Microbiology Primer

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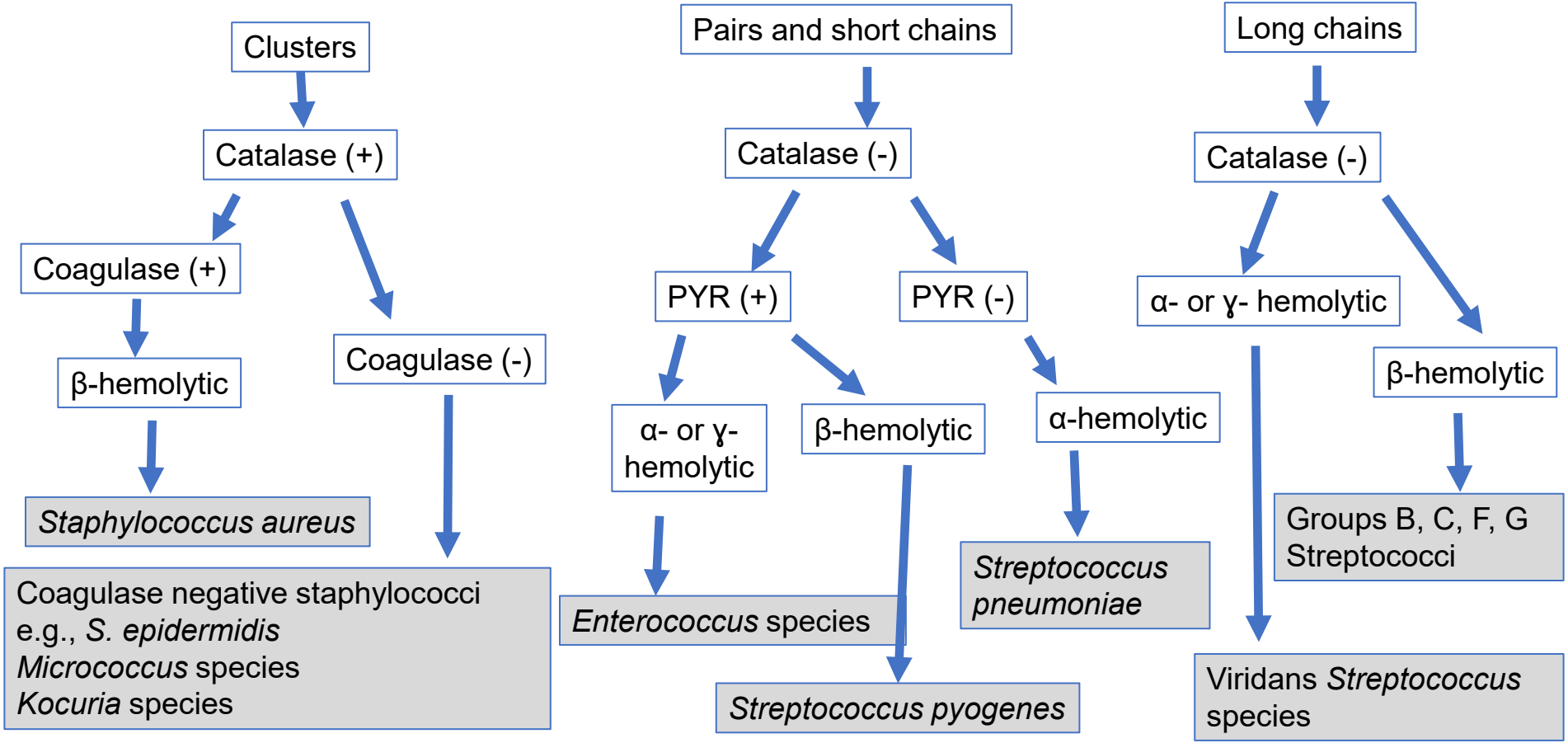
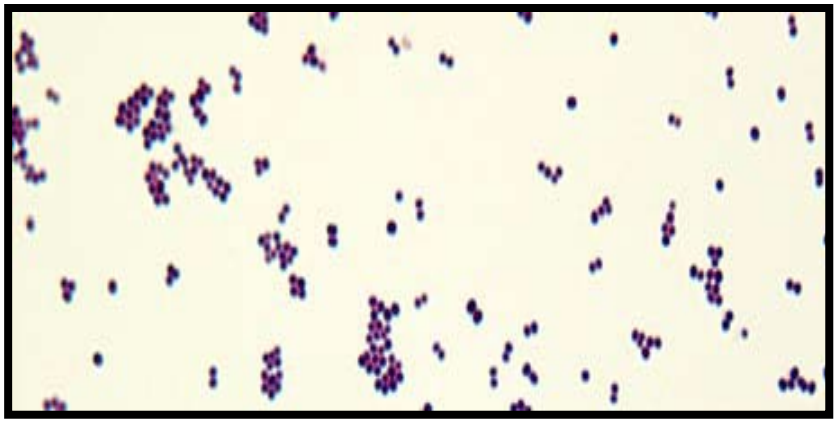
6/5/20

Bacteriology

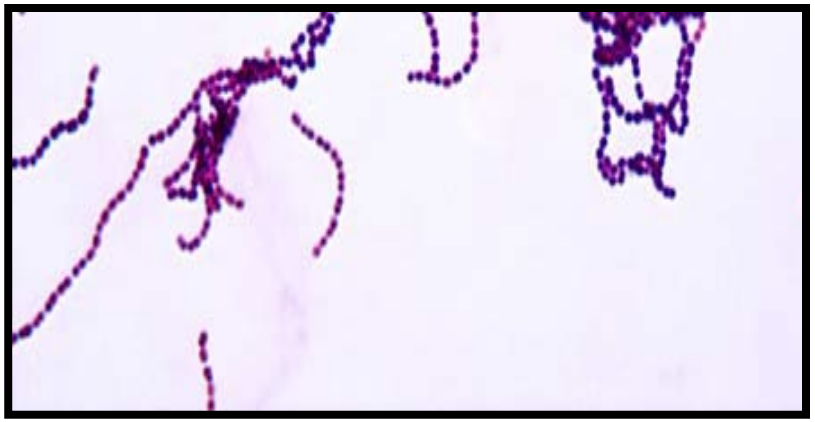
Gram Positive Cocci



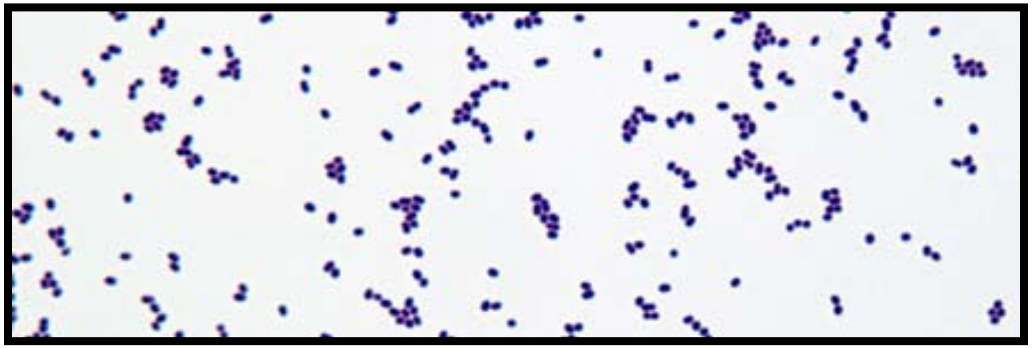
Gram-Positive Cocci



Staphylococci



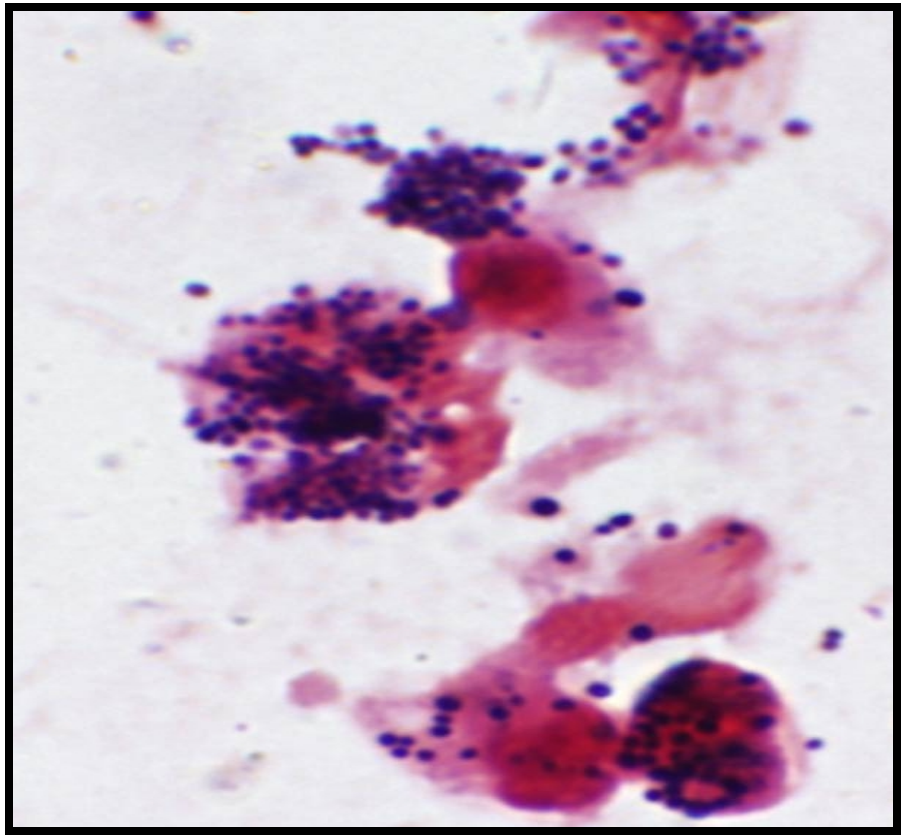
Streptococci



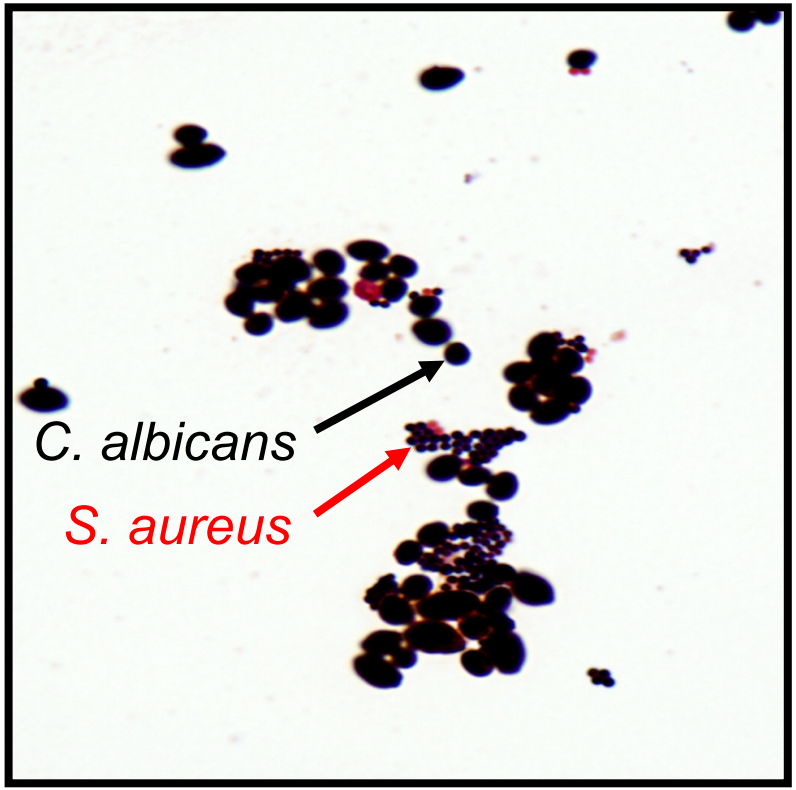
Enterococci

Staphylococcus aureus

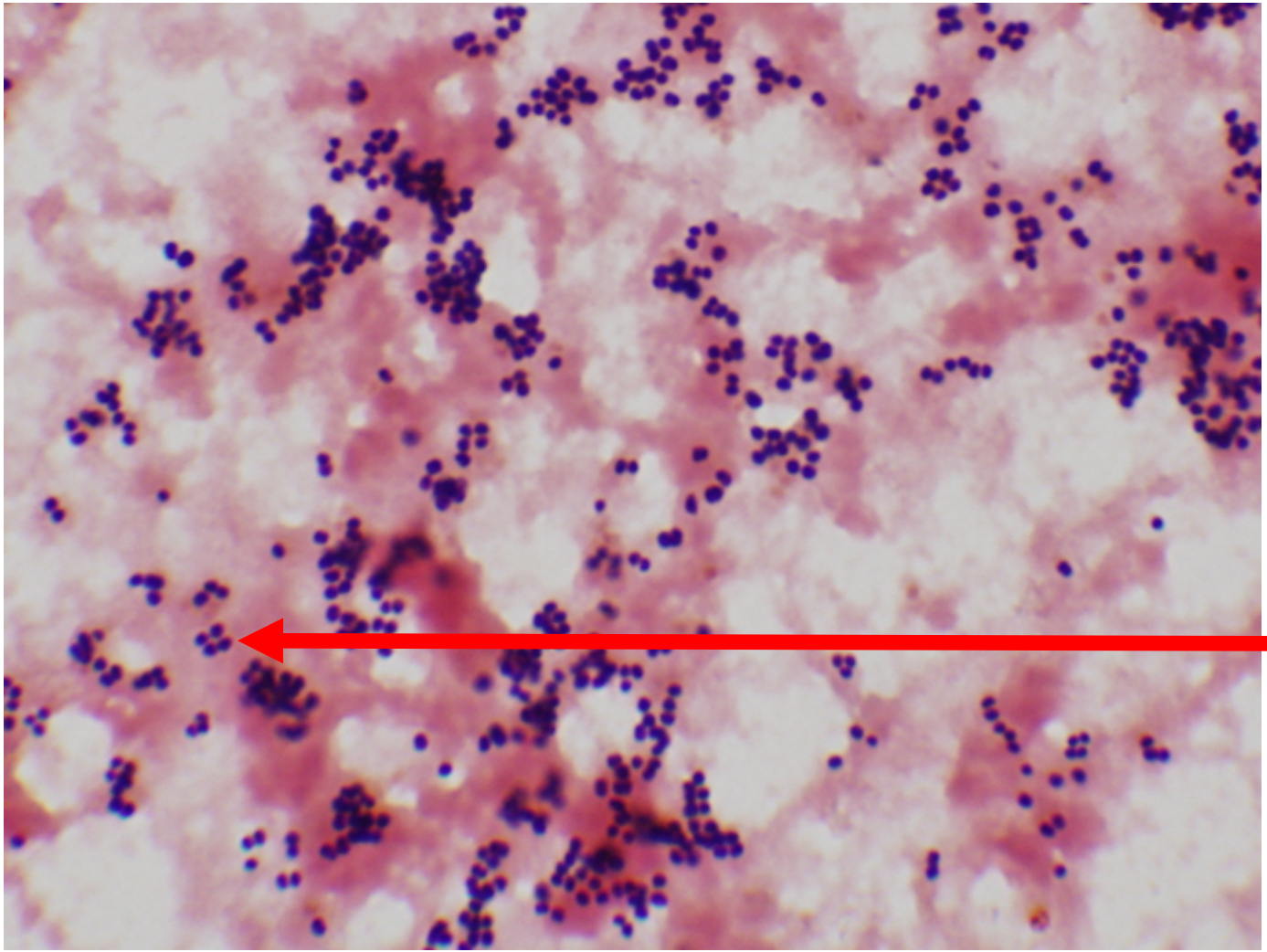
- In clinical specimens, *S. aureus* appears as pairs, small clusters, or within phagocytic cells



Yeasts May Appear Gram-Positive



Note size difference between *Staphylococcus aureus* and *Candida albicans*



Gram-positive cocci in clusters indicate *Staphylococcus*, *Micrococcus* or *Kocuria* species (plus others)

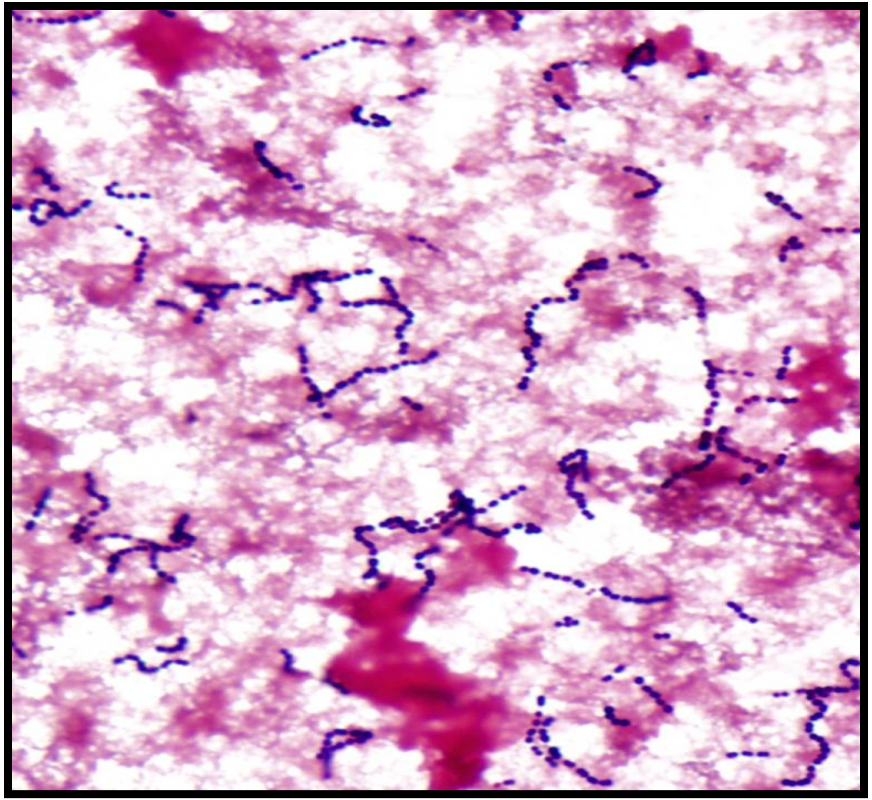
Tetrads may indicate coagulase negative staphylococci or *Micrococcus* species

Streptococcus species – β-hemolytic

Species	Lancefield	Special features
S. pyogenes	A	Small colony Large zone of β-hemolysis “String of pearls” Gram stain (round cells) PYR (+); Bacitracin susceptible
S. agalactiae	B	Large colony Small zone of β-hemolysis CAMP test (+); hippurate hydrolysis (+) May be confused with *Listeria monocytogenes*
S. dysgalactiae subsp. *dysgalactiae*	C	
S. dysgalactiae subsp. *equisimilis*	C, G, (A, L)	
S. anginosus group	A, C, G, F	Can be α- or β-hemolytic
S. porcinus	E, P, U, V	

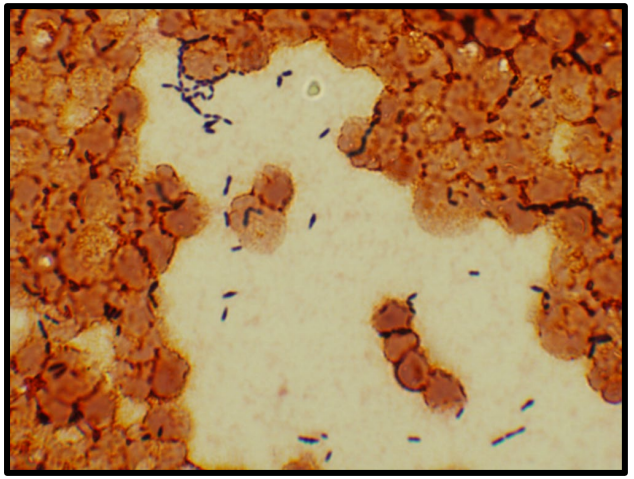
Streptococcus pyogenes
Group A Streptococcus

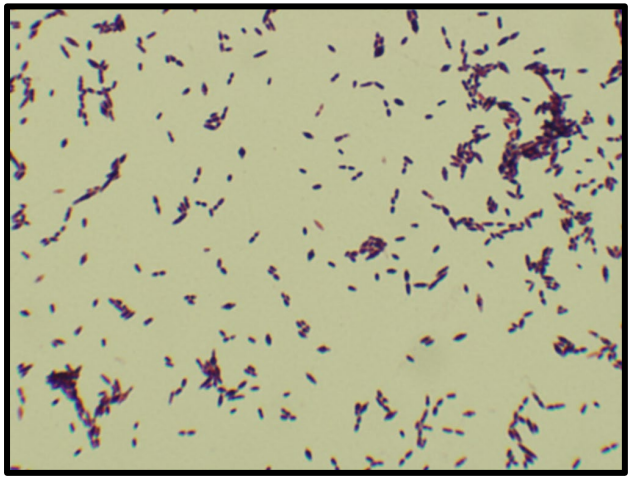
- Forms long chains of round cells, described as "string of pearls"
- β-hemolytic colonies
  - Only PYR (pyrrolidonyl arylamidase) positive *Streptococcus* species



Nutritionally Variant Streptococci

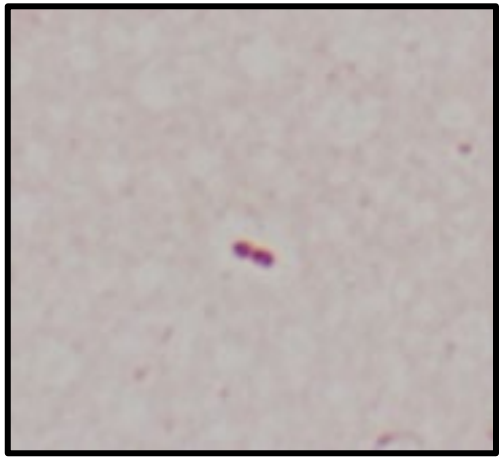
- Two genera
  - *Abiotrophia* species
  - *Granulicatella* species
- Require pyridoxal or cysteine to grow
- Will grow only on chocolate agar
- May be resistant to penicillin
  - *Granulicatella adiacens* may be ceftriaxone-resistant

