

MLAB 1331 – Parasitology

Lecture Guide

I. Introduction to Parasitology:

A. Diagnosis of parasitic infections requires knowledge of:

1. Types of patients at risk - travel, day care center attendance, refugee, etc.
2. Appropriate specimen - must be collected and processed correctly; Series of three specimens on alternate days
3. Accurate diagnosis - dependent on laboratory results utilizing appropriate procedures
4. Quality of results - dependent upon training

B. North Americans do not suffer from a multitude of parasites:

1. high standards of education - better housing, higher standard of living
2. general good health - poor health = more susceptible to disease
3. nutrition - adequate diet
4. sanitation - sewers and septic systems keeps raw sewage out of streams
5. temperate climate - parasites do better in the warmth of the tropics
6. absence of certain appropriate vectors - intermediate hosts such as the tsetse fly, certain snails, etc.

C. Parasitic infections in this country are generally due to:

1. increased travel - into areas of the world where parasitic disease is highly endemic
2. low level of understanding about parasitic infections - results in an increased likelihood of transmission

D. Definitions

1. Parasite - one animal deriving its sustenance from another without making compensation. The uncompensated animal is the host.
2. Parasitology - the science or study of host-parasite relationships.
3. Medical parasitology - study of parasites which infect humans.

4. Host - the partner providing food and/or protection. Some parasites require more than one host to complete their life cycle; or may not require a host during some stage(s).
 - a. definitive host - the host in which sexual maturity and reproduction takes place. Man is usually a definitive host.
 - b. intermediate host - the host in which the parasite undergoes essential development. More than one intermediate host may be required by some parasites. Man sometimes serves as an intermediate host (malaria).
 - c. reservoir (carrier) host - the host harboring a parasite in nature, serving as a source of infection for other susceptible hosts. Reservoir hosts show no sign or symptom of disease.
5. Vector - "carrier" of a parasite from one host to another. Often an insect.
6. Symbiosis - "living together," a close association between two organisms.
 - a. mutualism - both organisms are benefited (bacteria in bowel)
 - b. commensalism - "eating at the same table"; one organism is benefited, the other is unaffected.
 - c. parasitism - one organism is benefited at the expense of another (host).
 - 1) a parasite is successful - only when it is in delicate balance with the host. If the balance is upset, the host may destroy or expel the parasite; if the host is overly damaged, it may die - as will the parasite.
 - 2) parasitology is important - because this balance is not always maintained

E. Parasitic damage to host:

1. trauma - damage to tissues, intestine, liver, eye.
2. lytic action - activity of enzymes elaborated by organism.
3. tissue response - localized inflammation, eosinophilia.
4. blood loss - heavy infection with hookworm may cause anemia.
5. secondary infections - weakened host susceptible to bacterial infection, etc.

F. Modes of infection

1. filth-borne or contaminative - serious where personal hygiene and community sanitation lacking. Cysts or eggs remain infective for long periods in contaminated soil.
2. soil or water-borne - difficult to control. Children eat dirt which can contain eggs, etc.; certain larvae can penetrate skin of bare feet or enter skin in infested water. Education & sanitary control of waste is best means of prevention.
3. food-borne - inadequately cooked beef, pork, fish, shell fish & some vegetables can be sources of infection.

4. arthropod-borne - the most difficult of all to control; often produces diseases that are fatal. More commonly found in moist tropical areas of world. Mosquitoes transmit malaria, etc.

II. Collection, Processing, & Examination of Diagnostic Specimens

A. Overview:

1. Microscope - must be calibrated in order to insure accurate measurement of organisms
2. Centrifuges - Swinging bucket - the required type; should not use angle head centrifuges. Gravitational force (g-force) determined by revolutions per minute (RPM) and radius of the rotor. Use of flammable reagents hazard, prefer use of explosion proof centrifuge.
3. Types of specimens which can be examined for diagnosis of parasites -
 - a. "Natural secretions" - feces, sputum, and urine are used to detect lumen dwelling parasites of GI, pulmonary and genitourinary tracts.
 - b. Blood is usual specimen for detection of blood and tissue parasites, along with tissue biopsies, aspirates, etc.

B. Intestinal Lumen Dwelling Parasites - Fecal Specimens

1. Patient preparation - must avoid the presence of substances which can interfere with stool examination. No antimicrobial medications should be taken during the 10 days prior to collection of specimens. Certain substances can interfere with visibility of organisms, reduce their numbers, and damage or destroy their morphology.
 - a. Medications containing antimicrobial agents:
 - 1) Bismuth
 - 2) Barium
 - 3) Mineral oil
 - 4) Kaolin
 - 5) Anti-diarrheal preparations
 - 6) Laxatives
 - b. Contaminant free specimens - no urine, water or dirt - these may destroy organisms, or could contain confusing free-living organisms.
2. Fecal (stool) specimens - the most commonly submitted specimen for parasitology examination. These specimens must be initially examined for consistency in order to determine which procedures are to be utilized.
3. Types of stool specimens submitted and forms of parasites which may be detected in each -
 - a. Liquid specimens (loose & watery specimens) - trophozoite stages are more likely to be present.
 - 1) Must be examined within 30 minutes of passage or placed into

an appropriate preservative - trophozoites begin to deteriorate rapidly, when this happens, morphology is adversely affected.

