BIO173: GENERAL PARASITOLOGY LABORATORY MODULE

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Microscope

This activity gives the students the opportunity to use a basic instrument of biology – the microscope. The microscope is an instrument designed to make objects visible that are too difficult or too small to see with the unaided eye. There are various types of microscopes that have different functions and levels of magnification. The microscopes commonly used in the biology lab are usually compound binocular or monocular light microscope. Though there are many variations in the features of microscopes, they are all constructed on a similar plan.

In this exercise, students will familiarize themselves with the proper care and use of the compound microscope.

Objectives: At the end of the activity, the student should be able to

1. Identify the parts of a typical microscope and learn their functions
2. Focus specimens under the compound microscope
3. Measure specimens under the microscope

Materials

Compound microscope
Newspaper cut-out
Ocular micrometer
Dropper bottle with distilled water
Specimen slides (Blood smear, *Giardia lamblia, Trypanosoma* sp.)
Glass slide
Cover slip
Stage micrometer

Procedure

Section1. Parts of the microscope. Locate and examine each part of the microscope. You should be able to point out correctly to your instructor all the parts and their uses before labeling the illustration provided (Figure 1-1).

MECHANICAL PARTS. These consist of certain precise parts chiefly of metal to support and adjust the optical parts.

1. **Draw tube** is the hollow cylinder where eyepiece or ocular is placed.
2. **Body tube** forms the body of the microscope. The ocular and draw tube rests on the body tube.
3. Attached to the base of the body tube is the circular **revolving nosepiece.**
4. A metallic structure above the nosepiece which protects the lower lenses is the **dust shield.**
5. The microscope is focused by turning two knobs. The **coarse adjustment knob** consists of large knobs on the side of the tube. Turn the coarse adjustment knob and see what happens.

6. You will notice a pair of small knobs, the **fine adjustment knob.** The fine adjustment knob is used for sharp focus after the object has been brought into view with the coarse adjustment knob.

7. Illuminator control knob located on the side of the base just below the mechanical stage adjustment knobs controls the light output of the illuminator.

8. The platform where you put slides for observation is called the **stage.** It has a circular opening called an **aperture**. The opening permits the entry of light.

9. The slide is held in place on the stage with a pair of **stage clips** or the stage may be equipped with a mechanical device into which the slide is clipped called a **mechanical stage**.

10. The curved part of the microscope is the **arm.** When carrying the microscope, one hand should grasp the microscope by the arm and another hand supports the base. The base should always be held parallel to the floor.

11. A short vertical extension on which the arm connects with the base is the **pillar.**

12. The portion of the microscope in contact with the table is the **base.**

13. The body of the microscope is attached to the base by means of the **inclination joint**. This permits the tilting of the microscope.

OPTICAL PARTS. These consist principally of special types of carefully ground and polished glasses aligned in an optical axis for the enlargement of the image of the object under study.

1. Eyepiece or **ocular** is the removable cylinder on top of the microscope. It contains one set of lenses, the magnifying power of which is indicated on top of the eyepiece (e.g. 10X or 12.5X)

2. Objective lens gives the initial magnification. In general, the lower the magnification, the shorter is the objective lens and the farther is it positioned from the specimen when focused. The microscope may have four of these located on the revolving nosepiece. These are as follows:

Scanning lens is a short lens which magnifies the size of an object 4X. It has the broadest field of view of all the lenses, and is used for initial focusing of objects.
Low Power Objective (LPO) magnifies by 10X.
High Power Objective (HPO) magnifies by 40X.
Oil Immersion Objective (OIO) magnifies by 100X.

3. Substage condenser is a lens located under the stage aperture, and its distance from the stage is controlled by a knob. Its function is to condense the light and focus it on the specimen.

4. Iris diaphragm is beneath the condenser and functions to control the amount of light passing through the specimen. This can be moved from side to side to affect a change in light intensity. One type of diaphragm appears as a circular disc with a series of circular openings. One chooses a circular opening depending on the amount of light needed. The other type of diaphragm consists of several flat metal blades forming a singular opening whose size can be regulated by a lever.
5. Substage illuminator is the source of light which illuminates the object being viewed. This may be replaced by a mirror.

6. You will also see a two-sided mirror, one flat and one concave. When do you use the concave mirror?

Section 2. Use of the microscope

1. Place the microscope on the table giving an allowance of at least an inch from the edge of the table.
2. Gently rotate the nosepiece to bring the LPO directly above the opening of the stage. The LPO is in position when you hear a click as you turn the nosepiece.
3. Look through the ocular with one eye looking through and adjust the mirror for even illumination. In doing this and for all activities using the microscope, you should always keep both eyes open.
4. Regulate amount of light by adjusting the iris diaphragm.
5. Put a cut letter in the middle of a slide. Place a drop of water and cover with cover slip. Place slide on stage. Bring the tip of the objective about one or two millimeters above the cover slip and glass slide.
6. Put the letter into view by slowly turning the coarse adjustment knob counterclockwise. Use the fine adjustment knob to get a sharp image.

Section 3. Magnification in the microscope

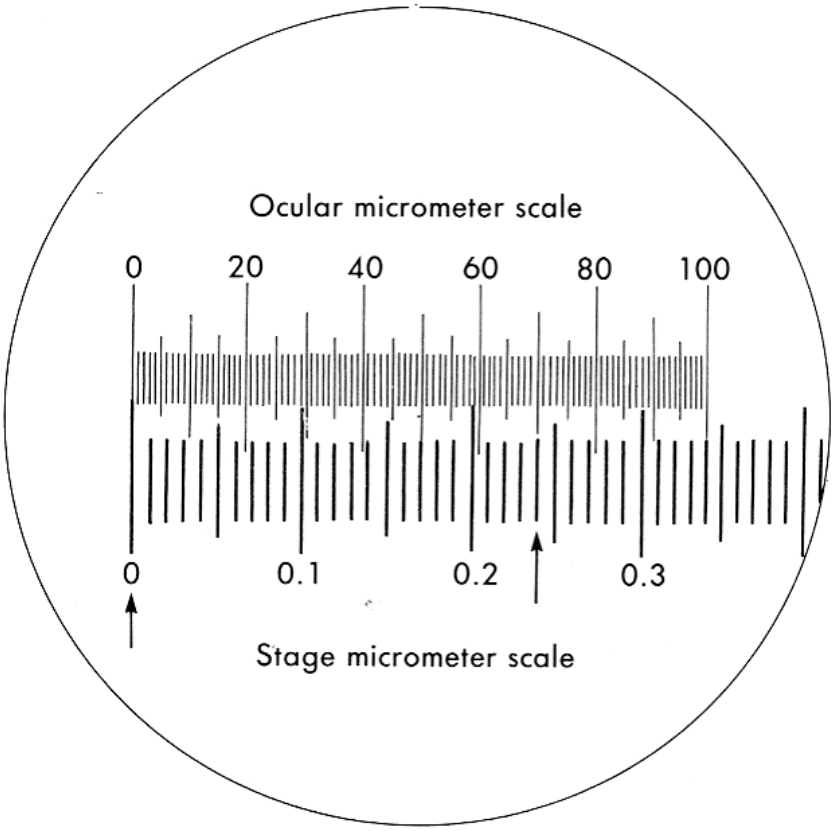
How much your microscope will magnify depends on the power of the combination of lenses you are using. Your microscope is probably equipped with a 10X ocular, which magnifies the object 10 times in diameter. The objectives may be designated, respectively, 4X (scanning objective), 10X (low power objective), 45X (high power objective), and 100X (oil immersion objective). The total magnifying power is determined by multiplying the power of the objective by the power of the ocular.