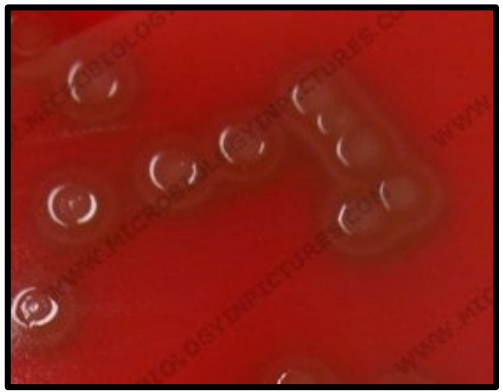




Streptococcus pneumoniae

- Member of *Streptococcus mitis* group
- Generally stains uniformly in clinical specimens but may decolorize, appearing Gram-negative in culture
- Individual cells appear elongated, arranged in pairs and short chains
- Refractile capsule
- Elongated, lancet shaped cells
- Doughnut-shaped colonies (raised edges, indented center)
- Cultured organisms rapidly identified by exposing cells to bile which “dissolves” bacteria (“bile soluble”)
  - Optochin susceptible



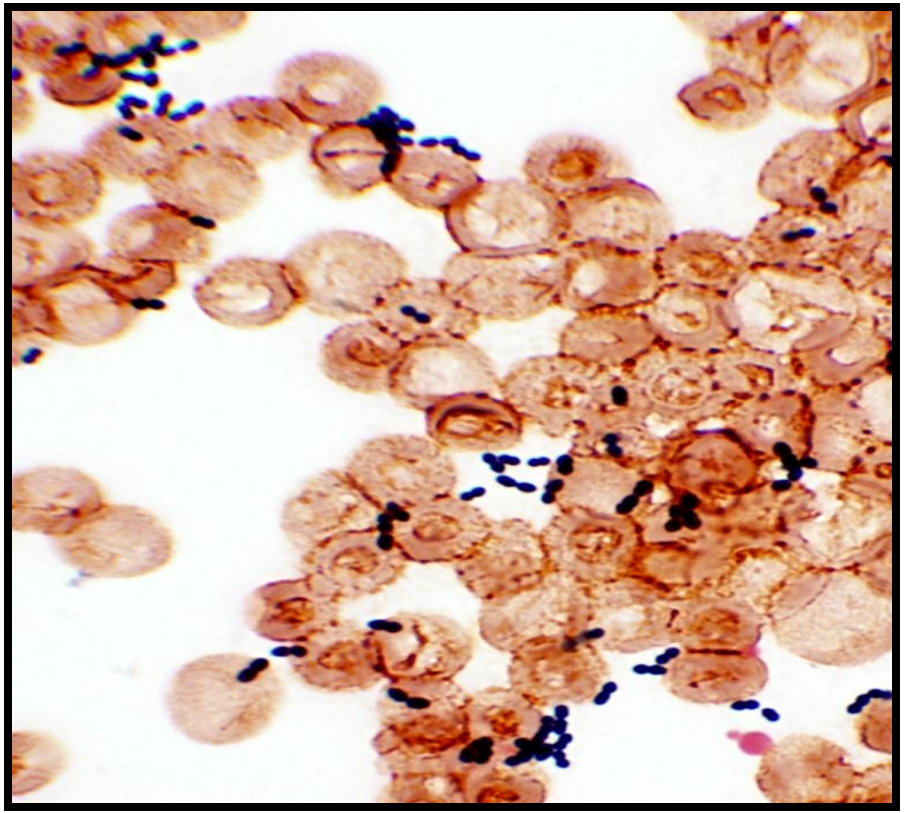


Viridans group Streptococci
>25 Species Divided into 5 groups

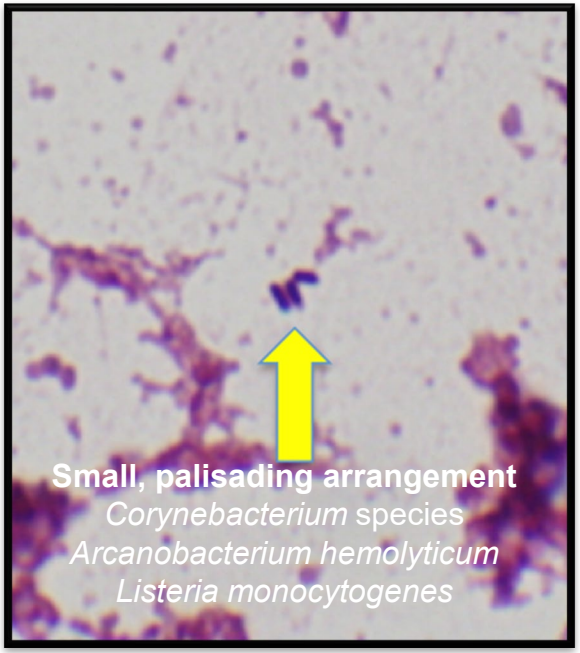
Group	Species
mutans	*S. mutans* *S. sobrinus*
anginosus (milleri)	*S. anginosus* *S. constellatus* *S. intermedius* *anginosus* group associated with abscesses
mitis	*S. mitis* *S. pneumoniae* *S. sanguinis* *S. gordonii* *S. oralis*
salivarius	*S. salivarius* *S. vestibularis*
bovis	*S. gallolyticus* subsp. *gallolyticus* (associated with gastrointestinal malignancies) *S. gallolyticus* subsp. *pasteurianus* (associated with neonatal meningitis) *S. gallolyticus* subsp. *macedonicus* *S. infantarius* subsp. *infantarius* and subsp. *coli* *S. alactolyticus* *S. equinus*

Enterococci

- Elongated Gram-positive cocci in pairs & short chains
  - Cultured organisms may be identified by positive PYR (pyrrolidonyl arylamidase) test, bile esculin (+), grow in 6.5% NaCl, most grow at 45°C, motile
- Most important species
  - *E. faecium* (often vancomycin and ampicillin resistant)
  - *E. faecalis* (typically vancomycin and ampicillin susceptible)
  - *E. gallinarum*
  - *E. casseliflavus* (yellow)

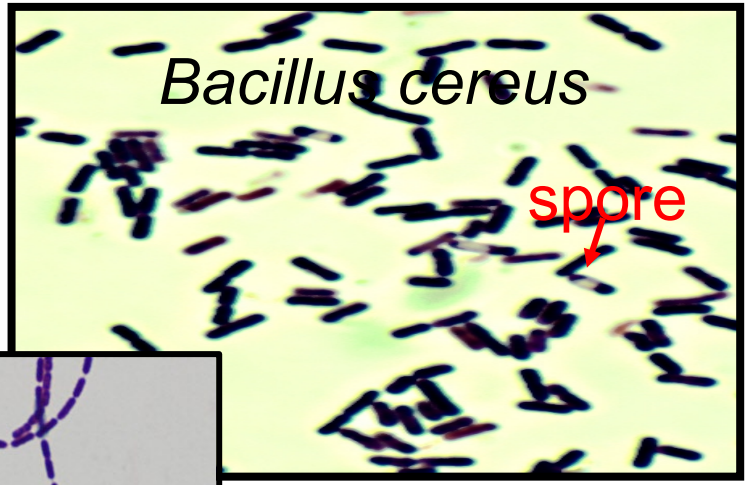


Gram Positive Rods - Gram Stain Morphologies



Spore-Forming Gram-Positive Rods

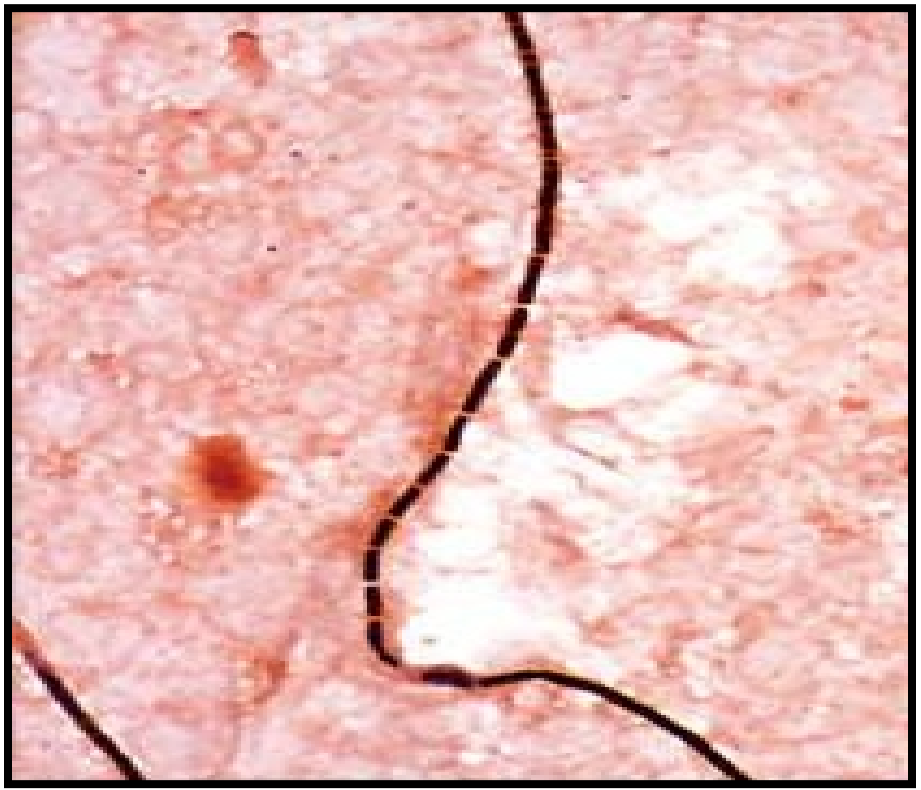
- Aerobes
  - *Bacillus* species
    - *B. anthracis* - ɣ-hemolytic & non-motile
    - *B. cereus* – β-hemolytic & motile
- Anaerobes
  - *Clostridium* species
    - *C. perfringens*
    - *C. septicum*
    - *C. difficile*
- Spores appear as clear areas



Note presence of spores indicated by cell clearing and/or swelling

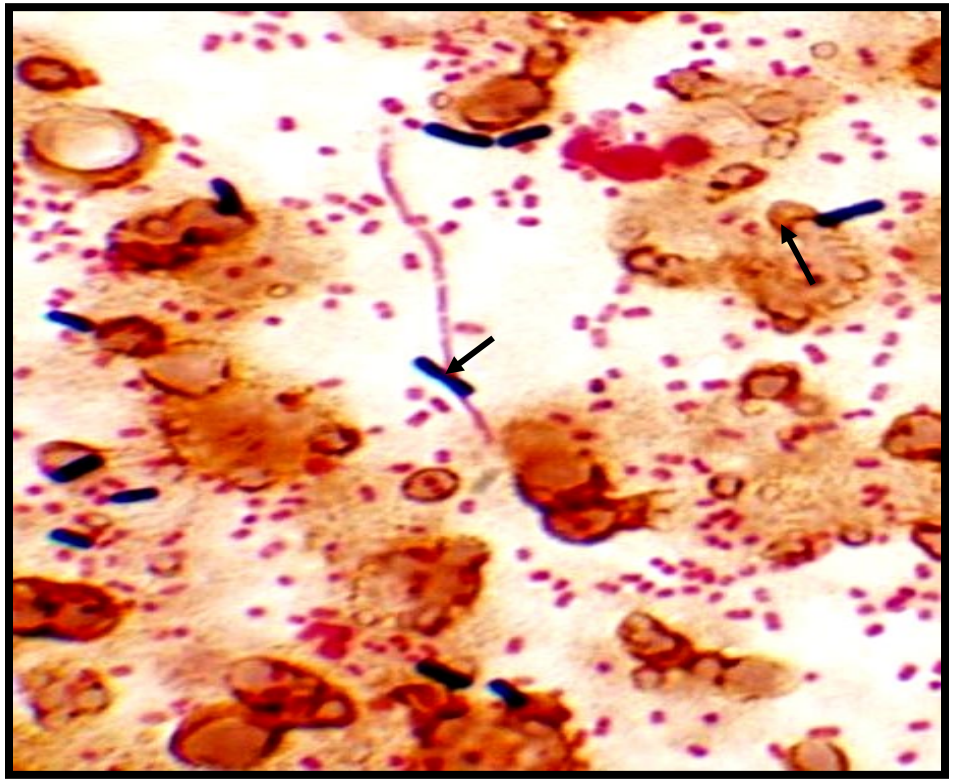
Bacillus anthracis

- Gram stain of *B. anthracis* in blood culture from bacteremic patient
  - Spores not seen in image
  - Bacteria form long chains



Clostridium perfringens

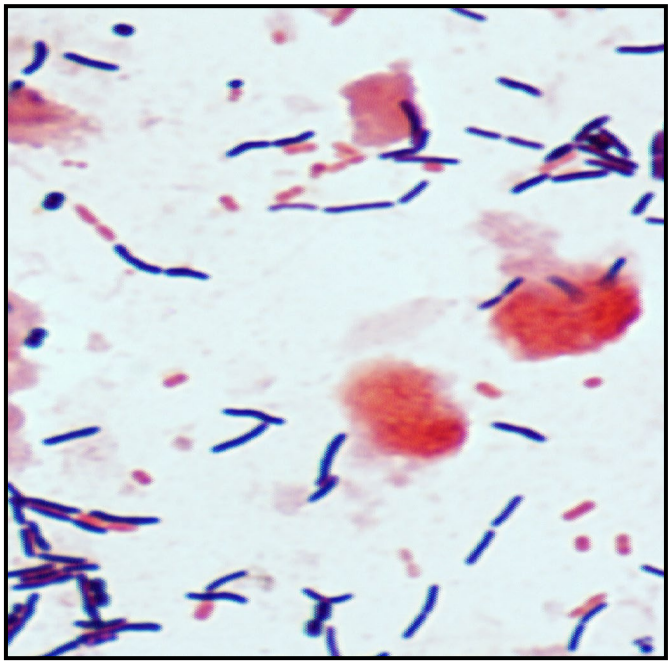
- Common species isolated from clinical specimens
- Spores almost never seen
- Rods “boxcar shaped” or rectangular
  - Larger than most bacteria



C. perfringens (arrows) in mixed culture with *Escherichia coli* and *Klebsiella pneumoniae*

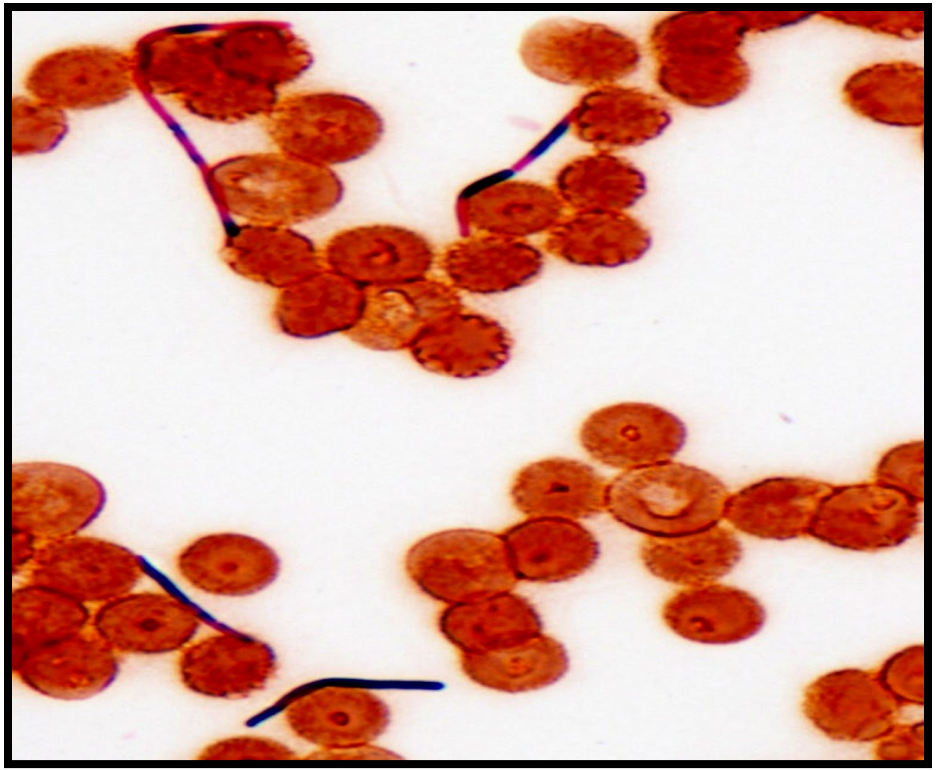
Clostridioides difficile

- Culture not recommended
  - Takes days
  - Toxin production must be documented
- Diagnostic approaches
  - Glutamate dehydrogenase (GDH) antigen followed, if positive, by toxins A/B enzyme immunoassay (EIA) or cytotoxin assay (possible NAAT if negative)
  - Nucleic acid amplification test (NAAT)
- Toxins A/B EIA
  - Less sensitive than NAAT



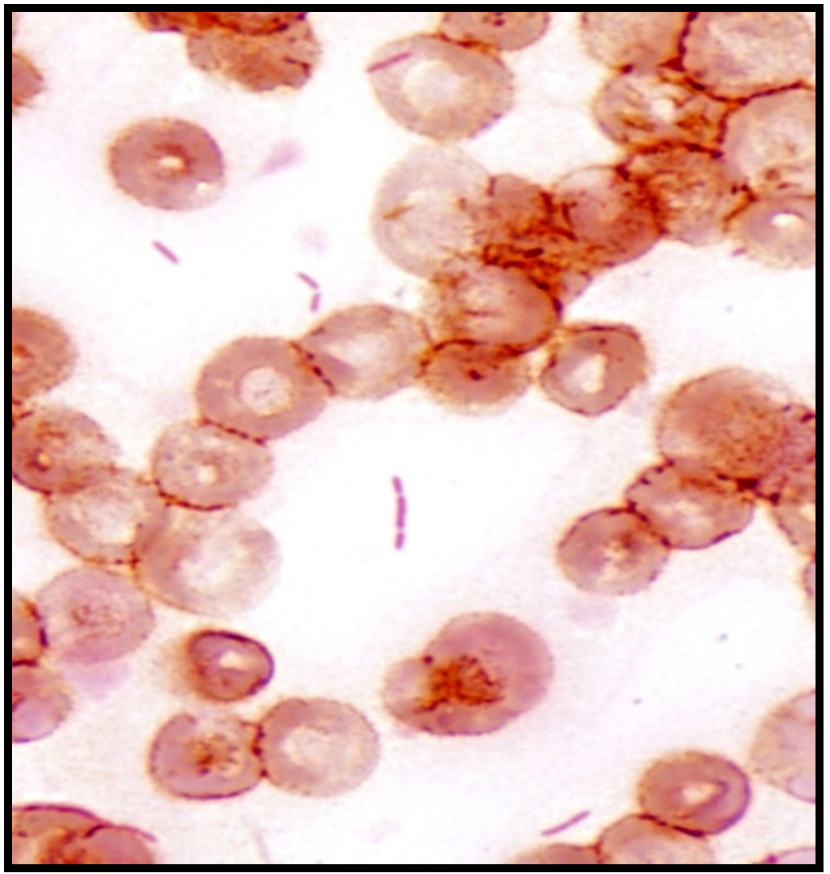
Clostridium septicum

- Grows rapidly
- Forms elongated rods arranged in chains
- Forms spores in specimens and culture (not seen in image)
- Almost always clinically significant when isolated in blood cultures
  - Overwhelming sepsis originating from large intestine
  - Associated with colon cancer, hematologic malignancy



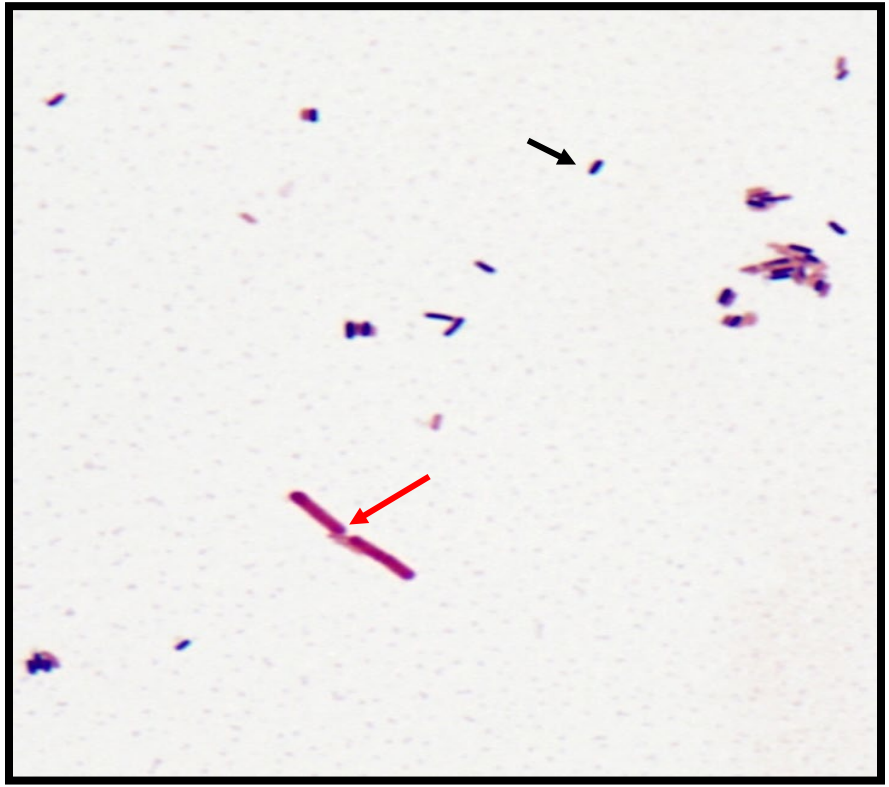
Clostridium tertium

- Characteristically stains Gram-negative
  - Clue that this is not a Gram-negative bacterium → will not grow on MacConkey agar
- May grow aerobically but grows better anaerobically
- May form spores (not seen in image)



