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.
Contrast Stain: India Ink Stain

- The India ink stain is a type of negative contrasting stain used primarily for detection of Cryptococcus neoformans.

- The ink is excluded by the fungal capsule so the fungi (arrows) are unstained and surrounded by a clear halo, while the ink particles provide a background contrast.

- Two cryptococcal cells are indicated by the arrows. These must be distinguished from the neutrophils in this photo.

Contrast Stain: Iodine Stain

- The iodine stain is a contrast stain used primarily for detection of intestinal parasites.

- This is an example of the nonpathogenic amoeba, Entamoeba coli.
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
**Differential Stain: Spore Stain**

- The spore stain is a differential stain used to detect spores produced by Bacillus and Clostridium.

- In this example, spores are stained by malachite green (arrows) and the bacterial cells have a pale color.

- This stain is used to rapidly distinguish between spores and clear intracellular vacuoles.

**Differential Stain: Giemsa stain**

- The Giemsa stain is a differential stain used with peripheral blood or buffy coat films.

- The organisms most commonly detected with the Giemsa stain are:
  - Parasites - Plasmodium (arrows), Babesia, Leishmania, Toxoplasma, microfilaria
  - Fungi – Histoplasma
  - Bacteria – Anaplasma, Ehrlichia, Borrelia
Differential Stain: Silver stains

- Silver stains are primarily used in anatomic pathology labs and not the microbiology lab.

- Fungal elements (hyphae [photo] and cells) are stained black with silver particles.

- The stain is also used to detect selected bacteria (e.g., Legionella) in tissues.

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: Acridine Orange Stain

- The acridine orange stain is an example of a fluorescent stain; that is, fluorescent dyes are used to stain organisms and then observed using a fluorescent microscope.

- At an acid pH, organisms will stain orange and background cells will be green.

- This is an example of trypanosomes in blood stained with acridine orange.

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
Gram-Positive Cocci

- The most common, clinically important gram-positive cocci are: Staphylococcus, Streptococcus, and Enterococcus
- The catalase test is used to separate Staphylococcus (catalase-positive) from Streptococcus and Enterococcus (catalase-negative)
- The coagulase test is used to separate Staphylococcus aureus (coagulase-positive) from most other species of Staphylococcus (coagulase-negative staphylococci)
- Streptococci are subdivided into beta-hemolytic streptococci (e.g., groups A, B, C, F, G) and viridans streptococci (nonhemolytic or alpha-hemolytic)
- The PYR test (test for the enzyme pyrrolidonyl arylamidase) is used to separate enterococci (PYR-positive) from most streptococci (PYR-negative; S. pyogenes [group A] is PYR-positive).
- Hemolysis on blood can be used to separated Enterococcus faecalis (typically nonhemolytic) and Enterococcus faecium (typically alpha hemolytic).

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
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. casseliiflavus.

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 (top) and blood culture (bottom).

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

- The malachite green stain will specifically stain spores green (bottom figure).

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.

---

**Clostridium septicum Gram stain of wound**

- C. septicum can sporulate readily in wounds so this organism would not be mistaken for C. perfringens.

- The spores in this photo are indicated with arrows. They appear as clear areas in the gram-stained cell.

- Specific spore stains are also available to demonstrate these structures.
**Clostridium clostridioforme**

- C. clostridioforme is another common clostridium isolated in the clinical lab although it is generally not associated with disease.

- Organisms typically stain gram-negative, and are arranged as single cells or in pairs – pairs with tapering ends that resemble “cat eyes”.

**Clostridium tertium**

- C. tertium is an anaerobe that characteristically stains gram-negative and can grow readily 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).
**Clostridium spiroforme and Lactobacillus**

- C. spiroforme is a clostridium with an interesting curly shape.
- It is part of the normal colonic bacterial population and is not associated with disease.
- The other long, thin gram-positive rods in this Gram stain of stool are lactobacilli. This is the typical morphology of Lactobacillus.

**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.
**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.

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. Note that these are very small bacteria (top figure).

- Long pleomorphic forms (bottom figure) can be seen in patients receiving antibiotics.

