Primer 01

Microbiology 101

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DISCLOSURES

Financial Relationships with Relevant Commercial Interests

- Becton Dickinson Diagnostics – Employee and stockholder/ownership interest
Overview – Microbiological Stains

• Because most organisms are colorless and transparent, various dyes (stains) are used to see the individual cells.

• A variety of different types of stains are used in the microbiology lab including –
  – Contrast stains (e.g., methylene blue, lactophenol cotton blue, India ink, iodine)
  – Differential stains (e.g., Gram stain, acid-fast stains, spore stains, Giemsa stain, silver stains, Trichrome stain)
  – Fluorescent stains (e.g., acridine orange, auramine-rhodamine, calcofluor white, antibody-conjugated fluorescent stains)
Contrast Stain: Methylene Blue Stain

- Contrast stains are nonspecific stains used to detect the normally transparent organisms such as bacteria and fungi.

- The methylene blue stain was one of the first ones developed (used to stain C. diphtheriae in this example).

- This stain has now been replaced for the most part with other contrast stains.
Contrast Stain: Lactophenol Cotton Blue (LCB) Stain

• LCB is a commonly used contrast stain used primarily for observing the morphology of fungal molds.

• This is an example of Aspergillus fumigatus.
Differential Stain: Gram Stain

- The Gram stain is the most commonly used differential stain for bacteria.

- It is used to distinguish gram-positive (purple; top figure) from gram-negative (red; bottom figure) bacteria.

- Some gram-positive bacteria can be easily decolorized (appear red) and some gram-negative bacteria can retain the crystal violet and appear somewhat gram-positive.

- The shape and spatial arrangement of cells is as important as the color of cells for interpreting this stain.
Differential Stains: Acid-Fast Stains

- One of the most common acid-fast stains is the Kinyoun (cold) stain.

- This is a variation of the older Ziehl-Neelsen (hot) acid-fast stain where the slides had to be heated during staining.

- Mycobacteria (top) stain with both stains.

- “Modified” acid-fast stain – if a weak decolorizing solution is used to remove the primary stain, then partially or weakly acid-fast organisms can be stained. Partially acid-fast organisms include:
  - Nocardia (bottom figure)
  - Rhodococcus
  - Tsukamurella
  - Gordonia

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Differential Stains: Trichrome stain

• The trichrome stain is used for the detection of intestinal protozoa.

• The parasite’s cytoplasm stains blue-green and the internal structure stain red or purplish red (arrow).

• Background debris stain blue-green so care must be taken to distinguish between the protozoa and the background.
Fluorescent Stain: Auramine Rhodamine Stain

• The AR stain is a fluorochrome stain used to detect acid-fast bacteria.

• This is essentially the same as a Kinyoun stain except the carbol fuchsin dye is replaced with fluorescent dyes (auramine and rhodamine) and the stained smears are examined under UV illumination using a fluorescent microscope.

• Because of the high contrast between the fluorescing rods and the black background, this stain is more sensitive than the Kinyoun stain.
Fluorescent Stain: Calcofluor White Stain

• The calcofluor white stain is used to detect yeasts and molds in clinical specimens.

• This fluorescent dye binds to chitin in the fungal cell wall.

• False-positive results can occur if cotton fibers are present in the specimen because the dye will also bind to cellulose.
Antibody-Conjugated Fluorescent Stains

- Fluorescent antibody stains are specific stains where antibodies are attached to a fluorochrome (such as fluorescein).

- The antibody-antigen binding is detected by the fluorescence.

- Two examples of this test are illustrated here – Pneumocystis (top figure) and tissue culture cells infected with Varicella-Zoster virus (bottom figure) and then stained with fluorescein-tagged antibodies.
Bacteriology
Gram-Positive Cocci

Staphylococcus

Streptococcus

Enterococcus
Staphylococcus aureus

• S. aureus differs from the coagulase-negative staphylococci –
  – Uniform size and shape
  – Stacked together in a symmetrical arrangement

• When observed in clinical specimens, S. aureus will commonly appear as pairs, small clusters, or within phagocytic cells (lower figure)
  – may be more difficult to distinguish among the staphylococcal species and other gram-positive cocci

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Staphylococcus aureus and Candida albicans

- This photo illustrates the size difference between S. aureus (black arrow) and yeasts, in this case Candida albicans (red arrow).

- Yeast can appear as gram-positive although they tend to decolorize readily.
Streptococcus pyogenes

• Most group A streptococci are Streptococcus pyogenes.

• Group A streptococci form long chains or round cells, described as a “string of pearls.”

