, and only parasite found




Vacuolar form
Size: 5-15µm
Unique: large central vacuole up to 6 nuclei pushed up against the plasma membrane

# Amebae Ova and Parasite Examination (OVAP)

Iodine wet preparation



**F:**

**K:**

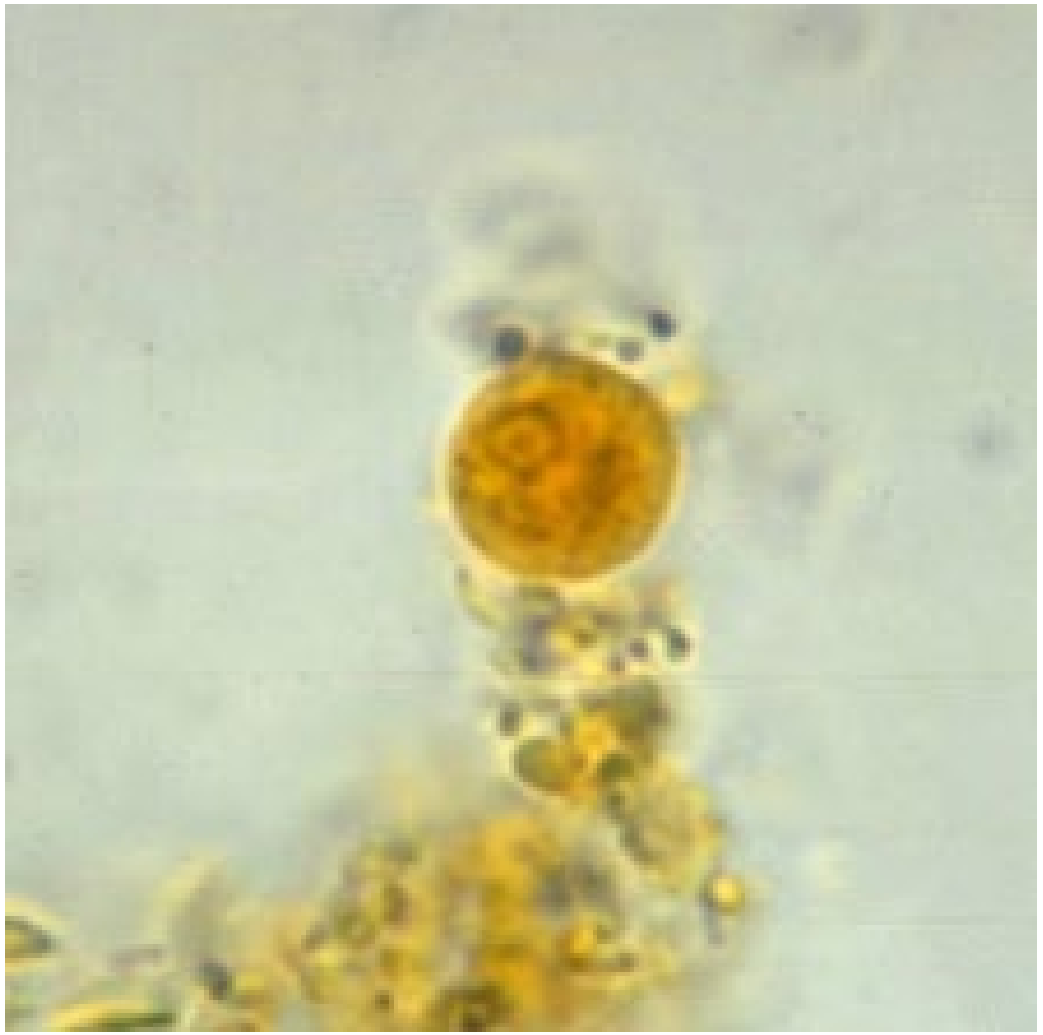
