



Prelim-PARA-LAB

Clinical parasitology (Our Lady of Fatima University)



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CLINICAL PARASITOLOGY LABORATORY

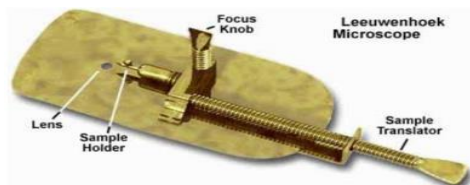
THE COMPOUND MICROSCOPE

KINDS OF MICROSCOPE

Microscope- an optical instrument consisting of a lens or combination of lenses for making enlarged images of minute objects. Most common tool used in the laboratory.

Simple Microscope

- ❖ Generally considered to be the first microscope.
- ❖ Created in the 17th century by Antonie van Leeuwenhoek, who combined a convex lens with a holder for specimens.
- ❖ Magnifying between 200 and 300 times, it was essentially a magnifying glass.
- ❖ Simple microscopes are not used often today because the introduction of a second lens led to the more powerful compound microscope.



Compound Microscope

- ❖ With two lenses, the compound microscope offers better magnification than a simple microscope; the second lens magnifies the image of the first.
- ❖ Bright field microscopes (the specimen is lit from underneath, and they can be binocular or monocular).
- ❖ Provide a magnification of 1,000 times, which is considered to be high, although the resolution is low. This high magnification, however, allows users to take a close look at objects too small to be seen with the naked eye, including individual cells.



Scanning Electron Microscope (SEM)

- ❖ Uses electrons rather than light for image formation. Samples are scanned in vacuum or near-vacuum conditions, so they must be specially prepared by first undergoing dehydration and then being coated with a thin layer of a conductive material.
- ❖ Surface of the specimen is first dried and spray-coated with a very thin layer of heavy metal (often gold) that reflects electrons in a beam scanning the specimen.
- ❖ After the item is prepared and placed in the chamber, the SEM produces a 3-D, black-and-white image on a computer screen.

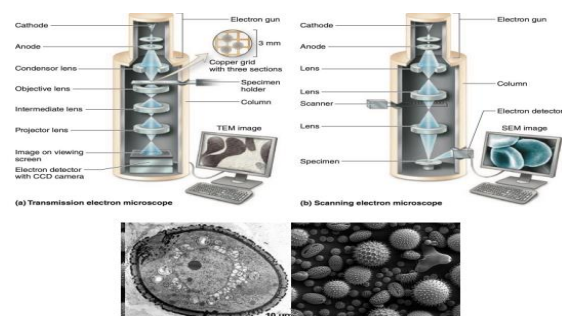


Transmission Electron Microscope (TEM)

- ❖ Like the scanning electron microscope, the transmission electron microscope (TEM) uses electrons in creating a magnified image, and samples are scanned in a vacuum so they must be specially prepared.
- ❖ Uses a slide preparation to obtain a 2-D view of specimens, so it's more suited for viewing objects with some degree of transparency.
- ❖ Offers a high degree of both magnification and resolution that allows isolated particles magnified as much as 400,000 times to be viewed in detail.



Pollen grain under TEM and SEM



COMPOUND MICROSCOPE

Mechanical Parts: used to support and adjust the parts

Magnifying Parts: used to enlarge the specimen

Illuminating Parts: used to provide light



MECHANICAL PARTS

- ❖ **Base:** bottom part portion that supports the entire microscope, provides stability and support for the microscope when it is upright. The base also typically holds the illuminator, or light source.
- ❖ **Pillar:** part above the base that supports the other parts.
- ❖ **Inclination joint:** allows for tilting for the convenience of the user
- ❖ **Arm:** connects the eyepiece tube to the base, curve or slanted part which is held while carrying the microscope.
- ❖ **Stage and stage clip:** is a platform for the slides, which hold the specimen. The stage typically has a stage clip on either side to hold the slide firmly in place. Some microscopes have a mechanical stage, with adjustment knobs that allow for more precise positioning of slides.
- ❖ **Body tube:** attached to the arm and bears the lenses.
- ❖ **Draw tube:** cylindrical structure on the top of the body tube that holds the ocular lenses.
- ❖ **Revolving nosepiece:** contains the objective lenses. Microscope users can rotate this part to switch between the objective lenses and adjust the magnification power.
- ❖ **Dust shield:** lies atop the nose piece and keeps dust from settling on the objectives.
- ❖ **Coarse Adjustment Knob:** moves stage (or body tube) up and down, used to focus the image on the microscope under low or medium power.

- ❖ **Fine Adjustment Knob:** small knob, used to fine-tune the focus of your specimen after using the coarse adjustment knob.

MAGNIFYING PARTS

- ❖ **Ocular/ Eyepiece:** contains the ocular lens, which the user looks through to see the magnified specimen, further magnify the image produced by the objective lenses by 5x to 15x.
- ❖ **Objective lenses:** combine with the eyepiece lens to increase magnification levels. Microscopes generally feature three or four objective lenses, with magnification levels ranging 4x to 100x.