Listeria monocytogenes

- Small, non-spore forming, Gram-positive rods
- Colonies weakly β-hemolytic
- Can grow at 4˚C
- Tumbling motility
- May be mistaken for either streptococci or corynebacteria and discounted as contaminant
  - CAMP (+) and hippurate hydrolysis (+)
    - May be confused with *S. agalactiae*
- Grows slowly



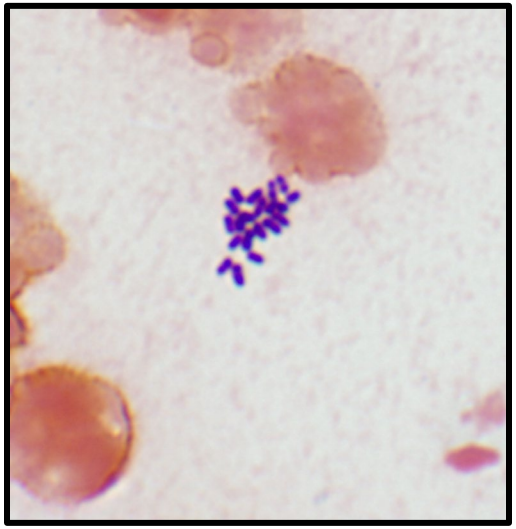
Compare size *Listeria* cells (black arrow) to pair of Gram-negative rods (red arrow)

Corynebacterium species, *Arcanobacterium hemolyticum*

- *C. diphtheriae* pathogenic; other species less pathogenic

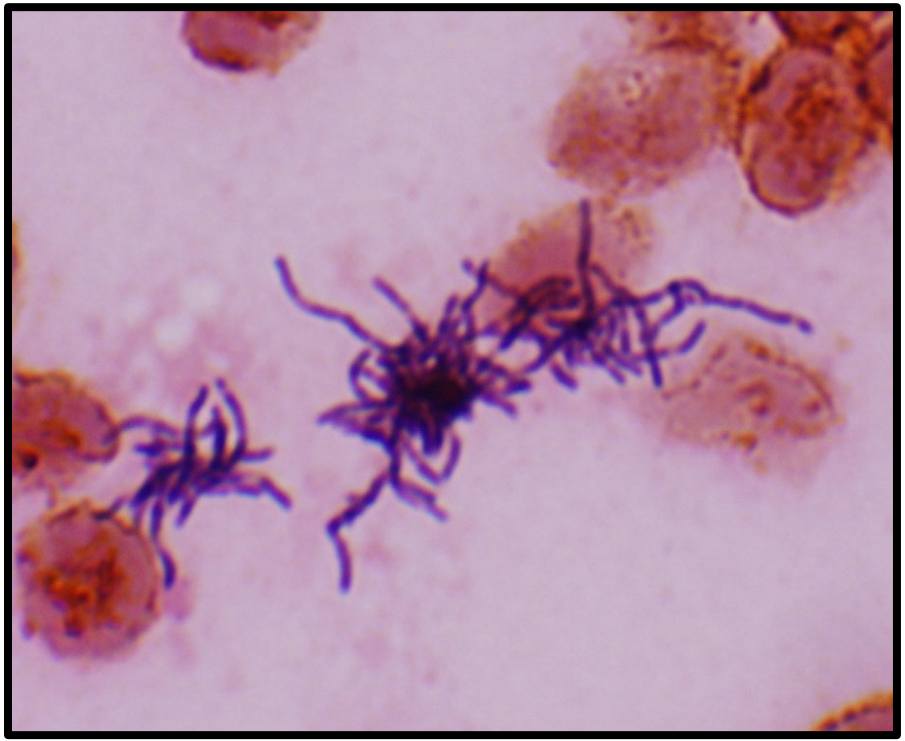
  Clump or palisade

  – Aerobes in contrast to *Cutibacterium/Propionibacterium* species which are anaerobes
- *Arcanobacterium hemolyticum*
  - Catalase (-); distinguishes from *Corynebacterium* species
  - Presents like streptococcal pharyngitis
  - β hemolytic
  - Negative on Lancefield grouping performed on β-hemolytic streptococci



Cutibacterium (formerly *Propionibacterium*) *acnes*

- **Common contaminant**
  - **Occasional cause of PJI, endocarditis, CSF shunt infection**
- **Anaerobic growth**
- **Slow growing – several days**
- **Forms clumps of Gram-positive rods**
  - **Anaerobic counterpart to *Corynebacterium* species**



Gram-Negative Cocci, Coccobacilli, and Rods

- Cocci
  - *Neisseria* species
  - *Moraxella catarrhalis*

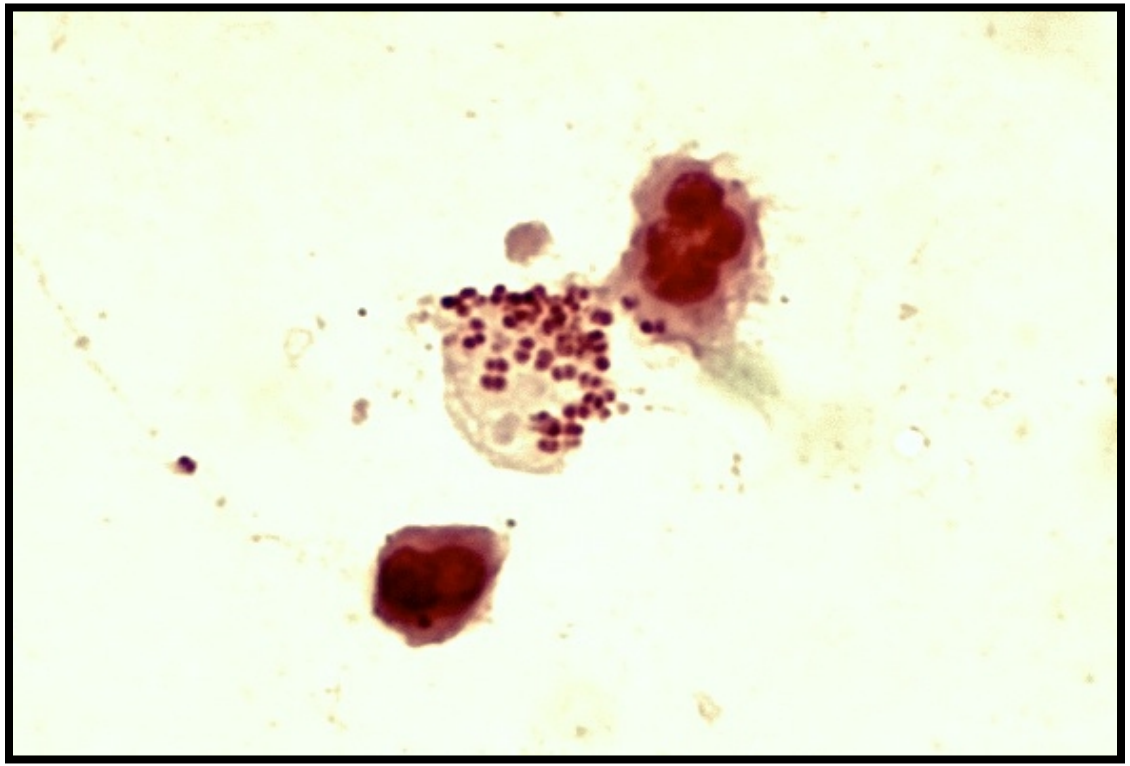
- Coccobacilli
  - *Moraxella* species
  - *Acinetobacter* species
  - *Haemophilus* species

- Rods
  - *Enterobacteriaceae* (e.g., *Escherichia coli*, *Klebsiella* species)
  - *Pseudomonas* species, *Stenotrophomonas maltophilia*, *Burkholderia* species
  - Miscellaneous (e.g., *Bacteroides* species, *Fusobacterium* species)

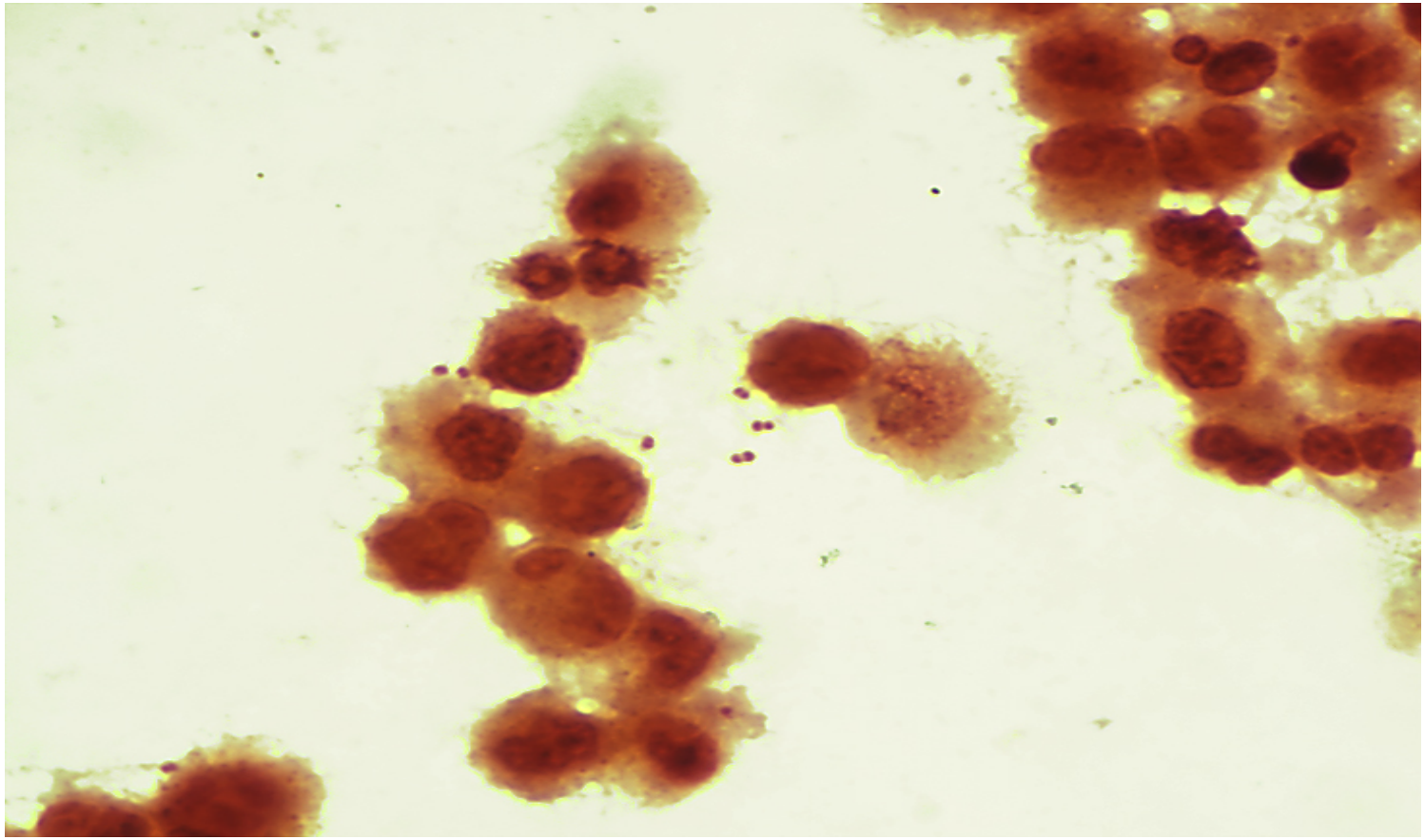
Neisseria gonorrhoeae and *Neisseria meningitidis*

- Typically arranged in pairs with adjoining sides flattened into shape resembling a “coffee bean”
- Gram-negative coccobacilli may be mistaken for *Neisseria* species
- Fastidious
- Modified Thayer Martin used for gonococcal culture
- No growth on MacConkey agar

Neisseria gonorrhoeae



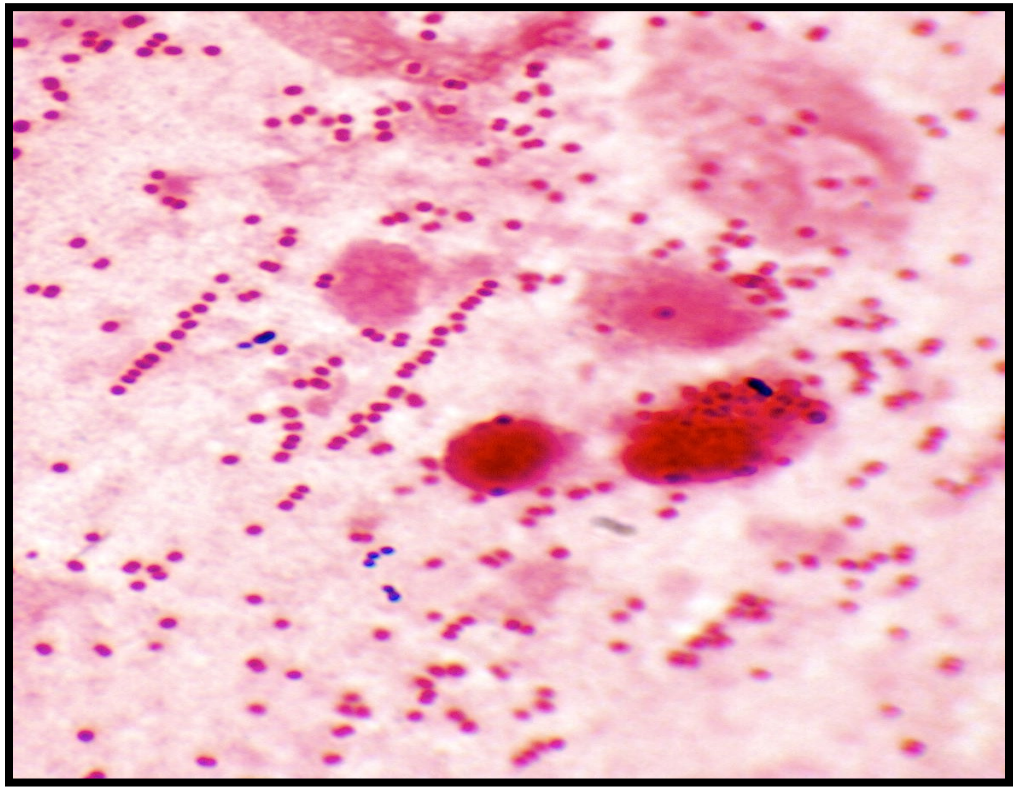
Neisseria meningitidis



Moraxella catarrhalis

- *Moraxella* species are typically Gram-negative rods

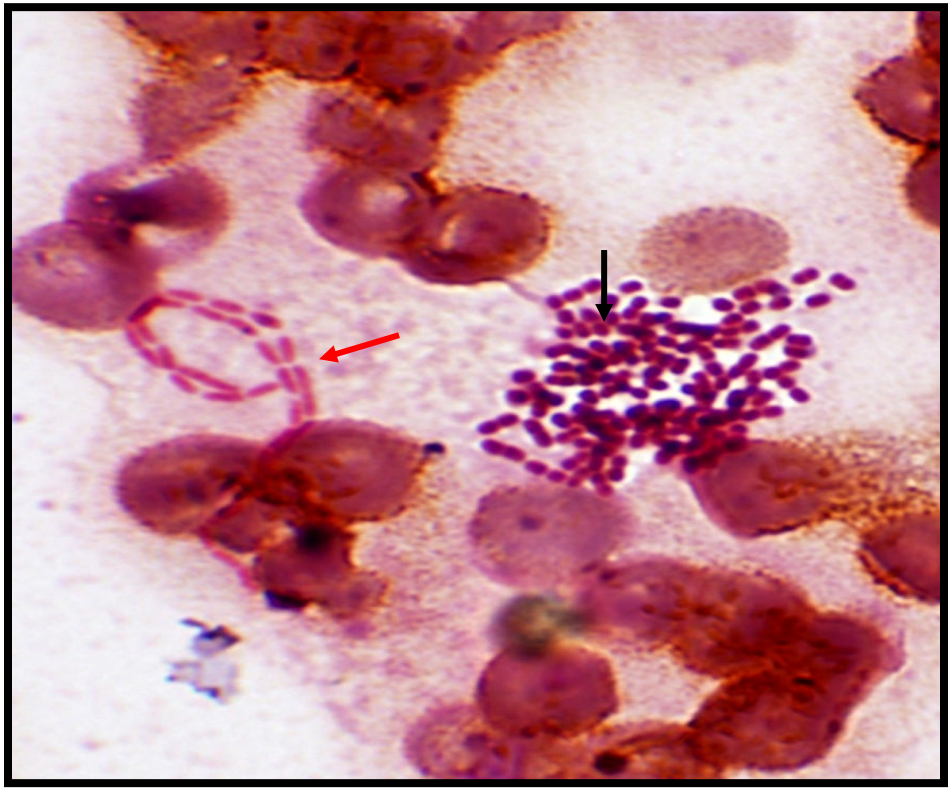
- *M. catarrhalis* originally classified as a *Neisseria* species because its morphology resembles *Neisseria* species



***M. catarrhalis* in sputum from patient with pneumonia - note the large number of neutrophils**

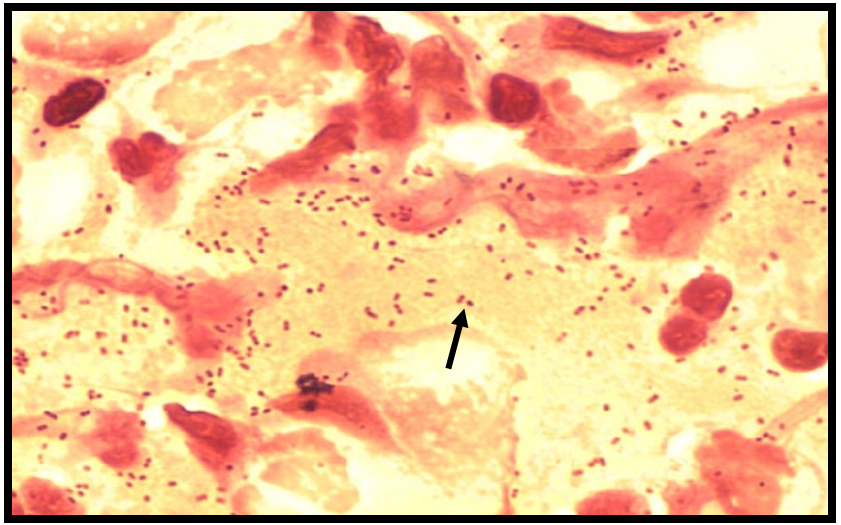
Acinetobacter species

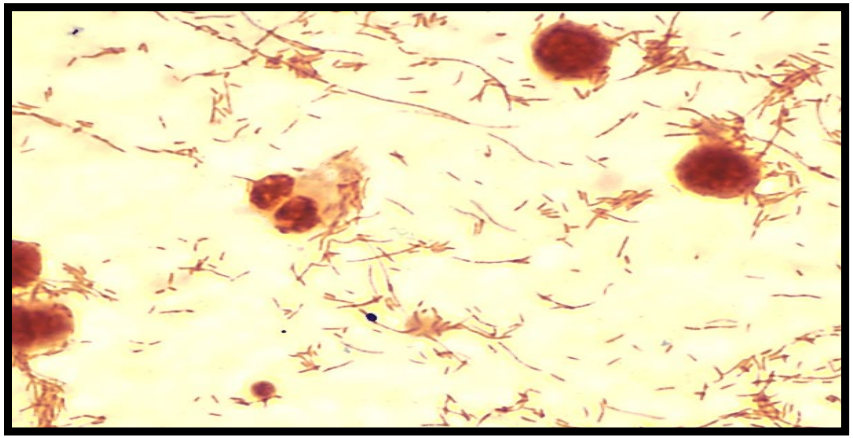
- Gram-negative coccobacilli that may retain crystal violet and resemble Gram-positive cocci in pairs (black arrow).
- Other bacterium shown is *Pseudomonas* species (red arrow), a more obvious Gram-negative rod
- *Acinetobacter* species are larger than *Neisseria* species; adjoining sides are not flattened



Haemophilus species

- Very small Gram-negative rods or coccobacilli (top figure)
- Long pleomorphic forms (bottom figure) may be seen in patients receiving antibiotics
- Bottom figure is Gram stain of spinal fluid from child with *H. influenzae* meningitis
- *H. influenzae* requires X and V factors to grow; will only grow in chocolate agar or as satellite colonies on sheep blood agar - tiny colonies around β hemolytic colony (e.g., *E. coli, S. aureus*)





Gram-Negative Rods

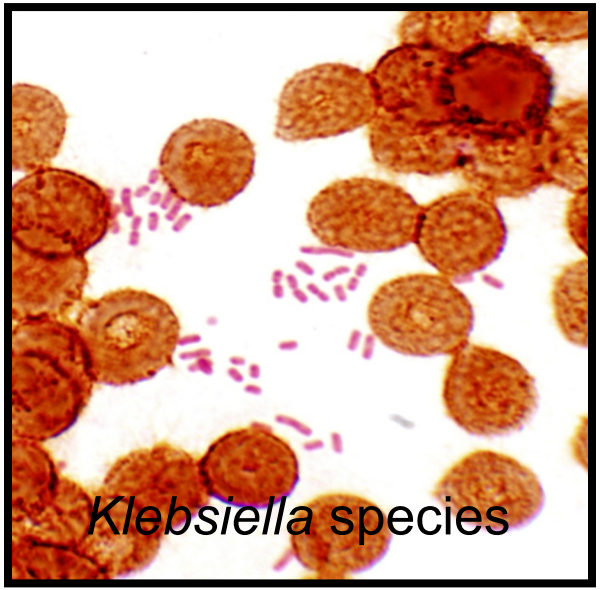
Subdivided here by spot oxidase test and growth on MacConkey agar*