Section 4. Measuring objects with ocular and stage micrometer calibration.

An **ocular micrometer** can be fitted into the microscope's ocular. It is a disc on which is engraved a scale of units. These units are arbitrary values that always appear the same distance apart no matter which objective is used in combination with the eyepiece. The ocular micrometer cannot be used to measure objects until it is calibrated with a **stage micrometer**. The stage micrometer resembles an ordinary microscope slide but bears an engraved scale on its upper surface, usually 1 or 2 mm long, divided into 0.1 and 0.01 mm divisions.

Place stage micrometer on microscope stage and focus on engraved scale with the LPO. Both scales should appear sharply defined. Rotate eyepiece until the two scales are parallel. Now move the stage micrometer to bring the 0 mark of the stage micrometer scale in exact alignment with the 0 marking of the ocular micrometer scale. The scales should be slightly superimposed (Figure 1-2). To calibrate the ocular scale for this objective, use the longest portion of the ocular scale that can be seen to coincide precisely with a line on the stage scale. Repeat calibration procedure for high power lens.

Figure 1-2. Calibrating an ocular micrometer



$$\text{Calibration Constant} = \frac{\text{Stage}}{\text{Ocular}} \times 0.01 \text{ mm}$$

Remove the stage micrometer and replace it with a prepared specimen slide. Use the calibration constant to determine the length and width of the specimen. Multiply the number of divisions in the ocular micrometer covered by the specimen by the calibration constant.

Length or width = number of spaces covered in ocular micrometer X calibration const

Activity 1
The Microscope

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw and Label the parts of the compound light microscope and stereoscopic dissecting microscope on a separate sheet.

II. Using the compound light microscope

Draw the position of letter as you placed it on the slide

Draw under low power objective

Draw under high power objective

Movement of image when paper is moved to right?

Movement of image when paper is moved away from you?

Movement of image when paper is moved towards you?

Is your microscope parfocal? Justify your answer.

What happens to the light intensity under high power?

III. Magnification of the microscope

	Ocular	X	Objective	=	Total Magnification (diameters)
Scanner		X		=	
LPO		X		=	
HPO		X		=	
Oil Immersion Objective		X		=	

IV. Measuring objects with ocular and stage micrometer calibration

Micrometer values for microscope number _____
Low-power lens, 1 ocular unit = _____µm
High-power lens, 1 ocular unit = _____µm

Show solutions:

Determine the width of the specimen (blood smear, *Giardia lamblia* and *Trypanosoma* spp.). Measure the object available to you in the laboratory. Show your solution for both LPO and HPO.

Specimen	Size in micrometers	
	LPO	HPO
Blood Smear		
Giardia lamblia		
Trypanosoma spp.		

How do the LPO and HPO sizes compare with each other?

2

Protista

This activity gives the students the opportunity to learn and explore the Protists. Protists are an extremely diverse group of organisms. In the animal-like Protista, collectively referred as protozoa, all the functions of life are performed within the limits of its single plasma membrane. These organisms have specialized organelles functioning as its skeletons, organs of locomotion, sensory systems, conduction mechanisms, defense mechanisms and contractile systems.

Protozoa are oftentimes erroneously referred as "simple" organisms but they are not simple organisms as many of them are exceedingly complex. Protozoa carry on all the basic functions of a multi-cellular animal. They are found in fresh, marine, brackish waters and in moist soils. Some are free living; others live as parasites or in some other symbiotic relationship.

Study Suggestions

Make detailed sketches and notes on specimens. This will help you in two ways: 1) when you attempt to draw a specimen, you are forced to look at it more closely, and 2) the drawings will help you study later.

The Protozoa are considered members of the Kingdom Protista. They may either be unicellular eukaryotes or multi-cellular organisms. The subkingdom Protozoa embraces those protists that share many animal-like characteristics with the metazoan, especially in the manner of ingesting food. Traditionally, there are four main groups of protozoa that have been recognized: the flagellates, the amebas, the spore formers and the ciliates.

In this activity, we will survey representatives from the many of the candidate kingdoms of protists.

Candidate Kingdom Diplomonadida

The diplomonads (and their relatives the parabasalids) represent a group of eukaryotes that probably diverged very early from the rest of Domain Eukarya. They have multiple flagella, two nuclei, and cytoskeletons that are simple compared to those of most eukaryotes. View the prepared slide showing cysts of *Giardia lamblia*, the parasitic protist that causes giardiasis, characterized by severe diarrhea and cramping. Although probably not visible in this specimen, a key feature of this species and other members of its candidate kingdom is that they lack mitochondria.

Candidate Kingdom Euglenozoa

All members of Euglenozoa are single-celled protozoans with flagella. Their mode of nutrition varies: some species are heterotrophic, some are photosynthetic, and some are mixotrophic. Some euglenozoans are free-living and others are parasitic. View the prepared slide showing the *Trypanosoma spp.*, the parasitic protist causes sleeping sickness.

Candidate Kingdom Alveolata

This is a diverse candidate kingdom that includes both autotrophs and heterotrophs. All are single-celled and are characterized by "**alveoli**" (sub-surface, membrane-bound cavities), but their mode of nutrition and locomotion varies from group to group. There are three distinctive groups of alveolates:

Dinoflagellates
Dinoflagellates are mostly photosynthetic marine phytoplankton with two **flagella**; they also have distinctive internal armor of **cellulose plates**. See the prepared slide of *Ceratium*. Note the armored appearance and look for the flagella -- can you see the transverse groove that houses one of the flagella? (The other flagellum would be sticking out from the body of the dinoflagellate, perpendicular to the first flagellum.)

Ciliates
The ciliates are unicellular heterotrophs with **cilia**, fine hairlike projections used for locomotion. See the prepared slide of *Balantidium coli*. Try to locate the following structures on the *Balantidium coli*: cilia, oral groove, macronucleus and micronucleus, and contractile vacuole. Observe the **contractile vacuole** closely under the oil immersion lens on your microscope. What do you think are the functions of each structure in this organism?

Apicomplexans
The organism that causes malaria (*Plasmodium*) is an apicomplexan, one of the three sub-groups within Alveolata. This group is characterized by the presence of an "apical complex" of organelles that is used to penetrate the tissues of the host organism. View the prepared slides of the *Plasmodium spp.* Try to differentiate the ring, trophozoite, schizont and gametocyte stages of the parasite.

Candidate Kingdom Stramenopila

The stramenopiles are a diverse group of algae and mold-like protists united by a common structural feature of their flagella (some of which bear fine hair-like projections) and their chloroplasts, if present (these are derived from endosymbiotic eukaryotes).

Candidate Kingdom Rhodophyta

Rhodophyta includes the red algae, so-called because of their red color; imparted by the pigment **phycoerythrin** (they also have chlorophyll *a*). Red algae may be differentiated from brown and green algae not only by their color, but also because they store food as **floridean starch** (another kind of carbohydrate) and because they have no flagellated cells at any point in their life cycle. Like phaeophytes, red algae are primarily multi-cellular and mostly thriving in marine environments.