ILLUMINATING PARTS

- ❖ **Illuminator or Electric Lamp:** microscopes require a light source for viewing. This can come in the form of a built-in, low-voltage illuminator light, or a mirror that reflects an external light source like sunlight.
- ❖ **Mirror:** has a concave and plane surface to gather and direct light in order to illuminate the object.
- ❖ **Condenser:** focus the light onto the specimen, concentrating light rays on the specimen.
- ❖ **Iris Diaphragm:** controls the amount of light reaching the specimen and regulates the light necessary to obtain a clear view of the object.

FOCUSING THE MICROSCOPE

1. Plug in the microscope into a power outlet and turn on the microscope using the main switch.
2. Turn on the light source by rotating the light intensity adjustment knob.
3. Place the specimen on the mechanical stage by placing it inside a bow-shaped lever. The specimen should be centered in the aperture of the stage.
4. Use the scanner objective for initial observation of the specimen.
 - a. Hold and rotate the revolving nosepiece until the 10x objective aligned to the specimen. A lock-in feature will be obtained once the objective is positioned.
 - b. Rotate the condenser knob to bring the condenser down and partially open the iris diaphragm.
 - c. Turn the coarse focus adjustment knob so that the stage moves upward towards the objective.

Move it as far as it can without touching the slide.

- d. Now, look into the eyepiece and adjust the coarse focus adjustment knob so that the stage moves away from the slide. Continue moving until a clear image of the slide comes into focus.
- e. Turn the fine adjustment knob to precise the focus of the image.
- f. Move the microscope slide until the object of focus is at the center of the field of view. Then readjust the illuminator or diaphragm in order to attain the maximum, or comfortable light intensity.

USING THE HIGH POWER OBJECTIVE

- a. Hold and rotate the revolving nosepiece until the 40x is obtained.
- b. Rotate the condenser knob to bring the condenser half-way up and open the iris diaphragm to attain a clear image.
- c. Look in the eyepiece, rotate the fine adjustment knob to focus the specimen. (**DO NOT USE THE COARSE FOCUS ADJUSTMENT KNOB!** Minimal use of focusing adjustment is necessary in a high-power objective once a clear image is obtained under the low-power objective.)

USING THE OIL-IMMERSION OBJECTIVE

- a. When changing the objective lens of the microscope into oil-immersion objective, a small drop of immersion oil is added into the slide under the low-power or scanning objectives. (If the HPO is still used, change the objective into LPO or scanning objective using the revolving nosepiece before dropping the immersion oil.)
- b. Hold and rotate the revolving nosepiece to change the objective into 100x. Wait for a "click" until it is positioned. The 40x objective must never come into contact with the immersion oil in the slide. The lens of OIO should be slightly dipped into the immersion oil.
- c. Rotate the condenser focus knob to bring the condenser up and adjust the iris diaphragm to get a clearest image. The light intensity may also be adjusted to maximum if necessary.
- d. Look into the eyepiece, rotate the fine adjustment knob to bring the image into precise focus. (**DO NOT USE THE COARSE FOCUS ADJUSTMENT KNOB!**)

- e. After use, remove the oil from the objective by wiping the front lens with lens paper or gauze with 70% alcohol or mixture of 70% ether – 30% alcohol.

PROPER POSITION AFTER USE

- a. Turn off the microscope and pull the plug out of the power supply.
- b. Rotate the objective into the lowest power objective.
- c. Remove the slide and clean the mechanical stage from any dust, debris, or oil that may be left.
- d. Coil the power cord around the base of the microscope or put it in microscope container if available.
- e. Place back the microscope into proper place in the cabinet for storage or equip the microscope with microscope coat if available.

DEFINITION OF TERMS

1. Magnification

- ❖ The ratio of the apparent size of an object as seen through the microscope and the actual size of the object.
- ❖ Total magnification: multiplying the magnifying power of the objective and ocular lenses

Objective Lens	Objective Lens Power	Ocular Lens Power	Total Magnification
Scanner	4x	10x	40x
Low power	10x	10x	100x
High power	40x	10x	400x
Oil immersion	100x	10x	1000x

2. Resolution/Resolving Power

- ❖ The ability of the lens to clearly separate or distinguish two points of two lines individually in the image.
- ❖ The smallest distance between two structures at which they can be seen as separate objects. It determines the quality of the image, its clarity and richness of detail.
 - Maximal resolving power: 0.2 μm , which can permit clear images magnified 1000-1500 times.
 - Objects smaller or thinner than 0.2 μm (single ribosome or cytoplasmic microfilament) cannot be distinguished.

3. Numerical Aperture

- ❖ A measurement of the ability of the condenser and the objective lens to gather light.