**P:**

**U:**

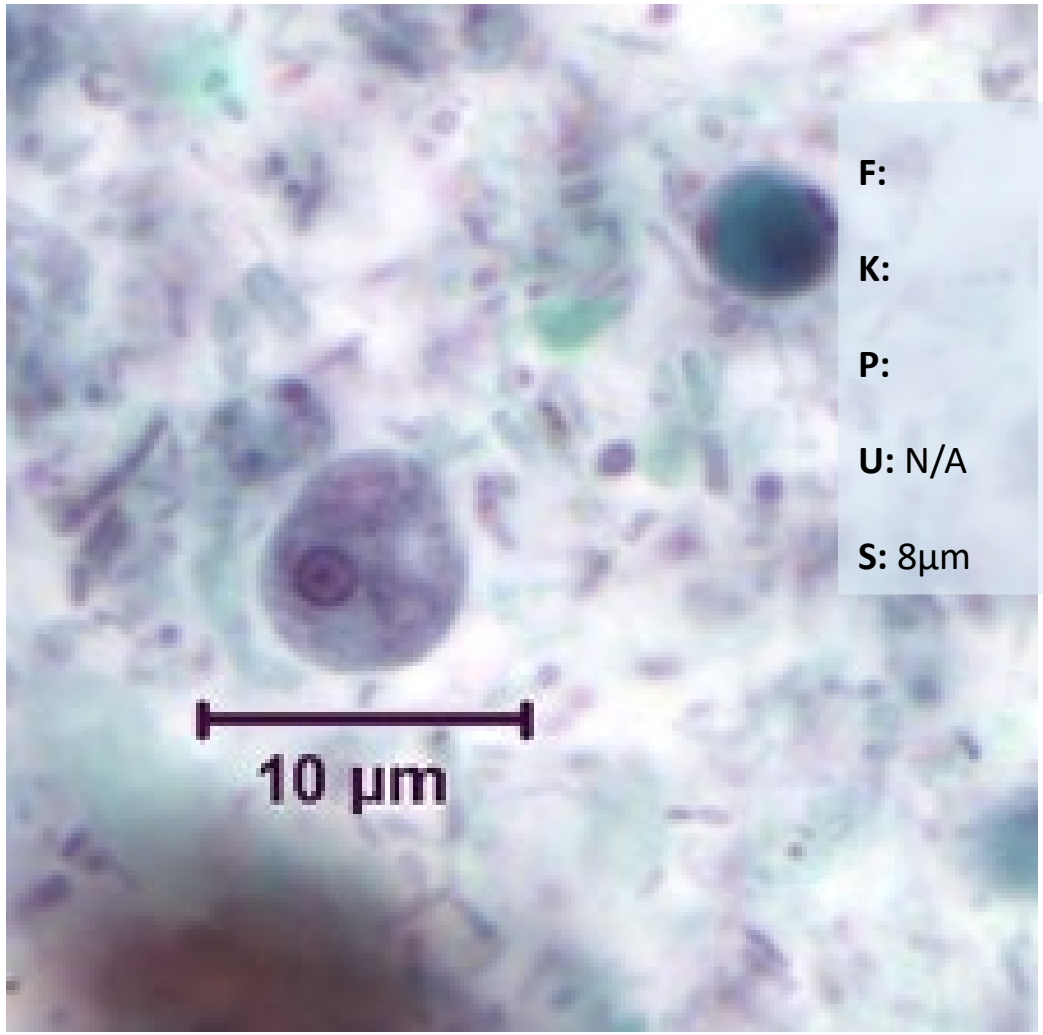
**S:** 15µm

Trichrome

# Amebae Ova and Parasite Examination (OVAP)



Iodine wet preparation




**F:**

**K:**

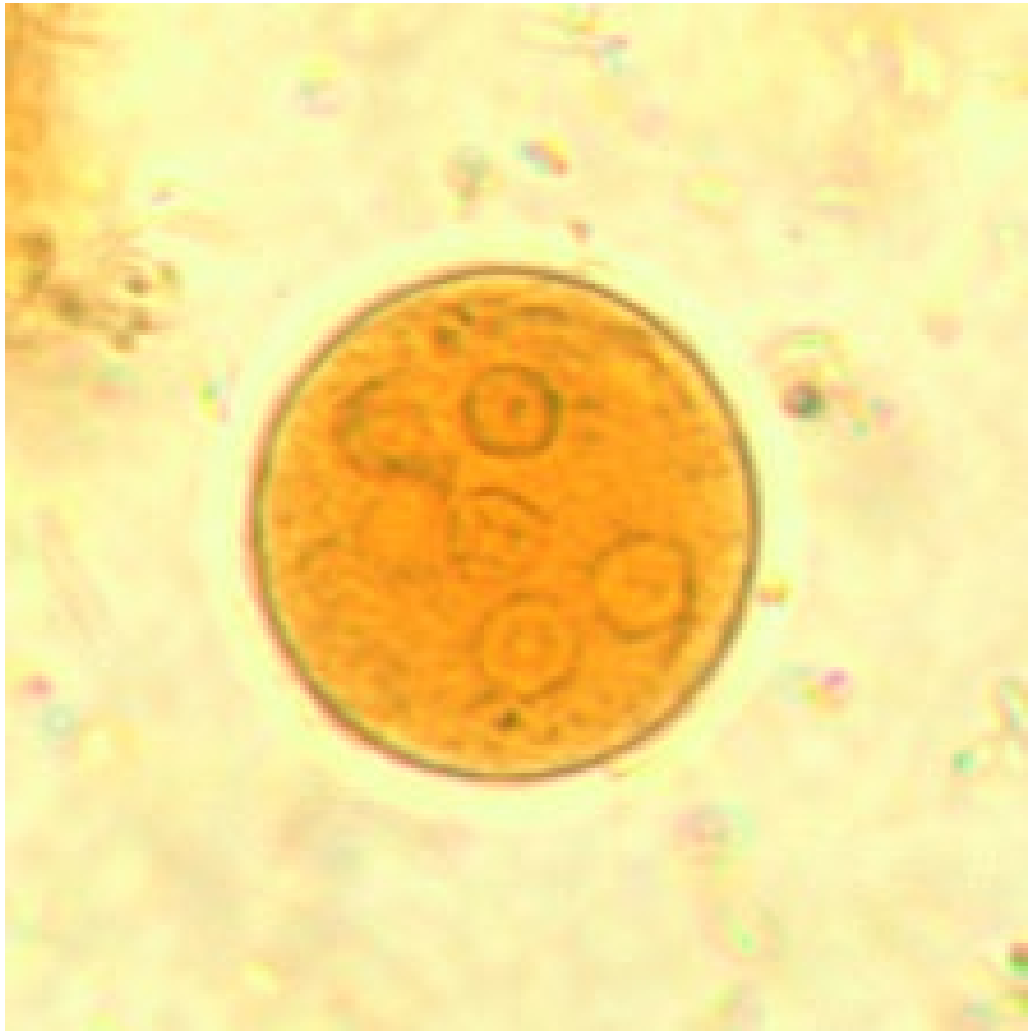
**P:**

**U:** N/A

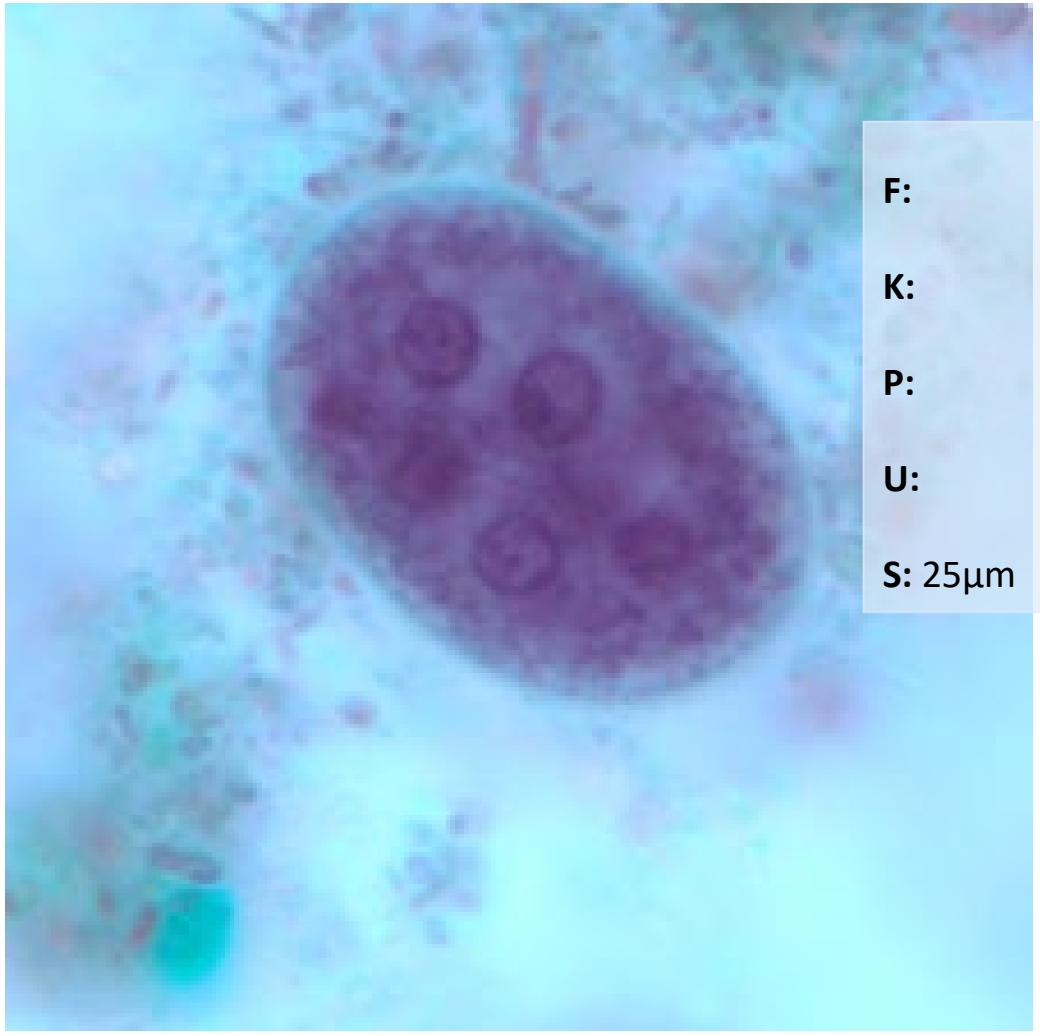
**S:** 8µm

Trichrome