• Streptococcus pyogenes is the only Streptococcus species that is PYR positive.
Streptococcus mitis

- The viridans streptococci consist of more than 25 species and are divided into 5 groups:
  - Streptococcus anginosus group
  - Streptococcus mitis group
  - Streptococcus salivarius group
  - Streptococcus mutans group
  - Streptococcus bovis group

- The viridans streptococci tend to form long chains of cells.

- Differentiation of the individual groups is important clinically because they are associated with different diseases (e.g. anginosus group = abscesses).
Streptococcus pneumoniae

• The most important member of the mitis group is S. pneumoniae.

• S. pneumoniae generally stains uniformly in clinical specimens but may rapidly decolorize and appear gram-negative in culture.

• The individual cells will appear elongated and will be arranged in pairs and short chains.

• A refractile capsule may be seen but is not obvious in this photo.
Enterococcus

- Enterococci will appear as elongated gram-positive cocci arranged in pairs and short chains.

- These bacteria cannot be distinguished from S. pneumoniae by Gram stain.

- S. pneumoniae can be rapidly identified by exposing the cell to bile (or another detergent) that will dissolve the bacteria (“bile soluble”).

- Enterococci can be identified by the PYR test (positive test for the enzyme pyrrolidonyl arylamidase).

- The most important enterococci are E. faecium, E. faecalis, E. gallinarum, and E. casseliflavus.
Gram-Positive Rods

The most common or important gram-positive rods are:

• **Spore-forming rods**
  – Aerobes – Bacillus species (e.g., B. cereus, B. anthracis)
  – Anaerobes – Clostridium species (e.g., C. perfringens, C. septicum, C. difficile)

• **Non-sporeforming rods**
  – Uniform shape – Listeria, Lactobacillus
  – Irregular (coryneform) shape – Corynebacterium, Propionibacterium

• **Acid-fast rods**
  – Acid-fast – Mycobacterium
  – Weakly (partially) acid-fast – Nocardia, Rhodococcus
Spore-Forming Rods

- Two genera of bacteria commonly isolated in the lab form spores: Bacillus (aerobe) and Clostridium (anaerobe).

- These images are Gram stains of B. cereus from a culture plate.

- Spores are not stained with the Gram stain and will appear as clear areas in the cell (red arrow).
Bacillus anthracis

- This is a Gram stain of B. anthracis in the blood culture from a bacteremic patient.

- Note that spores are not seen and the bacteria form long chains. This is characteristic of this pathogen.
Clostridium perfringens

- C. perfringens (arrows) in a mixed culture with E. coli and K. pneumoniae.

- This is one of the most common species of Clostridium isolated from clinical specimens.

- C. perfringens spores are almost never seen; rods are described as “boxcar shaped” or rectangular and are generally larger than most bacteria.
Clostridium difficile

- C. difficile is now the most important clostridium species associated with disease.

- As the name implies, the organism can be difficult to grow because it is highly sensitive to oxygen; however, spores are formed readily and can contaminate the hospital environment.

- A variety of tests have been used to diagnose C. difficile disease. Although commonly used, immunoassays are insensitive and nonspecific. The best diagnostic test currently is PCR and a number of rapid, commercial tests are available.
Clostridium septicum

- Clostridium septicum is virtually always clinically significant when isolated in blood cultures.

- Commonly associated with overwhelming sepsis originating from the large intestine.

- Typically the patient has colon cancer or a hematologic malignancy.

- C. septicum grows very rapidly and forms elongated rods arranged in chains; will readily form spores in specimens and culture (not seen in this photo).
Clostridium tertium

- C. tertium is an anaerobe that characteristically stains gram-negative and can grow on plates incubated aerobically.

- A clue that this is not a typical gram-negative bacterium is that it will not grow on a MacConkey agar plate.

- If the isolate is subcultured onto both aerobic and anaerobic blood agar plates, it grows much better on the anaerobic plate and can form spores (not seen in this image).
Listeria monocytogenes

• Listeria are small, non-sporeforming, gram-positive rods. Compare the size of the listeria cells (black arrow) to the pair of gram-negative rods (red arrow).

• Listeria can be mistaken for either streptococci or corynebacteria and may be discounted as a culture contaminant.

• Listeria colonies are beta-hemolytic but this may not be obvious.

• The organism also grows slowly so the lab should be notified if listeria is suspected.
Corynebacterium Species

- Corynebacteria are commonly isolated as contaminants in blood cultures.

- The organisms tend to clump together and have an irregular shape (“coryneform” shaped; top figure).

- The organism in the bottom figure is C. jeikeium. This species is important clinically, causing disease in hospitalized patients and is frequently resistant to most commonly used antibiotics except vancomycin.
Propionibacterium acnes

- This is a typical Gram stain of P. acnes, a common contaminant of blood cultures.

- These organisms will grow after 4-5 days of incubation and only in the anaerobic bottle.