Candidate Kingdom Chlorophyta

The green algae, Chlorophyta, are the protistan group most closely related to the Kingdom Plantae (true plants). Like true plants, the color of green algae is imparted by chlorophyll *a* and *b*. Like plants, their food is stored as **starch**. Green algae may be single-celled, colonial, or multi-cellular; they are mostly thriving in freshwater environments, but some thrive in marine or even terrestrial environments.

Candidate Kingdom Mycetozoa

This candidate kingdom includes two distinct groups of fungus-like organisms: the plasmodial slime molds (**Myxogastrida**) and the cellular slime molds (**Dictyostelida**). Although superficially similar to true fungi, both groups of slime molds have amoeboid stages that are much more similar to amoebas than they are to fungi. Both plasmodial and cellular slime molds have complex life cycles that involve both feeding and reproductive stages. A key distinction is that the feeding stage (**plasmodium**) of the plasmodial slime molds is a massive multinucleate cell, whereas the feeding stage of the cellular slime molds is made up of many independent amoeboid cells.

The following below are the condensed Linnean classification giving the three largest and most important of the seven protozoan phyla.

Kingdom Protista
Subkingdom Protozoa
Phylum Sarcomastigophora
These sarcomastigophorans have locomotor organelles of flagella, pseudopodia, or both; only one type of nucleus (no micronucleus) and no spore formation.
Subphylum Mastigophora
These organisms have flagella for locomotion; autotrophic, heterotrophic, or both; reproduction usually asexual by fission.

Class Phytomastigophorea. Autotrophic flagellates with chromoplasts containing chlorophyll. Examples: *Chilomonas, Euglena, Volvox, Ceratium, Peranema.*

Class Zoomastigophorea. Flagellates without chromoplasts; amoeboid forms with or without flagella in some groups; predominantly symbiotic. Examples: *Trypanosoma, Trichonympha, Leishmania*.

Subphylum Sarcodina
These organisms have pseudopodia for feeding and locomotion. Their body is naked or with external or internal skeletons; free living or parasitic.

Superclass Actinopoda. Pseudopodia with axial filaments; body often spherical; usually planktonic. Examples: *Actinosphaerium, Actinophrys, Thalassicolla, Radiolarians*.

Superclass Rhizopoda. Locomotion by lobopodia, filopodia, or reticulopodia, or by cytoplasmic flow without discrete pseudopodia. Examples: *Amoeba, Chaos, Arcella, Difflugia,* foraminiferans.

Phylum Apicomplexa
Characteristic set of organelles (apical complex) at anterior end in some stages; cilia and flagella usually absent; all species parasitic.

Class Sporozoea. Spores or oocysts typically present, which contain infective sporozoites; locomotion of mature organisms by body flexion, gliding, or undulation of longitudinal ridges; flagella present only in microgametes of some species; pseudopodia usually absent; one host or two host life cycles. Examples: gregarines, coccidians, malaria parasites.

Phylum Ciliophora

These organisms have cilia or ciliary organelles present in at least one stage of life cycle, usually two types of nuclei; binary fission across rows of cilia; budding and multiple fission occur; sexually involving conjugation, autogamy and cytogamy; heterotrophic nutrition; mostly free living; contractile vacuole typically present. Examples: *Paramecium, Colpoda, Tetrahymena, Stentor, Blepharisma, Epidinium, Vorticella, Euplotes, Didinium*.

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The Flagellates

This activity gives the students the opportunity to learn and explore the Subphylum Mastigophorans – The Flagellates. These organisms are characterized by their flagella allowing the organism to move efficiently in their environments. There are two major classes of flagellates: The phytoflagellates or "plant-flagellates," (Phytomastigophoran) and the zooflagellates or "animal-flagellates," (Zoomastigophoran).

Phytoflagellates are pigmented and autotrophic organisms. Zooflagellates are colorless, lack photosynthetic pigments, and are heterotrophic organisms. Many are free living but some are parasitic for humans and other animals.

In this exercise, students will familiarize themselves with the organisms under the Zoomastigophoran group.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Zoomastigophoran group

Materials

Compound microscope
Specimen slides (*Chilomastix mesnili; Retortamonas intestinalis, Giardia lamblia, Trichomonas spp.,Trypanosoma spp., Leishmania spp.*)

Procedure

1. View the specimens, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section1. General Flagellate Anatomy

Flagellate organisms have a pellicle (combination of plasma membrane and thin, translucent, secreted envelop) giving the organism a more defined shape. The flagellum is used for locomotion. It may be present or not; they may be long or short; they may have one or more and they arise from granules that may be attached or free. The flagella arise from the basal granule, blepharoplast and kinetosome.

Section 2. The Flagellates

Chilomastix mesnili. This organism is non-pathogenic and is endocommensal. This organism's lifecycle involves both trophozoites and cysts forms. They live in the cecum and divide by binary fission. They are oftentimes referred as water-borne parasites. The trophozoite is 6-24µm long and 3-20µm wide. There are 4

flagella that arise from its kinetosome at the anterior end. Three flagella extends anteriorly and 1 extends into the cytostome. The cyst is lemon-shaped ranging to 6-10µm in diameter. It contains a single nucleus, cytosome and retracted flagella.

Retortamonas intestinalis. This is a tiny protozoan that is similar to the *Chilomastix mesnili* but the trophozoite is only 4 to 9 µm long. It has 2 flagella, one extending anteriorly and the other emerging from the cytostomal groove and trails posteriorly. The cyts are small ovoid to pear-shaped containing a single nucleus.

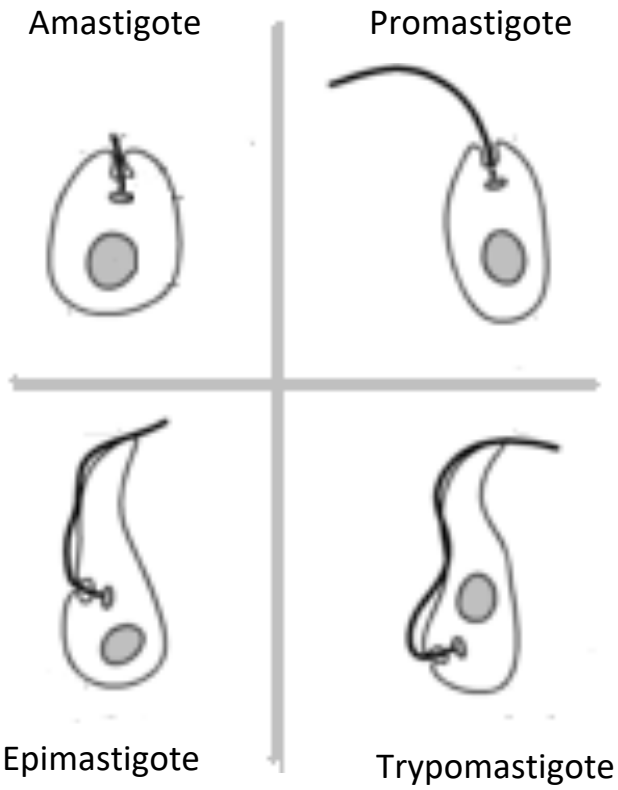
Giardia lamblia. This protozoan is also known as *Giardia intestinalis* or *Giardia duodenalis*. It was first discovered by Antony van Leeuwenhoek. A parasite known to cause Giardiasis. Its habitat is at the duodenum or at the upper jejunum. The trophozoite looks like a tennis racket. It is 12 to 15µm long, rounded at the anterior end and pointed at the posterior end. The organism is dorso-ventrally flattened and convex on the dorsal surface. The flattened ventral surface bears a concave, bilobed adhesive disc. The cyst is oval in shape and is 8 to 12µm by 7 to 10µm in size. Newly formed cysts have 2 nuclei but older ones have 4 nuclei.

