Diagnostic Parasitology

General Concepts in Parasitology Laboratory Methods

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Disclaimer

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- Most of the information was adopted from the Textbook of Diagnostic Microbiology by Mahon & Lehman (see citation) but condensed for bite sized learning.

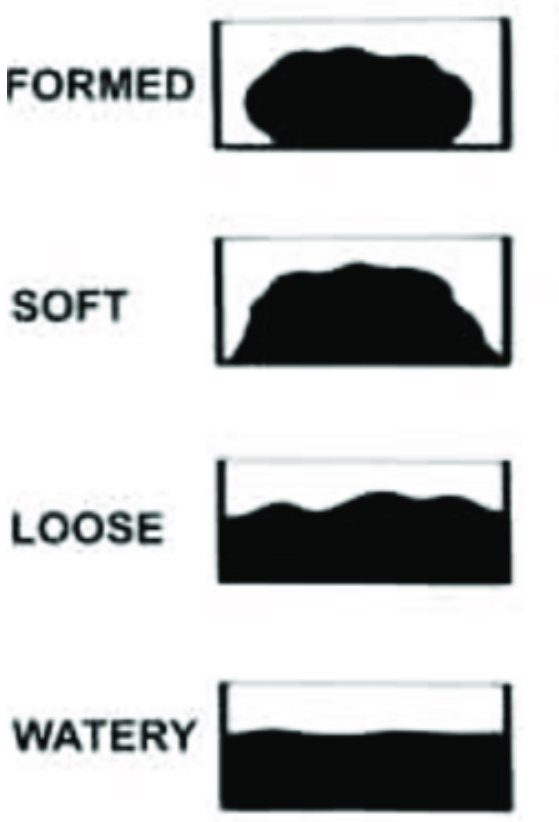
Diagnostic Parasitology

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

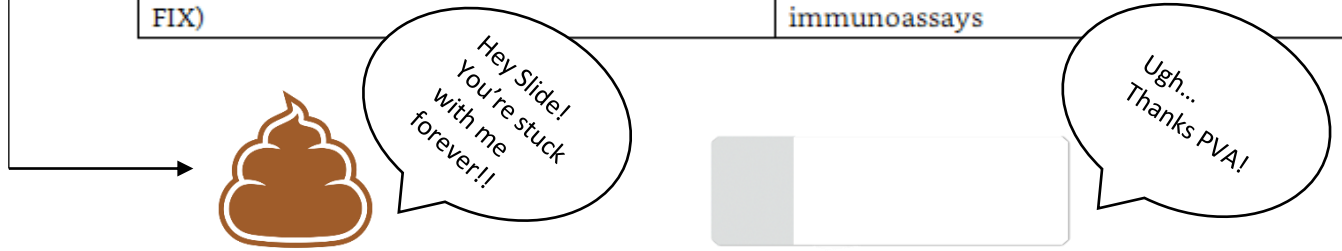


- Can only be done on unpreserved stool
- Look for
  - Intact worms
  - Consistency (formed, soft, loose, watery)
  - Color (brown, black, red)
- Following macroscopic examination, the specimen should be placed in preservative
- Blood or blood-tinged mucus areas should be selected for microscopic analysis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