	Growth on MacConkey agar	
Oxidase	**Yes**	**No**
Positive	***Aeromonas* species** ***Vibrio* species** ***Burkholderia* species** ***Elizabethkingia meningoseptica*** ***Pseudomonas* species**	***Aggregatibacter* species** ***Cardiobacterium* species** ***Eikenella corrodens*** ***Kingella kingae*** ***Pasteurella multocida*** ***Brucella* species**
Negative	***Chromobacterium* species** **Enterobacterales** ***Acinetobacter* species** ***Stenotrophomonas maltophilia***	***Capnocytophaga canimorsus*** ***Capnocytophaga cynodegmi*** ***Streptobacillus moniliformis*** ***Francisella tularensis***

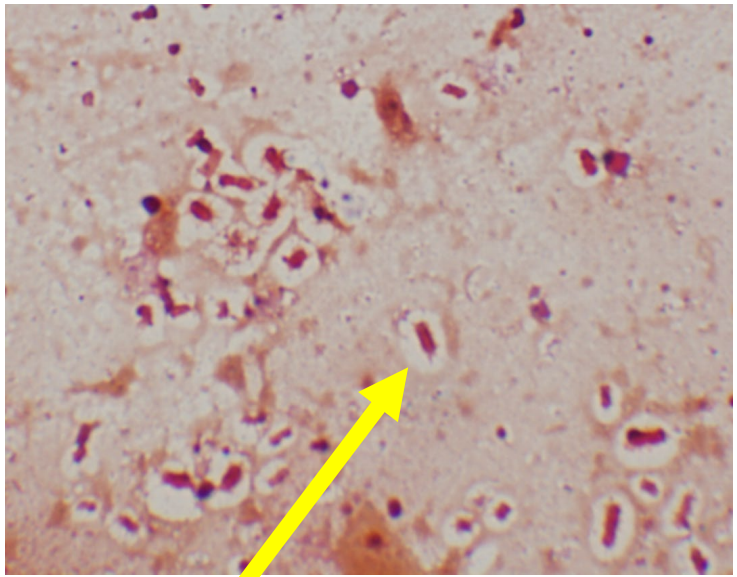
*Selective for Gram negatives; differentiates lactose fermenters (pink colonies) from non-lactose fermenters (colorless colonies)

Enterobacterales

- >100 genera
- Glucose fermenters
- Oxidase negative
  - Exception (*Plesiomonas* species)
- Grow on MacConkey agar
- Relatively large Gram-negative rods
  - May stain more intensely at ends ("bipolar" staining)

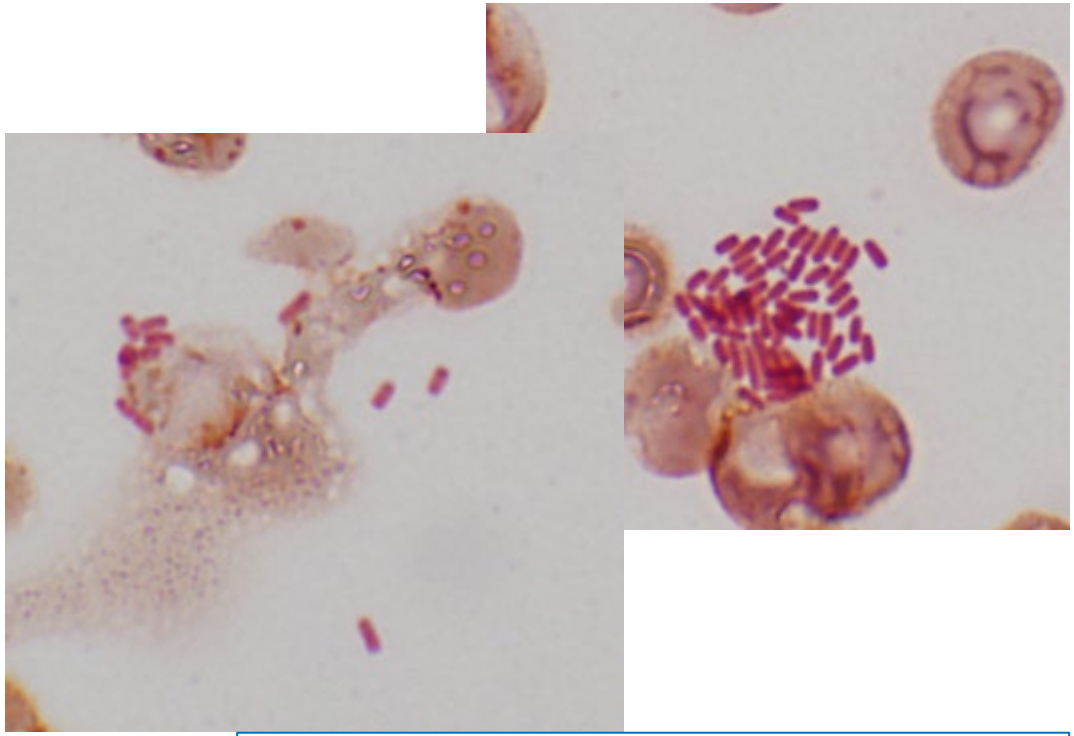


Lactose fermentors (pink colonies on MacConkey)	**Late lactose fermenters (pink colonies at 48 h)**	**Non-lactose fermenters (colorless on MacConkey)**
Escherichia coli	*Citrobacter* species	*Shigella* species
Klebsiella species (*pneumoniae, oxytoca, aerogenes*)	*Serratia* species	*Salmonella* species
Enterobacter species (*cloacae* complex)		*Yersinia* species
		Proteus species
		Providencia species



Example of encapsulated *K. pneumoniae*. Notice the clearing around the cells.

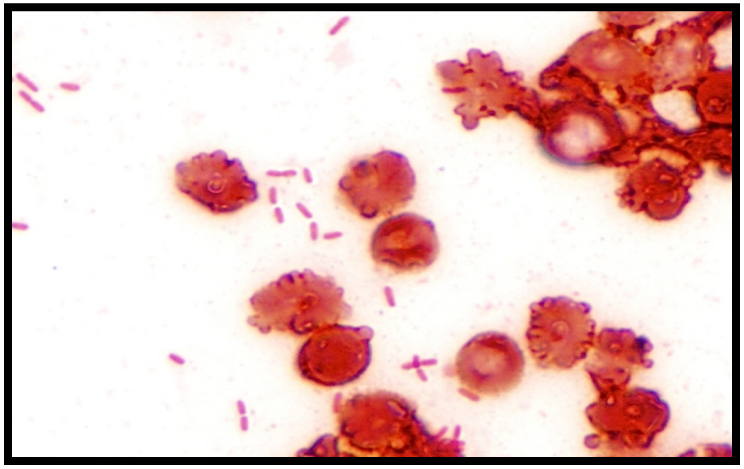
***K. pneumoniae* often has a "short, fat, chubby" appearance ± capsule**

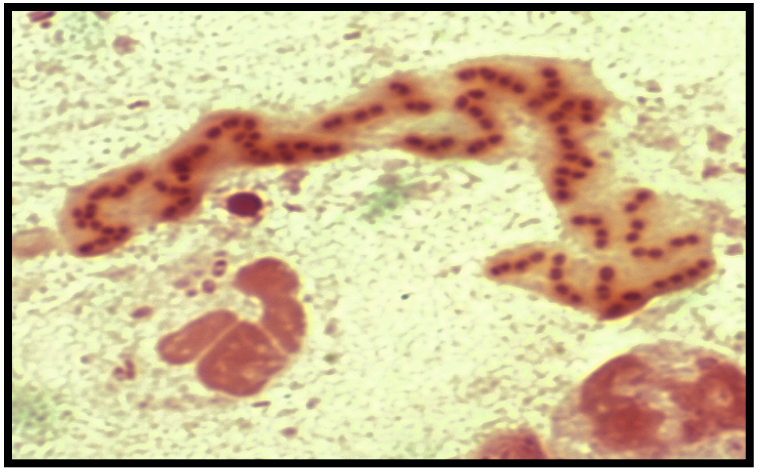


Gram stains of *E. cloacae* complex and *E. coli* from blood. Notice the **bipolar staining** (darkening at the ends) suggestive of the Enterobacterales

Gram-Negative Rods, continued

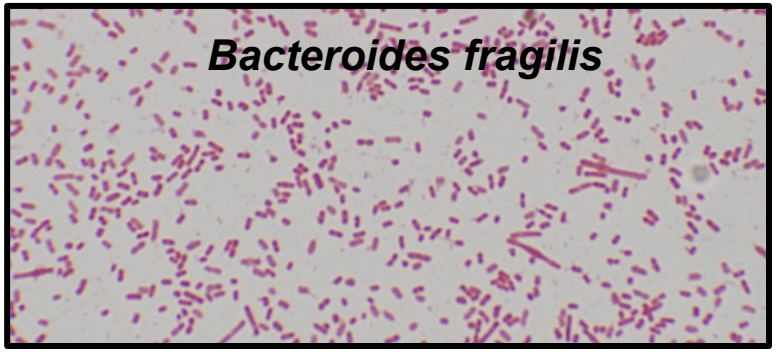
- *Pseudomonas* species, *Burkholderia* species, *Stenotrophomonas maltophilia*
  - Small Gram-negative rods typically arranged singly or in pairs
  - *Pseudomonas aeruginosa* may be surrounded by extracellular matrix/capsule (bottom figure)
- Non-glucose, non-lactose fermenters
- *P. aeruginosa* smells like grapes

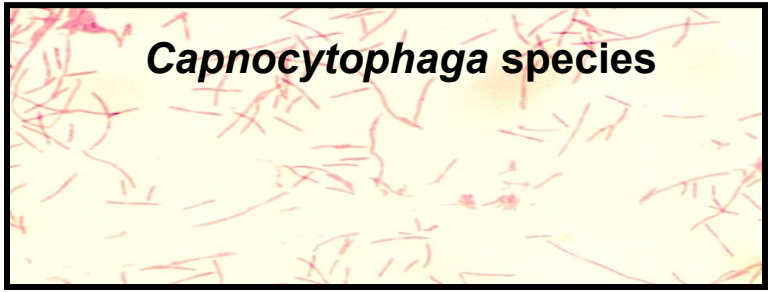




Gram-Negative Rods, continued

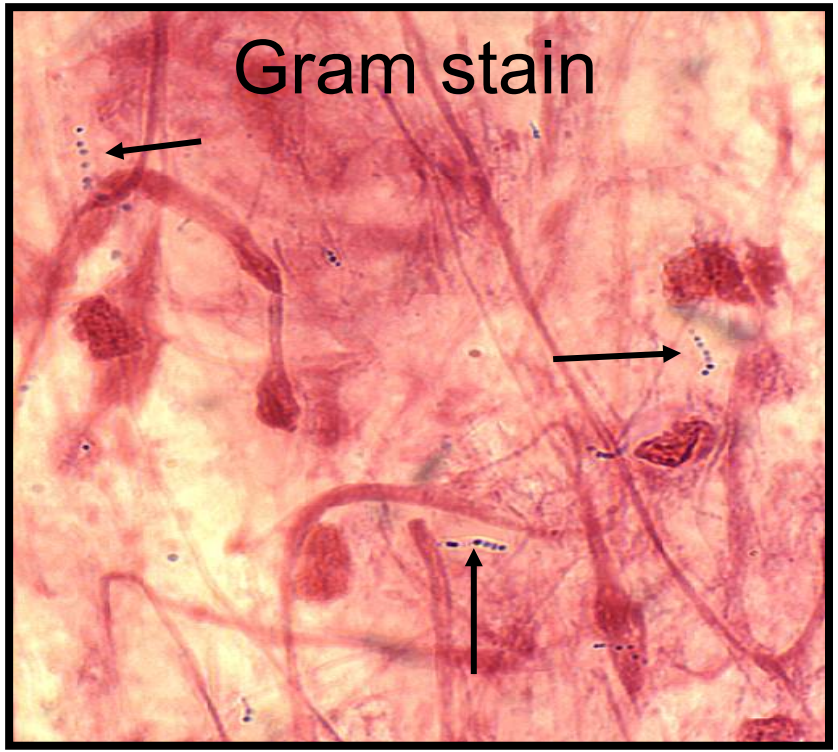
- *Bacteroides fragilis*
  - Anaerobe
  - Pleomorphic Gram-negative rods - short and long
- Thin and long Gram-negative rods
  - Anaerobe
    - *Fusobacterium* species
  - Aerobe
    - *Capnocytophaga* species





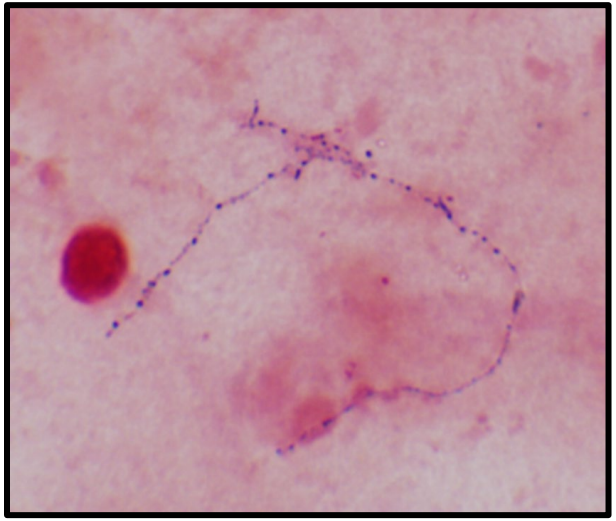
Mycobacterium species

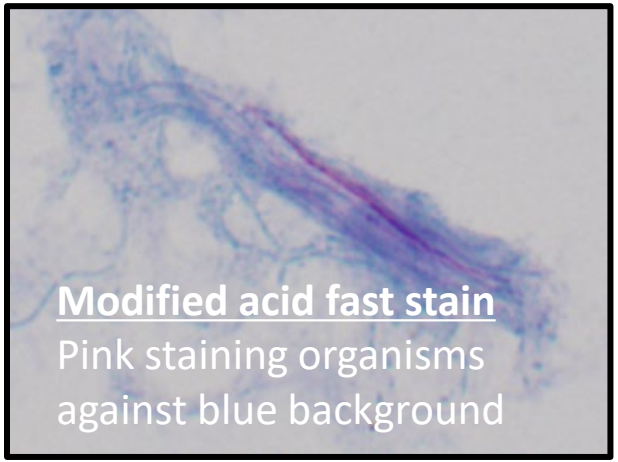
- Mycobacteria *may* retain crystal violet in Gram stain appearing as beaded rods (arrows)
- Rapid growers (growth within 7 days)
  - *M. chelonae*
  - *M. abscessus* group (includes *M. massiliensis*)
  - *M. mucogenicum*
  - *M. fortuitum*
- *M. avium* complex (growth 5-7 days)
- Slow growers (growth >10 days)
  - *M. tuberculosis* complex (includes *M. bovis*)
- Scotochromogens (colonies produce pigment without light exposure)
  - *M. gordonae, M. scrofulaceum*
- Photochromogens (colonies produce pigment upon exposure to light)
  - *M. marinum, M. kansasii*



Nocardia species

- Gram stain - **thin, filamentous, branching forms**, stain irregularly
- No other acid-fast organisms form long, branching structures
- Differentiation from streptococci:
  - True branching of filaments
  - “Beads” do not touch and are irregularly distributed
- Modified acid fast stain

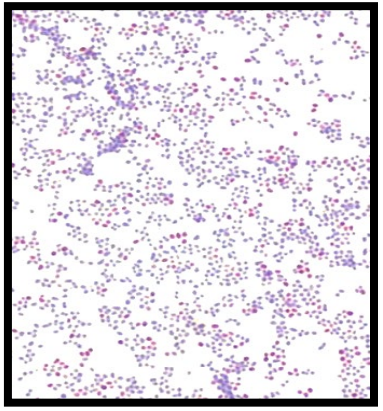




Rhodococcus species – Gram Stain

- Retain crystal violet dye more uniformly than *Mycobacterium* or *Nocardia* species
- After growth for few hours, stain well with Gram stain and appear rod-like
- With longer incubation, bacteria assume coccoid form
- Most cells decolorize easily with weak acid-alcohol solution, so only a few cells appear "red" (acid fast)

4 hours



24 hours*

*Photo was prepared from colony of *Rhodococcus* species growing on media used for mycobacteria

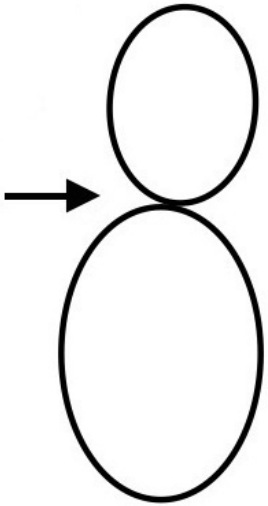
If colonies grown on the blood agar plates used in bacteriology, very few cells appear acid fast

Mycology

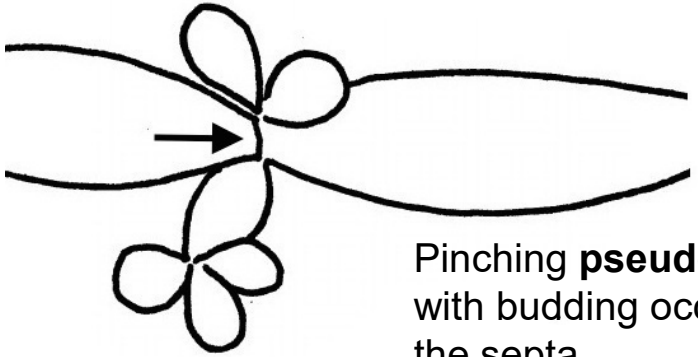
Yeasts and Molds

- **Yeasts** (single cell organisms)
  - Some produce pseudohyphae (pinching & budding at septa)
  - *C. albicans* can produce pseudohyphae AND true hyphae
  - *Candida* species (e.g., *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. auris*)
  - *Cryptococcus* species (e.g., *C. neoformans, C. gattii*)
  - *Malassezia* species (e.g., *Malassezia furfur*)
  - *Pneumocystis jirovecii*
- **Molds** (multicellular organisms)
  - Septate (true hyphae; parallel walls, branching before or after the septa) *versus* aseptate
  - Hyaline (colony reverse cream/tan/white) *versus* dematiaceous (colony reverse black/brown; presence of melanin)
- **Dimorphic fungi** (yeast phase 35˚C/mold phase 25˚C)
  - *Histoplasma capsulatum, Blastomyces dermatitidis, Sporothrix schenckii, Coccidiodes immitis/posadasii*

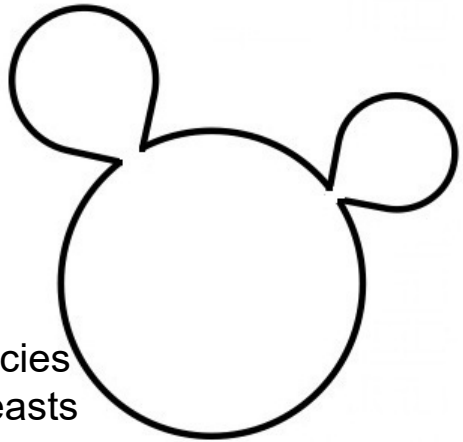
Yeasts



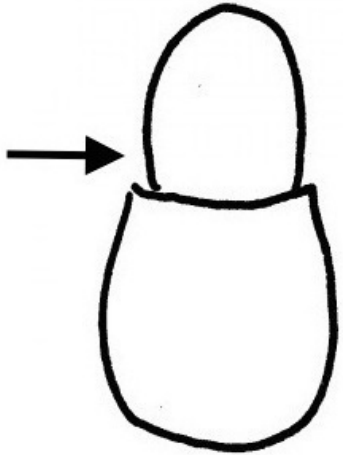
Candida species
Budding **oval** yeasts



Pinching **pseudohyphae** with budding occurring at the septa



Cryptococcus species
Budding **round** yeasts



Malassezia species with prominent **collarette** at point of budding

Candida species

- Top: *Candida albicans* isolated in blood culture; note **yeast cells** and **pseudohyphae**
- Bottom: Yeast colonies with “feet” indicative of pseudohyphae production


Gram stain of budding yeasts

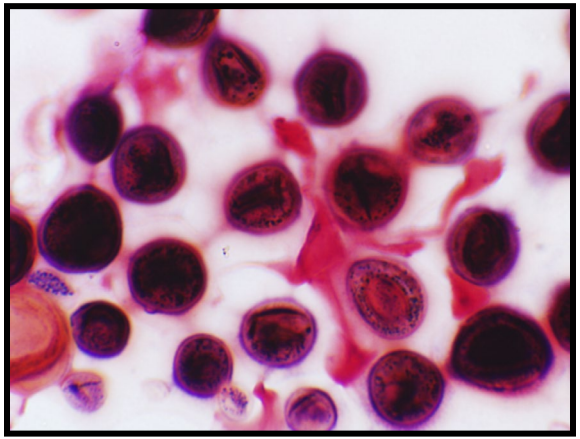

Yeast colonies with “feet”

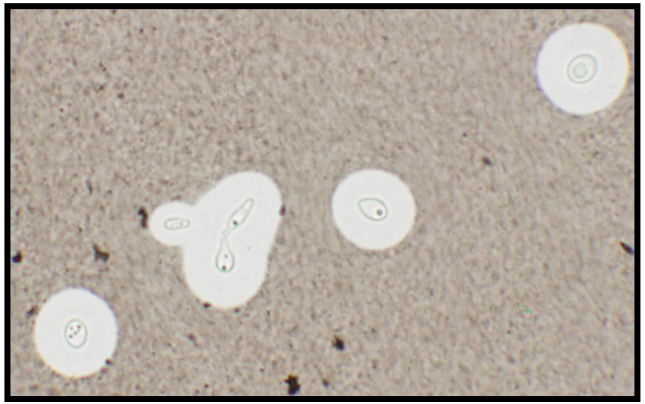
Germ Tube Test

- Rapid test for identification of *Candida albicans*
  - *Candida dubliniensis* also germ tube positive
- Yeast cells inoculated in serum form “germ tubes” within 2 hours
- “Tube” is continuous extension from yeast cell; no septum exists
  - Distinguishes germ tubes from pseudohyphae

Cryptococcus neoformans/gattii

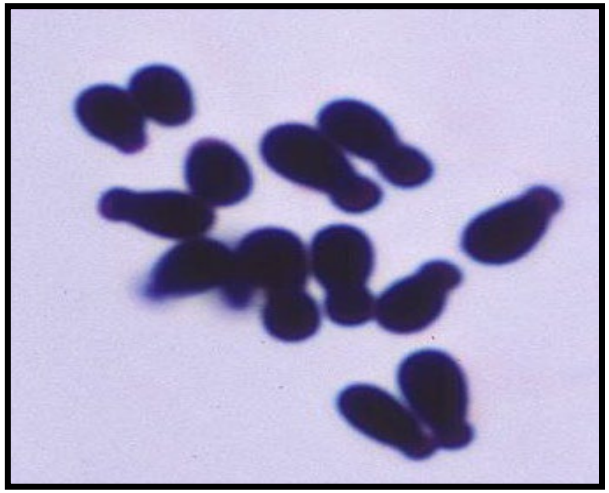
- Cells suspended in India ink appear as different sized round cells with clear halo (capsule)
  - Inflammatory cells uniform size
- Budding may be seen
- India ink stain not commonly used today
  - Replaced with more sensitive cryptococcal antigen test





Malassezia species

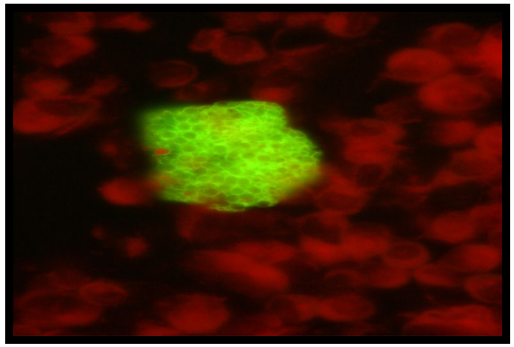
- Grow on skin surface
  - Pityriasis versicolor
  - Rarely, systemic infection
- Small yeast cells have prominent “collarette” that forms where daughter cells bud
- Short hyphal elements may be seen in skin scrapings (“spaghetti and meatballs”)
- *Malassezia furfur* culture requires supplementation of media with lipids (e.g., olive oil).