Trichomonas spp. There are 3 trichomonads in humans, *Trichomonas tenax, Trichomonas vaginalis* and *Pentatrichomonas* hominis. They have been considered to be similar morphologically until recently when differences between the *P. hominis* and the other 2 have been recognized. The trophozoite is the only stage in its life cycle. They are 7 to 32µm long and 5 to 12µm wide. The *Trichomonas tenax* was first discovered by O.F. Muller. It is an oblong cell 5 to 16µm long by 2 to 15µm wide with size varying according to strain. There are 4 free anterior flagella with a fifth flagellum curving back along the margin of an undulating membrane and ending posterior to the middle of the body. A costa arises in the kinetosome complex. This costa distinguishes this family from other families in its order. This organism only lives in the mouth and divides by binary fission. *Trichomonas vaginalis* was first found by Donne in purulent vaginal secretions and in male urogenital tract secretions. The organism is similar to *T. tenax* but differes in size as it is larger, 7 to 32µm long by 5 to 12µm wide. Its undulating membrane is shorter and there are more granules along its axostyle and costa. *Pentatrichomonas hominis* is a harmless commensal of the intestinal tract. This was founded by Davaine. Most specimens bear 5 anterior flagella. Its size is 8 to 20µm by 3 to 14µm/ A recurrent flagellum exists in all 3 species alongside the undulating membrane except that in *P. hominis*, the recurrent flagellum is long, free flagellum past the posterior end of the body.

Trypanosoma spp. These organisms are referred as hemoflagellates as they live in the blood or certain other tissues of all classes of vertebrates. Some trypanosomes are nonpathogenic but others produce severe disease especially the **sleeping sickness**. The *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause African sleeping sickness in humans and *Trypanosoma brucei brucei* causes a related disease in domestic animals. *Trypanosoma cruzi* causes Chaga's disease in humans in Central and South America. All species of Trypanosomatidae have single nucleus and are either elongated with single flagellum or rounded with a very short non-protruding flagellum. Various species pass through amastigote, promastigote, epimastigote and/or trypomastigote stages.

Leishmania spp. This organism causes leishmaniasis. Part of their life cycle occurs in the gut of the fly where they assume a promastigote stage and the remainder of their life is found in the vertebrate tissues where only the amastigote form is found.

Figure 3-1 shows the developmental stages of the *Trypanosoma spp.* and *Leishmania spp.*



Activity 2
The Flagellates

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the stages of each specimen viewed in the laboratory and properly label each with its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II. Tabulate the distinguishing features, locomotion, and mode of nutrition and reproduction of each specimen viewed. (Student may use separate paper)

III. Fill in the table below. Summarize the characteristics of the hemoflagellates.

Parasite	Morphologic Features	Developmental Stages	Vector	Disease
Trypanosoma brucei gambiense				
Trypanosoma brucei rhodesiense				
Trypanosoma cruzi				
Leishmania spp.				

4

The Amebas

This activity gives the students the opportunity to learn and explore the Subphylum Sarcodina – The Amebas. These organisms may be naked or enclosed in a shell. A few species in this group are found to be parasitic while others are commensals.

In this exercise, students will familiarize themselves with the organisms under the Subphylum Sarcodina that are known to be parasitic in humans. There are 6 species of amebas belonging to 4 genera and are known to be parasitic in man while there are 2 species that are free-living and are accidental parasites of man.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Subphylum Sarcodina

Materials

Compound microscope

Specimen slides (*Entamoeba histolytica, Entamoeba coli, Entamoeba gingivalis, Dientamoeba fragilis, Endolimax nana, Iodamoeba buetschlii, Naegleria spp., Acanthamoeba spp.*)

Procedure

1. View the specimens, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section1. General Ameba Anatomy

Ameba organisms have the pseudopodia (false foot) as their locomotory organelle. The organism has an irregular shape. It is bounded by an elastic cell membrane, the plasmalemma. The cytoplasm enclosed by the plasmalemma is differentiated into a thin peripheral rim of ectoplasm and an inner, more fluid endoplasm. A prominent nucleus in the endoplasm is found. Organisms may also have contractile and food vacuoles present.

Section 2. The Amebas

Entamoeba histolytica This organism is pathogenic and is responsible for the disease called amebiasis, amebic dysentery and amebic hepatitis. Several successive stages occur in the life cycle of this organism: the trophozoite, precyst, cyst, metacyst and metcystic trophozoite. The trophozoite is referred as the active vegetative stage. The organism size is as small as 10µm or as large as 60µm but most trophozoites fall into the range of 20 to 30 µm. The wide, clear, refractile, hyaline ectoplasm is sharply separated from the endoplasm and where it constitutes 1/3 of the entire animal. The RBC may be present in the granular endoplasm of the animal. The organism has a

single eccentric nucleus. The precystic amebas are colorless, round or oval cells. They are smaller than the trophozoite but larger than the cyst. The cyst is round or oval cells with slightly asymmetrical hyaline bodies. They are 10 to 20µm in diameter with smooth, refractile, thick, non-staining wall about 0.5µm. The cytoplasm of young cysts contains vacuoles with glycogen, sausage-shaped bars with rounded ends. Immature cysts have single nuclei while the mature infective cyst has 4 smaller nuclei, rarely more. This organism lives in the wall and lumen of the colon especially in the cecal and sigmoidal regions

Entamoeba coli. This organism is a commensal that lives in lives in the large intestine. It has a similar life cycle and is mistaken for the *Entamoeba histolytica*. The trophozoite is 15 to 50µm in diameter and its cyst vary in diameter from 10 to 33µm.The endoplasm of this organism contains ingested bacteria and debris and rarely contains RBC. It has a narrower, less differentiated ectoplasm. The pseudopodium is broader and blunter. The organism is characterized by a heavier, irregular peripheral chromatin and large eccentric karyosome in its nucleus. The organism has larger cysts with more granular cytoplasm, slender, splinter-like chromatoidal bodies and may contain as many as 8 nuclei.

Entamoeba gingivalis. This organism is a non-pathogenic inhabitant in the mouth. They are usually found in the tartar of teeth and in gingival pockets. Only the trophozoite has been found. The trophozoite is 10 to 20µm in diameter. Food vacuoles are numerous in the endoplasm of this organism containing cellular debris, bacteria and occasionally blood cells. The spheroid nucleus contains a small, nearly central endosome.