Scanner	4x	0.10
LPO	10x	0.25
HPO	40x	0.65
OIO	100x	1.25

4. **Focal length**

- ❖ Thickness of the object that maybe seen at one time under focus.
- ❖ Distance between the center of a lens or curved mirror and its focus.

5. **Working distance**

- ❖ Distance between the front lens of the objective lens and the top of the cover glass when the specimen is in focus.

6. **Parfocal**

- ❖ Refers to quality of the objectives and eyepiece where practically no change in focus has to be made when objective is substituted for another

7. **Refractive Index**

- ❖ Bending of light rays away from the objective lens when light passes from the glass of the microscope slide to the air.

MICROSCOPE CARE

- ✚ Always carry the microscope with two hands.
- ✚ Never touch the lenses with your fingers.
- ✚ Only use lens paper for cleaning.
- ✚ Keep desk clear of objects and cords.
- ✚ When you are finished with your "scope", rotate the nosepiece so that it's on the low power objective, roll the stage down to lowest level, rubber band the cord, then replace the dust cover.

CLINICAL PARASITOLGY LABORATORY

ROUTINE STOOL EXAMINATION

PHASES OF ROUTINE STOOL EXAMINATION

- A. Physical Analysis
- B. Chemical Analysis
- C. Microscopic Analysis
- ❖ Routine stool examination is useful in determining patients clinical status based on the stool sample collected.
- ❖ Those three results also correlate with one another in identifying parasitic infection in an individual.

ROUTINE STOOL EXAMINATION

A. Physical Examination

- ❖ Stool specimens submitted for parasitic study should first be examined macroscopically to determine the consistency and color of the samples and should be screened and examined for the presence of gross abnormalities.
- ❖ The Laboratory must receive a fresh, unpreserved stool specimen in order to perform the physical examination.
- ❖ Most laboratory received fecal sample that are already in the fixative, this step is often skipped because this macroscopic characteristic cannot be determined.
 - If you received a specimen that has preservative, you have to take note of the gross appearance, either on the actual specimen or on the requisition form, and it is recommended at the time of specimen collection

Consistency

- ❖ degree of moisture in a stool specimen may serve as an indication of the types of potential parasites present
 - Soft or liquid stools they may suggest the presence of protozoan trophozoites. Protozoan cysts are more likely to be found in fully formed stool. Helminth eggs and larvae are found in either liquid or formed stool.
- ❖ If the stools are fresh, the laboratory can classify the consistency as formed, semi-formed, soft, loose, or watery.



Color

- ❖ Color of a stool is important because it may indicate the condition of the patient.
 - There are pathological condition associated with the color of stool.
- ❖ The normal color of the stool is brown.

COLOR	POSSIBLE CAUSE
Black	Upper GIT bleeding (before it is passed out in the feces, your blood is being oxidized) Iron therapy Charcoal Bismuth (antacids)
Red	Lower GIT bleeding (within anus or rectum, the blood is fresh) Ingestion of beets and food coloring Rifampin (drug for Mycobacterium tuberculosis)
Green	Biliverdin Oral antibiotics Green vegetables
Pale yellow, gray, white	Bile duct obstruction Barium sulfate
Presence of mucus, blood streak	Dysentery Malignancy Presence of blood or mucus in loose or liquid stool it may suggest the presence of Amoebic ulceration in the large intestine

*Note: Children who have primary complex and adult who have Mycobacterium tuberculosis, if they are taking Rifampin, they can have RED COLORED STOOL even their urine can have a pinch of medication

**Bright red blood on the surface of formed stool is usually associated with irritation and bleeding. (constipation, hemorrhoids/almonaras)

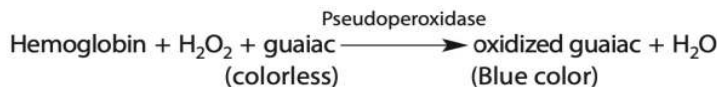
Gross abnormalities

- ❖ Adult worms, proglottids and parasitic indications (pus and mucus) They should be taken noted with

- ❖ Samples containing adult worms may be carefully washed through a wire screen. This process allows for the retrieval and examination of the parasites for identification purposes.

B. Chemical Examination

- ❖ By far the most frequently performed fecal analysis is the detection of occult **blood (hidden blood)**.
- ❖ **Principle:** detection of the pseudoperoxidase activity of hemoglobin.
- ❖ Bleeding of 2.5mL / 150g of stool (PATHOLOGIC)



Hema-screen Guiac Slide Test Kit

1. Hema-Screen Slide
2. Hema-Screen developer (<6% H₂O₂) (75% denatured ethyl alcohol in aqueous solution)

Results:

(+) any trace of blue coloration

(-) no detectable blue coloration

Interference of FOBT (Fecal Occult Blood Testing)

False-Positive

- ❖ Aspirin and anti-inflammatory medications (It can cause irritation to GIT leads to bleeding)
 - You should avoid taking aspirin and anti-inflammatory medications before FOBT
 - Aspirin and Non-steroidal anti-inflammatory drugs other than acetaminophen is the only medicine you can take because it can't irritate your GIT
 - Aspirin and Non-steroidal anti-inflammatory drugs should be taken 7 days before the

specimen collection to prevent possible gastrointestinal irritation.