- 2) Freshly passed specimens are necessary in order to recover motile trophozoites.
 - 3) Cyst formation will not occur once the organism is outside the body and trophozoites tend to disintegrate rapidly after passage.
 - 4) Procedures usually performed - direct wet mounts can aid in detecting motility; permanent stains exhibit the best morphology.
- b. Semi-formed specimens (soft specimens) - the entire range of stages may be present, therefore, one should perform all procedures (direct examinations, concentration, & permanent stain).
- c) Formed specimens - direct wet mounts will serve to detect those organisms which do not concentrate well. Concentration is necessary to detect light infections, but permanent stains are equivocal (if blood or mucous is present on the specimen, stain it).

C. Collection Methods:

1. Submit fresh specimens directly to lab - these must be examined within 30 min to one hour. Store these at room temperature if examined shortly after 30 minutes from collection. Refrigerate them otherwise, but never incubate these specimens
2. Use commercially prepared kits with preservatives if not able to examine in the above-mentioned time frame. Preservation will insure the integrity of the specimen.

D. Fixatives and Preservatives:

1. Introduction -
 - a. An ideal preservative would preserve all diagnostic stages, and not interfere with concentration & staining techniques.
 - b. If a specimen cannot be processed immediately, at least prepare the film for permanent staining on the day of receipt. Then it can be finished during the next work day, avoiding delay.
2. Commonly used preservatives
 - a. MIF (merthiolate - iodine - formalin) - for wet smear & concentration only. Cannot permanent stain.
 - b. SAF (sodium acetate - acetic acid) - OK for concentration, can permanent stain with iron hemotoxylin only, trichrome stain will not produce satisfactory results.
 - c. PVA (polyvinyl alcohol) / Formalin Kit – a popular method using two vials:
 - 1) 5 -10% formalin preserves helminth eggs and larvae, and protozoan cysts. Specimen can be used for direct wet mounts

and in a concentration procedure.

- 2) Polyvinyl alcohol fixative preserves protozoan cysts and trophozoites for permanent staining. There are a variety of "fixing agents" in PVA including mercury, zinc, and copper. Zinc is the best alternative to mercury which remains the "gold standard".

d. Others:

- 1) Schaudinn's Fixative - used for staining fresh specimens, contains mercury.
- 2) 10% buffered formalin - for concentration only.

E. Stool Collection Kits:

1. A kit should have three (3) containers -
 - a. Formalin (5% or 10%) for concentration
 - b. PVA-Fixative for permanent stains
 - c. Clean vial for unpreserved portion culture and assessment .
2. Considerations in the selection of a kit:
 - a. vial size - should be of adequate size to allow for a representative sample
 - b. child proof
 - c. stirrers - scoops, etc.
 - d. labels to indicate poison should be present in several languages.
 - e. patient label information - name; date and time of collection, etc.
 - f. instruction sheet in several languages.
 - g. mailers must meet postal regulations - triple barrier.
 - h. cost

F. Specimen Collection

1. Collect in a clean container - without urine or water (these may be damaging to trophozoites. The entire passage is desirable (this allows for a thorough macroscopic examination).
2. Minimum number of specimens - due to irregular shedding patterns of parasites, a series of three normally passed specimens is preferred.
3. Time frame of collection - Collect on alternate days. Never on same day.
4. Use of laxatives is not permitted - these can mask infection or damage organisms.

5. Exact date and time of collection - important, required information.
6. Proper performance of diagnostic testing is critical - no shortcuts! Follow the procedure, do not modify it for convenience.