- They form these clumps of gram-positive rods.

- This is the anaerobic counterpart to the corynebacteria.
Gram-Negative Cocci, Coccobacilli, and Rods

Gram-negative bacteria are among the most commonly isolated bacteria. Examples include:

• Cocci
  – Neisseria
  – Moraxella catarrhalis

• Coccobacilli
  – Moraxella, other species
  – Acinetobacter
  – Haemophilus

• Rods
  – Enterobacteriaceae (e.g., Escherichia, Klebsiella)
  – Pseudomonas, Stenotrophomonas, Burkholderia
  – Miscellaneous (e.g., Bacteroides, Fusobacterium)
Neisseria

- Neisseria gonorrhoeae (top) and N. meningitidis (bottom) are the most commonly isolated aerobic gram-negative cocci.

- They are typically arranged in pairs with the adjoining sides flattened into a shape that resembles a “coffee bean”.

- Care must be used to not mistaken gram-negative coccobacilli for Neisseria.
Moraxella catarrhalis

- Moraxella species are gram-negative rods. However, M. catarrhalis was originally classified as a Neisseria because the morphology of these bacteria closely resembles Neisseria.

- This photo is M. catarrhalis in a sputum from a patient with pneumonia. Note the inflammatory cells and large number of bacteria – this is typical of respiratory infections with this organism.
Acinetobacter

- Acinetobacter are gram-negative coccobacilli that can retain the crystal violet and resemble **gram-positive cocci in pairs** (black arrow).

- The other bacteria in this figure is Pseudomonas (red arrow), clearly gram-negative rods arranged in a chain (arranged in pairs is more common).

- Acinetobacter are larger than Neisseria and the adjoining sides are not flattened.
Haemophilus

- Haemophilus are very small gram-negative rods that could also be mistaken for a gram-negative cocci (top figure).

- Long pleomorphic forms (bottom figure) can be seen in patients receiving antibiotics. This is a Gram stain of CSF from a child with Haemophilus meningitis.
Other Glucose-Fermenting Gram-Negative Rods

- **Glucose-fermenting GNRs** can be subdivided by the spot oxidase test and growth on MacConkey agar.

<table>
<thead>
<tr>
<th></th>
<th>Growth on MacConkey</th>
<th>No growth on MacConkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase-positive</td>
<td>Aeromonas, Vibrio</td>
<td>Actinobacillus, Aggregatibacter, Capnocytophaga, Capnocytophaga, Cardiobacterium, Eikenella, Kingella, Pasteurella</td>
</tr>
<tr>
<td>Oxidase-negative</td>
<td>Chromobacterium, Enterobacteriaceae</td>
<td>Capnocytophaga (C. canimorsus, C. cynodegmi), Dysgonomonas, Streptobacillus</td>
</tr>
</tbody>
</table>

- Most GNRs that fail to grow on MacConkey agar originate in the mouth and are associated with oral infections, bite wounds, bacteremia, or endocarditis.
Enterobacteriaceae

• The Enterobacteriaceae is a large family of more than 100 genera of glucose-fermenting bacteria.

• With one exception (Plesiomonas), all Enterobacteriaceae are oxidase-negative; all grow on MacConkey agar.

• Escherichia, Klebsiella (figure), Enterobacter, Proteus, Salmonella, and Shigella are some common members of the family.

• All members of this family are relatively large gram-negative rods that tend to stain more intensely at their ends (“bipolar” staining).
Other Glucose-Oxidizing Gram-Negative Rods

Glucose-oxidizing **GNRs** can also be subdivided by their oxidase reaction and ability to grow on MacConkey agar.

<table>
<thead>
<tr>
<th></th>
<th>Growth on MacConkey</th>
<th>No growth on MacConkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase-positive</td>
<td>Achromobacter, Burkholderia, Elizabethkingia, Ochrobactrum, Oligella, Pseudomonas, Roseomonas, Sphingomonas</td>
<td>Brucella, Methylobacterium, Moraxella (M. catarrhalis)</td>
</tr>
<tr>
<td>Oxidase-negative</td>
<td>Acinetobacter, Pseudomonas (P. oryzihabitans, P. luteola), Stenotrophomonas</td>
<td>Acidovorax, Bartonella, Francisella</td>
</tr>
</tbody>
</table>
Glucose-Oxidizing Gram-Negative Rods

• “Pseudomonads” (e.g., Pseudomonas, Burkholderia, Stenotrophomonas) are small gram-negative rods typically arranged singly or in pairs.

• The Gram stain morphology is characteristic of this group of organisms but individual genera cannot be reliably differentiated by Gram stain.