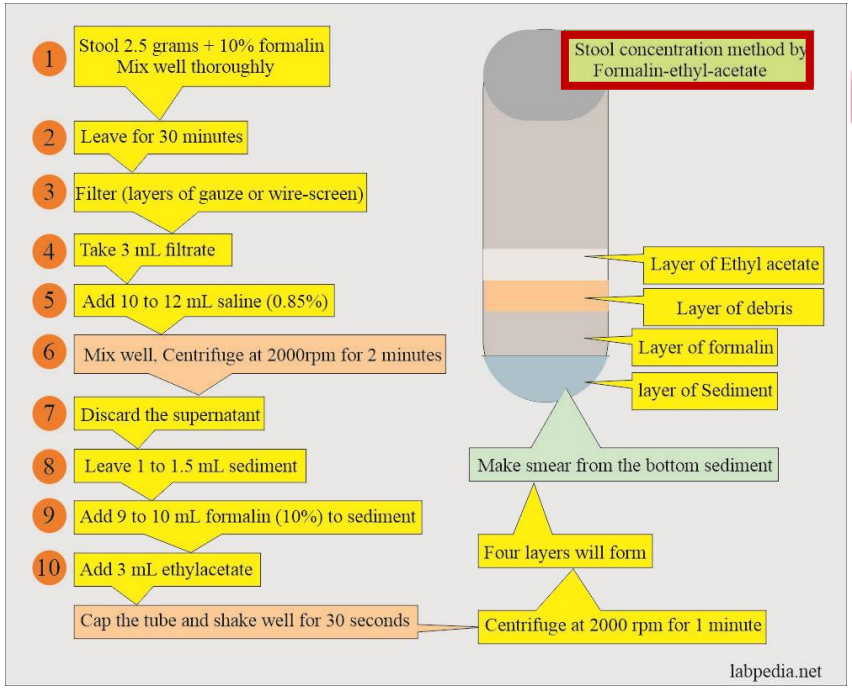


Fecal Specimens: OVAP-Microscopic Examination

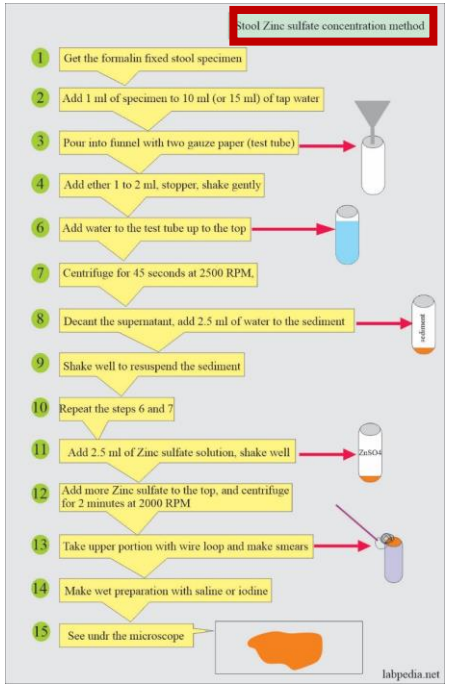
- Wet mount
  - BONUS Direct <u>unpreserved, unconcentrated</u> stool
    - With saline
      - Used to describe motility patterns (best if done within 30 mins of passage)
    - Iodine:
      - Provides contrast (but kills the motile form of protozoa)
  - ★ <u>Preserved, concentrated</u> stool(↑sensitivity)
    - Iodine
- ★Preparation of permanently stain smears.
  - <u>Preserved, unconcentrated</u>
  - Helpful for definitive identification of protozoa
  - Eggs do not stain well

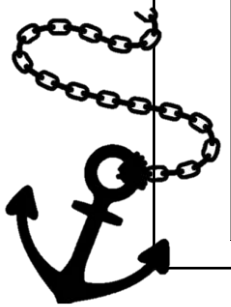
Fecal Specimens: Stool Concentration

Sedimentation



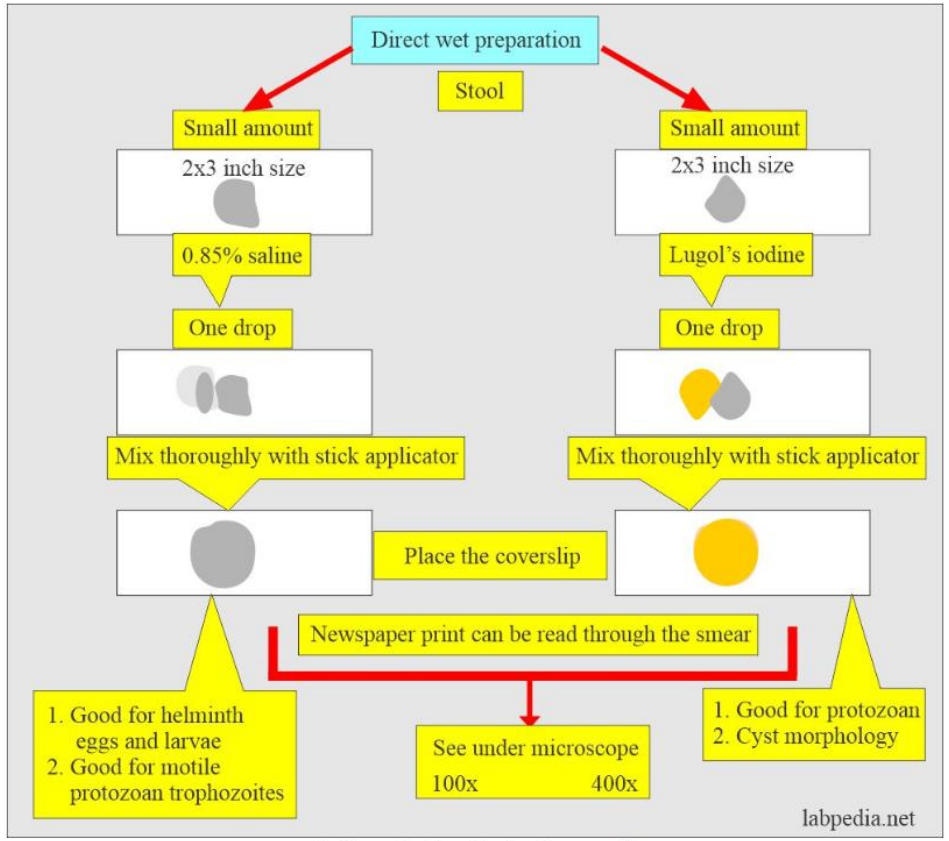
Floatation





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.