Other Glucose-Fermenting Gram-Negative Rods

- Glucose-fermenting GNRs can be subdivided by the 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, 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, 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,</td>
<td>Brucella, Methylobacterium,</td>
</tr>
<tr>
<td></td>
<td>Elizabethkingia, Ochrobactrum,</td>
<td>Moraxella (M. catarahallis)</td>
</tr>
<tr>
<td></td>
<td>Oligella, Pseudomonas, Roseomonas,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sphingomonas</td>
<td></td>
</tr>
<tr>
<td>Oxidase-negative</td>
<td>Acinetobacter, Pseudomonas (P.</td>
<td>Acidovorax, Bartonella, Franciscella</td>
</tr>
<tr>
<td></td>
<td>oryzihabitans, P. luteola),</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stenotrophomonas</td>
<td></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 this 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).
Mixed Bacteria

- This is a photograph of a stool specimen.

- Note the gram-positive cocci in pairs (enterococci); short, fat enteric-like gram-negative rods; long, pleomorphic gram-negative rods (e.g., Bacteroides), and very thin, curved gram-negative rods (Campylobacter; black arrows).

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.

<table>
<thead>
<tr>
<th>Acid-Fast Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary stain</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Staining conditions</strong></td>
</tr>
<tr>
<td><strong>Decolorizer</strong></td>
</tr>
<tr>
<td><strong>Counterstain</strong></td>
</tr>
<tr>
<td><strong>Examination</strong></td>
</tr>
</tbody>
</table>
Mycobacterium avium – Gram stain

- Mycobacteria are gram-positive but do not stain very well.
- In this photo the organisms appear as “ghosts” or unstained areas (arrows).

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 beads will appear to 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 hyphal 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 along the hyphal structure.

(c) 2012 Infectious Disease Board Review
Nocardia – Modified Acid-Fast Stain

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

- Note the branching, hyphal 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.

Gordonia – Acid-Fast Stain

• Like Rhodococcus, Gordonia does not stain well with even the modified acid-fast stain. The organisms in this photo stain very faintly.

• A few rods in this photo appear red (acid-fast) but most are blue (appear as background material and are not acid-fast staining).
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. beigelli has been subdivided into 6 species and 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

- 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 jirovecii

**Staining Properties**

<table>
<thead>
<tr>
<th>Stain</th>
<th>Cysts</th>
<th>Trophozoites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram</td>
<td>Unstained wall; purple intracystic bodies</td>
<td>Faintly staining</td>
</tr>
<tr>
<td>Methenamine silver</td>
<td>Brown to black cyst wall</td>
<td>Unstained</td>
</tr>
<tr>
<td>Giemsa</td>
<td>Unstained wall; purple intracystic bodies</td>
<td>Red-purple nuclei, light to dark blue cytoplasm</td>
</tr>
<tr>
<td>Calcofluor white</td>
<td>Blue-white or green (depends on filter) with walls intensely staining</td>
<td>Unstained</td>
</tr>
<tr>
<td>Fluorescent antibody</td>
<td>Cyst wall green; contents usually unstained</td>
<td>Polygons or sphere outlined in green; nuclei may/may not stain</td>
</tr>
</tbody>
</table>
Pneumocystis – Gram stain

• Although not commonly used to detect Pneumocystis, the fungi will be seen in Gram stained specimens from patients with an overwhelming infection.

• The cyst wall is unstained but the intracystic bodies stain purple.

• The trophozoites stain faintly.

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 – Giemsa stains (e.g., Diff-Quik)**

- Typically performed in surgical pathology
- Cysts walls unstained around stained intracystic bodies
- Nuclei of trophozoites stain red-purple, and cytoplasm stains light to dark blue

**Pneumocystis – Calcofluor white (Fungifluor)**

- Typical stain used to detect fungi
- Cysts stain blue-white with the wall intensely fluorescent; trophozoites are unstained.
- Organisms observed using this stain, or other nonspecific stains such as Gram or Giemsa, should be confirmed with a specific stain such as DFA.
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.

- The yeasts and conidia can be darkly pigments so this fungus is classified as a dematiaceous 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 zygomycete.

• 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 multicelled macroconidia of **Fusarium**.

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

Alternaria  Bipolaris  Curvularia

Dermatophytes

Trichophyton  Epidermophyton  Microsporum

(c) 2012 Infectious Disease Board Review
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>
<thead>
<tr>
<th>Parasite</th>
<th>Pathogenic</th>
<th>Nonpathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amebae</td>
<td>Entamoeba histolytica</td>
<td>Entamoeba dispar</td>
</tr>
<tr>
<td></td>
<td>Blastocystis hominis (?)</td>
<td>Entamoeba hartmani</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entamoeba coli</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endolimax nana</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Iodamoeba butschlii</td>
</tr>
<tr>
<td>Flagellates</td>
<td>Giardia lamblia</td>
<td>Chilomastix mesnili</td>
</tr>
<tr>
<td></td>
<td>Trichomonas vaginalis</td>
<td>Trichomonas hominis</td>
</tr>
<tr>
<td></td>
<td>Dientamoeba fragilis (?)</td>
<td>Pentatrichomonas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enteromonas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retortamonas</td>
</tr>
<tr>
<td>Ciliates</td>
<td>Balantidium coli</td>
<td></td>
</tr>
</tbody>
</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 protozoan 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.