- ❖ Red meat
- ❖ Horseradish
- ❖ Raw broccoli, cauliflower, radishes, turnips
- ❖ Melons (avoid eating those foods 3 days before specimen collection for FOBT)
 - This foods contain dietary pseudoperoxidase, which can cause interference and result to False-Positive
- ❖ Menstrual and hemorrhoid contamination

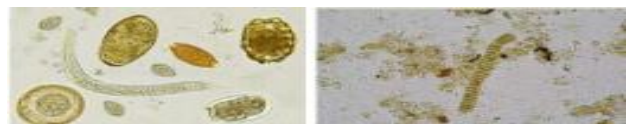
False-Negative

- ❖ Vitamin C >250 mg/d
- ❖ Iron supplements containing vitamin C (should be avoided 3 days before the collection)
 - Ascorbic acid is a strong reducing agent; it interferes with peroxidase reaction thus causing a False-Negative result.
- ❖ Failure to wait specified time after sample is applied to add the developer reagent (wait for the chemical reaction to take place)
 - You should follow the instruction

C. Microscopic Examination

- ❖ To detect the presence of parasites in a stool specimen, microscopic examinations are performed.
- ❖ It can also reveal many elements present in the intestinal tract aside from parasites and normal fecal constituents (artifacts).
- ❖ It should be performed on a fresh specimen.
- ❖ Involves three distinct procedures:
 - Direct wet preparations/Saline-Iodine preparation
 - Concentrated technique
 - Permanently stained smear
- ❖ Reporting:
 - **NOPS**- No ova and/or parasite seen
 - **NIPS**- No intestinal parasite seen
 - **If Positive:** You will write the name of the parasite, the morphology, counted parasite per (power field)

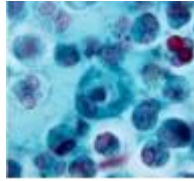
Example. *Entamoeba histolytica* cyst. 2-4 per high-power field. *Ascaris lumbricoides* eggs/ova. 10-15 per high-power field



Artifacts	Pseudoparasites	and Confusers
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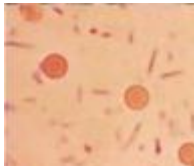
White Blood Cells

- ❖ Polymorphonuclears (PMNs), which may indicate inflammation.
- ❖ Eosinophils, which may indicate an immune response to a parasitic infection.



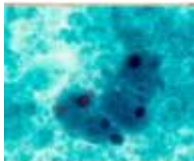
Red Blood Cells

- ❖ May indicate ulcerations or bleeding
- ❖ Smaller than WBC



Macrophages

- ❖ Usually present in both bacterial and parasitic infections.
- ❖ One can mistake the active macrophages for amoebic trophozoites (inside RBC)
- ❖ Active macrophage (performs phagocytosis just like Amoebic tro)



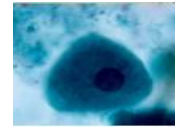
Charcot-Leyden Crystals

- ❖ Released with the disintegration of eosinophils
- ❖ May indicate presence of hypersensitivity or parasitic infections, especially amebiasis.



Epithelial Cells

- ❖ From the intestinal tract.



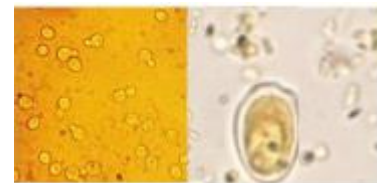
Eggs of Athropods, Plant Nematodes, and other Spurious Parasites

- ❖ May be mistaken for human parasites



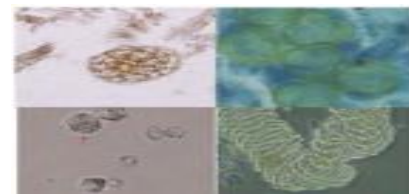
Fungal Spores

- ❖ Candida spp., yeast, and yeast-like fungi



Elements of Plant Origin

- ❖ Plant cells/fibers
- ❖ Pollen grains (contamination of soil)
- ❖ Starch granules (gloves)
- ❖ Vegetable spirals



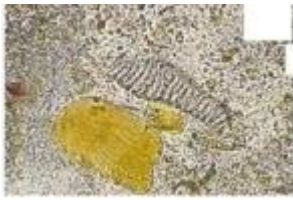
Plant and Animal Hairs

- ❖ May look like helminth larvae.