# Amebae Ova and Parasite Examination (OVAP)



Iodine wet preparation



**F:**

**K:**

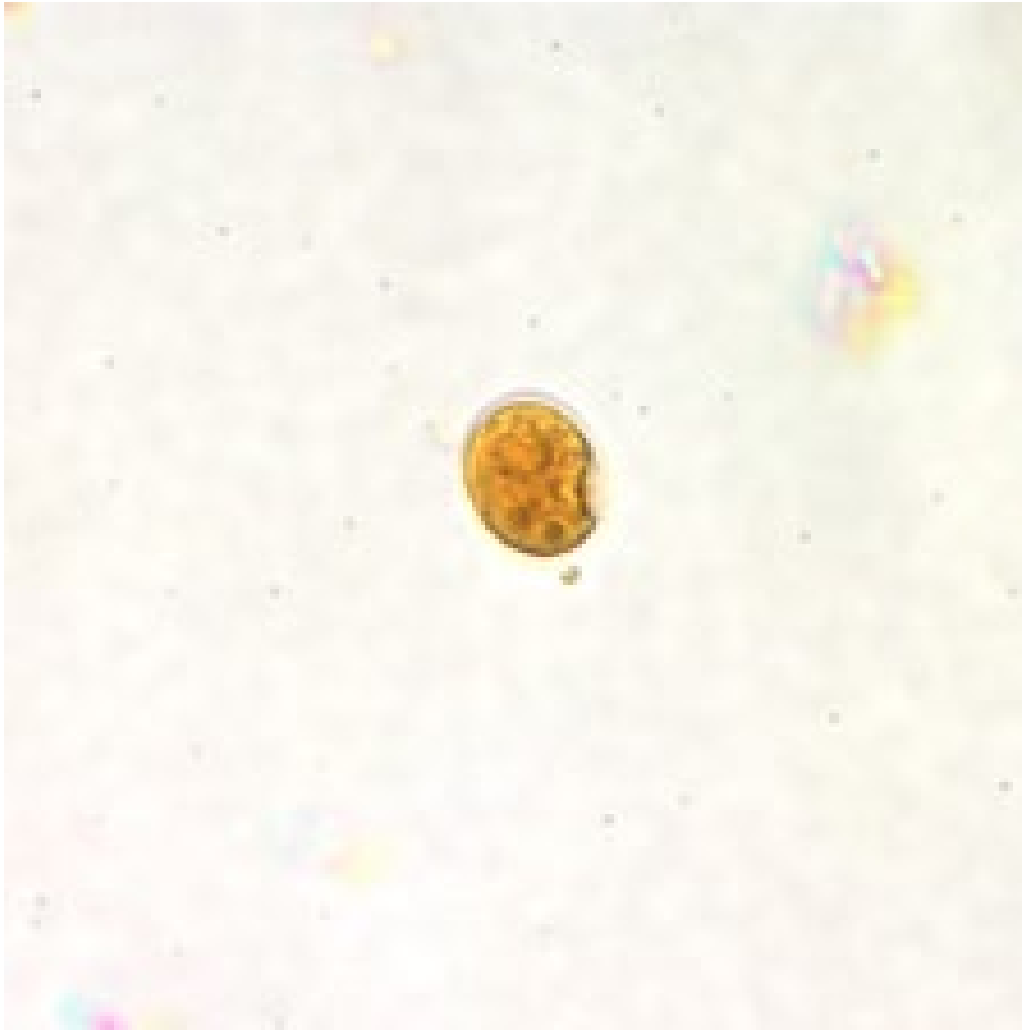
**P:**

**U:**

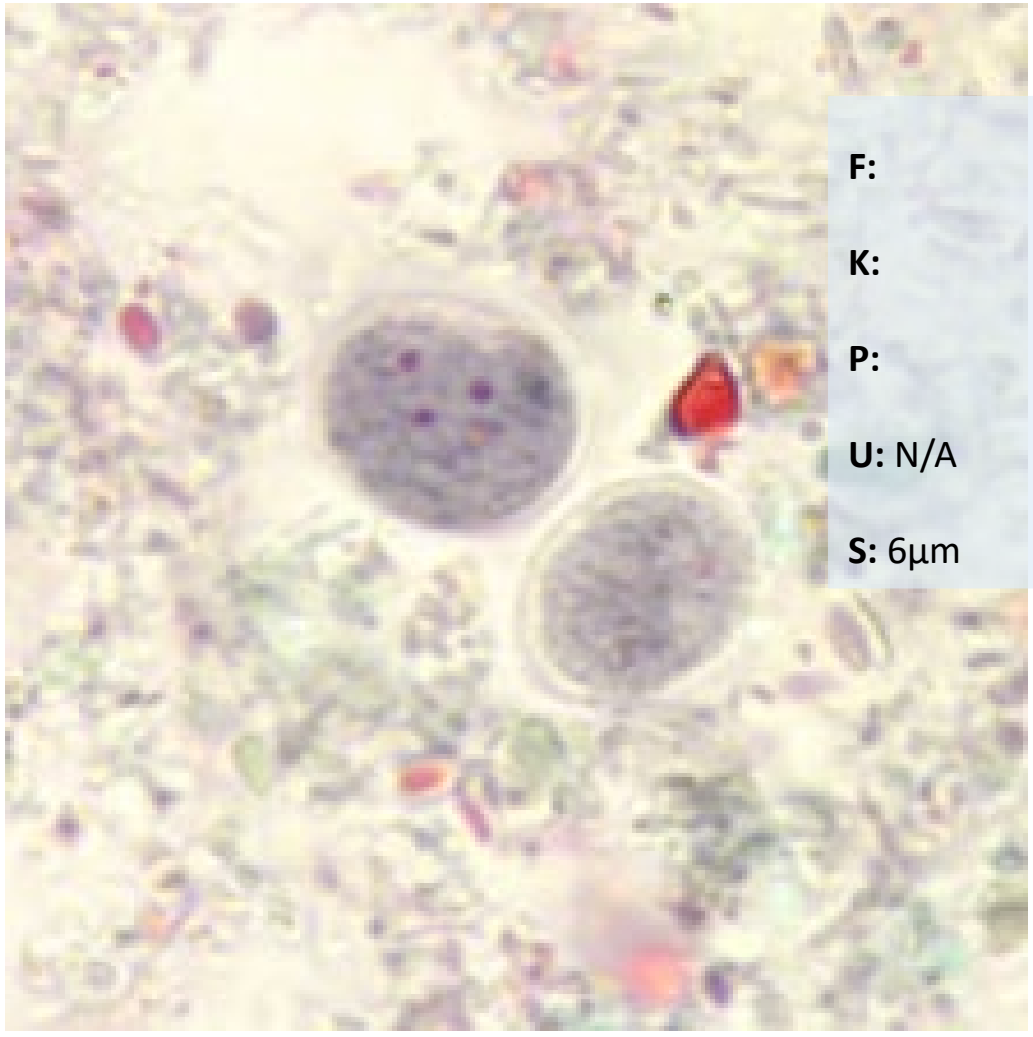
**S:** 25µm

Trichrome

# Amebae Ova and Parasite Examination (OVAP)



Iodine wet preparation