Endolimax nana. This commensal organism lives in the large intestine mainly at the cecum and feeds on bacteria. The trophozoite measures 6 to 15µm in diameter. The ectoplasm is thin and surrounds the granular endoplasm. The pseudopodium is short and blunt. The nucleus is small and contains a large centrally or eccentrically located endosome. Large glycogen vacuoles are present and food vacuoles contain bacteria, plant cells and debris. The mature cyst is 5 to 14µm in diameter and contains 4 nuclei.

Iodamoeba buetschlii. This organism is the commonest ameba in swine that is known to infect humans, other primates and pigs. The trophozoite is 9 to 14µm long but may range from 4 to 20µm. It has short, blunt pseudopodia. This organism has a characteristic nucleus that is relatively large and vesicular, containing a large endosome. Food vacuoles contain bacteria and yeasts. The mature cyst is uni-nucleated and oblong measuring 6 to 15µm long. It has a large conspicuous glycogen vacuole stains deeply with iodine (iodinophilous vacuole).

Dientamoeba fragilis. This organism is a small parasitic amebo-flagellate thriving the intestinal tract and only found in a trophozoite form. The trophozoites are delicate and easily disintegrate in feces or water. They are 6 to 12µm in diameter. A single broad pseudopodium is usually present. The organism is binucleated that resembles the trichomonads antigenically and ultrastructurally. They are only recognized in fresh liquid or soft stools. The organism may be circular in appearance but may give a stellate appearance. The endosome of this organism is eccentric, sometimes fragmented or peripheral in the nucleus. Individuals infected with this organism may produce moderate, persistent diarrhea and gastrointestinal symptoms but in some individuals, they may show no apparent harmful effect.

Naegleria fowlerii. This organism is also known as *Naegleria aerobia*. It is a major cause of the disease called primary amebic meningoencephalitis (PAM). The transformation of the ameboid form to the flagellated form is rapid. The flagellated form bears 2 long flagella at one end, elongated and does not form pseudopodia. The ameboid stage has a single blunt pseudopodium (lobopodium). The nucleus of

organism is vesicular and has large endosome and peripheral granules. A contractile vacuole is present in free-living forms. Food vacuoles contain bacteria in free-living stages but are filled with host cell debris in parasitic forms.

Acanthamoeba spp. This organism is a facultative parasite in humans. The free-living forms are similar to the *Naegleria spp.* except that the flagella are not known to be produced. This organism cannot tolerate water as hot as the *Naegleria spp.* can. They usually cause chronic infections of the skin or central nervous system among the immune-compromised patients. They may even cause Acanthomoeba meningitis and corneal ulceration leading to blindness and chronic granulomatous infections of the skin. The trophozoites have small spiky acanthopodia.

Activity 3
The Amebas

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the stages of each specimen viewed in the laboratory and properly label each with its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II. Tabulate the distinguishing features, locomotion, and mode of nutrition and reproduction of each specimen viewed. (Student may use separate paper)

III. How would you describe and explain ameboid movement?

5

The Apicomplexa

This activity gives the students the opportunity to learn and explore the Phylum Apicomplexa. All apicomplexans are parasitic and possess an apical complex structure. These organisms have a single type of nucleus and no cilia or flagella, except for the flagellated microgametes in some groups. The phylum contains 2 classes: Gregarinea, primarily parasitizing invertebrates and Coccidea, parasitizing both vertebrates and invertebrates. All apicomplexans have a life cycle stage that functions in transmission as a "spore" and the development of the sporozoites is completed within an invertebrate vector.

In this exercise, students will familiarize themselves with the organisms under the Phylum Apicomplexa particularly those under the Class Coccidea.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Phylum Apicomplexa

Materials

Compound microscope
Specimen slides (*Plasmodium spp., Isospora belli, Cryptosporidium spp., Toxoplasma gondii, Blastocystis hominis, Monocystis sp., Gregarina sp.)*

Procedure

1. View the specimens, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section1. General Feature

These sporozoans are entirely endoparasitic. They lack special locomotor organelles, although some can move by gliding or changing their body shape and some may have flagellated gametes. The generalized life cycle processes of the Apicomplexans are schizogony (asexual process), gamogony (sexual reproduction) and the sporogony (multiple fission of a zygote).

Section 2. The Apicomplexans

Plasmodium spp. This is the genus of sporozoan parasites causing malaria. This organism requires 2 hosts, one a vertebrate and the other an invertebrate. The organisms are transmitted to a human by a bite of a female *Anopheles* mosquito, which introduces the sporozoites. The sporozoites travel to the liver cells to undergo merogony and in the process become merozoites. Merozoites continue merogony in liver cells or infect RBC to undergo schizogony (production of schizonts,

asexual cleavage multiplication). These schizonts can undergo further schizogony by infecting new RBC or produce gametocytes that are eventually taken in by a hungry Anopheles mosquito. The summarized characteristics of the *Plasmodium spp.* are shown in Table 5-1.

Table 5-1. Characteristics of the *Plasmodium spp.* in humans

Stage	***P. vivax***	***P. falciparum***	***P. ovale***	***P. malariae***
Early trophozoite	1/3 diam of RBC; chromatic dot heavy; vacuole prominent	1/5 diam of RBC; chromatin dot small; 2 dots frequentl marginal forms frequent	Similar to *P. vivax* and *P. malariae*	Single, heavy chromatin dot; cytoplasmic circle often smaller, thicket, heavier than *P. vivax*; vacuole fills in early
Growing trophozoite	Pseudopodia common; 1 or more food vacuoles	Stage not usually seen in peripheral blood smear	Compact, little vacuolation	Cytoplasm usually compact; little or no vacuole; sometimes in band form across RBC
Late trophozoite	Large mass of chromatin; fine brown hemozoin; almost fill RBC	Stage not usually seen in peripheral blood smear		Chromatin often elongated, less definite in outline than in *P. vivax*; cytoplasm dense, rounded, oval or band shaped almost filling the RBC

Stage	***P. vivax***	***P. falciparum***	***P. ovale***	***P. malariae***
Hemozoin	Short delicate rods, irregularly scattered; yellowish brown	Granular, has tendency to coalesce; coarse in gametocytes	Hemozoin lighter than in *P. malariae*; similar to *P. vivax*	Granules rounded; larger, darker than in *P. vivax*; tendendcy to peripheral arrangement
Appearance of erythrocyte	Larger than normal, Schuffner's dots at all stages but young rings; multiple infection occasional	Normal size; Mauer's spots common in cells with later trophozoites (not usually seen in peripheral blood)	Schuffner's dots often present in ring & later stages; RBC larger than normal, oval, often with irregular edge	About normal or slightly smaller; stippling rarely seen; multiple infections rare
Schizont	12-24 merozoites	8-24 or more merozoites; rare in peripheral blood	4-16 but usually 8 merozoites	6-12 but usually 8 or 10 merozoites in rosette or cluster arrangement; often found in peripheral blood
Microgametocyte	Rounded or oval; almost fill the RBC; dark hemozoin throughout cytoplasm chromatin diffuse, no vacuoles	Crescent or sausage shaped; length about 1.5x diam. RBC, chromatin diffuse, hemozoin granules in central portion, cytoplasm pale blue	Similar to *P. vivax* but somewhat smaller; mature macrogametocyte fills infected cell; microgametocyte smaller	Similar to *P. vivax* but smaller; pigment more conspicuous
Macrogametocyte	Cytoplasm stains darker blue; chromatin more compact,	Size and shape same as microgametocyte; chromatin more compact; cytoplasm darker; hemozoin concentrated		Pigment abundant; round, dark brown granules; coarse than *P. vivax*

Toxoplasma gondii. This organism causes toxoplasmosis. The tachyzoites or sporocyst is the asexual form that is pyriform in shape, measuring 3-6µm and contains a nucleus and some organelles. The oocyst is 10 to 13µm by 9 to 11µm and is similar in appearance to those of the Isosporan species.