• Pseudomonas aeruginosa, the most commonly isolated pseudomonad, may be surrounded by a thick mucus capsule (bottom figure).
Other Gram-Negative Rods

• Bacteroides fragilis is an anaerobic gram-negative rod that is pleomorphic—short and long rods can be seen in the top image. One way to determine that these are the same organisms is to note that the diameter of the cells is the same even though the length is variable.

• Some gram-negative rods are thin and long. Species of Fusobacterium (middle figure) and Capnocytophaga (bottom figure) are the most common, although other bacteria may look like this if the patient is receiving antibiotics (refer to the Haemophilis photo previously shown).
Acid-Fast and Partially Acid-Fast Bacteria

• Very few bacteria stain with acid-fast stains.

• Members of the genus Mycobacterium are acid-fast and members of the other genera listed here are “weakly or partially acid-fast”.

• Some of the rapidly growing mycobacteria are also weakly acid-fast.

• Acid-fast and partially acid-fast bacteria include:
  – Mycobacterium
    • Rapid-growers
    • Slow-growers
  – Nocardia
  – Rhodococcus
  – Gordonia
  – Tsukamurella
Acid-Fast Stains

- The **Ziehl-Neelsen** is the original stain and requires heating the slide after basic fuchsin is added so the stain penetrates into the bacteria.

- The **Kinyoun** stain and **modified Kinyoun** stain are referred to as a **cold acid-fast stains**. Heating is not needed because the concentration of basic fuchsin is increased as well as the concentration of phenol.

- The modified Kinyoun stain differs from the Kinyoun stain by using a weak acid solution in alcohol. Nocardia, Rhodococcus, Gordonia, and Tsukamurella will retain some of the basic fuchsin stain when this weak solution is used but not when the higher concentration of acid is used.

- The **fluorochrome** stain replaces basic fuchsin with two fluorescent dyes, auramine and rhodamine. The fluorochrome is a weak acid-fast stain so all acid-fast organisms will stain.
Mycobacterium avium – Gram Stain

• This is an example of mycobacteria that retained a little of the crystal violet in the Gram stain.

• They appear as beaded rods (arrows). Care must be used to not confuse them with streptococci (strep tend to stain more uniformly and the cells will be touching and uniform in shape).
Mycobacterium

• Top figure: Kinyoun (carbol-fuchsin) stain of mycobacteria. Note the beaded appearance.

• Bottom figure: Fluorochrome (auramine-rhodamine) stain of mycobacteria.

• Acid-fast bacteria are much easier to detect using the fluorochrome stain because of the contrast with the dark background.
Nocardia – Gram Stain

- Nocardia is the second most commonly isolated acid-fast organism.

- This Gram stain illustrates the **thin, filamentous, branching forms** that stain irregularly with the Gram stain (note the blue and red sections of the structures).

- No other acid-fast organism forms long, branching structures.

- Important ways to differentiate this organism from streptococci include: (1) true branching of the filaments, and (2) the observation that the “beads” (arrows) do not touch and are irregularly distributed.
Nocardia – Modified Acid-Fast Stain

• Modified acid-fast stain of Nocardia in a sputum specimen.

• Note the branching forms and the fact the organism does not stain uniformly.

• If a regular Kinyoun stain was used, most of the basic fuchsin stain would be removed by the strong acid-alcohol solution and the organism would stain very weakly.
Rhodococcus – Gram Stain
4 hour Broth Culture

• Rhodococcus was originally classified as a Corynebacterium.

• These organisms retain the crystal violet dye more uniformly than either mycobacteria or nocardia.

• After growth for a few hours, they stain well with the Gram stain and appear rod-like.
Rhodococcus – Modified Acid-Fast Stain
24 hour Culture

• When Rhodococcus is incubated in culture for 24 hours or longer, the bacteria assume a coccoid form (hence the name red coccus for cocci that form red colonies).

• Most of these bacteria decolorize easily with the weak acid-alcohol solution so only a few appear “red” or acid-fast.

• This photo was prepared from a colony of rhodococcus growing on media used for mycobacteria. If the colonies are grown on the blood agar plates used in bacteriology, then very few cells will retain the basic fuchsin dye.
Mycology
Yeasts and Molds

- Fungi can be subdivided into **yeasts** (single cell organisms) and **molds** (multicell organisms). A few important fungi (**dimorphic fungi**) can exist in both forms (e.g., Histoplasma, Blastomyces, Sporothrix)

- The most important genera of yeast are:
  - Candida (e.g., C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei)
  - Cryptococcus (e.g., C. neoformans)
  - Trichosporon (e.g., T. asahii, T. mucoides; the former species T. beigelii has been subdivided into 6 species and the old name is not currently accepted)
  - Malassezia (e.g., M. furfur)
  - Pneumocystis (maybe more appropriately called a nonmold rather than a yeast cell)
Candida Species

- Top figure: *Candida albicans* isolated in blood culture; note the **yeast cells** and **pseudohyphae** or pseudo branched forms.