III. Techniques of Stool Examination:

- A. Gross examination - grade consistency of the specimen; decide on best methods for the examination to allow for detecting most likely stages/parasites. Look for worms, segments of worms (blood and/or mucous should be examined with wet mounts & permanent stains).
- B. Direct Wet Mounts (fresh or formalin preserved specimens) -
 1. Used primarily to detect motility - fresh specimens are examined for motility; preserved specimens are examined for organisms which do not concentrate well.
 2. Procedure - use large slide & coverslip; density - should be able to just read newsprint through smear.
 3. Advantages
 - a. will reveal many helminth eggs and larvae
 - b. may reveal motile trophozoite and nonmotile cysts
 - c. addition of iodine may reveal more morphology
 - d. will reveal other indicator cells of an inflammatory process, (macrophages, leukocytes, and epithelial cells)
 4. Disadvantages
 - a. does not lend itself to oil immersion examination
 - b. may not reveal adequate morphology causing mis-interpretation
 - c. if preparation is too thick, organisms will be missed.
 - d. don't use on PVA-preserved specimens (iodine stain coagulates the PVA).
- C. Stained Wet Mounts -
 1. For liquid specimens likely to contain trophs, Quensel or Nair buffered methylene blue may be used. Iodine is too harsh for trophs- often damages morphology of nucleus.
 2. For formed specimens likely to contain only cysts, popular stains include Dobell, Lugol, or D'Antoni iodine stains. Do not make smears too thick; examine them systematically.
- D. Concentration Techniques -

1. Purposes -
 - a. Eliminate fecal debris
 - b. Increase density / concentration of parasites
 - c. Preserve morphology
2. Types of concentration procedures -
 - a. Flotation Procedures -
 - 1) Floats parasites free of fecal debris by using a solution having a specific gravity greater than the parasites, and less than background fecal matter. Can lose operculated eggs, and other larger eggs since they are too heavy to float.
 - 2) Zinc sulfate flotation (Sheather's sugar flotation)
 - a) Advantages -
 - (1) can provide clean concentrate
 - (2) reagents have long shelf life, & available commercially
 - (3) morphology adequate
 - b) Disadvantages -
 - (1) specific gravity must be checked frequently
 - (2) a number of helminth eggs will not float
 - (3) errors can occur if directions are not followed exactly (must read quickly or organisms will begin to settle)
 - b. Sedimentation Procedures -
 - 1) Concentrates diagnostic stages as sediment while keeping some elements of the fecal matter suspended.
 - 2) Formalin-Ether or Formalin-Ethyl Acetate -
 - a) Most commonly used - good for either fresh or preserved specimens.
 - b) Reagents
 - (1) Formalin
 - (2) Ethyl Ether or Ethyl Acetate
 - b) Advantages -

(1) allows the recovery of all helminth eggs, larvae, and protozoan cysts

(2) easy to perform (can be read anytime following concentration; several stopping places exist in the procedure)

c) Disadvantages

(1) once required use of ether (ethyl acetate now recommended as safe substitute)

(2) preparation not as clean as zinc sulfate

(3) subject to variables in material preparation

(4) amount of specimen used in relation to reagents must be carefully monitored (too much specimen can interfere with efficiency of cleansing & concentration)

E. Permanently Stained Smears:

1. Introduction: These procedures are aimed at trophozoite stages, and are most useful in the identification of organisms.

2. Factors Affecting Permanent Staining -

a. age - organisms deteriorate with time

b. consistency - mucousy specimens can be difficult in getting "stuck" onto the slide.

c. composition

d. lag time between preparation of smears and staining

e. fixation - must thoroughly mix specimen with preservatives.

f. smear preparation - must not be too thick or thin.

g. staining reagents - must replace every 40 slides or weekly.

3. Iron Hematoxylin Procedure -

a. excellent morphology

b. time consuming and difficult to perform

4. Wheatley's Trichrome Stain – most popular

a. rapid procedure and technically easier (stable reagents, can be used repeatedly).

b. appearance after staining - cytoplasm is greenish; nuclear material is dark bluish-black to purplish-red.

5. Pros and cons - trichrome is the stain of choice for most organisms, it is more easily performed and more reproducible. Specimens must be mixed thoroughly to insure complete fixation. Take care to not overly destain.

IV. Special Techniques

A. Special stains:

1. *Pneumocystis carinii* - methenamine-silver; Giemsa; Periodic acid Schiff
2. *Cryptosporidium parvum* - modified acid fast; direct fluorescent microscopy

B. Immunoserologic Detection (parasitic serology tests)

1. Most tests detect presence of antibody; a few detect antigen. Those detecting antigen are acceptable, but do not increase the amount of antigen present. Other procedures may demonstrate the antigen as well, and less expensively.
2. Test methodologies include
 - a. Enzyme Immunoassay (EIA)
 - b. Complement Fixation (CF)
 - c. Latex Agglutination (LA)
 - d. Direct & Indirect Immunofluorescence (DIF & IIF)
 - e. Indirect hemagglutination (IHA)
 - f. Bentonite flocculation (BF)
 - g. Immunoblot (IB)
3. Procedures & testing could improve - Standardization of antigens, reference reagents, and procedures would improve interpretation of results.

C. Identification by Molecular Methods - Nucleic acid probes and molecular techniques to aid in detection of malaria, toxoplasmosis, amebiasis and leishmaniasis are being developed for diagnostic and epidemiologic purposes.