- 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).

Amebae: Identification Clues

- The keys for identification of amebae is their size, nuclear structure, and composition of cytoplasm.

- Characteristics of *E. histolytica* –
  - Nucleus with evenly distributed chromatin along the periphery of the nucleus and a small centrally located dot (karyosome)
  - Cytoplasm appears homogeneous and contains RBCs
  - 4 nuclei in mature cysts

- Characteristics of *E. dispar* –
  - Identical to *E. histolytica* except no RBCs in cytoplasm

- Characteristics of *E. hartmanni* –
  - Identical to *E. histolytica* except (1) no RBCs, and (2) much smaller

- Characteristics of *E. coli* –
  - Cysts and trophozoites are generally larger than *E. histolytica*
  - Nucleus with uneven chromatin and eccentric karyosome
  - Cytoplasm “foamy” appearing and no RBCs
  - 8 nuclei in mature cysts
<table>
<thead>
<tr>
<th>Organism</th>
<th>Trophozoites</th>
<th>Cysts</th>
</tr>
</thead>
</table>
| E. histolytica| **Size:** 12-60 um  
*Nucleus:* 1; evenly distributed peripheral chromatin;  
karyosome centrally located  
**Cytoplasm:** with RBCs | **Size:** 10-20 um  
*Nucleus:* 4  
**Cytoplasm:** elongated,  
blunt (chromotoidal) bodies may be present |
| E. dispar     | Identical to E. histolytica except not RBCs in cytoplasm                    | Identical to E. histolytica                                           |
| E. hartmanni  | Identical to E. histolytica except smaller (5-12 um) and no RBCs in cytoplasm | Identical to E. histolytica except smaller (5-10 um)                  |
| E. coli       | **Size:** 15-50 um  
*Nucleus:* 1; unevenly distributed chromatin;  
karyosome ecentric  
**Cytoplasm:** vacuolated; no RBCs | **Size:** 10-35 um  
*Nucleus:* 8  
**Cytoplasm:** Chromatoidal bodies with rough, pointed ends |

- **Trophozoites**
- **Cysts**

(c) 2012 Infectious Disease Board Review
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 Pentatrichomonas hominis, Enteromonas hominis, and Retortamonas intestinalis 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 ellipsoid; 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.
**Dientamoeba fragilis**

- The top figure is a line drawing of the D. fragilis trophozoite. The bottom figure is a photo of the same. No cyst stage exists for this parasite.

- Even though this parasite is a flagellate, flagella are not seen.

- Most trophs have 2 nuclei (arrows). The nucleus has a central mass of karyosome material typically arranged in granules. No peripheral chromatin is seen.

- The cytoplasm has many vacuoles and granules, as well as bacteria and yeasts; no RBCs are present.

---

**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
  - Cryptosporidium
  - Cyclospora
  - Isospora

- 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.

### Summary: Microsporidia and Coccidia

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Microscopy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid-fast stain</td>
<td>Size (μm)</td>
<td>Autofluorescence</td>
<td>Trichrome stain</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>+</td>
<td>1-4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>+</td>
<td>4-6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cyclospora</td>
<td>+</td>
<td>8-10</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Isospora</td>
<td>+</td>
<td>14 x 32</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

(c) 2012 Infectious Disease Board Review
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")
Intestinal Roundworms: General Comments

- Intestinal roundworm infections are the most common helminth infections seen in the US
- Most infections are acquired during travel to developing countries.
- Although most infections are diagnosed by detection of the worm eggs in fecal specimens, there are some exceptions:
  - Pinworm adults deposit their eggs in the perianal area so these eggs are detected on the skin surface
  - Strongyloides eggs will hatch in the intestine so the larvae and not eggs are detected in feces
  - If the fecal specimen is left at room temperature overnight, hookworm eggs can hatch and larvae similar in morphology to Strongyloides will be seen in the feces

