

The background of the slide is a blurred photograph of a laboratory rack filled with numerous test tubes. The test tubes contain liquids of various colors, including yellow, orange, red, and purple, suggesting a variety of chemical or biological tests being performed. The rack itself is a standard laboratory equipment, and the overall scene is brightly lit, giving it a clean, professional appearance.

# Diagnostic Parasitology

General Concepts in Parasitology Laboratory Methods

**Kaitlin Landfried, MLS(ASCP)<sup>CM</sup>**

# Disclaimer

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

# Diagnostic Parasitology

Medically Important Human Parasites		
Unicellular	Protozoa	Amebae
		Ciliates
		Flagellates
		Sporozoa
Multicellular	Helminths (worms)	Trematodes (flukes)
		Cestodes (tapeworms)
		Nematodes (roundworms)
	Ectoparasites (arthropods)	Insects (lice, bedbugs, kissing bugs)
		Arachnids (ticks & mites)

# Fecal Specimens: Collection, Handling, and Transport

- Intestinal parasites are shed irregularly
  - 3 stool specimens collected within a 10-day period
- Collect feces...
  - In a clean, dry, waterproof container with a lid
  - Before barium enema, procedures using dyes, and the start of antimicrobial therapy.
  - Purged specimens should be collected with saline or phosphosoda purgative
  - Note the time stool was passed and the time it was placed in the fixative



# Fecal Specimens: OVAP-Macroscopic Examination



- Can only be done on unpreserved stool
- Look for
  - Intact worms
  - Consistency (formed, soft, loose, watery)
  - Color (brown, black, red)
- Following macroscopic examination, the specimen should be placed in preservative
- Blood or blood-tinged mucus areas should be selected for microscopic analysis and preservation.



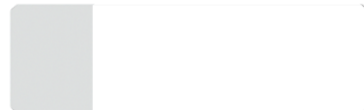
# Fecal Specimens: Preservation

- Stool must be preserved if the specimen will not be delivered immediately to the laboratory and/or after macroscopic examination
- Ratio of three parts preservative to one-part feces

Preservative	Laboratory examination method
Modified polyvinyl alcohol	Permanently stained smear, DNA-PCR
10% formalin	Formalin-ethyl acetate concentration, direct wet mount, and most immunoassays
Sodium acetate-acetic acid-formalin	Permanently stained smears, concentration, and most immunoassays
Merthiolate-iodine-formalin	Concentration and direct wet mount
Single-vial systems (EcoFix, Parasafe, PROTO-FIX)	Concentration, direct wet mount, permanently stained smears, and most immunoassays



Hey Slide!  
You're stuck  
with me  
forever!!



Ugh...  
Thanks PVA!



# Fecal Specimens: OVAP-Microscopic Examination

- Wet mount



- Direct unpreserved, unconcentrated stool

- With saline
      - Used to describe motility patterns (best if done within 30 mins of passage)
    - Iodine:
      - Provides contrast (but kills the motile form of protozoa)



- Preserved, concentrated stool(↑sensitivity)

- Iodine



- Preparation of permanently stain smears.

- Preserved, unconcentrated
  - Helpful for definitive identification of protozoa
  - Eggs do not stain well



# Fecal Specimens: Stool Concentration

## Sedimentation

- 1 Stool 2.5 grams + 10% formalin  
Mix well thoroughly
  - 2 Leave for 30 minutes
  - 3 Filter (layers of gauze or wire-screen)
  - 4 Take 3 mL filtrate
  - 5 Add 10 to 12 mL saline (0.85%)
  - 6 Mix well, Centrifuge at 2000rpm for 2 minutes
  - 7 Discard the supernatant
  - 8 Leave 1 to 1.5 mL sediment
  - 9 Add 9 to 10 mL formalin (10%) to sediment
  - 10 Add 3 mL ethylacetate
- Cap the tube and shake well for 30 seconds
- Stool concentration method by Formalin-ethyl-acetate
- Layer of Ethyl acetate  
Layer of debris  
Layer of formalin  
layer of Sediment
- Make smear from the bottom sediment
- Four layers will form
- Centrifuge at 2000 rpm for 1 minute