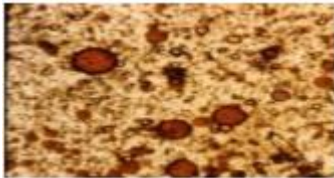
Muscle fibers

- ❖ Undigested striated muscle fibers are associated with pancreatic insufficiency



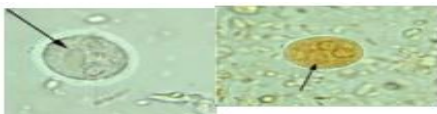
Fat Globules

- ❖ Found in greasy stools



Direct Fecal Smear

- ❖ Most common examination in Parasitology laboratory
- ❖ About 2 mg of stool (amount forming a low cone at the tip of an applicator stick) is comminuted thoroughly with a drop of 0.85% sodium chloride solution (NSS) and then covered with cover slip.
- ❖ Primary useful in the detection of motile protozoan trophozoites (pale and transparent).
 - Gram's iodine is used in Gram's staining. for Parasitology we use Weak Iodine Solution such as Lugol's and D' Antoni
- ❖ Weak iodine solution (Lugol's and D' Antoni) are temporary stain to demonstrate nuclei. Helminth eggs and larvae can be detected using this preparation.
 - **Cytoplasm:** Golden yellow
 - **Nucleus:** Pale and Refractile
 - **Glycogen (Iodine loving):** Deep Brown



Direct Fecal Smear

Kato-Thick Smear

- ❖ Qualitative Analysis
- ❖ Katho-katz (Quantitative Analysis)
- ❖ About 50-60 mg of stool (approximately the size of two munggo bean) is placed over a glass slide and covered with cut cellophane paper soaked in a mixture of glycerine and malachite green solution.
 - **Glycerine:** Clearing solution
 - **Malachite Green:** To give color to the cellophane in order to give a pale green background to the eggs and to minimize the brightness of the microscope.
- ❖ Preparation is best examined within 10 to 20 minutes.
- ❖ Simple, economical and useful for mass stool examination
- ❖ Very good in detecting eggs with thick shells (Ascaris and Trichuris) but not with thin shells (hookworm eggs, become distorted and transparent).
- ❖ Usefulness is limited if stools are diarrheic and watery, notable to detect protozoan cysts and trophozoites.



KATO-THICK SMEAR

ROUTINE STOOL EXAMINATION PROCEDURE

Direct Fecal Smear

1. Label slide and place one drop of NSS and one drop of Lugol's iodine.
2. Place a match head size portion of the stool sample and make a smooth uniform emulsion by rotary motion of the applicator stick.
3. Place the coverslip.
4. Examine under the microscope.



DIRECT FECAL SMEAR

Kato-Thick Smear

1. Place 50-60 mg stool (size of 2 munggo bean) at the center of the slide and place a piece of cellophane soaked in pretreated glycerin-malachite green solution.
2. Using a test tube press cellophane gently in order to spread the stool sample.
3. Leave at room temperature (10-20 minutes) or 1 hour

CLINICAL PARASITOLOGY LABORATORY

SPECIMEN COLLECTION AND HANDLING

- Most parasitic diseases cannot be established base of clinical sign and symptoms
- The sign and symptoms that the patient experiencing they are not specific to tell that the condition of the patient is parasitic infection or parasitic in nature

LABORATORY DIAGNOSIS

- Accurate diagnosis of parasitic infections can help decrease the prevalence and incidence of a parasitic infection.
- A parasitology laboratory should be able to:
 - ✓ **confirm clinical impression that the condition has a parasitic nature**
 - To confirm the disease if it is really parasitic in nature, we need to do laboratory diagnosis
 - We have to established that the causative agent of a particular pathology or diseases is parasitic in nature
 - ✓ **rule out differential diagnoses**
 - To aid the clinician in the proper medication or in the choice of treatment
 - We have to specify the exact or the particular parasite that is causing the pathology
 - ✓ **aid a clinician in the choice of proper medication**
 - ✓ **help in monitoring the effect of treatment regimen**
 - patient can go back and have the specimen retested

The ability of a parasitology laboratory to generate reliable results is dependent on the following:

- ✓ **proper collection, handling, and processing of specimens prior to examination**
 - Depends on the integrity of the sample collected or submitted inside the laboratory
 - Most important and most crucial part
 - We have to assure primarily that the specimen where going to use is suitable for processing or for testing.

- ✓ **skill of the laboratory analyst (examiner)**
 - we should have the necessary skills and we should know the knowledge of laboratory test
- ✓ **quality of equipment used in the examination**
 - in PH setting most of parasitic diseases are intestinal in nature (they are being passed out in the feces
 - parasites are microscopic- they are too small to seen by the naked eye

Diagnostic parasitology is done by:

A. Demonstration of parasites or parasite component

- eggs, larvae, adults, cysts, oocysts, trophozoites and antigens
 - It is already true positive if you have seen parasitic morphology on the sample
 - Demonstration of parasite is possible only during the patent stage (Patent Stage- It is where the evidence of infection appears)
 - **Schistosomiasis** – Not demonstrable even on active infection. Stool and Urine sample comes out negative during laboratory diagnosis
 - When there are light infections, parasite is immature, and no parasite on your sample, it will give you false negative result. In this cases Immunoassay is maybe useful
- provides a definitive diagnosis

B. Detection of host immune response to the parasites

- Antibodies- produced against particular antigen from the parasite.
 - For Entamoeba histolytica
 - Counter immune electrophoresis (CIEP), ELISA, IHAT, IFAT
- humoral immune response provides only presumptive evidence of infection

PHASES OF LABORATORY IDENTIFICATION OF PARASITES:

Pre-analytic phase

- Specimens received in the laboratory that are compromised because of improper collection, labeling, or transport should be rejected and a new specimen should be requested.