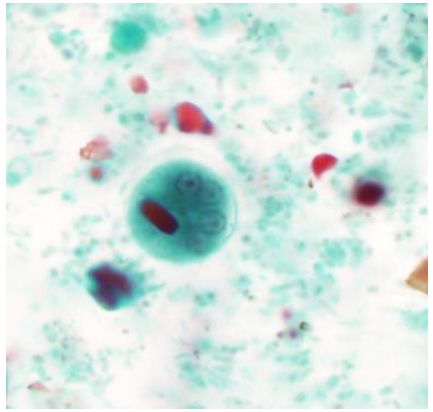
How to make stool smear with Lugol's Iodine:

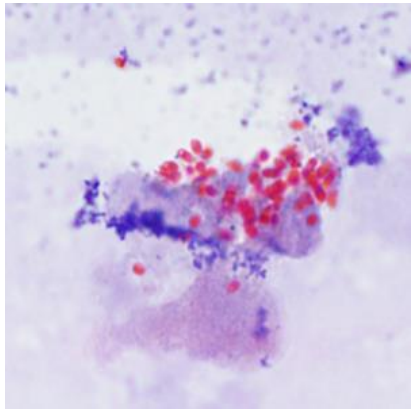
1. Take a drop of Lugol's iodine solution.
    1. Take a small amount of stool and mix it well.
    2. Make a thin smear.
2. Put the cover glass on it and gently press it to get an evenly thin smear.
3. See under 100 x and 400 x objective lenses.
    1. Too weak iodine solution; in that case, organisms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

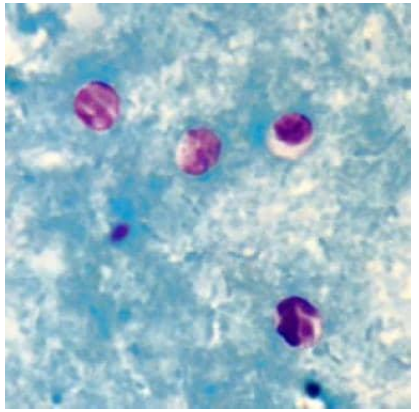
Fecal Specimens: Microscopic Examination

NOTE: Some labs use an iron hematoxylin stain instead

Trichrome	Modified Trichrome	Modified Acid-fast
• Identification of protozoa • Cytoplasm stains blue-green and nucleic acid stains reddish-purple	• Identification of Microsporidia • Spore wall stains pink	• Identification of intestinal sporozoa • Stain pinkish-red
Slides are fixed with Schaudinn's fixative or methanol before staining		