- Bottom figure: *Candida glabrata*; these are **smaller** than other yeasts; they also do not form pseudohyphae; common cause of urinary tract infections and second most common cause of fungemia.
Germ Tube Test

• This is a rapid test for the identification of C. albicans. Only one other Candida species (C. dubliniensis) is germ tube positive.

• Yeast cells inoculated in serum will form “germ tubes” within 2 hours.

• Note the “tube is a continuous extension from the yeast cell and no septum exists. This distinguishes germ tubes from pseudohyphae.
Cryptococcus neoformans

- C. neoformans cells (arrows) suspended in India ink will appear as different sized round cells with a clear halo (capsule). Inflammatory cells will generally be uniform in size.

- Budding may be seen (no budding cells in this photo)

- The India ink stain is not commonly used now – replaced with the more sensitive and specific cryptococcal antigen test.
Malassezia

• Malassezia species grow on the skin surface and can cause pityriasis versicolor and rarely systemic infections.

• The small yeast cells have a prominent “collarette” (arrow) that forms where the daughter cells bud.

• Short hyphal elements may also be seen in skin scrapings (referred to as “spaghetti and meatballs”).

• Culture of the most important species in this genus require supplementation of media with lipids (e.g., olive oil).
Pneumocystis

• The taxonomy of this group of organisms has changed.
  – Previously classified as a parasite, it is now accepted as a fungus.
  – Previously a single genus and species was recognized, Pneumocystis carinii. It is now recognized that there are multiple species, each with a specific mammalian host. P. jirovecii is the human pathogen

• The two developmental forms that are observed in human tissues are the trophozoite and the cyst (terminology is a hold over from when these organisms were classified as parasites).
Pneumocystis – Methenamine Silver Stains

- Stain performed in surgical pathology
- Cysts stain brown to black; trophozoites are unstained
- Nonspecific staining in the background may make interpretation difficult if only a few cysts are present
Pneumocystis – Direct Fluorescent Antibody Test

• Fluorescein-conjugated monoclonal antibodies for Pneumocystis stain the cyst wall green with the contents usually unstained

• Trophozoites stain and appear as small polygons or spheres outlined in green
Dimorphic Fungi

- Dimorphic fungi exists in two forms – typically as yeasts at body temperature and molds at room temperature.
- The most commonly isolated dimorphic molds in the US are:
  - Histoplasma capsulatum
  - Blastomyces dermatitidis
  - Coccidioides immitis
  - Sporothrix schenckii
Histoplasma

- H. capsulatum forms small (2-4 um) yeast cells in tissue (top figure, silver stain) and filamentous forms in culture (bottom figure, LCB stain).

- Note the thin hyphae, microconidia, and large macroconidia (arrows) with knobby surfaces (tuberculate conidia).
Blastomyces

- B. dermatitidis forms large (8-15 um) yeast cells in tissue and hyphal forms in culture at ambient temperatures.

- The yeasts have thick wall and form a broad base where the daughter cell buds.

- The mold form has thin hyphae with numerous small microconidia attached to the hyphae by thin branches (resembles lollipops).
Coccidioides

- C. immitis forms large (up to 120 µm), endospore filled “spherules” (top figure) in tissues and filamentous forms at room temperature.

- Barrel shaped, spore-like structures (arthroconidia) are formed in alternate hyphal cells (bottom figure, arrow).
Sporothrix

- S. schenckii forms narrow based yeast cells in tissue (top figure) and delicate hyphae with a cluster ("flowerette") of conidia (spores) at the end of a narrow stalk (bottom figure).

- The yeasts and conidia can be darkly pigmented so this fungus is classified as a dematiaceous (dark) mold.
Filamentous Fungi - Molds

• The taxonomic classification of molds is complex and generally confusing for nonmycologists. Traditionally these fungi are classified by morphologic features and some clinical properties:
  – Nonseptated molds (e.g., Rhizopus, Mucor, Rhizomucor, Absidia)
  – Lightly colored or hyaline (moniliaceous), septated molds
    • Opportunistic fungi (e.g., Aspergillus, Fusarium, Paecilomyces, Scopulariopsis, Penicillium)
    • Dermatophytes (e.g., Trichophyton, Epidermophyton, Microsporum)
  – Darkly pigmented (dematiaceous), septated molds (e.g., Alternaria, Bipolaris, Curvularia, Exophiala)

• Current work is underway to classify these organisms by gene sequencing and mass spectrometry (MALDI). It is anticipated that these approaches will be more rapid and objective.