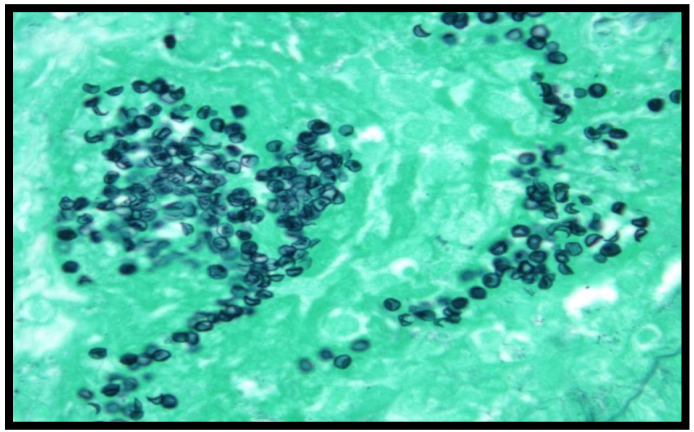


Fluorescent calciifluor white stain of *Malassezia furfur* showing prominent collarette (arrow)

Pneumocystis jirovecii

- Formerly considered a parasite, now classified as a fungus
- Two developmental forms observed in human tissues
  - Trophozoite
  - Cyst





Direct Fluorescent Antibody Test

- Fluorescein-conjugated monoclonal antibodies against *Pneumocystis* stain cyst wall green with contents being unstained
- Trophozoites appear as small polygons or spheres outlined in green

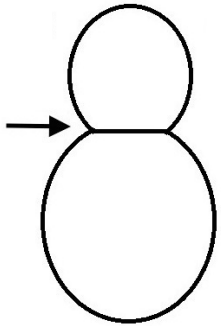
Methenamine Silver Stain

- Performed in surgical pathology
- Cysts stain brown-black
- Trophozoites do not stain

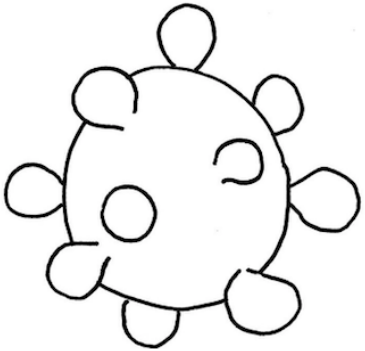
Dimorphic Fungi

- Exist in two forms
  - Yeasts at body temperature (except *Coccidioides immitis/posadasii* which forms a spherule)
  - Molds at room temperature (or 30ºC)
- Most commonly isolated dimorphic fungi, US:
  - *Histoplasma capsulatum*
  - *Blastomyces dermatitidis*
  - *Coccidioides immitis/posadasii*
  - *Sporothrix schenckii*

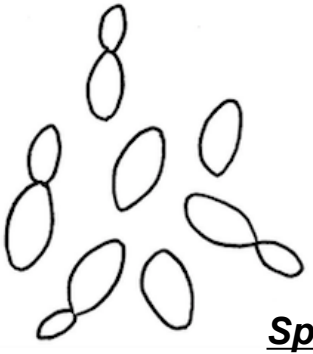
Dimorphic Fungi (35˚C)



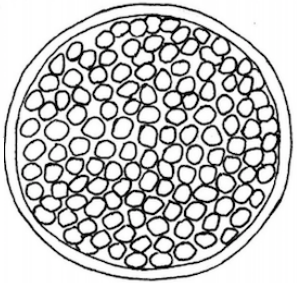
Blastomyces dermatitidis
Broad-based budding yeast



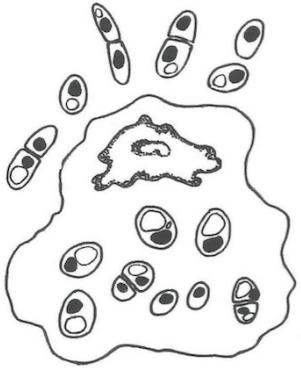
Paracoccidioides brasiliensis
Mariner's wheel

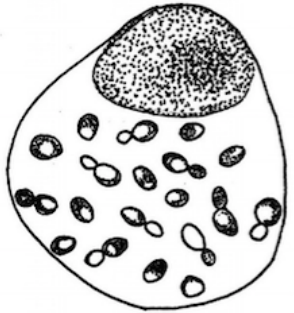


Sporothrix schenckii
Elongated cigar-shaped yeasts



Coccidioides immitis/posadasii
Spherules containing endospores

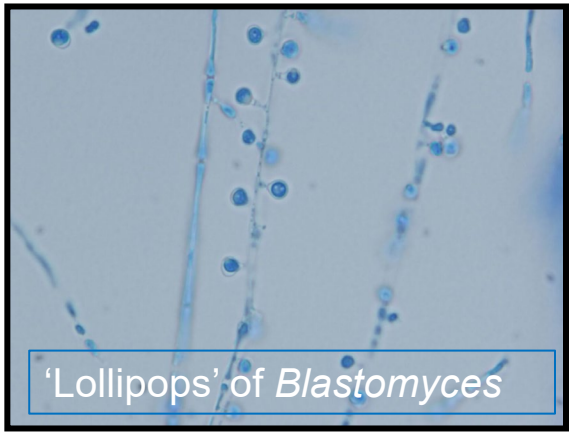


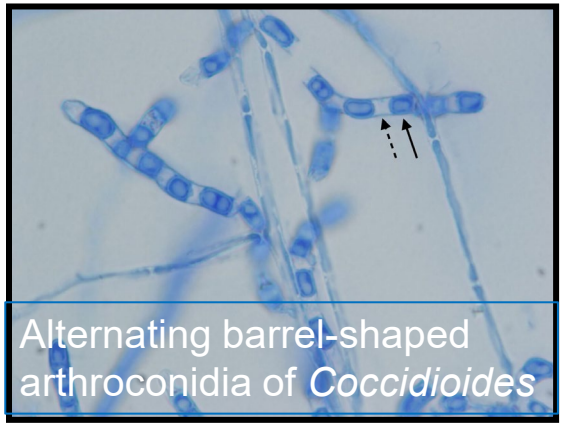


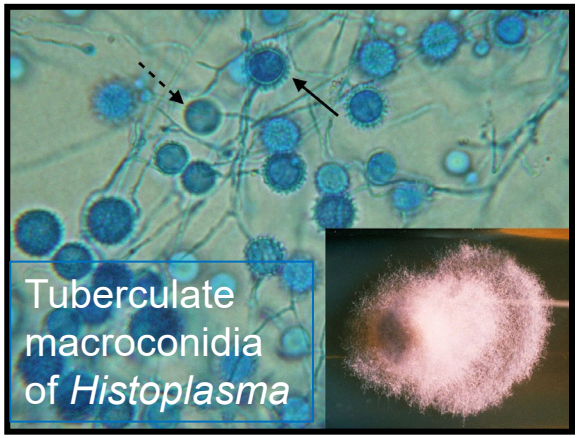
Histoplasma capsulatum
Small, intracellular yeasts

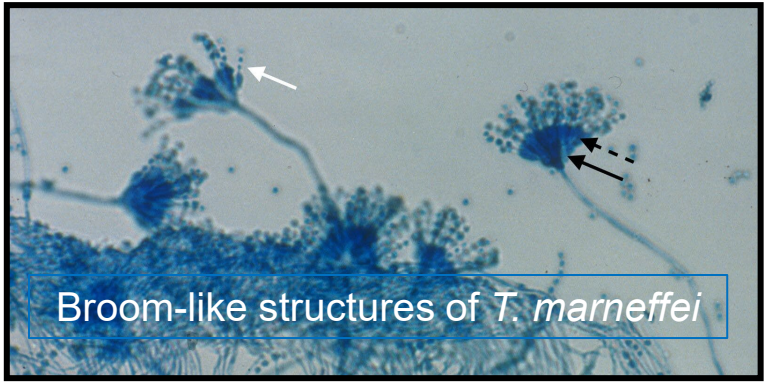
Talaromyces marneffei
Elongated non-budding yeasts, divides by binary fission

Dimorphic Fungi (25˚C)



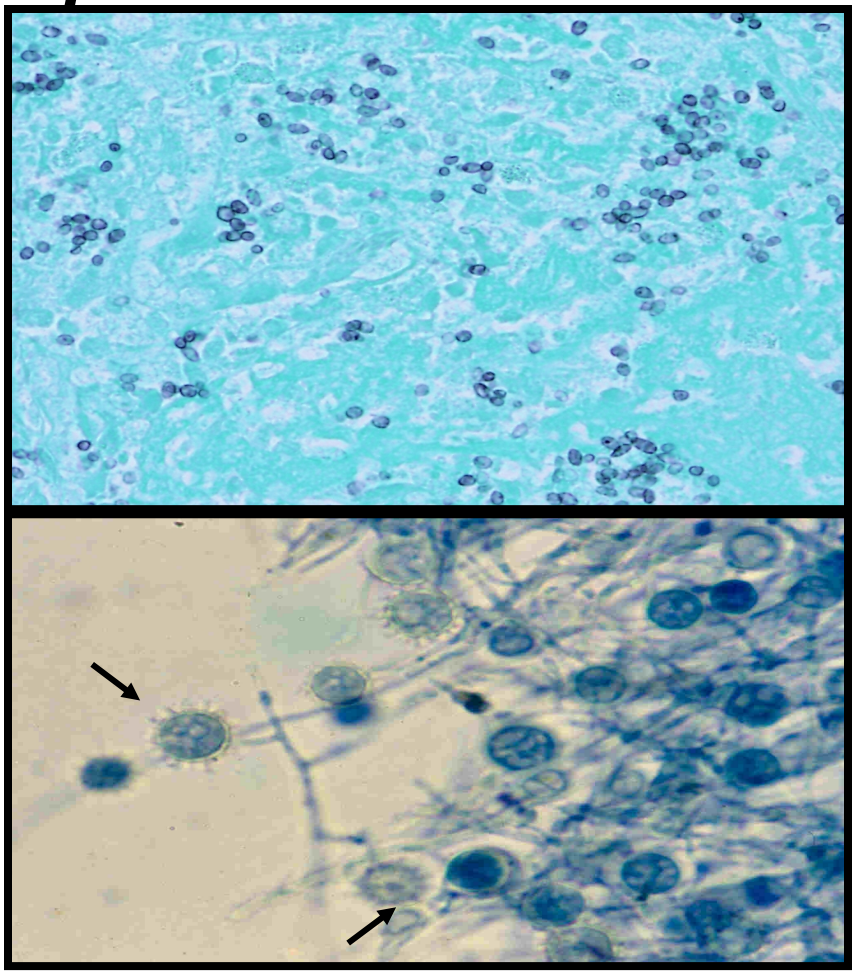






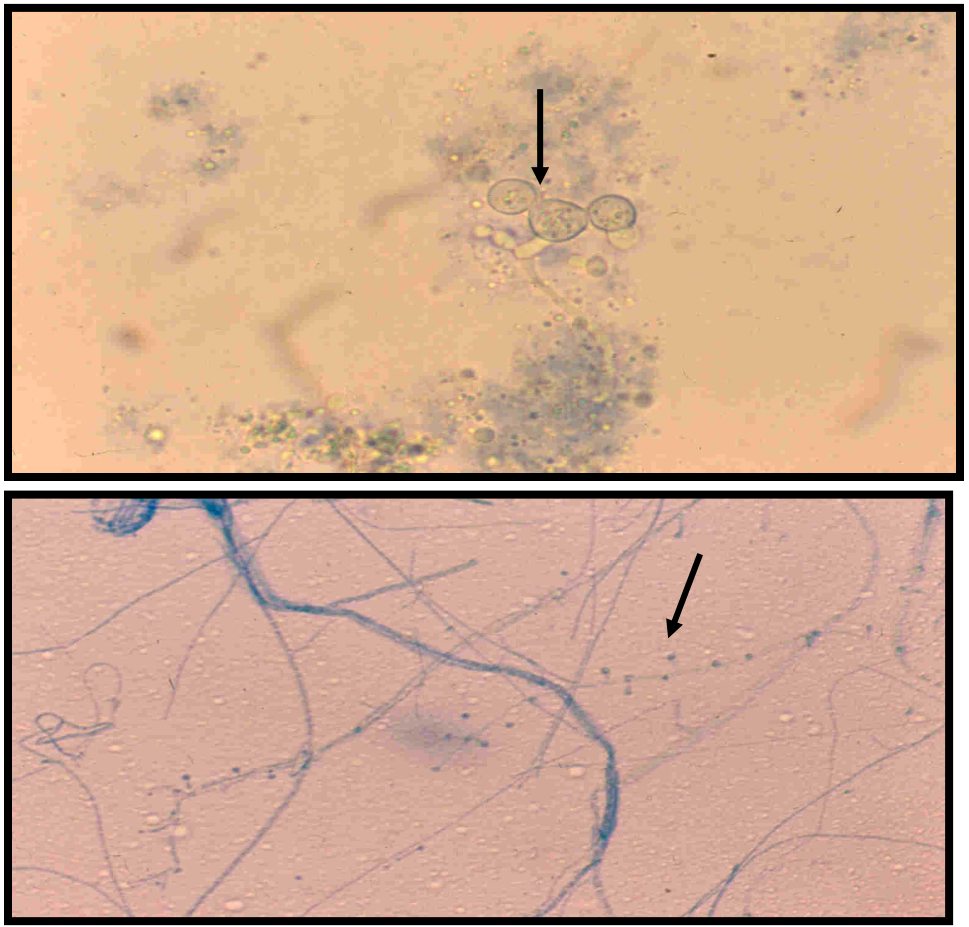
Histoplasma capsulatum

- Forms small (2-4 μm) yeast cells in tissue (top, silver stain)
- Forms filamentous forms in culture (bottom, lactophenol cotton blue stain)
  - Note thin hyphae, microconidia, and large macroconidia (arrows) with knobby (tuberculate) surfaces



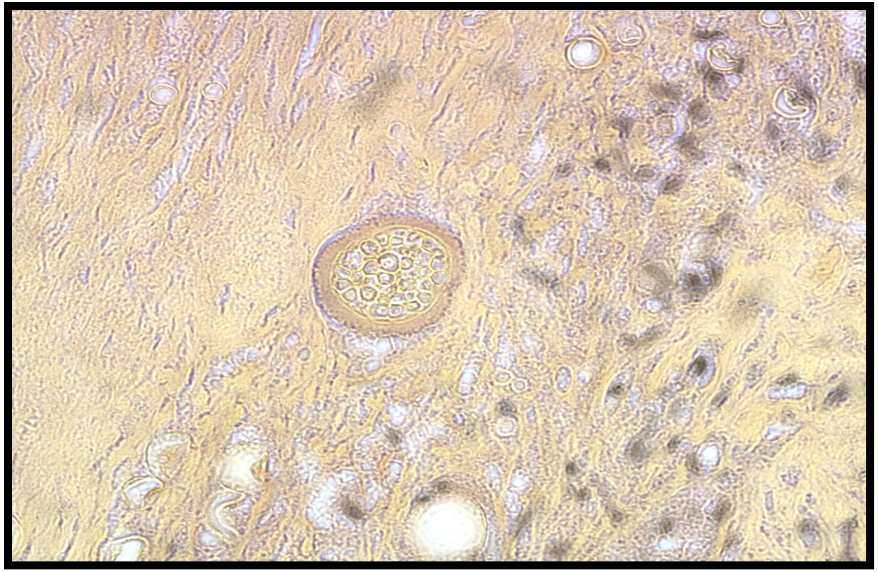
Blastomyces dermatitidis

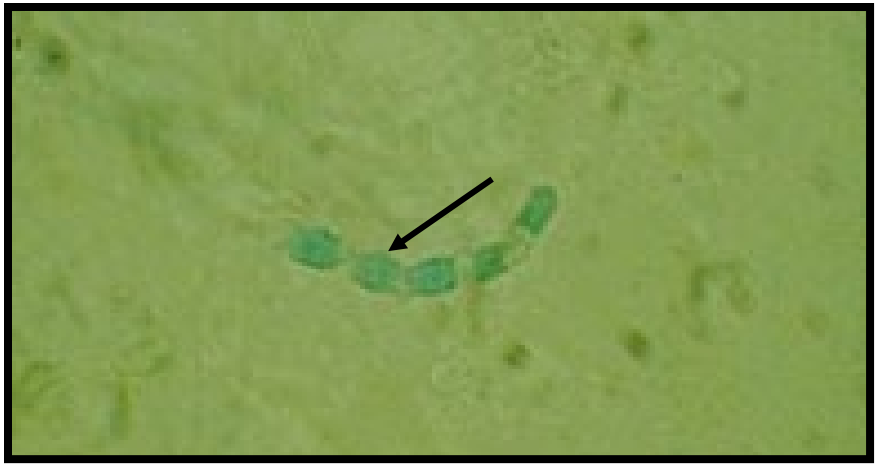
- Forms large (8-15 µm) yeast cells in tissue and hyphal forms in culture at ambient temperature
- Yeasts (top) have thick wall and form a broad base where daughter cell buds
- Mold form (bottom) has thin hyphae with numerous small microconidia attached to hyphae by thin branches (resembles lollipops)



Coccidioides immitis/posadasii

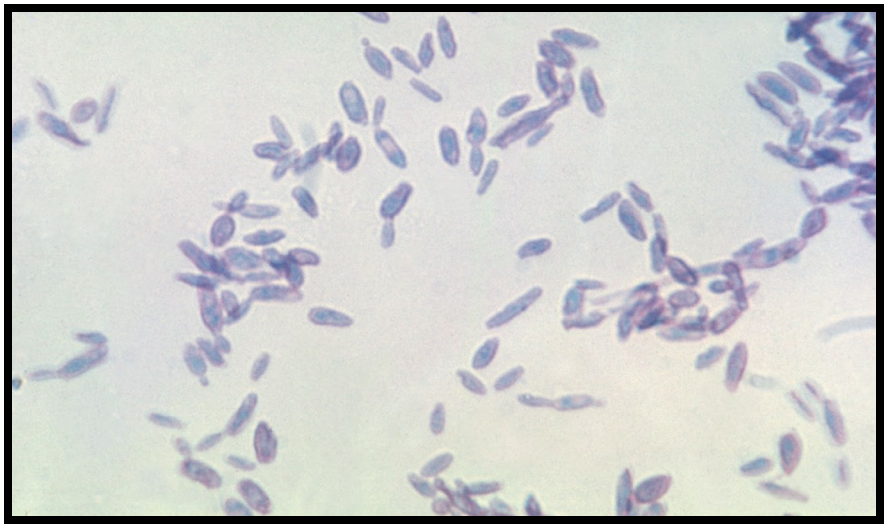
- Forms large (up to 120 µm), endospore filled "**spherules**" (top) in tissues
- Forms filamentous forms at room temperature (30ºC)
- Barrel shaped, spore-like structures (**arthroconidia**) formed in alternate hyphal cells (bottom, arrow)