- Specimen should be perfect
- If the specimen is not suitable based on the grounds or procedure you have the rights to reject it
- First thing to consider is the integrity of the specimen

Analytic phase

- Laboratory techniques performed in the testing of the samples should be completed with care to ensure that accurate results are obtained.
 - You must know what the procedure for the sample
 - Accurate diagnosis
 - Bawal mag kamali
 - Human nature can commit mistake

Post-analytic phase:

- Interpretation and reporting of results obtained should be accurately reported in a timely manner.
 - Process the test fastest

SPECIMENS AVAILABLE FOR PARASITIC EXAMINATIONS:

- Stool- Most common utilize specimen in the parasitology laboratory.
- urine
- blood
- sputum
- cerebrospinal fluid- For Acanthamoeba and Naegleria
- tissue aspirate
- tissue biopsies
- orifice swabs- Ex. Perianal swab

FACTORS TO CONSIDER IN SPECIMEN COLLECTION

- **Life Cycle (Parasite species and stage of development)**
 - You need to know the parasite species and their stages of development
- **Frequency and timing collection**
 - Periodicity- Blood parasite has a specific time when going out to peripheral circulation
 - Loa loa Worm has microfilariae and it is diurnal (daytime only)
 - Wuchereria bancrofti's microfilariae are nocturnal (night only)

FACTORS THAT SHOULD PRECIPITATE TESTING

- **Travel history**
 - If you acquired the parasite from the place you have been.
- **immune status of patients**
- **Documented previous infection**
 - Relapse of the parasite
- **Contact with infected individuals**
- **Screening outbreak situations**
- **Occupational screening for food handlers**
 - Health card for the vendors or food handlers
- **Therapeutic failure (retested after therapy)**

SPECIMEN FOR FECAL SAMPLE

- The most common method of diagnosis of intestinal parasites is through the demonstration of eggs, larvae, adults, trophozoites, cysts, or oocysts in the stool.
- 1. Container**
 - Clean, wide-mouthed containers made of waxed cardboard or plastic with tight fitting lid (screw-capped tops). For the sample not to leak and prevent contamination.
 - Not necessary sterile but it should be clean
 - Zip lock is a must when collected at home and it will be transported to the laboratory.
- 2. Label**
 - Patient Name
 - Age
 - Sex
 - Date/time of collection
 - Requesting physician
 - Requested procedure
 - Presumptive diagnosis
 - Prior infections
 - Travel history
 - ✚ In writing the specimen, middle name must also be written.
 - ✚ Patient name, age, sex, time and date of collection are the basic details
 - ✚ The label must be placed in the body of the container so that the sample will not be interchanged to the other samples
 - ✚ Universal precaution must be applied at all times (To treat all bodily fluid as infectious)

FACTORS TO CONSIDER FOR STOOL MATERIALS TO BE USEFUL IN PARASITIC DIAGNOSIS

- **Intake of drugs/medicinal substances (antacids, anti-diarrheals, barium, bismuth, mineral oil and laxatives)**
 - can leave crystal residues on stool samples, it can interfere laboratory diagnosis
 - Stool sample must be collected a (7 days) after the last intake of any of this drugs, so that it can't interfere to stool examination
 - (5-7 days after for barium, bismuth, mineral oil intake.)
- **Intake of antibiotics and antimalarial drugs**
 - Decrease the number of protozoan for several weeks. Wait for 2 weeks before stool examination
- **Amount of stool to be collected is dictated by the techniques that will be used.**
 - ✚ **Routine stool examination:** thumb-sized specimen of formed stool
 - Instruct your patient to submit a thumb-sized stool specimen
 - ✚ **Watery stool:** 5 to 6 tablespoons
- **Contamination with toilet water, urine, or soil must be prevented.**
 - Can destroy protozoan trophozoite when in contact with urine or toilet water because they are fragile
 - Soil and toilet water can contain other free living parasite
- **Age of stool sample**
 - **For urine:** trophozoite died at 30 mins-1hr after passage
 - After 1hr after passage is maximum age of stool sample if there is no preservative
 - **Watery stool:** 30 mins after passage of the stool sample
 - **Soft stool-** 1hr
 - **Formed stool-** 24hr
 - **When reporting-** integrity of stool is compromised
 - **For Patients-** The stool is spoiled and it can't be used for diagnosis
- **Delay in examination of specimens may require preservation to ensure that parasites are present in the identifiable stage.**

- If there are delay you need to preserve the specimen so that it won't compromise the integrity of the sample.