• It is impractical to give a comprehensive summary of all molds here; rather, I will illustrate the diversity of morphologic forms with selected photographs.
Mucor

- Mucor is an example of a zygomycetes.

- These fungi are characterized by a lack of septae (divisions) within the hyphae.

- In tissue (top figure; silver stain) the hyphae appear ribbon-like.

- The bottom figure is the mold in culture.
• **Aspergillus** in tissue stained with silver stain; note uniform diameter and branching of septated hyphae

• **Aspergillus** in culture with characteristic fruiting bodies – conidiophore covered with conidia
• Fusiform or sickle shaped multicelld macroconidia of *Fusarium*.

• Fruiting structures and thin hyphae of *Penicillium* in culture.
Dematiaceous Molds

Alternaria  Bipolaris  Curvularia
Dermatophytes

Trichophyton  Epidermophyton  Microsporum
Parasites

• Parasites can be subdivided into protozoa and helminths.

• Protozoa are subdivided into:
  – Amoeba (including intestinal ameba and free-living ameba)
  – Flagellates and ciliates
  – Coccidia and Microsporidia
  – Plasmodium and Babesia
  – Leishmania and Trypanosomes

• Helminths (worms) are subdivided into:
  – Nematodes or roundworms
  – Trematodes or flatworms (flukes)
  – Cestodes or tapeworms
Intestinal and Urogenital Amebae, Flagellates, and Ciliates

<table>
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<tr>
<th>Parasite</th>
<th>Pathogenic</th>
<th>Nonpathogenic</th>
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<tbody>
<tr>
<td>Amebae</td>
<td>Entamoeba histolytica</td>
<td>Entamoeba dispar</td>
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<td></td>
<td>Blastocystis hominis (?)</td>
<td>Entamoeba hartmanni</td>
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<td></td>
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<td>Entamoeba coli</td>
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<td>Iodamoeba butschlii</td>
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<td>Flagellates</td>
<td>Giardia lamblia</td>
<td>Chilomastix mesnili</td>
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<td>Trichomonas vaginalis</td>
<td>Trichomonas hominis</td>
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<td>Dientamoeba fragilis (?)</td>
<td>Pentatrichomonas</td>
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<td>Enteromonas</td>
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<td></td>
<td></td>
<td>Retortamonas</td>
</tr>
<tr>
<td>Ciliates</td>
<td>Balantidium coli</td>
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</table>
Amebae

• One intestinal ameba is a clear human pathogen, *Entamoeba histolytica*, and must be differentiated from 3 other nonpathogenic species: E. dispar, E. hartmanni, and E. coli.

• **Blastocystis hominis** is a common protozoa that has occasionally been associated with human disease.

• Two stages exists for most amebae: actively replicating **trophozoite** and dormant, stable **cyst**. The cyst stage is infectious because it is not destroyed by gastric acids when ingested.

• Detection and identification of most amebae is by recognition of the cyst or trophozoite forms in stool specimens. The exception is E. histolytica where antigen detection tests have also been developed (most commonly enzyme immunoassays, EIAs) as well as PCR molecular tests.
E. histolytica

E. hartmanni

E. coli

Trophozoites

Cysts

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Flagellates

• Two flagellates are well-recognized human pathogens: Giardia lamblia and Trichomonas vaginalis

• A third flagellate, Dientamoeba fragilis, has been implicated as an “occasional” human pathogen; however, most isolates of this organism represent insignificant colonization

• If a urogenital specimen is contaminated with fecal matter, then nonpathogenic flagellates (e.g., Pentatrichomonas, Enteromonas, and Retortamonas) may be confused with T. vaginalis.

• Chilomastix mesnili is another nonpathogenic flagellate that is occasional found in fecal specimens.
Giardia lamblia

- Trophozoites (top figure) are pear-shaped with 2 nuclei (black arrows) within a concave “sucking disk” (hard to see in this figure); 2 curved rods (red arrow) lie below the nuclei and 8 flagella are present.

- Cysts (middle figure) are ovoid to ellipsoidal; 4 nuclei are present in mature cysts; central fibrils (red arrow) are also seen.