**F:**

**K:**

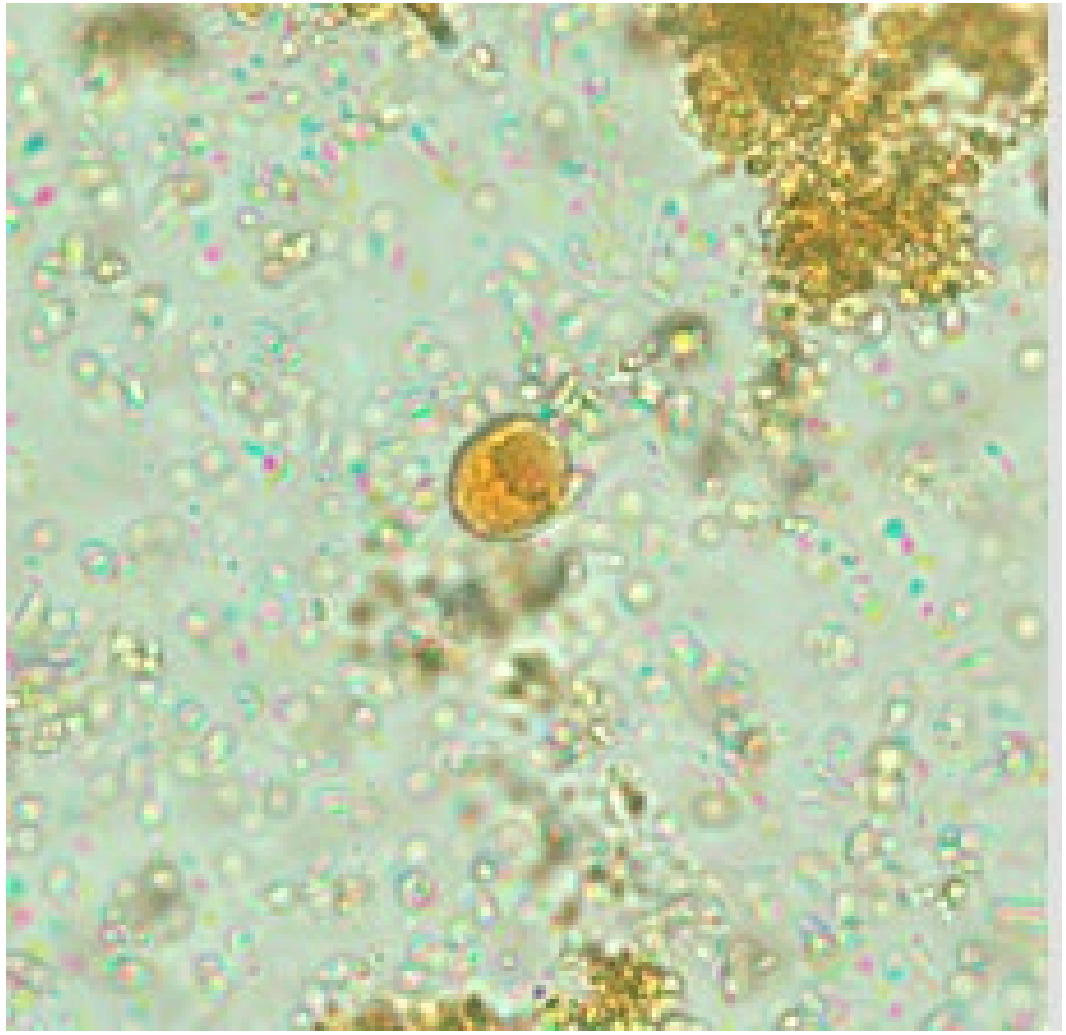
**P:**

**U:** N/A

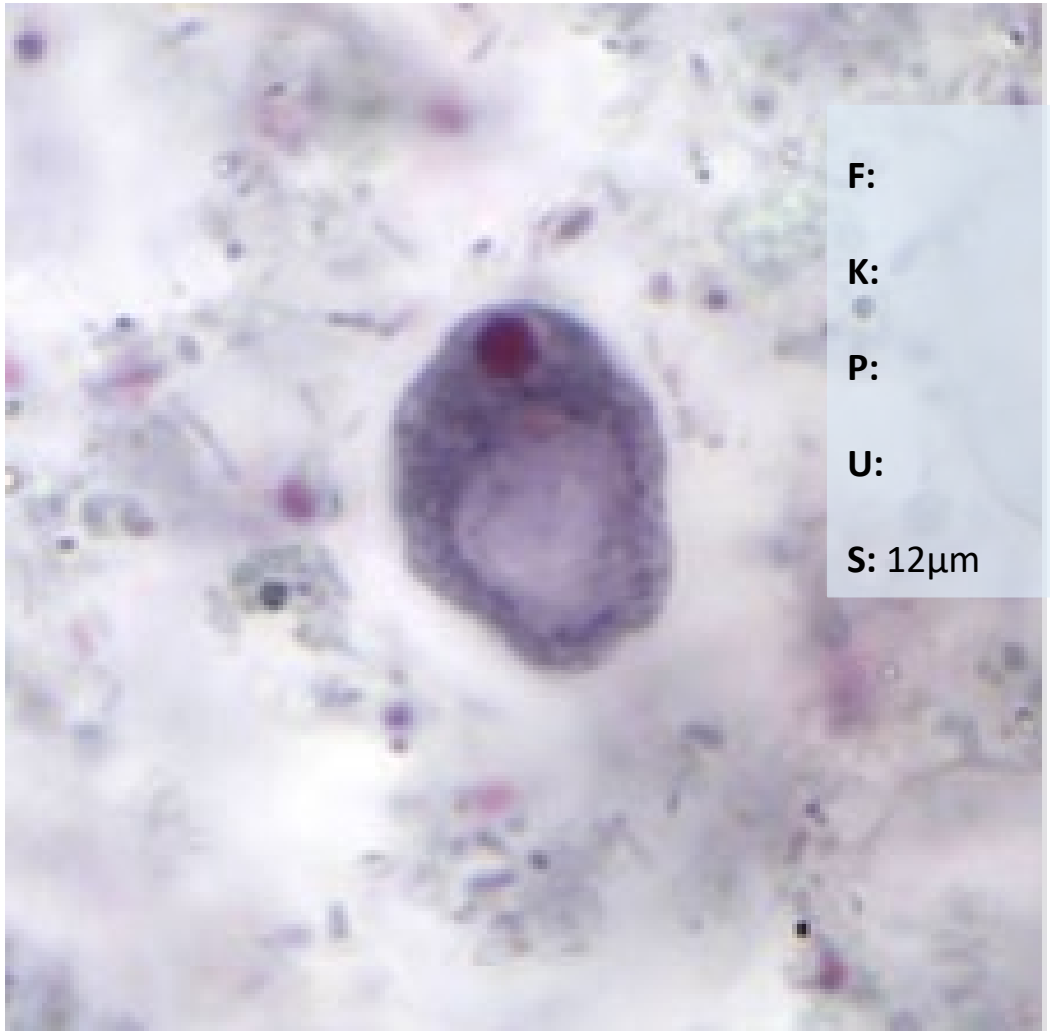
**S:** 6µm

Trichrome

# Amebae Ova and Parasite Examination (OVAP)



Iodine wet preparation



**F:**

**K:**

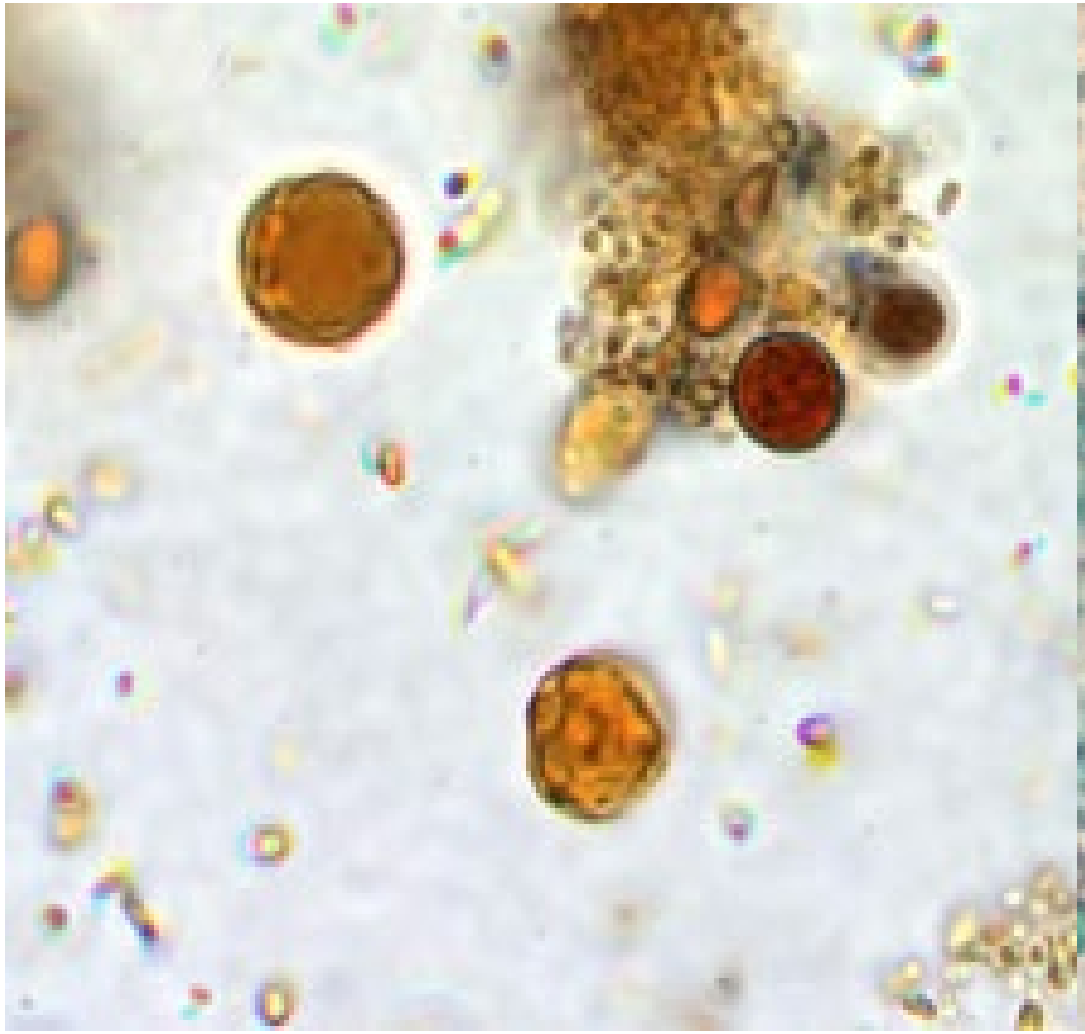