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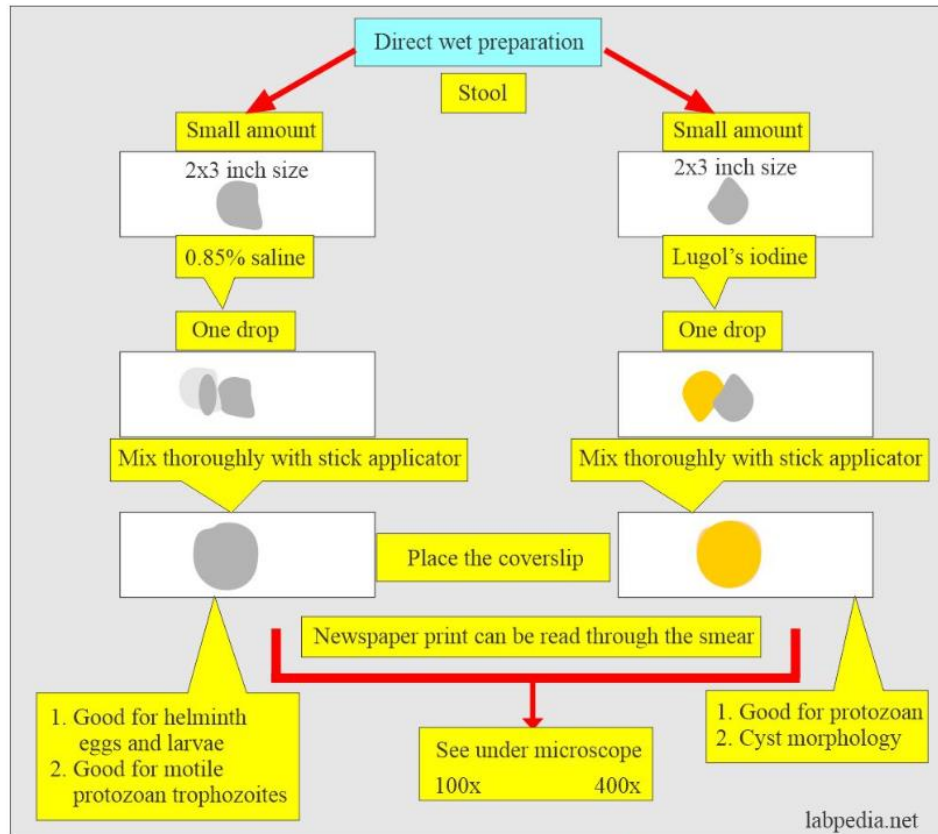
## Floatation

- 1 Get the formalin fixed stool specimen
  - 2 Add 1 ml of specimen to 10 ml (or 15 ml) of tap water
  - 3 Pour into funnel with two gauze paper (test tube)
  - 4 Add ether 1 to 2 ml, stopper, shake gently
  - 6 Add water to the test tube up to the top
  - 7 Centrifuge for 45 seconds at 2500 RPM.
  - 8 Decant the supernatant, add 2.5 ml of water to the sediment
  - 9 Shake well to resuspend the sediment
  - 10 Repeat the steps 6 and 7
  - 11 Add 2.5 ml of Zinc sulfate solution, shake well
  - 12 Add more Zinc sulfate to the top, and centrifuge for 2 minutes at 2000 RPM
  - 13 Take upper portion with wire loop and make smears
  - 14 Make wet preparation with saline or iodine
  - 15 See under the microscope
- Stool Zinc sulfate concentration method
- Zinc sulfate
- labpedia.net

Based on differences in specific gravity between the parasites and the concentrating solution.



# Fecal Specimens: OVAP-Microscopic examination



Stool examination: Stool wet preparation

## Saline wet preparation:

1. Take one drop of 0.85% saline.
2. Take a small amount of stool and mix well.
3. The smear should be thin to see the newsprint under the slide.
4. Put cover glass and see under the microscope 100x and 400x objective.
  1. This is best to see helminth eggs, larvae, and trophozoites.

## How to make stool smear with Lugol's Iodine:

1. Take a drop of Lugol's iodine solution.
  1. Take a small amount of stool and mix it well.
  2. Make a thin smear.
2. Put the cover glass on it and gently press it to get an evenly thin smear.
3. See under 100 x and 400 x objective lenses.
  1. Too weak iodine solution; in that case, organisms will not stain properly.
  2. Too strong an iodine solution will clump the stool.