➤ **Temporary storage of fecal sample**

- Refrigeration 3 to 5 degree Celsius (preservation of stool) for the mean time. trophozoite are killed by refrigeration. Protozoan cyst and helminths egg can live at refrigeration
- **NEVER FREEZE YOUR STOOL SAMPLE!**
And never keep them in incubator

SPECIMEN TYPE	STABILITY	AND PRESERVATION
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- Fresh specimen is mandatory for recovery of motile trophozoites.
- A. SPECIMEN TYPE AND STABILITY**
 - 1. Liquid stool**
 - Should be examined or preserved within **30 minutes** of passage.
 - Trophozoite form are found in this stool.
 - 2. Soft stool**
 - should be examined or preserved within **1 hour** of passage,
 - 3. Formed stool**
 - Should be examined or preserved within **24 hours** of passage.
 - Cyst form are found in this stool.
 - if the stool is in the toilet it can't proceed for stool examination
- B. STOOL PRESERVATIVES (fixative)**
 - In case of delay you need to apply preservatives, to preserve the integrity of the specimen. Appropriate fixation of the parasites in the stool will preserve the protozoan morphological features and prevent possible destruction of the helminths eggs
 - **Fixatives** are substances that preserve the morphology of the protozoa and prevent further development of a certain helminth
 - When selecting a fixative, the possibility of preparing a permanently stained slides should be considered. "Do you want to stain the slide permanently or not". "You want to use the slide for future references"
 - Some fixatives are not suitable for permanent staining
 - Stool samples must be adequately mixed with selective preservative in a portion 1 part of the stool, 3 parts of the preservative. 1:3 ratio.

1. Formalin

- all purpose fixative
- 5% formalin for protozoan cyst
- 10% formalin for helminth eggs and larvae
- solution may be buffered with sodium phosphate to preserve the morphological characteristics of the organisms

Advantages:

- it is easy to prepare
- it preserves specimens for up to several years
- it has a long shelf life

Disadvantages:

- it does not preserve parasite morphology adequately for permanent smears
 - not compatible if incorporate permanent smear
 - trophozoites usually cannot be recovered and morphologic details of cysts and eggs may fade with time
 - Trophozoites don't survive in formalin
 - considered a potential health hazard
2. Schaudinn's solution – most often combined with PVA, preserve fresh stool for staining purposes, contains HgCl₂ (highly toxic)
- Shadinn's is the one who makes the fixative and the PVA is an additional
3. Polyvinyl Alcohol (PVA) – plastic resin, which serves to adhere a stool sample onto a slide.

Advantage:

- Main advantage of using PVA is related to the preservation of protozoan cysts and trophozoites for permanent staining. (Trichrome stain)
- PVA-preserved specimens have a long shelf life when stored at room temperature.

Disadvantage:

- contains **mercuric chloride** which can cause potential health problems
4. Merthiolate-Iodine Formalin (Thimerosal) – useful for fixation of intestinal protozoans, helminth eggs and larvae.
- Thimerosal and Iodine- stain
 - Formalin- preservative
5. Sodium Acetate-Acetic Acid Formalin (SAF) – viable alternative to the use of **PVA and Schaudinn fixative**, can be used for performing

concentration techniques and permanent stained smears

- For the fixation of the samples for the staining with modified fast stained to detect the coccidial
- Coccidial- part of protozoans and they are acid fast organism
- Acid fast organism- incorporating modified acid fast stain

Advantages:

- mercury-free
- easy to prepare
- long shelf life
- can be used for preparing smears for staining with the modified acid-fast stain to detect coccidian oocysts.

Disadvantage:

- not as sharp as PVA and Schaudinn's fluid
 - adhesive properties of SAF are not good, the addition of albumin to the microscope slide may be necessary to ensure adhesion of the specimen to the slide
6. Modified Polyvinyl Alcohol – alternatives to mercury-based PVA are the use of substitute compounds containing copper sulfate or zinc sulfate

Advantage:

- can be used for concentration methods and permanent stained smears

Disadvantage:

- do not provide the same quality of preservation for adequate protozoan morphology on a permanent stained slide as the mercury-based fixatives
7. Alternative Single-Vial Systems – non-toxic fixatives that are free of formalin and mercury and can be used for concentration techniques and permanent stained smears. Some of these products can also be used for performing fecal immunoassays.
- It can detect immunoassays