- Immunoassays and direct fluorescent antibody tests (lower figure) have been developed to more easily detect the parasites in clinical specimen. The DFA test shown here uses a mixture of fluorescein labeled antibodies to Giardia (large oval cells) and Cryptosporidium (small round cells).
Trichomonas vaginalis

- The figure shows two *T. vaginalis* trophozoites; no cyst stage exists.
- The trophozoites are pear-shaped.
- The nucleus contains many chromatin granules and a small karyosome.
- An undulating membrane (black arrows) extends half way down the parasite (this membrane extends the length of *P. hominis* – key differential characteristic).
- Flagella (red arrows) extend beyond the bottom of the parasite.
- Diagnosis can be made by microscopy or culture; however, the most sensitive and specific test is PCR.
Ciliates: Balantidium coli

• One ciliated ameba causes human disease: B. coli.
• Trophozoite and cyst stages exist
• This parasite can be recognized by its very large size and the presence of cilia (arrow) that coat the surface
• A macronucleus and small micronucleus may also be observed, as well as cytoplasmic vacuoles.
Coccidia and Microsporidia

- Four groups of parasites stain with acid-fast stains:
  - Microsporidia (1-4 um)
  - Cryptosporidium (4-6 um)
  - Cyclospora (5-10 um)
  - Isospora (14 x 25 um)

- These parasites do not stain uniformly acid-fast (some stain weakly, and some cells do not stain)

- These parasites are most easily differentiated by their size: Microsporidia are the smallest, Isospora the largest.

- Most of these parasites do not stain with the traditional stains used for Ova and Parasite (O&P) examinations (e.g., Trichrome stain).

- Two parasites (Cyclospora and Isospora) will autofluoresce when examined under a UV light.
Panels A and B – Cryptosporidia; Panel C – Cyclospora; panel D - Isospora
Blood and Tissue Protozoa

• The most common blood and tissue protozoa are:
  – Plasmodium: P. falciparum, P. vivax, P. ovale, P. malariae
  – Babesia: B. microti
  – Leishmania: L. donovani, L. tropica, L. major (and many others)
  – Trypanosoma: T. cruzi, T. brucei rhodensiense, T. brucei gambiense

• These parasites are most commonly detected by Giemsa staining of blood and hematoxylin and eosin (H&E) staining of tissues.

• Representative examples will be shown on the next few slides.
*P. falciparum* ring forms (left) and gametocyte (middle); *B. microti* (right). Care must be taken to differentiate these two protozoa.
P. vivax

P. malariae
Leishmania amastigotes in tissue (left); Leishmania promastigote in culture (middle); Trypanosoma typomastigote in blood (right)
Nematodes (Roundworms)

• Examples of **intestinal nematodes** include:
  – Enterobius vermicularis (“pinworm”)
  – Trichuris trichiura (“whipworm”)
  – Ascaris lumbricoides (“roundworm”)
  – Strongyloides stercoralis (“threadworm”)
  – Necator americanus and Ancylostoma duodenale or hookworms

• Examples of **blood nematodes** include:
  – Brugia malayi (“Malayan filariasis” or “elephantiasis”)
  – Loa loa (“African eye worm”)
  – Onchocerca volvulus (onchocerciasis or “river blindness”)
  – Wuchereria bancrofti (“Bancroft’s filariasis” or “elephantiasis”)

• Examples of **tissue nematodes** include:
  – Trichinella spiralis (“trichinosis”)
  – Toxocara canis (“visceral larva migrans”)
  – Ancylostoma caninum (“cutaneous larva migrans”)

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Enterobius vermicularis ("pinworm") eggs are deposited by adult at night in perianal area; eggs collected by pressing tape on anal surface and examining microscopically; appear as embryo surrounded by a colorless shell; shell flattened on one side.

Trichuris trichuria ("whipworm") eggs present in stool specimens; naturally stained yellow-brown, with thick wall and clear polar plugs at each end.

Ascaris lumbricoides ("roundworm") eggs found in stool specimens, either fertile (here) or infertile; yellow-brown color with thick, rough shell; infertile eggs larger and more oval shaped; adult worms occasionally passed in stool specimens.
**Strongyloides stercoralis**

- Eggs, which resemble hookworm eggs, are not commonly seen in stool specimens

- Larvae exists in two forms: **rhabditiform** (passed in stools) and **filariform** (infectious stage that develops in soil and occasionally in patient (leads to autoinfection).

- Larvae can be detected microscopically or by placing feces on plate and detecting migrating larvae when they leave a trail of bacterial colonies.
Ancylostoma ("Old World") and Necator ("New World") Hookworms

- Hookworm eggs have a thin, colorless shell surrounding the developing larva.

- If stool specimens are left at room temperature, the larvae can hatch and will resemble Strongyloides larvae.
Blood and Tissue Nematodes: General Comments

• Infections caused by these roundworms are uncommon in the US; most infections are in residents of endemic areas or international travelers

• In contrast with other roundworm infections, disease is most commonly diagnosed by detection of larval forms in blood or tissues

• Diagnosis is also aided by knowledge of the patient’s travel history because these parasites exist in restricted geographic area (in large part determined by the distribution of the vectors)
**Wuchereria bancrofti**

Large microfilariae covered with a transparent sheath (black arrow) and nuclei extending to tip of tail (red arrow); transmitted by **mosquito** bite
Brugia malayi