**P:**

**U:**

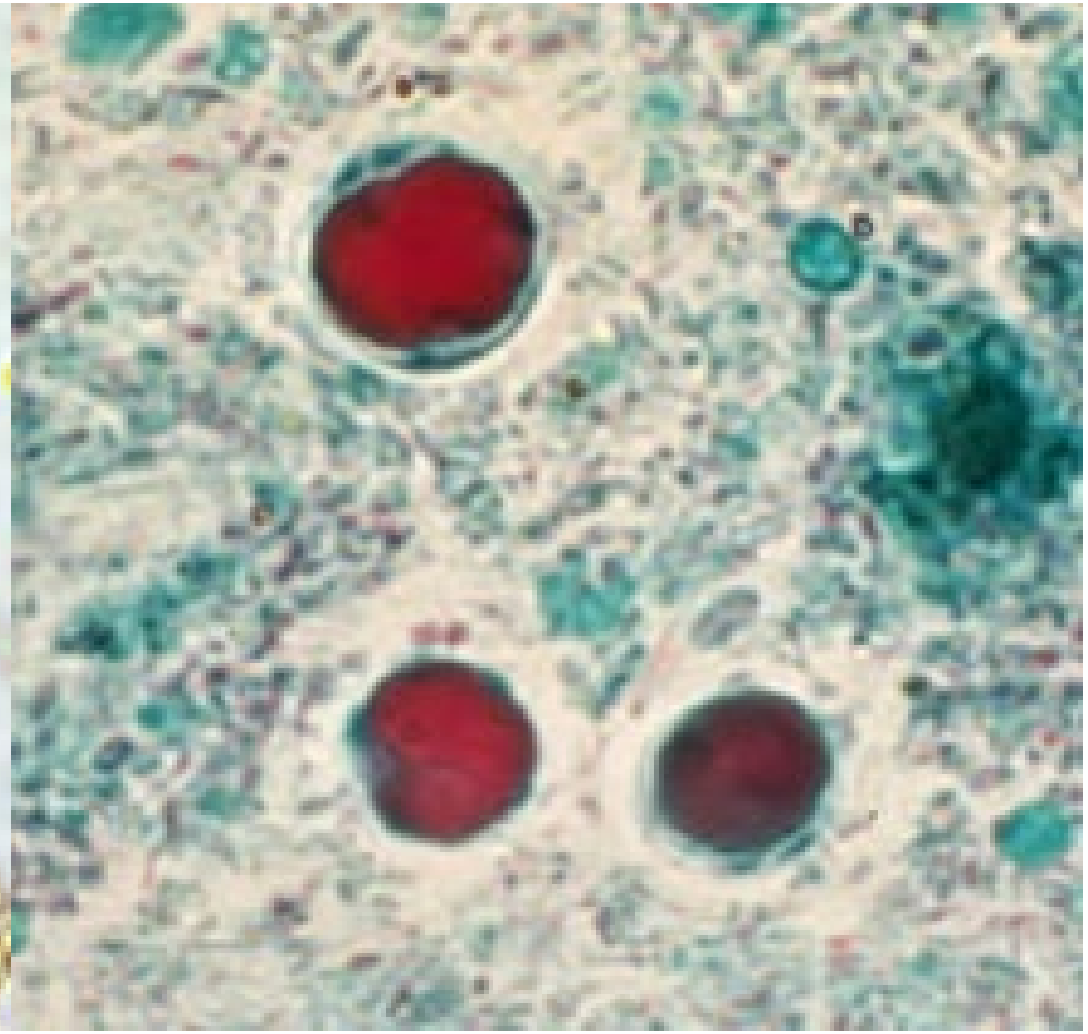
**S:** 12µm

Trichrome

# Amebae Ova and Parasite Examination (OVAP)



Iodine wet preparation



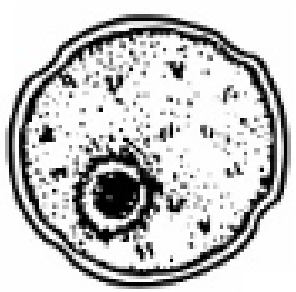
Trichrome

# Tissue Amebae: *Naegleria fowleri*

- Primary amebic meningoencephalitis (PAM) in immunocompetent & immunocomprimised
  - Trophozoite gains access to CNS by inhalation into the upper respiratory tract followed by the penetration of the nasal mucosa, which allows them to travel along the olfactory nerve to the brain
  - Headache, fever, stiff neck, nausea
  - Rapidly fatal
  - CSF will have increased neutrophils and proteins with decreased glucose

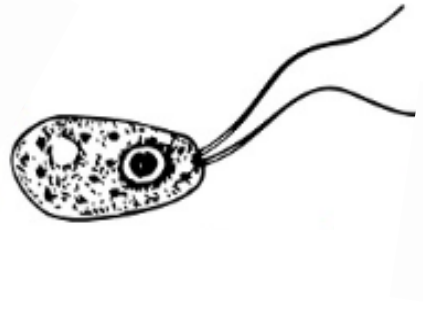
# Tissue Amebae: *Naegleria fowleri*

Nucleus: regular blot-like centrally located karyosome without peripheral chromatin