Cryptosporidium spp. This organism causes cryptosporidiosis commonly occurring in patients with AIDS. This organism's spherical oocysts are 4 to 5µm wide, highly refractile, contain 1 to 8 prominent granules. Sporocysts are absent. Each oocyst contains 4 slender, fusiform sporozoites.

Isospora bellii. This organism's oocyst contains 2 sporocysts, each with 4 sporozoites. This organism causes severe disease especially in AIDS patients.

Blastocystis hominis. This is an intestinal commensal unicellular protozoan commonly found among immunocompromised patients. This organism is strictly anaerobic and varies morphologically (vacuolar, granular, amoeboid and cyst forms) and in size where it measures between 2 to 200µm. The organism is commonly transmitted through an oral-fecal route. The life cycle of the organism begins with the ingestion of the cyst form. After its ingestion, the cyst develops to other forms, which may all re-develop into cyst forms.

Monocystis sp.
This organism thrives on the seminal vesicles of the earthworm, *Lumbricus terrestris*. The parasite enters the earthworm gut via a sporozoite-laden sporocyst. Juveniles penetrate the gut wall and proceed to the dorsal aorta where it eventually is pumped by the primitive heart to the seminal vesicles. Trophozoites feed on sperm morula, undergo syzygy and produce viable sporocysts that are released through the earthworm genital pore.

Gregarina sp. This organism is a coelozoic parasite of orthopterans, usually occurring along the digestive tract of cockroaches and grasshoppers. The body is divided into anterior protomerite and posterior deutomerite, with nucleus located at the latter. An epimerite may be present for attachment of the parasite to the host cell, and disappears upon release in the gut lumen. Sporont pairs in a process called syzygy and eventually develop into gamont. The orientation of gamonts during syzygy differ depending on the species (e.g. side-to-side and head-to-tail). Each pair of gamont becomes surrounded by gametocyst wall followed by division through multiple fission producing micro- and macrogametes. Pairs of the gametes fuse to form zygotes. Individual zygotes become surrounded by oocyst wall that develop into infective sporozoites.

Activity 4
The Apicomplexa

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the stages of each specimen viewed in the laboratory and properly label each with its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II. Tabulate the distinguishing features, definitive host, intermediate host, and disease of each specimen viewed. (Student may use separate paper)

6

The Ciliates

This activity gives the students the opportunity to learn and explore the Phylum Ciliophora – The Ciliates. These organisms have an outer elastic membrane, the pellicle surrounded by hair-like cilia enabling the organism to move. Most ciliates are free-living, but many are commensals of vertebrates and invertebrates. Only a few of these organisms are parasitic.

In this exercise, students will familiarize themselves with the organisms under the Phylum Ciliophora particularly those under the Class Litostomatea.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimen

Materials

Compound microscope
Specimen slides (*Balantidium coli)*

Procedure

1. View the specimen, noting the key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section1. General Feature

These organisms move using cilia. The cilia are generally short projections from the cell surface. These structures are generally shorter and more numerous than flagella. An oral groove extends from the mouth into the body. The cytoplasm is made up of 2 zones: ectoplasm and endoplasm. Outside the ectoplasm is a pellicle and underneath the pellicle is a delicate plasma membrane. Contractile vacuoles are located in each end of the body. The organism contains 2 nuclei, a large macronucleus and a small micronucleus.

Section 2. The Ciliates

Balantidium coli. This parasite causes Balantidiasis. This organism is the largest protozoan parasite found in humans. The trophozoites are oblong, spheroid or more slender, measuring at 30 to 150µm long by 25 to 120µm wide. Cysts measure at 40 to 60µm in diameter. The 2 nuclei, macronucleus and micronucleus are evident in both the cyst and trophozoite forms. The macronucleus is a large, sausage-shaped structure and the single micronucleus is small. Two contractile vacuoles are situated near the middle of the body and the other near the posterior end. The cytostome is at the anterior end. Food vacuoles contain RBC, cell fragments, and starch granules, fecal and other debris.

Activity 5
The Ciliates

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the specimen's cyst and trophozoite forms and properly label each with its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II. What are the specific functions of the macronucleus and micronucleus?

III. Compare the appearance and rate of locomotion in amoeboid, flagellated and ciliated organisms observed in this and the previous exercises.

IV. What are the other structures and the functions of these structures that you see in the cytoplasm of the *Balantidium coli*?

7

Platyhelminthes

This activity gives the students the opportunity to learn and explore the Phylum Platyhelminthes. The Platyhelminthes or flatworms are dorso-ventrally flattened organisms. They are referred as acoelomates since these are animals that do not have coelom (body cavity). This group of organisms is important as they include the free-living planarians, and the parasitic tapeworms and flukes.

As acoelomates, these organisms are said to be advanced over the radiate animals in several ways. These organisms have (1) bilateral symmetry, (2) tissues well defined and organized into complex functional organs, (3) nervous system is highly organized, (4) excretory system of specialized flame cells and tubules, and (5) gastrovascular system.

Section 1. General Features

Flatworms are group of animals that have three well-defined embryonic tissues layers: ectoderm, endoderm, and mesoderm. These embryonic tissue layers enable the organism to have a variety of tissues and organs. The respiratory, circulatory and skeletal systems are lacking for these organisms.

Section 2. Classification in the earlier literature

These phyla are subdivided into 4 classes based on earlier literatures. These classes are the following:

Class Turbellaria. These organisms are mostly free-living flatworms. They are not parasitic and their body is dorso-ventrally flattened. They are found under rocks, leaves and debris in freshwater ponds and creeks. These organisms are known to have ciliated epidermis. Example: *Dugesia tigrina*.

Class Monogenea. These are the monogenetic flukes. The adult bodies of these organisms are covered with a syncytial tegument without cilia. They are leaf-like to cylindrical in shape. Their posterior attachment organ may have hooks, suckers, or clamps, and are usually in combination. All monogenetic flukes are parasitic and are mostly found on the skin or gills of fishes. They inhabit a single host. They are monoecious. These organisms usually have a free-swimming ciliated larva. Examples: *Polystoma, Gyrodactylus*.

Class Trematoda. These are the digenetic flukes. The adult bodies of these organisms are covered with nonciliated syncytial tegument. They are leaf-like to cylindrical in shape. They usually have oral and ventral suckers with no hooks. The development is indirect where the first host is a mollusk and the final host is usually a vertebrate. These organisms are parasitic in all classes of vertebrates. Examples: *Fasciola, Clonorchis, Schistosoma.*

Class Cestoidea. These are the tapeworms. The adult bodies of these organisms are covered with nonciliated syncytial tegument. The scolex of these organisms has suckers or hooks or the combination of both that are used for attachment. They have long ribbon-like bodies usually divided into several proglottids. They do not have digestive organs. They are parasitic in the digestive tract of all classes of vertebrates. Their first host may either be an invertebrate or a vertebrate. Examples: *Taenia, Diphyllobothrium*.