Small microfilariae with sheath (black arrow) and a gap between the last and next to last nuclei in tail (red arrow); transmitted by mosquitoes
Loa loa

Small microfilariae covered with a sheath (black arrow); nuclei extend to the tip of the tail (red arrow); transmission by bite of tabanid flies (Chrysops)
Onchocerca volvulus

Adults mature in subcutaneous nodules and microfilariae are found in skin; microfilaria do not have a sheath and the nuclei do not extend to tip of tail; transmission by bite of black fly (Simulium)
Trichinella spiralis

Infection (trichinosis) is acquired by ingestion of meat with encysted larvae; larvae mature in intestine and then enter blood and are carried to muscles where they encyst; pigs and rats are important reservoirs; human infections acquired by eating undercooked pork or bear meat
Toxocara canis

Visceral larval migrans –
Dogs are normal host with adult worms in small intestine; eggs (figure) released in feces; eggs large (75 x 80 um) and covered with thick, pitted shell; humans accidental hosts – ingest eggs, larvae released in intestines and migrate extensively before encapsulation in various organs.
Trematodes or Flatworms

• Trematode infections are relatively uncommon in the US; most infections in residents of endemic areas who migrate to the US.

• Example of intestinal trematodes include:
  – Fasciolopsis buski (“porcine liver fluke”)

• Examples of tissue trematodes include:
  – Fasciola hepatica (“sheep liver fluke”)
  – Clonorchis sinensis (“Chinese liver fluke”)
  – Paragonimus westermani (“oriental lung fluke”)

• Examples of blood trematodes include:
  – Schistosoma mansoni (“intestinal bilharziasis”)
  – Schistosoma japonica (“oriental blood fluke”)
  – Schistosoma haematobium (“urinary bilharziasis”)
Fasciolopsis buski

- The “liver fluke” is the largest and most pathogenic human intestinal fluke.
- Pigs and humans are the primary hosts; infection acquired by ingestion of metacercariae encysted on aquatic vegetation (e.g., water chestnuts)
- Large eggs (130-140 um x 80-85 um) with inconspicuous operculum
Fasciola hepatica

- Infection with the "sheep liver fluke" is acquired by ingestion of aquatic vegetation (e.g., watercress) where the metacercariae have encysted.
- Eggs are large (130-150 um x 63-90 um) with inconspicuous operculum
Clonorchis sinensis

- Infection with the “Chinese liver fluke” is acquired by ingestion of metacercariae in the flesh of fish.

- Eggs 17-30 um x 13-18 um, with operculum (black arrow) at small end and knob (red arrow) at opposite end.
Paragonimus westermani

- Infection with the “lung fluke” is acquired by eating raw or inadequately cooked crabs and crayfish

- Eggs 80-120 um x 45-70 um; prominent operculum (arrow)
Schistosoma japonicum and Schistosoma mansoni

- Infection with schistosomes is acquired when cercariae released into water from infected snails penetrate through the skin of humans.

- Large, thin-shelled eggs without operculum; lateral spine on *S. mansoni* (top); inconspicuous spine on *S. japonicum* (bottom); terminal spine on *S. haemotobium* (not shown).
Cestodes or Tapeworms

• Cestode infections are relatively uncommon in the US.

• Examples of intestinal cestodes include:
  – Diphyllobothrium latum ("fish tapeworm")
  – Taenia solium ("pork tapeworm")
  – Taenia saginata ("beef tapeworm")
  – Hymenolepis nana ("dwarf tapeworm")
  – Hymenolepis diminuta ("rat tapeworm")
  – Dipylidium caninum ("dog tapeworm")

• Examples of tissue cestodes include:
  – Echinococcus granulosus “unilocular hydatidosis” or “hydatid disease”
  – Taenia solium ("cysticercosis")
Diphyllobothrium latum

- Human infection with the “**fish tapeworm**” is acquired by ingestion of fish with larvae in muscle
- This tapeworm is unique because it has 2 intermediate hosts (copepod, fish)
- Unique properties of egg are oval shape, presence of operculum (arrow), absence of hooks
Taenia saginata and T. solium

- Human infection with T. saginata (beef tapeworm) or T. solium (pork tapeworm) acquired by ingestion of larvae in beef or pig muscle; also acquired by ingestion of T. solium eggs (cysticercosis)

- Eggs identical with thick wall characteristic, outer membrane (arrow) may or may not be present
**Hymenolepis nana** and **H. diminuta**

- Human infection is acquired by accidental ingestion of larvae in beetles. **H. nana** infection also by ingestion of eggs or autoinfection.

- Eggs with hooks (arrows) and characteristic outer membrane; **H. nana** (*dwarf tapeworm*; top) eggs smaller than **H. diminuta** (*rat tapeworm*; bottom) eggs.