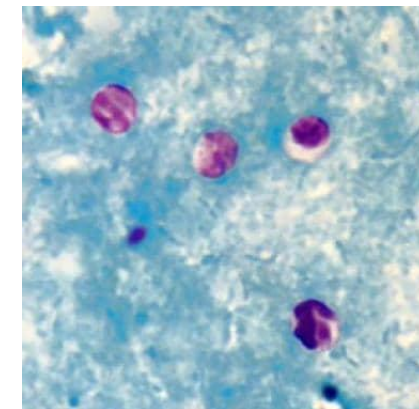
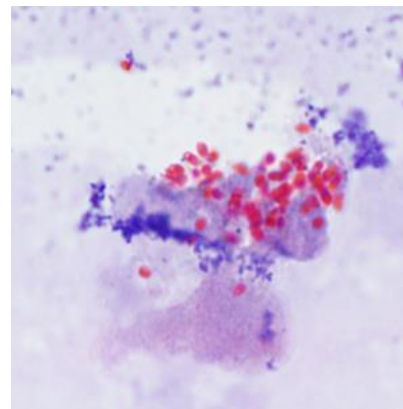
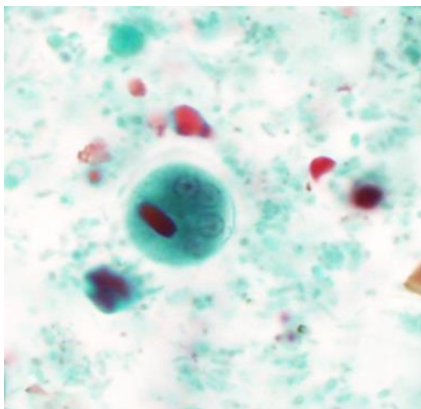


# Fecal Specimens: Microscopic Examination



NOTE: Some labs use an iron hematoxylin stain instead

Trichrome	Modified Trichrome	Modified Acid-fast
<ul style="list-style-type: none"><li>• Identification of protozoa</li><li>• Cytoplasm stains blue-green and nucleic acid stains reddish-purple</li></ul>	<ul style="list-style-type: none"><li>• Identification of Microsporidia</li><li>• Spore wall stains pink</li></ul>	<ul style="list-style-type: none"><li>• Identification of intestinal sporozoa</li><li>• Stain pinkish-red</li></ul>
Slides are fixed with Schaudinn's fixative or methanol before staining		



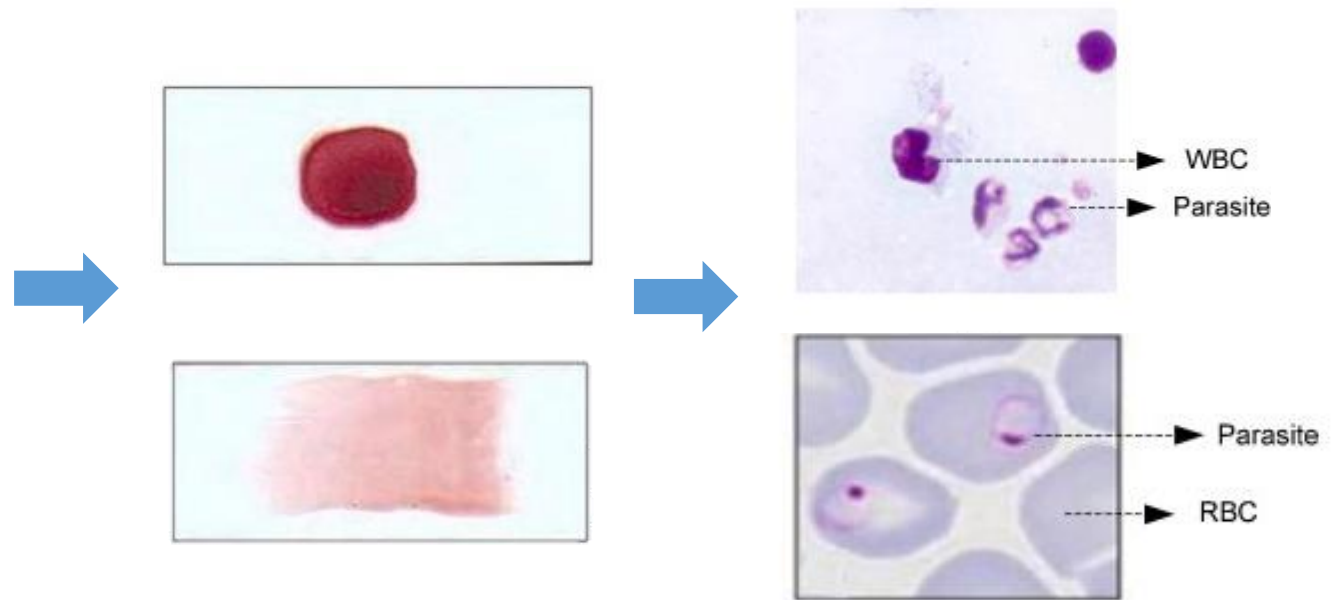
# Other Specimens: Blood

- Blood taken directly from a finger stick is the ideal specimen
- Can also use blood collected in ethylenediamine tetraacetic acid (EDTA), but permanent smears should be made within 1 hr