Section 3. Activity

Class Turbellaria (free-living flatworms)

1) Examine the prepared slide of *Planaria*. The planaria is a dorso-ventrally flattened and elongate organism. It has a triangular head bearing two black eyespots. Find the **light-sensitive "eyes."** It has 2 lateral projections, the auricles. The mouth is located mid-ventrally. Continuous with the mouth is the highly muscular reversible pharynx. Observe the three-branched intestine, one directed anteriorly and the other 2 directed posteriorly. The ventral surface of the planaria has numerous cilia. Sketch the planaria and label all the parts observed in this specimen.
2) Examine a slide with a cross-section of a planarian. Find the **intestine** and the **mesenchyme**, a loose aggregation of cells that fills the space between the outer body wall and internal organs. Note the total absence of a body cavity.

Class Trematoda (Digenetic flukes)

1) Examine the prepared slides of *Fasciola* (sheep liver fluke) and *Schistosoma* (blood fluke). The *Fasciola* is a parasitic flatworm inhabiting the bile ducts of sheep. Its body appears leaf-like. It has 2 suckers: anterior sucker, which surrounds the mouth and a ventral sucker or acetabulum located a little distance away from the anterior sucker. A pharynx, esophagus and a 2-branched intestine immediately follows the mouth. Male and female organs are found in a single liver fluke hence, they are referred as hermaphroditic. A black convoluted organ located near the acetabulum is the uterus. The testis appears like a tree-like network occupying more than half of the body. Lateral margins of the body contain the yolk glands.
   The *Schistosoma* is a parasitic blood fluke inhabiting the venules of the small intestine. Sexes are separate with the male being heavier and broad with a large ventral groove otherwise known as the gynecophoric canal. The gynecophoric canal embraces the long and slender female during copulation. Sketch both trematodes and properly label all the parts identified.

Class Cestoidea (Tapeworms)

Examine the prepared slides of *Taenia* (tapeworm). The body of the tapeworm appears like a long segmented ribbon. However, there is no true segmentation despite the segmented appearance. It has a small head called a scolex, which bears hooks or no hooks on an elevated rostellum. The short neck joins the scolex to the long strobila, which consists of numerous proglottids. The farther away the proglottid from the neck, the more mature is the segment. The closer the proglottid from the neck, the more immature is the segment. It is at the neck where new proglottids are formed by transverse budding. Sketch the specimen and properly label all the parts identified.

8

Trematodes

This activity gives the students the opportunity to learn and explore the Class Trematoda – The Digenetic Flukes. The trematodes are all parasitic organisms found in a variety of animals. Many of these organisms have 3 different hosts in their life cycle. Flukes exhibit a variety of shapes and sizes. Most flukes are dorso-ventrally flattened and oval in shape but some are thick and wide.

In this exercise, students will investigate the diversity of the Class Trematoda.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Class Trematoda

Materials

Compound microscope

Specimen slides (*Clonorchis sinensis, Opisthorchis sp., Schistosoma spp., Fasciola spp., Fasciolopsis buski, Paragonimus westermanii, Heterophyes spp., Euparyphium ilocanum*)

Procedure

1. View the specimens, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section 1. General Features

Digenetic flukes are group of animals that have complicated life cycles involving an alternation of generations or hosts. Their primary host may be terrestrial and or aquatic mollusks while their reservoir hosts may be other animals. They inhabit the circulatory system, intestine, liver and lungs. Sizes of these organisms vary from minute to large. Most species are known to be hermaphroditic. These organisms alimentary canal is incomplete with the mouth leading to a short pharynx and esophagus bifurcating to a pair of blind intestinal ceca (simple or branched). These organisms reproductive system is highly developed. Eggs are operculated except for the schistosomes. The life cycle involves: egg, larval (miracidium, sporocyst, redia, cercaria, metacercaria) and adult.

Section 2. Trematodes

Fasciola hepatica. This organism is also referred as sheep liver fluke. It inhabits the liver. The eggs are large, ovoid and operculated measuring 130 to 150µm long by 63 to 90µm wide. The adult is large, broad, leaf shaped, flat, cephalic cone with shoulders pointed posteriorly and wide anteriorly. The oral sucker is small and the ventral sucker is large. The tegument is covered with large, scale-like spines. The organisms

intestinal ceca is highly branched and extend to near the posterior end of the body. Testes are large and greatly branched, arranged in tandem behind the ovary. The smaller, branched ovary lies on the right side shortly behind the ventral sucker and the uterus is short, coiling between the ovary and the preacetabular cirrus pouch. Vitelline follicles are extensive filling most of the lateral body and are confluent behind the testes. This organism is the cause of Fascioliasis or "liver rot."

Fasciola gigantica. This organism is also referred as a liver fluke. The organism is longer and more slender but is very similar to the *Fasciola hepatica* morphologically, biologically, and pathologically. The only difference between the two is on the necessary snail hosts they inhabit.

Fasciolopsis buski. This organism is a common parasite in pigs but is also known to parasitize humans. This organism is the cause of Fasciolopsiasis. This organism inhabits the small intestine of its definitive host. The egg is operculated measuring 130 to 140 µm long by 80 to 85µm wide. Eggs are almost identical to the *Fasciola hepatica.* The adult is elongated, oval, and measures 20 to 75mm long by up to 20 mm wide. No cephalic cone or "shoulders" is present. The ventral sucker is larger than the oral sucker and is situated close to the oral sucker. This organism has unbranched ceca. The branched testes are in tandem at the posterior half of the worm. The ovary is branched and lies at the midline anterior to the testes. Vitelline follicles are extensive filling most of the lateral parenchyma all the way to the posterior end. The uterus is short.

Paragonimus westermanii. This organism is referred as the Oriental lung fluke and is known to be the cause of Paragonimiasis or Pulmonary distomiasis. This organism inhabits the lungs. The egg is oval, golden brown, with flattened operculum, and thick abopercular end measuring 80 to 118µm long by 48 to 60µm wide. The adult worm is 7.5 to 12 mm long and 4 to 6 mm wide. They are very thick, and reddish brown in color. The tegument is densely covered with scale-like spines. The oral and ventral suckers are equal in size. The excretory bladder extends from posterior end to near the pharynx. The lobated testes are at same level, located at junction of the posterior fourth of the body. A cirrus and cirrus pouch are absent. The genital pore is postacetabular. The ovary is lobated and found to the left of midline, slightly postacetabular. Uterus is tightly coiled into a rosette at the right of the acetabulum and opens to the common genital atrium with the vas deferens. Vitteline follicles are extensive in the lateral fields.

Clonorchis sinensis. This organism is referred as the Chinese liver fluke or the Oriental liver fluke. It inhabits the liver and or the bile ducts. The egg is operculated, convex, small protuberance looking like a small knob at the abopercular end. Eggs measure 26 to 30µm long. The adult worm measures 8 to 25mm long by 1.5 to 5mm wide. The tegument lack spines. The oral sucker is slightly larger than the ventral sucker, which is about a fourth of the way from the anterior end. The 2 testes are large, branched and are in tandem near the posterior end. The organism has a large serpentine seminal vesicle leading to the genital pore. Cirrus and cirrus pouch are absent. The ovary is small and is tri-lobed. The oviduct receives 2 yolk ducts from yolk glands located laterally in the worm's body. The oviduct connects to an ootype surrounded by a loosely organized Mehlis gland. Note that the ootype and the Mehlis gland are difficult to see. Vitelline follicles are small and dense and are confined to the level of the uterus.