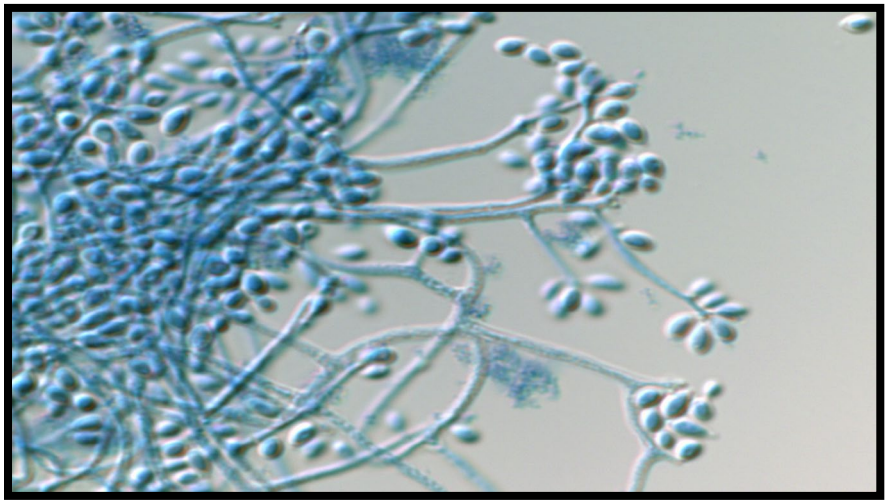




Sporothrix schenckii

- Forms narrow based yeast cells in tissue (top)
- Forms delicate hyphae with cluster (“**flowerette**”) of conidia (spores) at end of narrow stalk (bottom)
- Conidia can be darkly pigmented

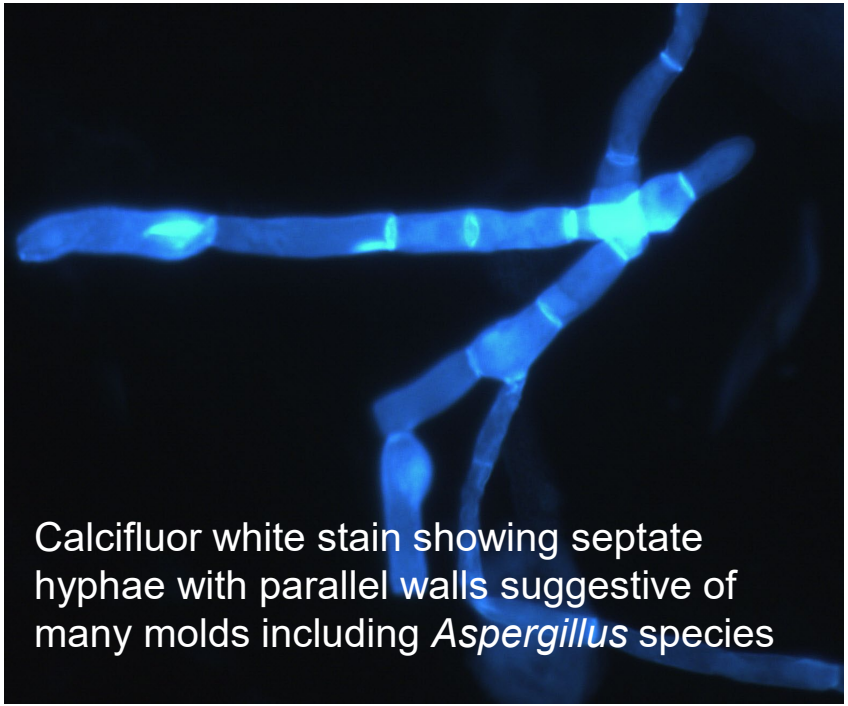




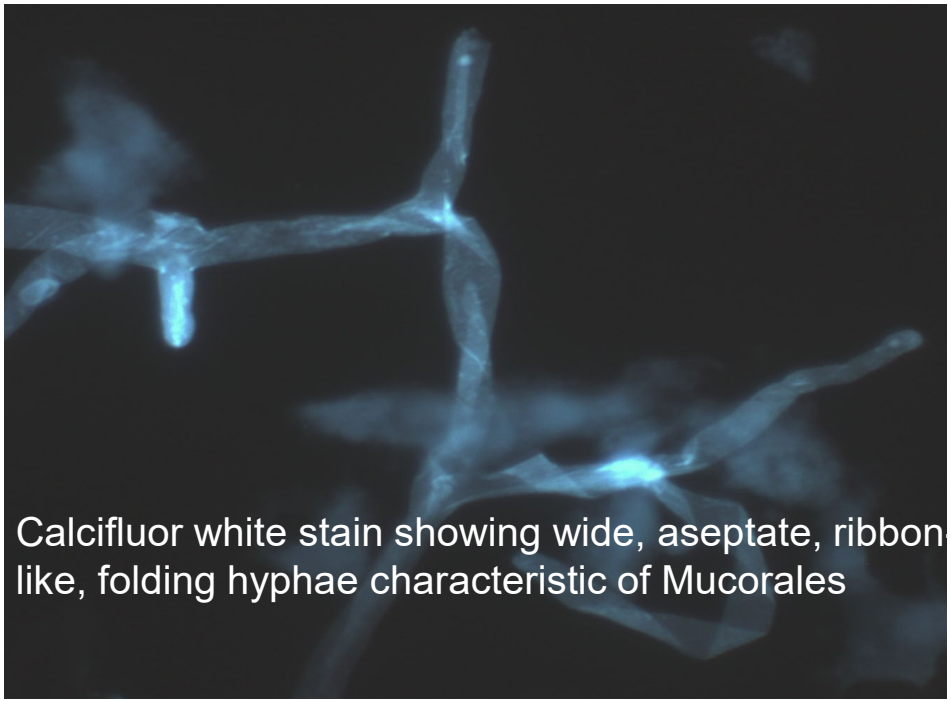
Filamentous Fungi – Molds (Selected)

- Nonseptated molds (Mucormycetes - e.g., *Rhizopus* species, *Mucor* species, *Rhizomucor* species, *Lichtheimia* species, *Cunninghamella* species)
- Lightly colored or hyaline, septated molds
  - Opportunistic fungi (e.g., *Aspergillus* species, *Fusarium* species, *Paecilomyces* species, *Scopulariopsis* species, *Penicillium* species)
  - Dermatophytes (e.g., *Trichophyton* species, *Epidermophyton floccosum*, *Microsporum* species)
- Darkly pigmented (dematiaceous), septated molds (e.g., *Alternaria* species, *Bipolaris* species, *Curvularia* species, *Exophiala* species, *Exserohilum* species, *Scedosporium* species, *Cladosporium* species, *Phialophora* species, *Cladophialophora* species, *Ochrochonis* species)

Septate *versus* Aseptate Hyphae



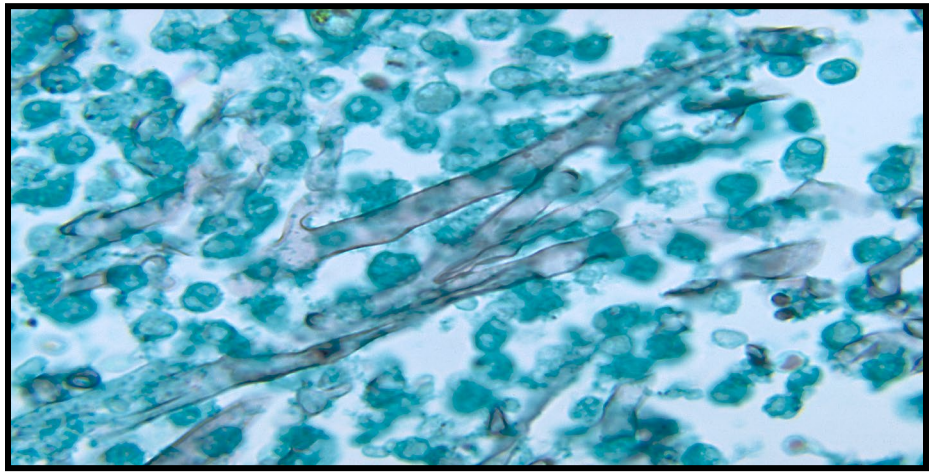
Calcifluor white stain showing septate hyphae with parallel walls suggestive of many molds including *Aspergillus* species

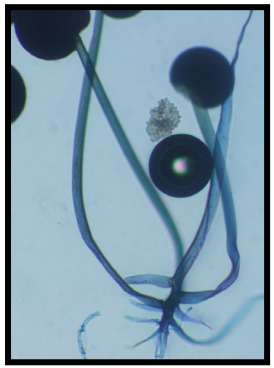


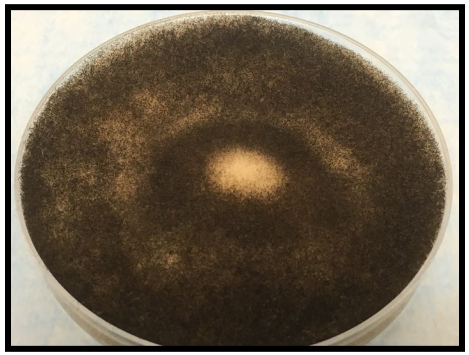
Calcifluor white stain showing wide, aseptate, ribbon-like, folding hyphae characteristic of Mucorales

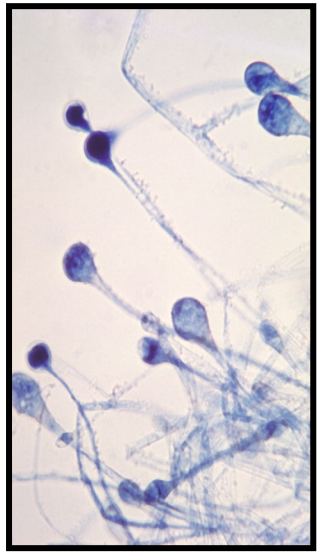
Mucormycetes

- No (or just a few) septae (divisions) within hyphae
- Grow quickly
  - "Lid lifters", "cotton candy"
- In tissue (top; silver stain), hyphae appear broad and ribbon-like with irregular walls and irregular branching
- Bottom figures shows *Mucor* species in culture



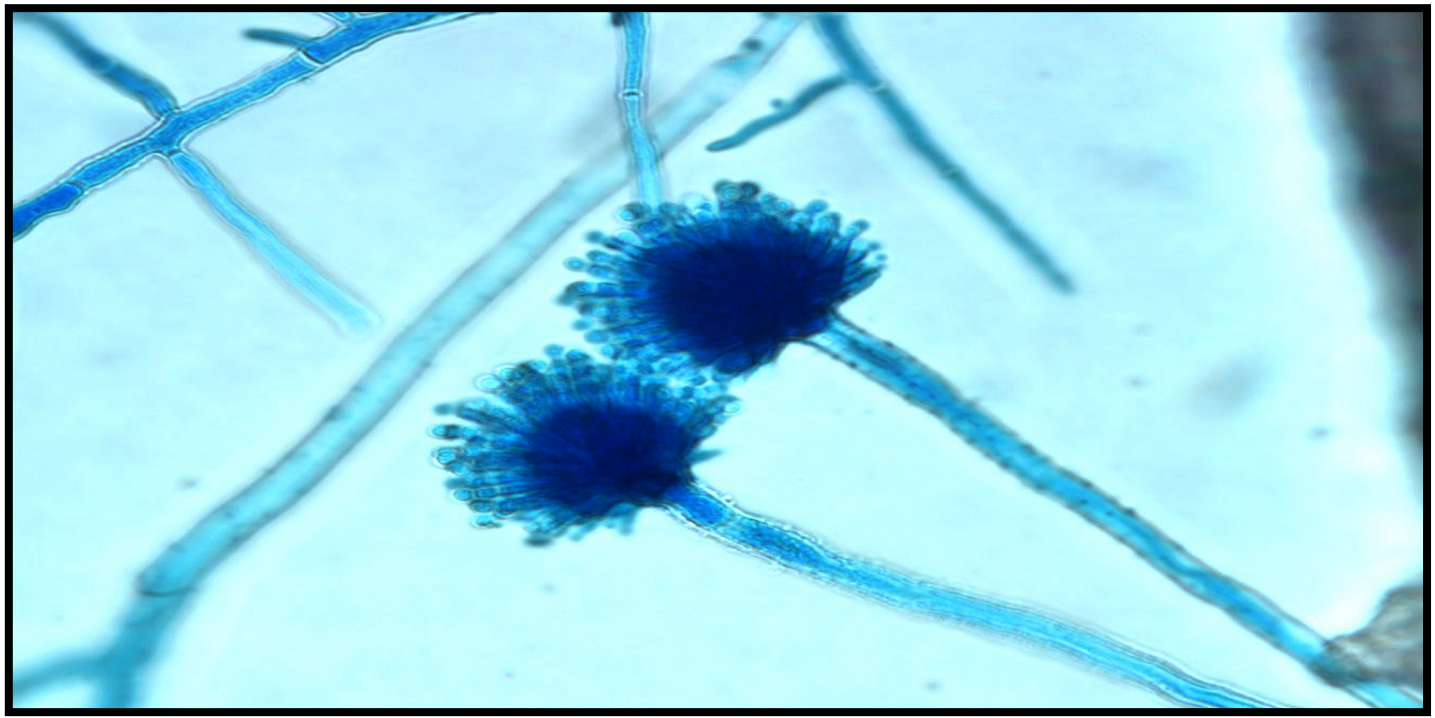






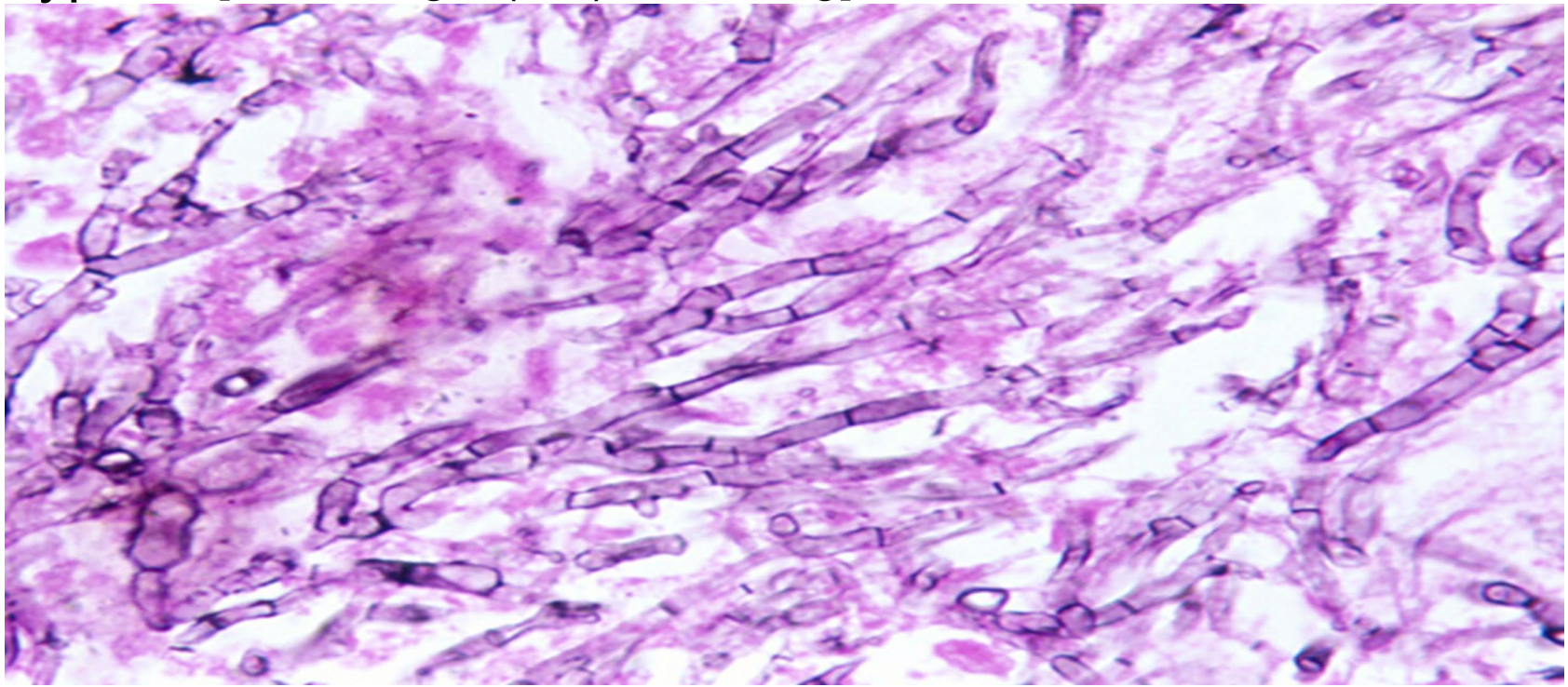
Aspergillus species

- In culture with characteristic fruiting bodies (bottom) – conidiophore covered with conidia

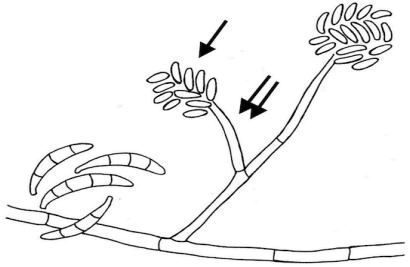


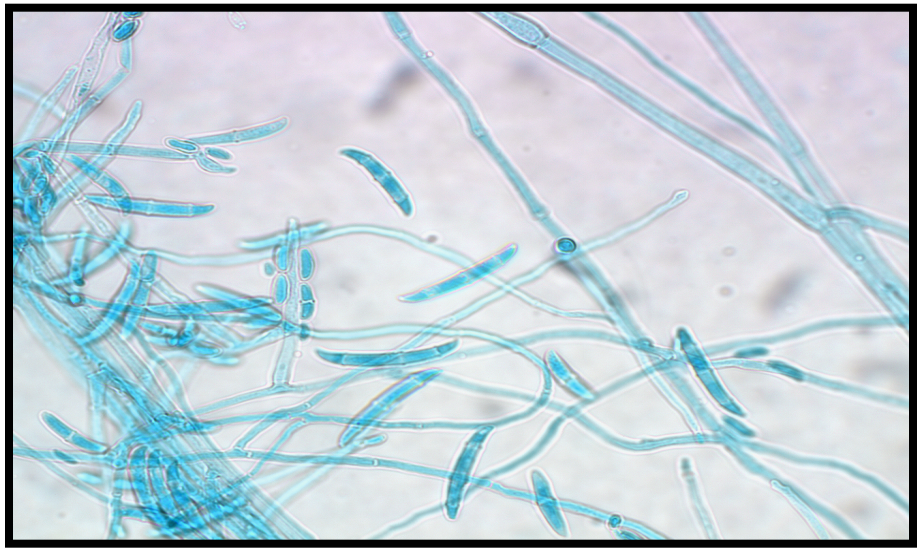
Aspergillus species

- In tissue - note uniform diameter and branching of septated hyphae [acute angle (45º) branching]

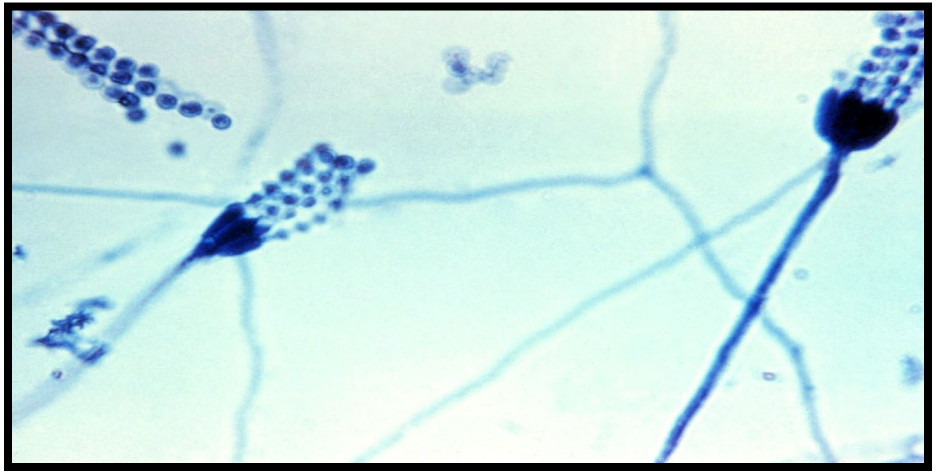


- Fusiform or sickle shaped multicelled macroconidia of *Fusarium* species (top)
  - Most likely mold to grow from blood culture bottles

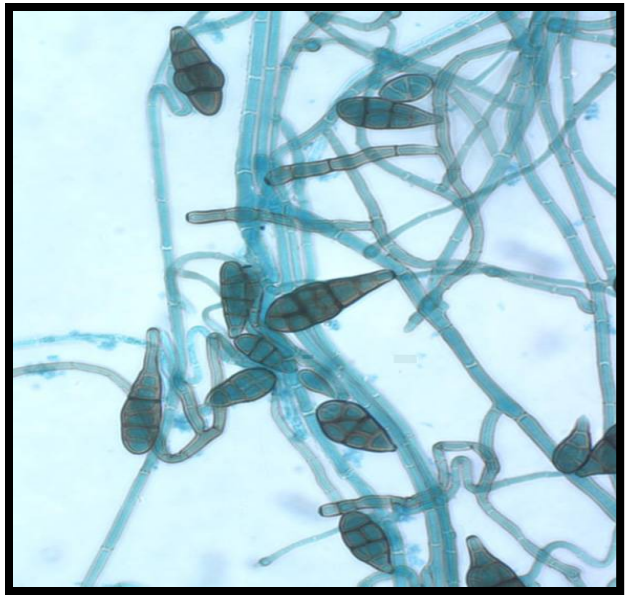




- Fruiting structures and thin hyphae of *Penicillium* species in culture (bottom)