D. Quantitative Worm Egg Count - can assess effectiveness of therapy, but best used to estimate worm burden. This must be performed on unpreserved specimens. Preservatives dilute the specimen, eliminating the ability to calculate "eggs per gram" of feces.

E. In Vitro Cultivation of Parasites - primarily used for blood & tissue protozoa. Can culture intestinal protozoa, but not generally done.

F. Animal Inoculation

1. Not routinely done - expensive, time consuming, lacks sensitivity.
2. Use - primarily used for isolation of blood and tissue parasites (trypanosomes, etc.)
3. Xenodiagnosis - may be considered a "special case" of animal inoculation; the term was originally applied to the diagnosis of Chagas' disease. After placing uninfected reduviid bugs on a patient suspected of having the disease, and allowing them to feed, the bugs are examined for developmental stages of the parasite *Trypanosoma cruzi*.

V. Use of Other Specimens:

- A. Anal Swabs / Scotch Tape Preparation for *Enterobius vermicularis* - must be collected in the morning prior to bathing or bowel movement.
1. For diagnosis of pinworm infections (other helminth eggs can be seen also, especially *Taenia* spp. eggs)
 2. Female worm migrates out of the host's anus at night
 2. Procedure - make impressions with sticky paddle or clear cellophane tape around the anus of the patient; examine for eggs under the microscope.
- B. Genital Specimens for *Trichomonas vaginalis* - vaginal, urethral, prostatic exudates are examined via wet mounts, looking for motile organisms.
- C. Urine Specimens -
1. *T. vaginalis*
 2. *Schistosoma hematobium* - inhabits blood vessels around the urinary bladder, eggs "pop" into bladder as result of expansion and contraction of the bladder along with the aid of a terminal spine on the egg.
- D. Sputum Specimens -
1. Tissue-dwelling trematode, *Paragonimus westermani* (the lung fluke). The worm lives in lung tissue; eggs are shed into alveoli, and are present in the sputum.
 2. Larval stages of *Ascaris*, *Strongyloides*, and Hookworm can be present in sputum as a result of lung migration.
 3. The trophozoite form of *Entamoeba histolytica* may be present as a result of pulmonary amebic abscesses.
- E. Aspirates and Biopsies
1. Aspiration of duodenal contents - Can be examined for *Giardia lamblia* and *Strongyloides stercoralis*.
 2. Entero-Test - a capsule containing a free-wheeling piece of yarn used to sample duodenal contents.
- F. Sigmoidoscopy smears and tissue biopsies - gets material directly from mucosa.
- G. Abscess aspirates - usually for extra-intestinal amoebiasis - wall of abscess is best area to examine
- H. Biopsies
1. Direct microscopic examination of muscle (*Trichinella spiralis*) or intestinal / bladder mucosa (*Schistosoma* eggs).
 2. Specimen submitted to histopathology laboratory for special staining and pathologist examination.

VI. Procedures for Detecting Blood Parasites:

A. Collection of Blood Samples

1. Finger, heel or earlobe sticks - preferred for blood smears.
2. EDTA samples - best if smear made within one hour.
3. Sodium citrate specimens - used for larger amounts of blood to be used in concentration or cultivation.
4. Clot tubes - for serological procedures; lets clot retract.

B. Examination of Blood Samples

1. Wet Mounts - screening for motile organisms (trypanosomes & filariae)
2. Permanent Stained Smears
 - a. Stains used - various Romanovsky stains which are methylene blue-eosin based.
 - 1) Wright's - alcohol based.
 - 2) Giemsa - water based, preferred stain, all things considered.
 - b. Thick Blood Films
 - 1) Used in the identification of malaria parasites, trypanosomes, and microfilariae.
 - 2) Preparation of smear - 3 - 4 drops of blood stirred together to size of a dime; newsprint just legible through smear. Must dry overnight before staining.
 - 3) "Laking" smear - dehemoglobinize in buffered water prior to staining with Wright's stain (not necessary in the water-based Giemsa stain).
 - 4) Advantage - concentrates blood, picks up light infections. Examine for 5 minutes or ~100 fields.
 - 5) Disadvantages - infected red blood cells are lost to lysis; more experience is needed to recognize organisms. More time is required to prepare & stain preparations.
 - c. Thin Blood Films
 - 1) Used in the identification of malaria parasites, trypanosomes, and microfilariae.
 - 2) Preparation - same as for WBC differential.
 - 3) Advantages - allows for study of "infected" red blood cell morphology. Morphology of organisms is generally better.

- 4) Disadvantages - must examine for 30 minutes or 100 fields.
Light infections easy to miss.
2. Concentration Techniques - Thick Blood smears; fractional centrifugation for culture.