Cyst

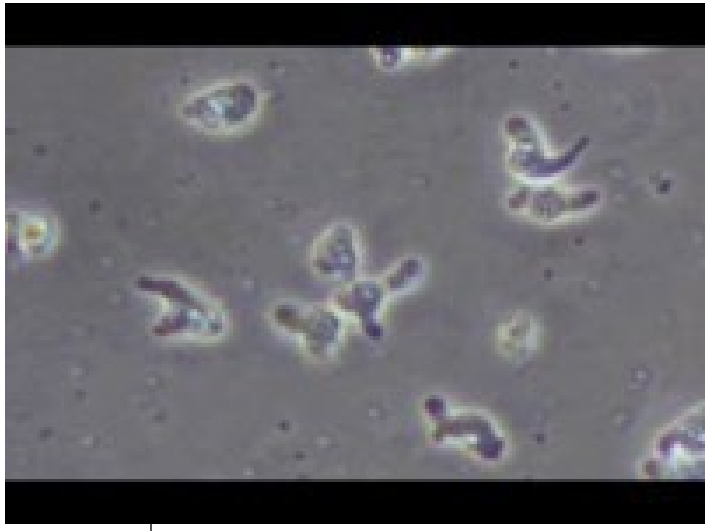
Trophozoite
Size: 10-35µm



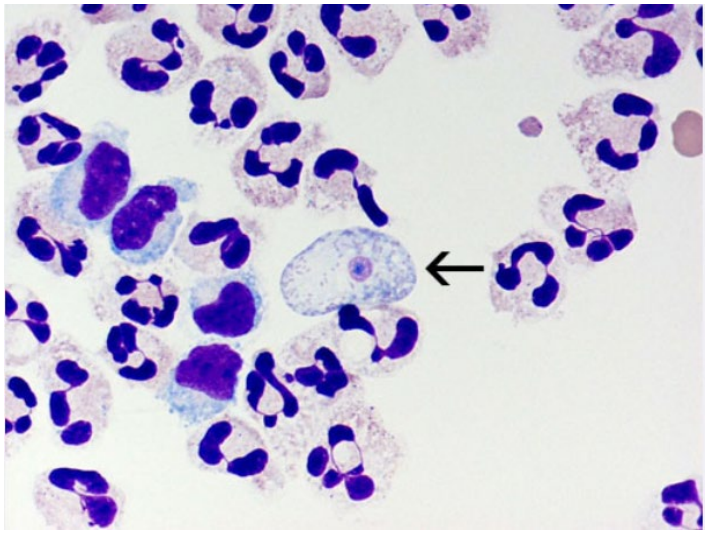
Transient flagellate

# Tissue Amebae: *Naegleria fowleri*

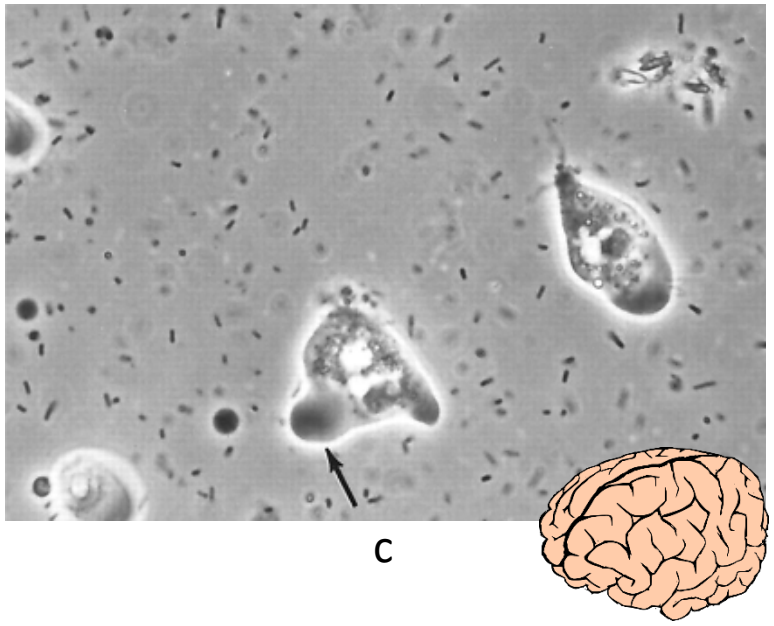
- Tests for identification
  a) Motile trophozoites can been seen in a wet mount of CSF
  b) Trophozoites can be seen on a Wright stain of CSF
  c) Can be cultured on nonnutrient agar overlayed with *Escherichia coli*



a



b



c

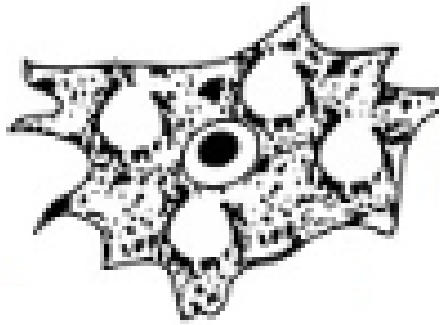
https://www.youtube.com/watch?v=2r8iJXwJQas

# Tissue Ameba: *Acanthamoeba* species

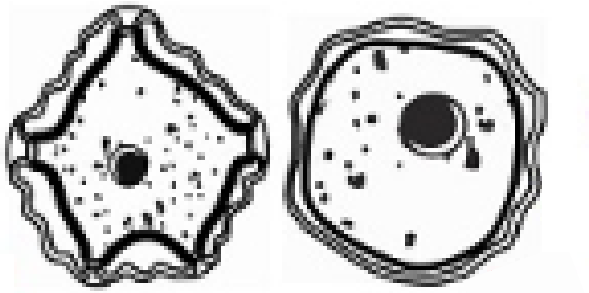
- Granulomatous amoebic encephalitis (GAE) in immunocompromised hosts
  - Months to years
- Amebic keratitis in immunocompetent due to improper storage and disinfection procedures (ex. wearing contacts while swimming)
- Cutaneous infections in AIDs patients
  - Chronic nonhealing lesions

# Tissue Ameba: *Acanthamoeba* species

Nucleus: regular blot-like centrally located karyosome without peripheral chromatin



Trophozoite
Size: 15-45µm
Unique: Single central nucleus with blunt pseudopods with spinelike projections



Cyst
Size: 15-20µm
Unique: Single central nucleus with doubled walls and a wrinkle appearance

# Tissue Ameba: *Acanthamoeba* species

- Tests for identification
  - Motile trophozoites can been seen in a wet mount of clinical specimens
  - Trophozoites and cysts can be seen on a permanent smears
  - Can be cultured on nonnutrient agar overlayed with *Escherichia coli*


https://www.youtube.com/watch?v=qBIdfJEJMK0

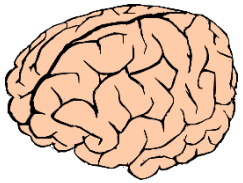
# Tissue Ameba: *Balamuthia mandrillaris*

- Primarily found in soil
- GAE and cutaneous infections  in immunocompetent and immunocomprimsed hosts.

Nucleus: regular blot-like centrally located karyosome without peripheral chromatin

Trophozoite
Size: 30-60µm
Unique: single central nucleus with broad pseudopods

Cyst
Size: 10-30µm
Unique: single central nucleus multiwalled



# Citations


- Mahon, C. R., & Lehman, D. C. (2023). *Textbook of Diagnostic Microbiology* (7th ed., pp. 639-707). Elsevier.
- Centers for Disease Control and Prevention (2019, November 20). DPDx-Laboratory Identification of Parasites of Public Health Concern. Retrieved November 13, 2023, from https://www.cdc.gov/dpdx/az.html