Dematiaceous Molds

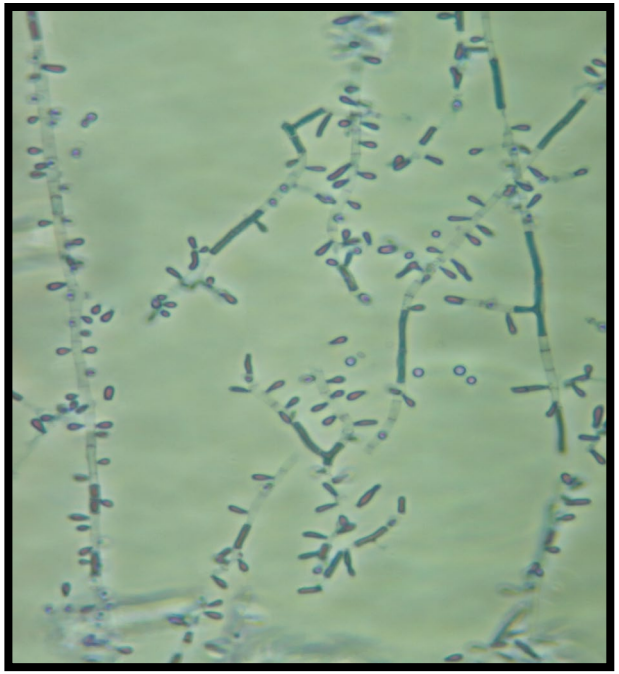


Alternaria species

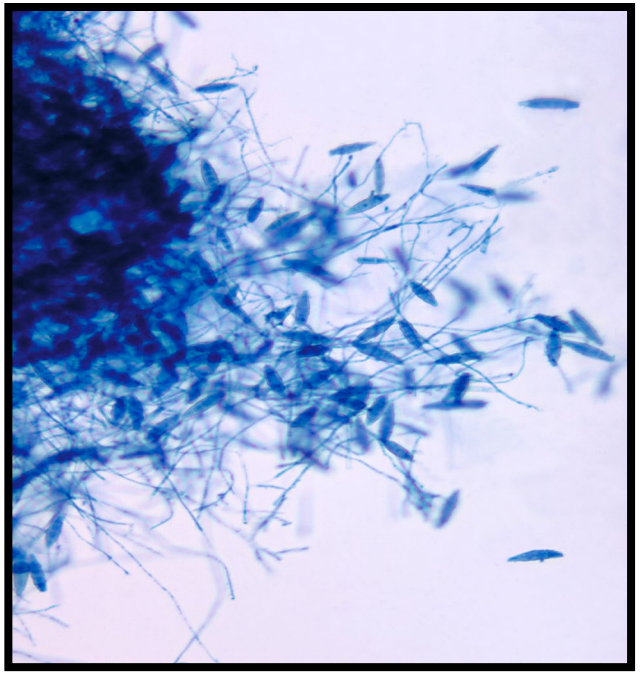
Bipolaris species

Curvularia species

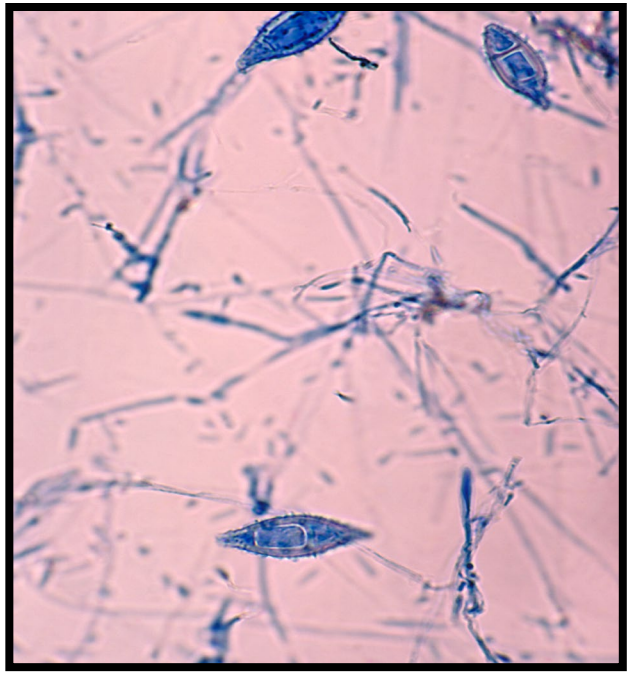
Dermatophytes



Trichophyton **species**



Epidermophyton floccosum



Microsporum **species**

Parasitology

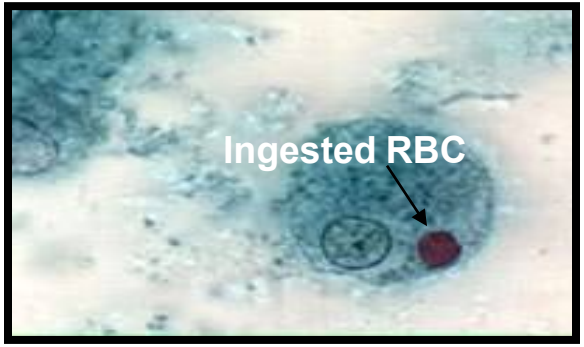
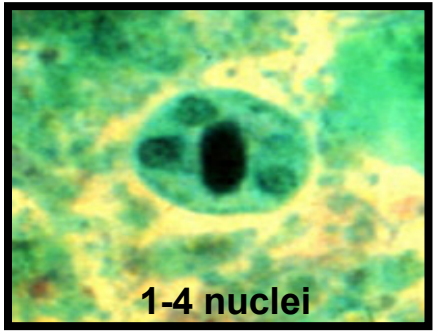
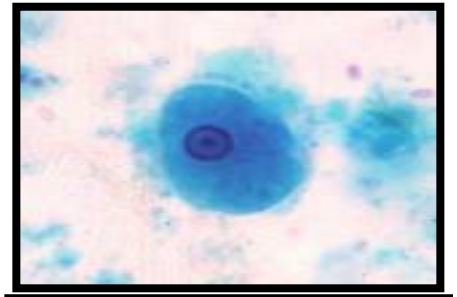
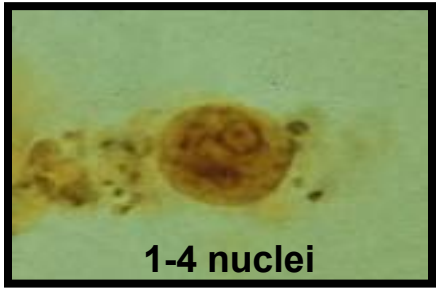
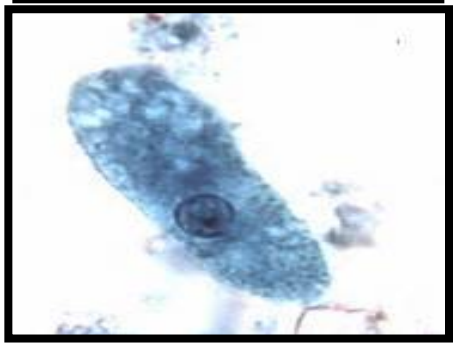
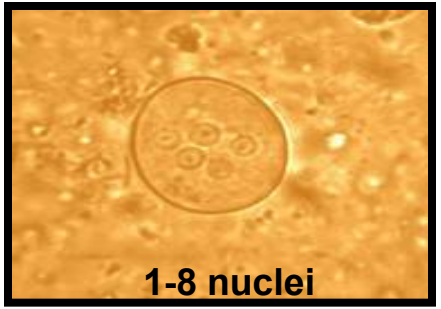
- Protozoa
  - Amoebae (intestinal and free-living)
  - Flagellates and ciliates
  - Coccidia and microsporidia
  - *Plasmodium* species and *Babesia* species
  - *Leishmania* species and trypanosomes
- Helminths (worms)
  - Nematodes or roundworms
  - Trematodes or flukes (flatworms)
  - Cestodes or tapeworms

Intestinal and Urogenital Amoebae, Flagellates, and Ciliates

Parasite	Pathogenic	Nonpathogenic
Amoebae	*Entamoeba histolytica*	*Entamoeba dispar* *Entamoeba hartmanni* *Entamoeba coli* *Endolimax nana* *Iodamoeba butschlii* *Entamoeba moskvoskii* *Entamoeba bangladeshi*
Flagellates	*Giardia* species *Trichomonas vaginalis* *Dientamoeba fragilis* (?) (amoeboflagellate)	*Chilomastix mesnili* *Pentatrichomonas hominis* *Enteromonas* *Retortamonas*
Ciliates	*Balantidium coli*	
Other	*Blastocystis hominis* (?)	

Amoebae

- *Entamoeba histolytica*, human pathogen
  - Nonpathogenic species
    - *E. dispar, E. hartmanni, E. nana, E. coli, E. bangladeshi*
- Most amoebae have two stages
  1. Trophozoite - actively replicating
  2. Cyst - dormant, stable (not destroyed by gastric acids, infectious)
- Detection and identification most amoebae by recognition of cyst or trophozoite forms in stool specimens
  - Exception *E. histolytica*
    - Antigen detection tests, NAATs, serology (for extraintestinal disease)
- *Blastocystis hominis* (not an amoeba) common, occasionally associated with disease

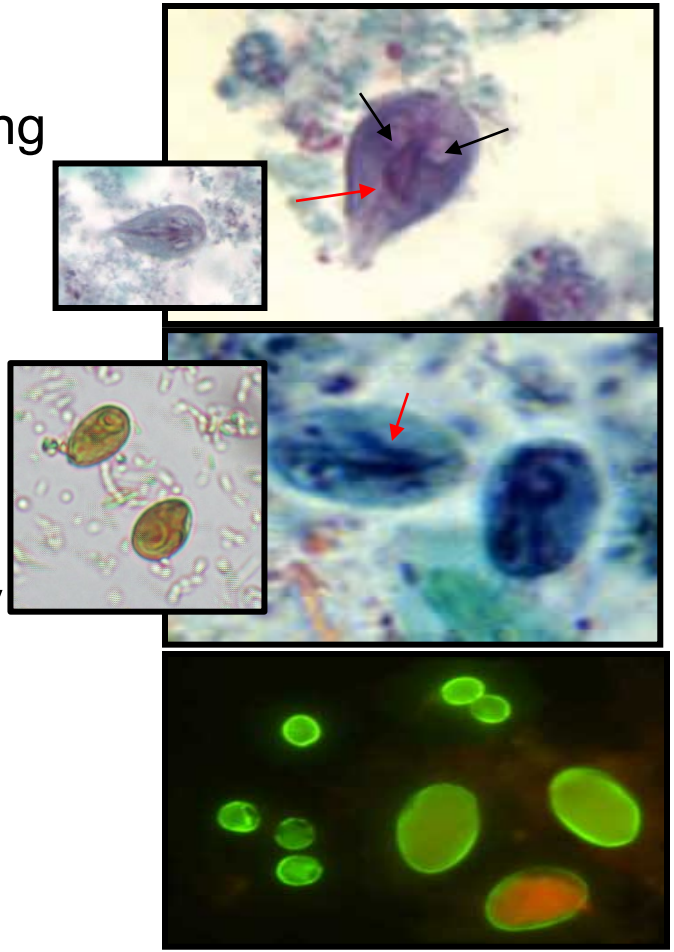
	Trophozoites	Cysts
Entamoeba histolytica		
Entamoeba hartmanni		
Entamoeba coli		

Flagellates

- Human pathogens
  - *Giardia* species*, Trichomonas vaginalis*
- *Dientamoeba fragilis* “occasional” human pathogen - mostly insignificant colonization
- Urogenital specimen contaminated with fecal matter may contain nonpathogenic flagellates (e.g., *Pentatrichomonas, Enteromonas, Retortamonas*) which can be confused with *T. vaginalis*
- *Chilomastix mesnili* nonpathogenic flagellate occasionally found in fecal specimens

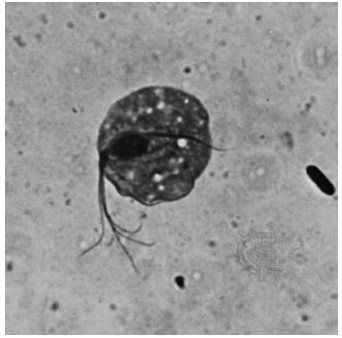
Giardia species

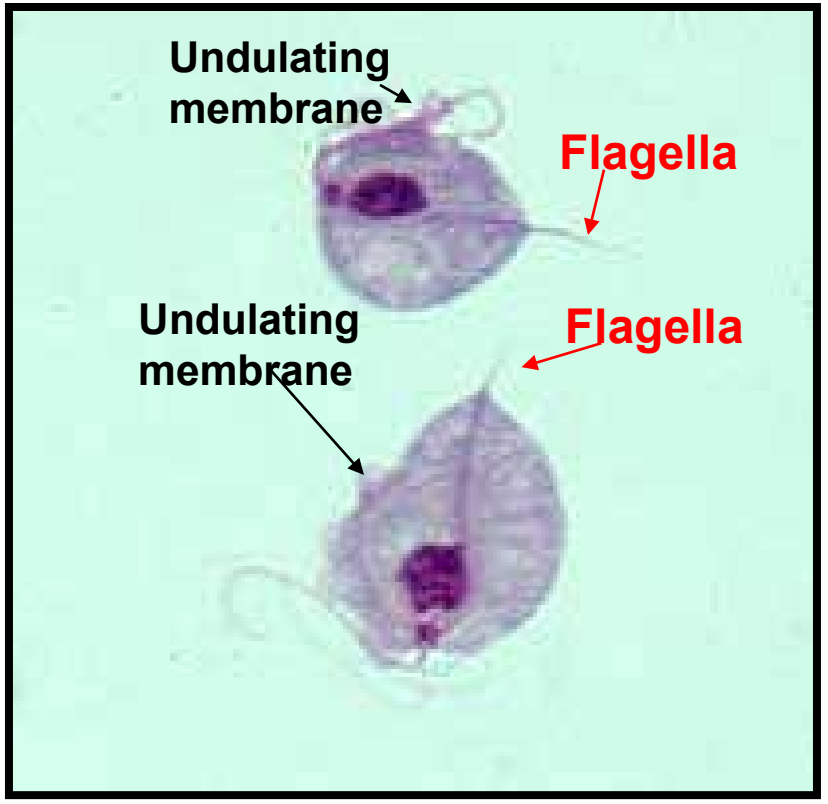
- Trophozoites (top) - pear-shaped with 2 nuclei (black arrows) within concave “sucking disk”; 2 curved rods (red arrow) lie below nuclei, 8 flagella
- Cysts (middle) - ovoid to ellipsoid; 4 nuclei present in mature cysts; central fibrils (red arrow)
- Immunoassays, direct fluorescent antibody tests (bottom), and NAATs more sensitively detect parasites in clinical specimens
  - DFA test shown uses a mixture of fluorescein-labeled antibodies to *Giardia* species (large oval cells) and *Cryptosporidium* species (small round cells)



Trichomonas vaginalis

- Trophozoites only (no cyst stage)
  - Pear-shaped
- Diagnosis
  - Microscopy or culture
  - NAATs - most sensitive, specific test



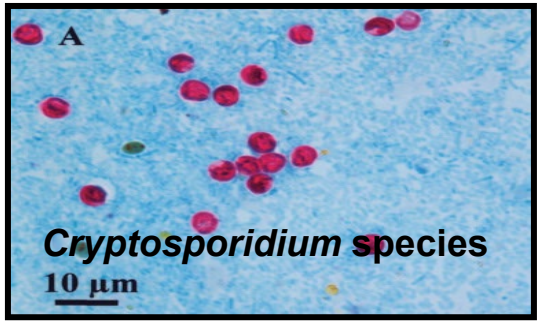


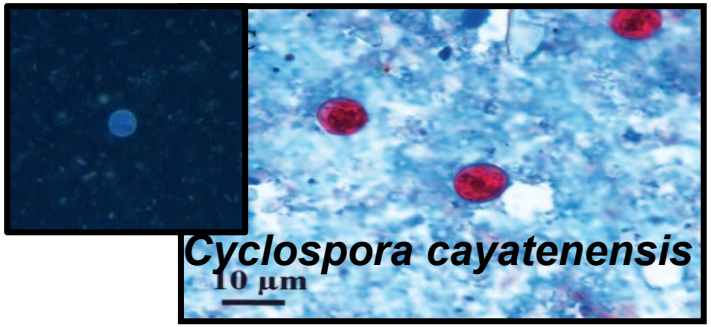
Balantidium coli

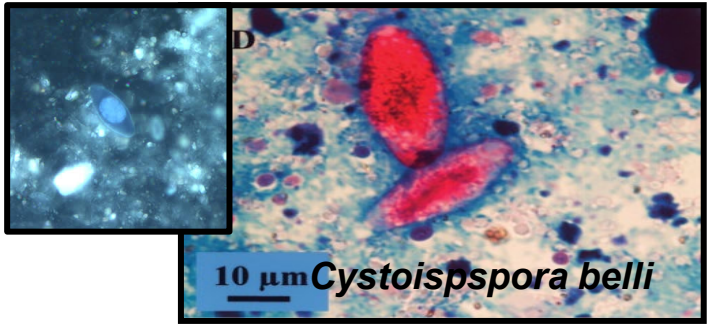
- Only ciliated amoeba to cause human disease
- Trophozoite and cyst stages
- Very large

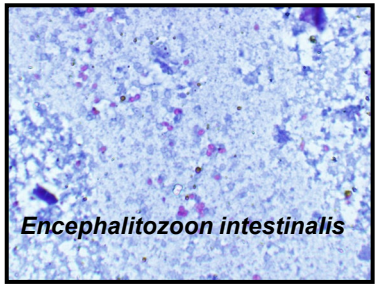
Coccidia and Microsporidia

- Stain (weakly, non-uniformly) with acid-fast stains:
    1. *Cryptosporidium* species (4-6 µm)
    2. *Cyclospora cayatenensis* (8-10 µm)
    3. *Cystisospora belli* (14x30 µm)
- Most do not stain with traditional Ova and Parasite (O&P) stains (e.g., Trichrome stain).
- *C. cayatenensis* and *Cystisospora belli* autofluoresce with ultraviolet light
- Microspordia (1-4 µm – similar to bacteria) stain with chromotrope stains (e.g., strong Trichrome blue - shown); NAATs or electron microscopy









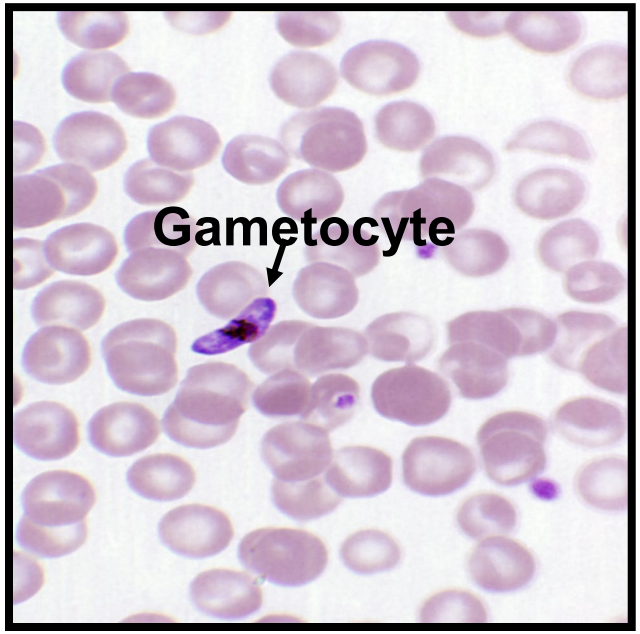
Blood and Tissue Protozoa

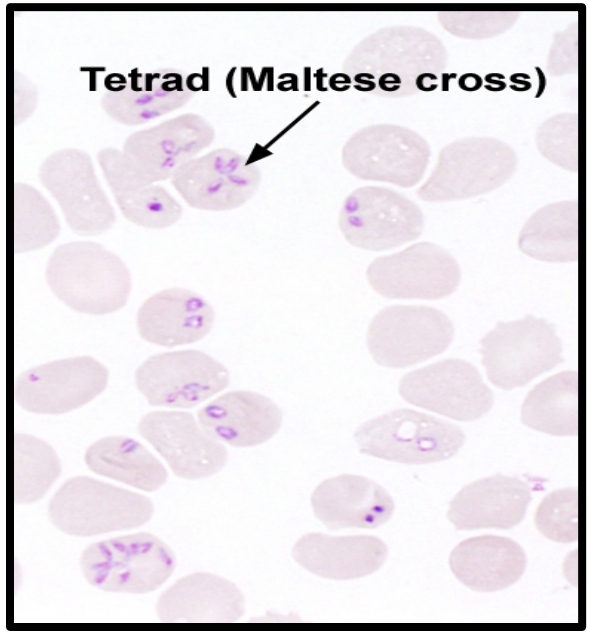
- *Plasmodium* species
  - *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*
- *Babesia* species
  - *B. microti, B. duncani,* MO-1, *B. divergens* (Europe)
- *Leishmania* species
  - *L. donovani, L. tropica, L. major*, etc.
- *Trypanosoma* species
  - *T. cruzi, T. brucei rhodesiense, T. brucei gambiense*
- Commonly detected by
  - Giemsa stain of blood
  - Hematoxylin and eosin (H&E) stain of tissues

Plasmodium species

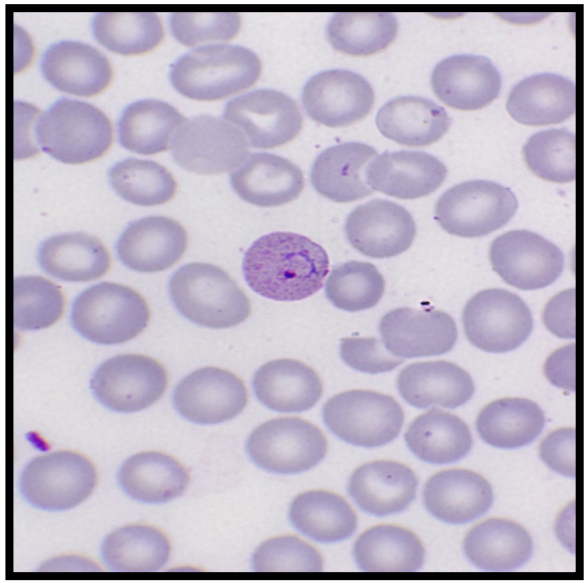
- Gold standard = thick and thin smears, **Giemsa stain**
- **Thick smear for increased sensitivity** (more blood volume examined because RBCs lysed)
- **Thin smear for organism species identification** (structures preserved in RBCs)