## Giemsa

- Thick smear
  - Lyses red blood cells
  - Increased sensitivity
  - Used to screen for blood parasites
- Thin smear
  - Fixed in methanol
  - Speciation of Plasmodium and Trypanosoma
  - Identification of Babesia and microfilariae

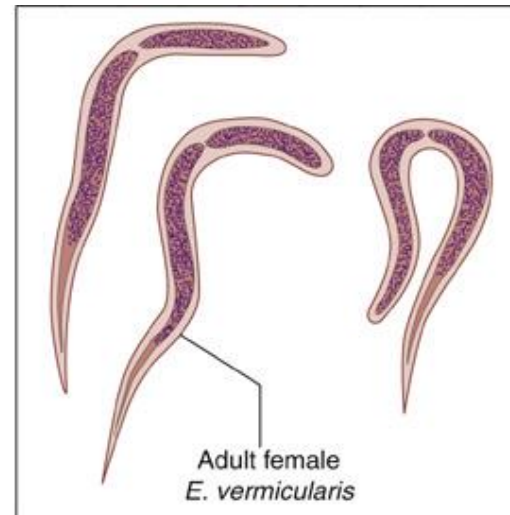
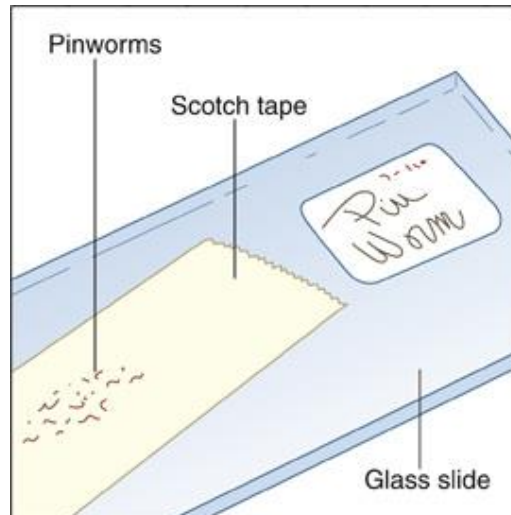


# Other Specimens: Cellophane Tape Preparation



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*Enterobius vermicularis*  
(pinworm)



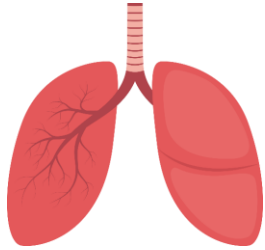
## Other Specimens: Entero-Test (String Test)



Test for *Giardia duodenalis* and *Strongyloides stercoralis*

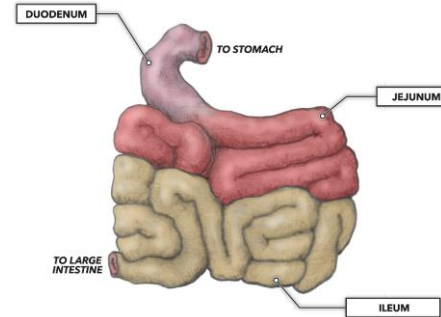


# Other Specimens: Summary



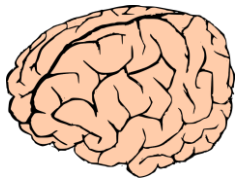
## SPUTUM

*Ascaris lumbricoides*  
*Cryptosporidium species*  
*Entamoeba histolytica*  
*Microsporidia*  
*Paragonimus westermani*  
*Strongyloides stercoralis*



## DUODENAL ASPIRATES

*Giardia duodenalis*  
*Strongyloides stercoralis*



## CSF

*Acanthamoeba species*  
*Balamuthia mandrillaris*  
*Naegleria fowleri*  
*Trypanosoma brucei*



## URINE/VAGINAL/URETHRAL

*Enterobius vermicularis*  
*Schistosoma haematobium*  
*Trichomonas vaginalis*



## BIOPSY

*Echinococcus granulosus*  
*Leishmania species*  
*Mansonella streptocerca*  
*Onchocerca volvulus*  
*Toxoplasma gondii*  
*Trichinella spiralis*

# Citations

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