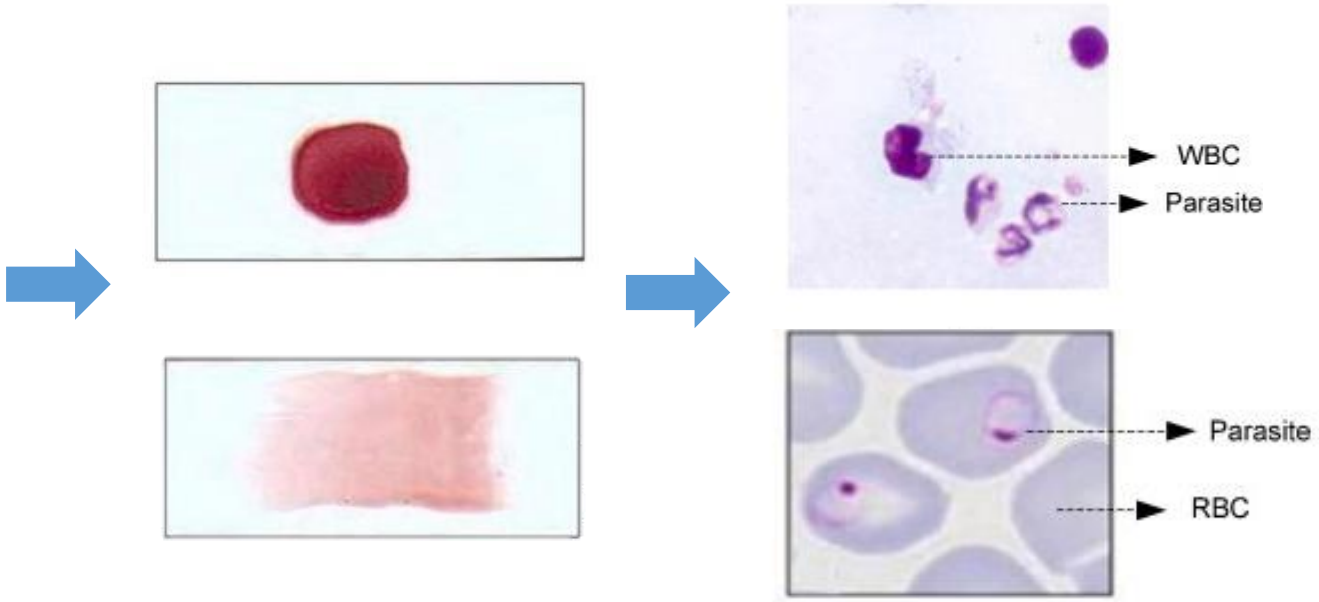


Other Specimens: Blood

- 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

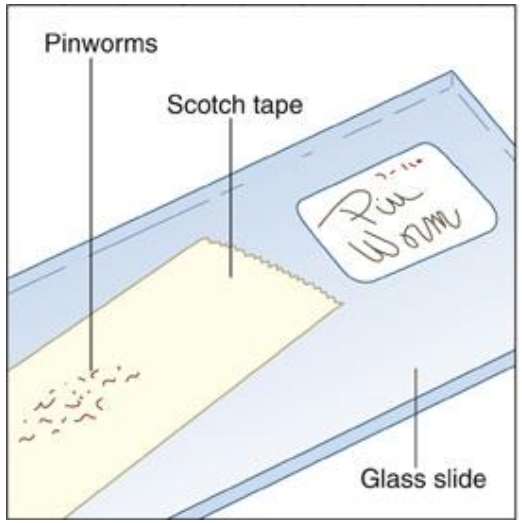
Giemsa

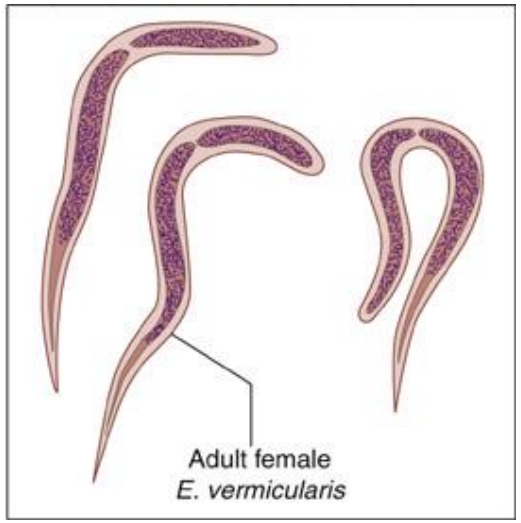
• Thick smear
  • Lyses red blood cells
  • Increased sensitivity
  • Used to screen for blood parasites
• Thin smear
  • Fixed in methanol
  • Speciation of Plasmodium and Trypanosoma
  • Identification of Babesia and microfilariae



Other Specimens: Cellophane Tape Preparation

Enterobius vermicularis (pinworm)

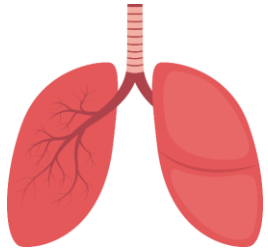




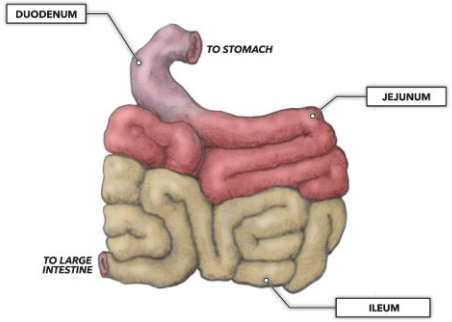
Other Specimens: Entero-Test (String Test)

Test for *Giardia duodenalis* and *Strongyloides stercoralis*

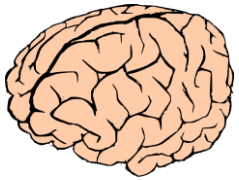
Other Specimens: Summary



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



DUODENAL ASPIRATES
Giardia duodenalis
Strongyloides stercoralis



CSF
Acanthamoeba species
Balamuthia mandrilaris
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