	P. falciparum	***P. vivax***	***P. ovale***	***P. malariae***	***P. knowlesi***
RBCs	Any	Young	Young	Old	Any
RBC Size	Normal	Bigger	Normal to Big	Small to Normal	Normal
Stippling		**Schuffners Dots**	**Schuffners Dots**		
Stages	**Rings and Gametocytes**	All	All	All	All
Multiple Rings	**Common**				Common
Troph	**Delicate; 2 chromatin dots; applique form**			**Band shape**	2 chromatin dots
Schizonts		12-24	8-12	**6-12 (rosette)**	10-16
Gametocyte	**Banana shaped**				

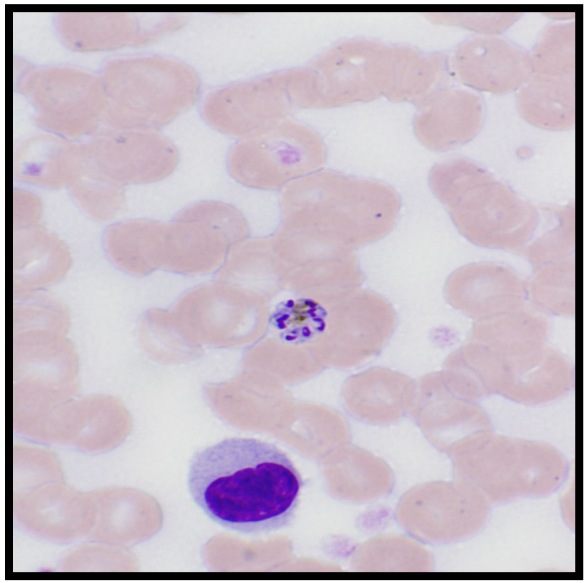




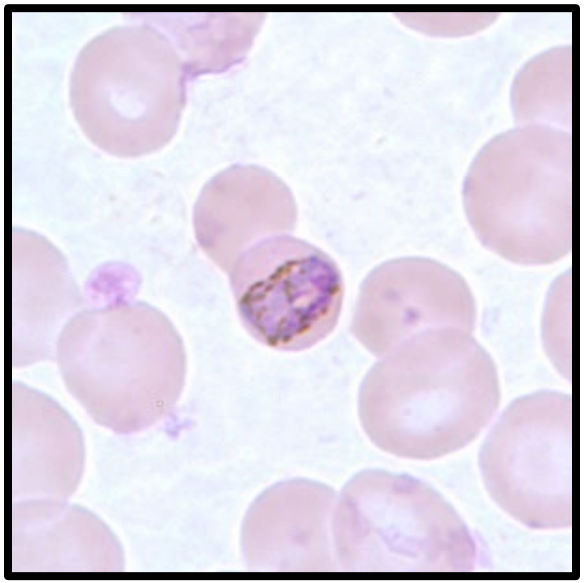
P. falciparum ***P. falciparum*** ***Babesia microti***



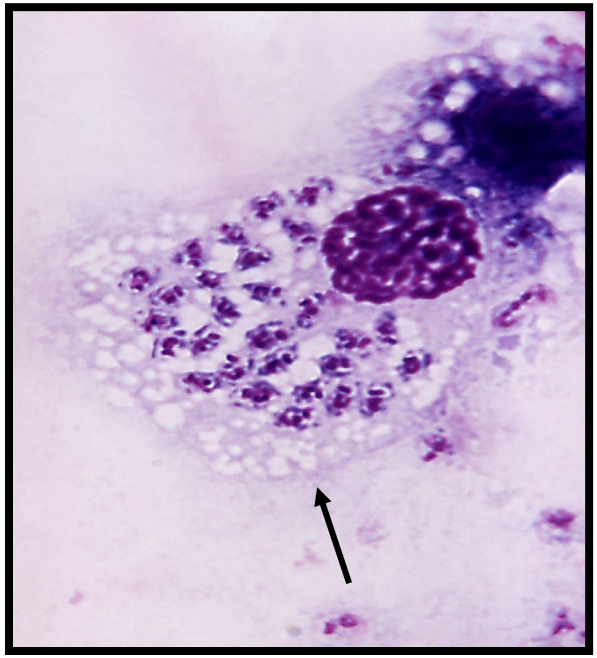
P. vivax
(amoeboid trophozoite)



P. malariae
(“rosette” schizont)

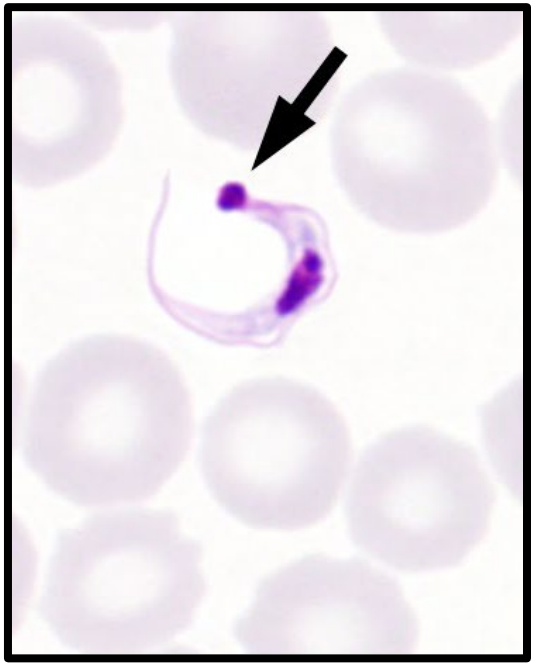


P. malariae
(band form trophozoite)



Leishmania **amastigotes in tissue**

→ may be confused with *Histoplasma* – look for strongly staining kinetoplast



“C” curve and large kinetoplast of *Trypanosoma cruzi* promastigote in peripheral blood

“S” curve and small kinetoplast of *Trypanosoma brucei* promastigote in peripheral blood

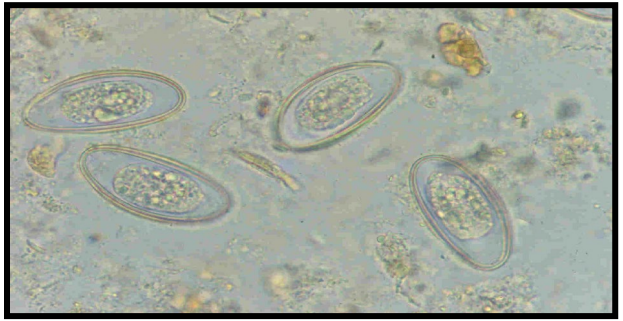
Trypanosoma photos courtesy of Bobbi Pritt, M.D.

Nematodes (Roundworms)

- **Intestinal nematodes**
  - *Enterobius vermicularis* (pinworm)
  - *Trichuris trichiura* (whipworm)
  - *Ascaris lumbricoides*
  - *Strongyloides stercoralis* (threadworm)
  - *Necator americanus, Ancylostoma duodenale* (hookworms)
- **Blood nematodes**
  - *Brugia malayi, Wuchereria bancrofti* (elephantiasis)
  - *Loa loa* (African eye worm)
  - *Onchocerca volvulus* (onchocerciasis or river blindness)
- **Tissue nematodes**
  - *Trichinella spiralis* (trichinosis)
  - *Toxocara canis* (visceral larva migrans)
  - *Ancylostoma caninum* (cutaneous larva migrans)

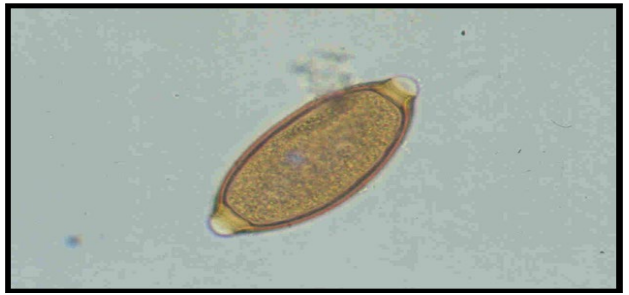
Enterobius vermicularis (pinworm) eggs

- Anal cellophane tape test
- Embryo surrounded by colorless shell, shell flattened on one side



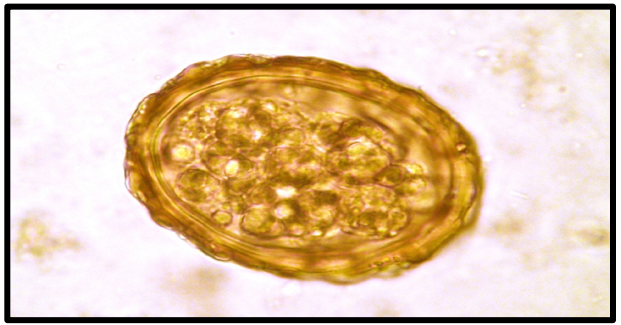
Trichuris trichiura (whipworm) egg

- Stool
- Naturally stained yellow-brown, with thick wall, clear polar plugs at each end



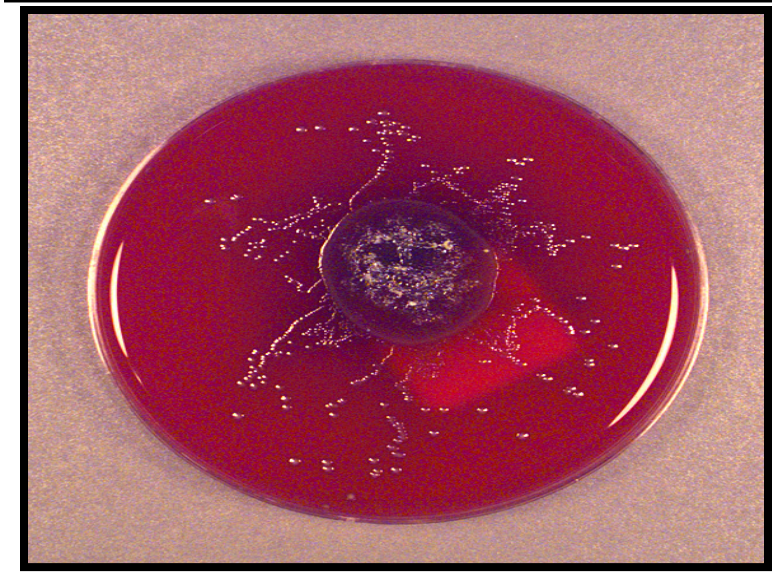
Ascaris lumbricoides egg

- Stool
- Either fertile (shown) or infertile
  - Infertile eggs larger and more oval shaped
- Yellow-brown color with thick, rough shell
- Adult worms occasionally passed in stool



Strongyloides stercoralis

- Larvae - two forms
  1. **Rhabditiform** (in stool)
  2. **Filariform** (infectious stage that develops in soil and occasionally in patient (leads to autoinfection)
- Larvae can be detected microscopically (top) or by placing feces on plate and detecting migrating larvae when they leave a trail of bacterial colonies (bottom)



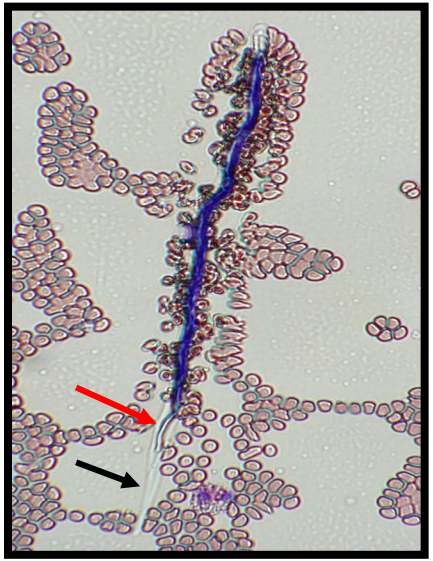
Ancylostoma duodenale (Old World) and *Necator americanus* (New World) Hookworms

- Eggs have thin, colorless shell surrounding developing larva
- If stool specimens left at room temperature, larvae can hatch and will resemble *Strongyloides* larvae

Wuchereria bancrofti

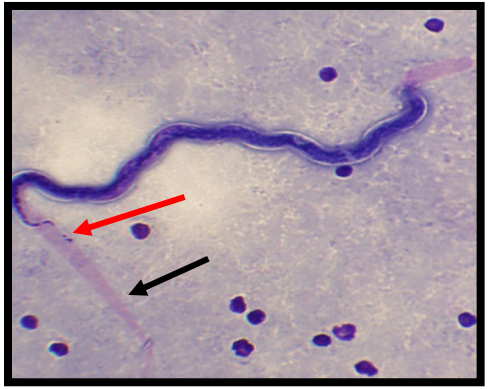
- Large microfilariae
- Transparent sheath
- Nuclei do not extend to tip of tail (red arrow)

Loa loa



- Small microfilariae
- Sheath (black arrow)
- Nuclei extend to tip of tail (red arrow)

Brugia malayi



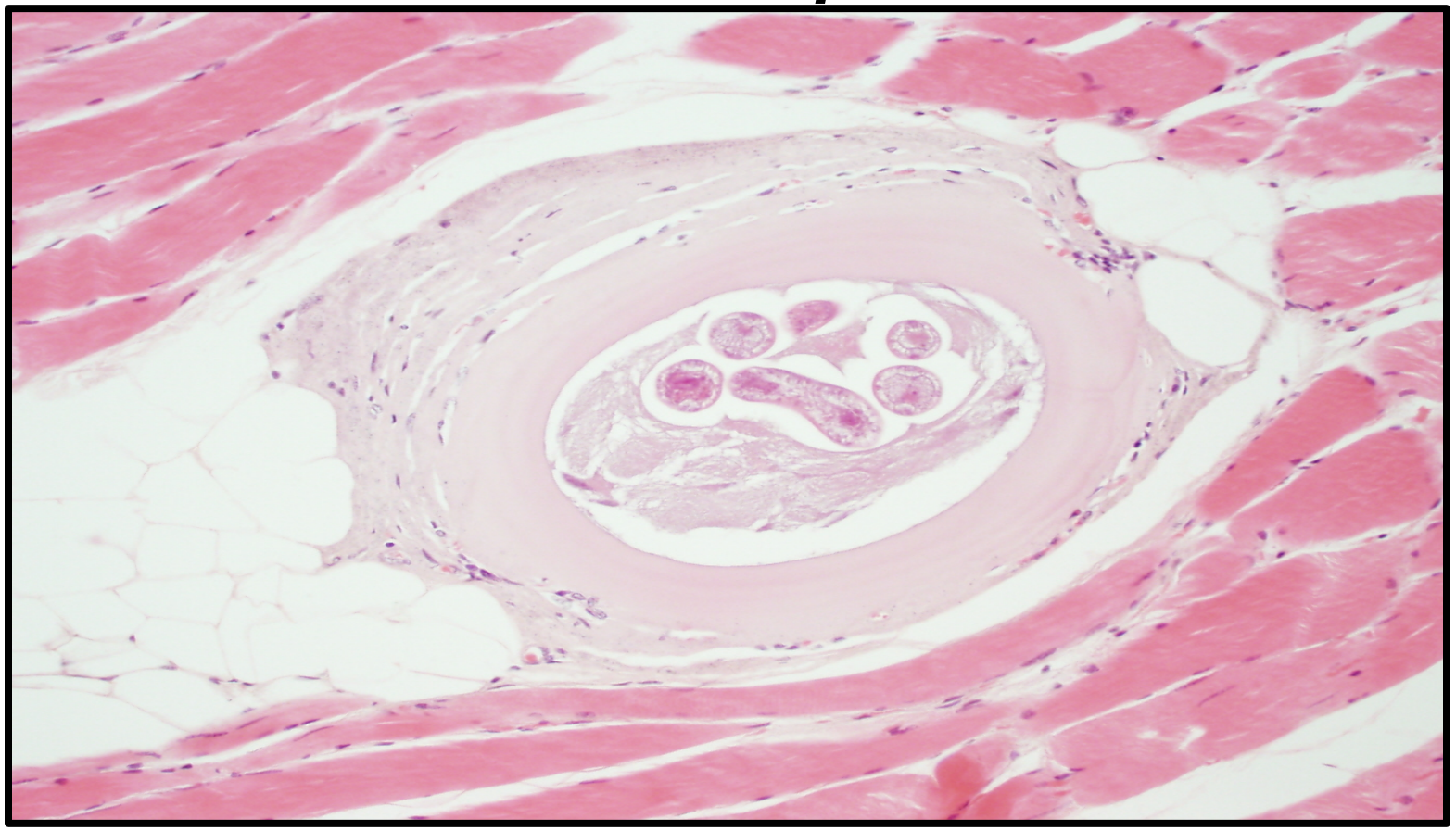
- Small microfilariae
- Sheath (black arrow)
- Gap between last and next to last nuclei in tail (red arrow)

Onchocerca volvulus

Adults mature in subcutaneous nodules

- Microfilariae in skin
  - No sheath
  - Nuclei do not extend to tip of tail

Trichinella spiralis



Trematodes (Flatworms)

- **Intestinal trematode**
  - *Fasciolopsis buski*
- **Tissue trematodes**
  - *Fasciola hepatica*
  - *Clonorchis sinensis* (Chinese liver fluke)
  - *Paragonimus* species (lung fluke)
- **Schistosomes**
  - *Schistosoma mansoni*
  - *Schistosoma japonica*
  - *Schistosoma haematobium*

Parasites with Opercula

Clonorchis sinensis

Acquired by ingestion of raw/undercooked fish

- Eggs
  - 17-30x13-18µm
  - Prominent operculum (black arrow) at small end
  - Knob (red arrow) opposite end

Fasciola hepatica

Acquired by ingestion of aquatic vegetation (e.g., watercress)

- Eggs
  - 130-150x63-90µm
  - Inconspicuous operculum

Fasciolopsis buski

Acquired by ingestion of aquatic vegetation (e.g., water chestnuts)

- Eggs
  - 130-140x80-85µm
  - Inconspicuous operculum

Paragonimus species

Acquired by eating raw or inadequately cooked crabs and crayfish

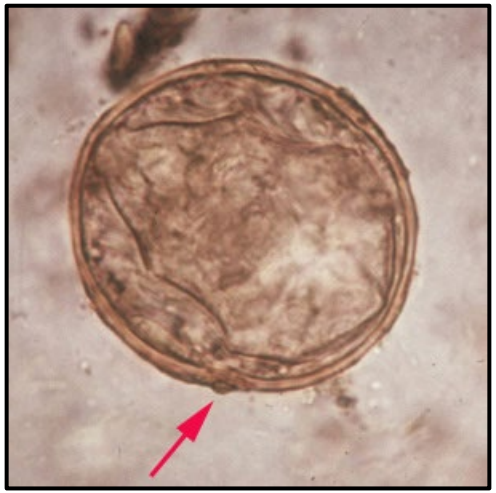
- Eggs
  - 80-120x45-70µm
  - Prominent operculum (arrow)

Schistosoma species

Large, thin-shelled eggs with no operculum

Schistosoma mansoni
(prominent lateral spine)

Schistosoma hematobium
(prominent terminal spine)

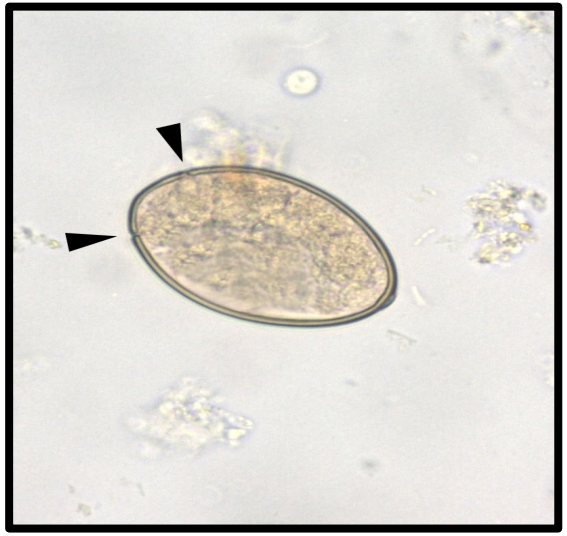


Schistosoma japonicum
(inconspicuous spine)

Cestodes (Tapeworms)

- **Intestinal cestodes**
  - *Diphyllobothrium latum* (fish tapeworm)
  - *Taenia solium* (pork tapeworm)
  - *Taenia saginata* (beef tapeworm)
  - *Hymenolepis nana* (dwarf tapeworm)
  - *Hymenolepis diminuta* (rat tapeworm)
  - *Dipylidium caninum* (dog tapeworm)
- **Tissue cestodes**
  - *Echinococcus granulosus* (hydatid disease)
  - *Taenia solium* (cysticercosis)

Diphyllobothrium latum



Egg

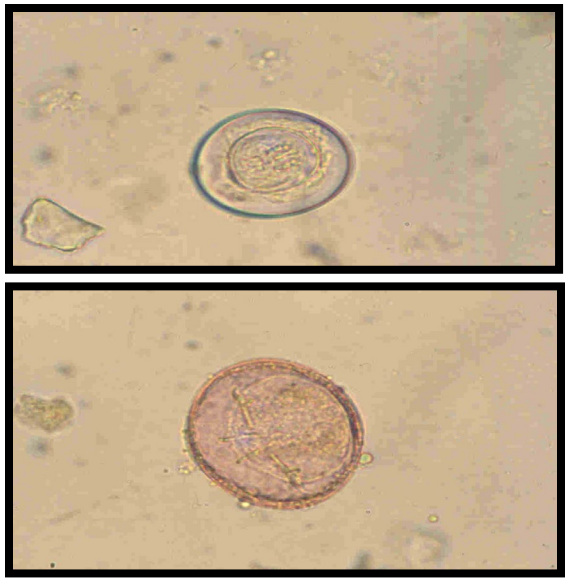
- **Oval shape**
- **Operculum (arrowheads)**
- **Absence of hooks**

Taenia saginata, Taenia solium

Eggs identical

- **Thick wall**
- **Outer membrane may or may not be present**

Hymenolepis nana, H. diminuta



Eggs

- **Hooks**
- **Outer membrane**
- ***H. nana* (top) eggs smaller than *H. diminuta* (bottom) eggs**