Roundworms: General Properties

<table>
<thead>
<tr>
<th>Properties</th>
<th>Enterobius</th>
<th>Trichuris</th>
<th>Ascaris</th>
<th>Hookworm</th>
<th>Strongyloides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Large intestine</td>
<td>Large intestine</td>
<td>Small intestine</td>
<td>Small intestine</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Eggs: Size (um)</td>
<td>20-30 x 50-60</td>
<td>22-24 x 50-55</td>
<td>35-50 x 55-75</td>
<td>36-40 x 55-75</td>
<td>---</td>
</tr>
<tr>
<td>Color</td>
<td>Colorless</td>
<td>Yellow-brown</td>
<td>Yellow-brown</td>
<td>Colorless</td>
<td>---</td>
</tr>
<tr>
<td>Shell</td>
<td>Thin</td>
<td>Thick</td>
<td>Thick</td>
<td>Thin</td>
<td>---</td>
</tr>
<tr>
<td>Polar plugs</td>
<td>None</td>
<td>Present</td>
<td>None</td>
<td>None</td>
<td>---</td>
</tr>
<tr>
<td>Flattened side</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>---</td>
</tr>
</tbody>
</table>
**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).
Blood and Tissue Nematodes

Summary

<table>
<thead>
<tr>
<th>Adults – location</th>
<th>Wuchereria</th>
<th>Brugia</th>
<th>Loa</th>
<th>Onchocerca</th>
</tr>
</thead>
<tbody>
<tr>
<td>location</td>
<td>lymphatics</td>
<td>lymphatics</td>
<td>subcut. tissues</td>
<td>subcut. nodules</td>
</tr>
<tr>
<td>Vector</td>
<td>mosquito</td>
<td>mosquito</td>
<td>Tabanid fly (Chrysops)</td>
<td>Black fly (Simulium)</td>
</tr>
<tr>
<td>Microfilariae - size (um)</td>
<td>7.5-10 x 260</td>
<td>5-6 x 220</td>
<td>6-8.5 x 240</td>
<td>5-9 x 310</td>
</tr>
<tr>
<td>sheath</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>Absent</td>
</tr>
<tr>
<td>nuclei</td>
<td>Not at tip of tail</td>
<td>Gap between 2nd and last nuclei in tail</td>
<td>At tip of tail</td>
<td>Not at tip of tail</td>
</tr>
<tr>
<td>periodicity</td>
<td>night</td>
<td>night</td>
<td>daytime</td>
<td>none</td>
</tr>
<tr>
<td>source</td>
<td>blood</td>
<td>blood</td>
<td>blood</td>
<td>Skin tissue</td>
</tr>
</tbody>
</table>

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)

---

(c) 2012 Infectious Disease Board Review
**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 the tip of the 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 westerman ("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”)
Cestodes: General Comments

- All cestodes are worldwide except Diphyllobothrium (temperate regions with cold lakes) and Echinococcus (sheep and cattle raising countries)
- Adults of all intestinal cestodes found in small intestine
- Most human infections acquired by ingestion of larvae in tissue (fish, beef, pork, beetles, flea); exceptions are cysticercosis (T. solium eggs), autoinfection with H. nana, and echinococcosis (E. granulosus eggs)
- Adult worms are identified by their head or scolex (shape and presence of suckers, hooks, rostellum), body segments or proglottids, and length (Diphyllobothrium 4-10 meters, Taenia 2-8 m, others <1 m)
- Eggs are used for laboratory identification

Cestodes: Egg Characteristics

<table>
<thead>
<tr>
<th>Properties</th>
<th>D. latum</th>
<th>Taenia</th>
<th>H. nana</th>
<th>H. diminuta</th>
<th>Dipylidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Ovoid</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
</tr>
<tr>
<td>Size (um)</td>
<td>45 x 65</td>
<td>31-43</td>
<td>30-47</td>
<td>60-85</td>
<td>25-40</td>
</tr>
<tr>
<td>Operculum</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hooks</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Shell</td>
<td>Thick</td>
<td>Thick</td>
<td>Thin</td>
<td>Thick</td>
<td>Thin</td>
</tr>
<tr>
<td>Outer membrane</td>
<td>No</td>
<td>No (+/-)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
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.

Echinococcus granulosus

- The dog is the definitive host of E. granulosus and herbivores (e.g., sheep) are the intermediate host.

- Humans acquire infection by the accidental ingestion of infective eggs from the feces of an infected dog.

- Cysts (hydatid cyst; photo) filled with the parasites form most commonly in the liver or lungs.