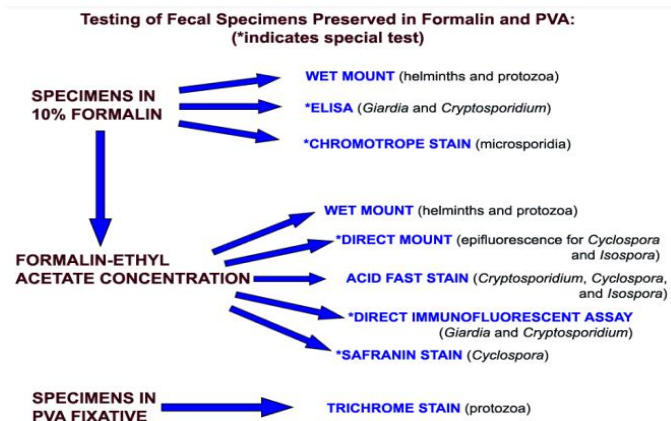
CONCENTRATION TECHNIQUES

- They are important in case of in light infection. Konti lang yung parasitic elements ma isolate

- Detecting light parasitic infection

Sedimentation- bottom of the solution

Floatation- at the top of the solution



ALTERNATIVE SINGLE	VIAL SYSTEM
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- No heavy metals and provides an alternative for laboratories seeking a less toxic fixative without compromising accuracy.
- Its innovative formula enables permanent stain from a concentrated sample without losing trophozoites.
- It provides extraordinary clear organism identification.

Wet Mount

- Prepare the wet mount by placing 1 drop of the sediment onto a clean glass slide
- Add a drop of iodine solution to the specimen, mix gently, and coverslip.

Permanent stain

- Spread the sample over the microscope slide.
- Chop the specimen with an applicator stick to create thick and thin areas.
- Allow the slide to remain flat for 1 to 2 minutes.
 - Allow the remaining excess liquid to drain at a 45 Degree angle. Air dry for 10-15 minutes.
- Stain using Wheatley's trichrome stain procedure or staining procedure of your choice.

STOOL COLLECTION PROCEDURE

- A typical stool collection protocol is consisting of 3 specimens
- It is collected in different days for much accurate diagnosis

- Give the patient a clean, dry glass jar or plastic cup with wide- mouthed and tight-fitting lid specimen container.
- Instruct the patient to directly pass the stool specimen on the specimen container or pass the stool in a clean pan and transfer the stool to the specimen container with a spatula.
- For specimen with no preservatives, tighten the lid to prevent leakage and refrigerate the sample if will not be transported immediately. The stool should be about thumb size of forming or soft specimen and 5-6 spoonful of watery specimen. In formed stool, try to collect a small amount of stool from each end and the middle part. Do not overfill the cup.
- For specimen with preservatives, use spoon to build into the lid for sampling to raise the sample into "Fill to here" line. Do not overfill the vial with preservative. Mash the formed stool against the side of the vial with a spoon. Tighten the lid and shake firmly until the specimen is well mixed.
- The stool must not contain any contaminant of soil, urine, or toilet bowl water. Sample areas of stool which appears bloody, slimy, or watery should have represented the sample.
- The specimen should be transported to the laboratory within half an hour after the passage for unpreserved specimen. Stability of sample with preservatives depends on the type of preservative is present to them.
- Each specimen should be labeled with basic details:

- Patient name
- Age
- Sex
- Date/Time of Collection

LABORATORY SAFETY IN PARASITOLOGY

1. Handle all fresh specimens carefully. Use the universal precaution. Fecal specimen may contain parasites and many potential pathogenic bacteria and contagious viral agents.
2. Handling specimens and disposal of waste material are of the utmost importance to maintain good health for the laboratory personnel and the community.
3. No food and drink allowed in the laboratory. They can be contaminated.
4. Smoking is not allowed in the laboratory.

5. Frequent hand washing can help to prevent the spread of parasites.
6. Laboratory gown and uniform must be washed at the working site. Special attention should be given to avoid contamination of other items.
7. Obtain immunizations to prevent pathogenic organisms and treat such.
 - 1 part of bleach and 9 parts of water or 1:9 ratios
8. Dispose contaminated materials on properly labelled waste containers with instructions for the waste collection.
9. Container for used slides must be filled with one third to one half disinfectant. The container should be autoclaved and emptied.
10. Specimen autoclaving before discarding is suggested.
11. Applicator sticks, tongue depressors and pipettes may be discarded in the same container as slides.
12. Spillage must be soaked with disinfectant. Residue should be cleaned and discarded as contaminated trash.
13. Chemicals must be considered poisonous, flammable and corrosive. Distinct labels must be affixed.
14. Mercuric compounds cannot be disposed as regular trash. A closed steel container is suitable.
15. Sandbox is required for strong acids and base. Avoid storing strong acids and bases together.
16. Flammables are considered to be explosives. Laboratory should have adequate ventilation and no open flames.
17. Poisons can intoxicate to the extent of death when ingested or inhaled. Pipetting by mouth should be avoided.
18. Prolonged contact with xylene and formaldehyde can be carcinogenic.