Opisthorchis sp.
Heterophyes heterophyes. This is a small fluke whose adults are 1 to 1.7mm long and 0.3 to 0.4mm wide. The body is covered with slender scales and most numerous at the anterior end. The oral sucker is slightly smaller than the ventral sucker and is located at the end of the first third of the body. The 2 oval testes lie side by side near the posterior end of the body. The ovary is small, medio-anterior to the testes.

A seminal receptacle and Laurer's canal are present. Uterus coils between the ceca and constricts before joining the ejaculatory duct to form the short common genital duct, which opens into the genital sinus. Lateral vitteline follicles are few and are confined at the posterior third of the worm. Eggs are 28 to 30 µm long by 15 to 17µm wide. Eggs closely resemble those of *Clonorchis sinensis.*

Euparyphium ilocanum. This organism was formerly known as *Echinostoma ilocanum.* Eggs are operculated, large and are typically about 100 µm long by 60µm wide. The adult has circumoral collar spines arranged in 2 rows. The number of spines has 49 to 51 spines, with five or six corner spines on each side. The double row of spines is continuous dorsally and the testes are deeply lobate. The genital pore is median and preacetabular, and the cirrus pouch is large, passing dorsal to the ventral sucker. The short uterus has an ascending limb only.

Schistosoma spp. These organisms are referred as blood flukes and are known as the cause of schistosomiasis or commonly known as "bilharzia." The schistosomes are of medical importance. There are 3 species of schistosomes: *Schistosoma mansoni, Schistosoma haematobium,* and the *Schistosoma japonicum.* The *Schistosoma mansoni* inhabits primarily the venules draining the large intestine. The *Schistosoma haematobium* inhabits the venules of the urinary bladder. The *Schistosoma japonicum* inhabits the venules of the small intestine. The males are stouter than the females and possess a ventral gynecophoric canal where the thinner female is embraced by the male. These organisms possess both an oral sucker and a ventral sucker. Eggs are elliptical shape, bearing a sharp spine (spine is terminal in *Schistosoma haematobium,* lateral in *Schistosoma mansoni,* lack a spine or knob-like protuberance laterally in *Schistosoma japonicum*).

Activity 6
The Trematodes

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the stages of each specimens belonging to the Class Trematoda and properly label all its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II.Fill in the table and compare the morphological characteristics of the *Schistosoma spp.*

Characteristic	*Schistosoma haematobium*	*Schistosoma mansoni*	*Schistosoma japonicum*
Tegumental papillae			
Number of testes			
Position of ovary			
Uterus			
Vitellaria			
Eggs			
Habitat in Definitive Host			
Intermediate Host			
Infective stage			

III. How does the *Schistosoma* differ from that of the other digeneans in terms of their life cycle?

9

Cestodes

This activity gives the students the opportunity to learn and explore the Class Cestoidea – The Tapeworms. The tapeworms are endoparasitic organisms. Most of these organisms require two hosts of different species, with the adult tapeworm living in the digestive tract of a vertebrate.

In this exercise, students will investigate the diversity of the Class Cestoidea particularly those belonging to the Orders Pseudophyllidea and Cyclophyllidea.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Class Cestoidea
3. Differentiate the Pseudophyllidean and Cyclophyllidean tapeworms

Materials

Compound microscope
Specimen slides (*Taenia spp., Diphyllobothrium latum, Hymenolepis nana, Hymenolepis diminuta, Dipylidium caninum, Echinococcus granulosus*)

Procedure

1. View the specimens, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section 1. General Features

Tapeworms are group of animals that possess holdfast organs called a scolex, a region of growth called a neck, a body called a strobila that is made up of several segments of proglottids. Sizes of these organisms vary. Each segment or proglottid is indicated to have a complete reproductive unit with male and female sex organs. Proglottids of these organisms are differentiated distinctly into immature, mature and gravid forms. The life cycle of these organisms involve an egg or an oncosphere, larva (coracidium, procercoid, plerocercoid, cysticercoid, cysticercus) and adult stages.

Section 2. Pseudophyllidean and Cyclophyllidean tapeworms

Pseudophyllidean and Cyclophyllidean are the two Orders of the Class Cestoidea that are known to parasitize man. The Pseudophyllidean tapeworms are those organisms that have spatulate scolex with bothria while the Cyclophyllidean tapeworms are those organisms that have globular scolex with 4 cup-like suckers. Pseudophyllidea larvae look fairly similar to the adult while the Cyclophyllidea larvae look differently from their adult forms. Other differences between the 2 orders are shown below in Table 9-1.

Table 9-1. Differences between the Pseudophyllidean and Cyclophyllidean tapeworms

Differentiating Feature	Pseudophyllidea	Cyclophyllidea
Scolex	Two sucking grooves (bothrial)	Four muscular suckers (acetabula)
Genital pore	Center of each proglottid	Margin(s) of each proglottid [may be located On both sides in an irregular pattern (Taenia spp); all on the same side (Hymenolepis spp); or each proglottid may have a pore on each side (dipylidium caninum)]
Uterine pore	Center of proglottides on ventral surface	Absent; uterus ends blindly
Uterus (gravid)	Relatively long and coiled	Saclike, highly branched
Eggs	Operculate	Nonoperculate
Oncosphere	Ciliated (coracidium)	Nonciliated
Larvae	Procercoid and plerocercoid; both forms solid	Cysticercoid, cysticercus, hydatid; all forms cystic

Section 3. Cestodes

Diphyllobothrium latum. This organism is also referred as the fish tapeworm causing Diphyllobothriasis. The most common infection of this parasite is due to the ingestion of raw or inadequately cooked fish containing the plerocercoid larva or sparganum. This organism belongs to the Order Pseudophyllidea where the scolex is an unarmed spatulate with dorsal and ventral sucking organs called a bothrium. The adult worms are usually ivory white in color, measuring 3 to 10m or more in length and may have 3000 proglottids. This organism has an unsegmented neck, and the mature and gravid segments form 4/5 of the entire worm. The mature segments are broader than long containing both male and female sex organs. The uterus is in rosette formation and opens in uterine pore situated at the ventral side of the segment. Eggs are broadly ovoid, operculated and are golden brown in color.

Dipylidium caninum. This organism is referred as the dog tapeworm causing Dipylidiasis. The organism has a rhomboidal scolex with 3 prominent oval suckers and retractile conical rostellum armed with 30 to 150 rose-thorn-shaped hooks arranged in transverse rows. The mature proglottid is vase-shaped and double set of reproductive organs and the genital pore is found midway on each lateral margin. The gravid proglottid is packed with membranous eggs. Eggs are globular in shape. The egg contains an oncosphere with 6 hooklets.

Hymenolepis nana. This organism is referred as the *Vampirolepis nana*. This organism is the cause of Hymenolepiasis and is also called as the dwarf tapeworm. The adult worms measure 10 to 45mm long by 0.5 to 1mm wide. The scolex bears a retractable rostellum armed with a single circle of 20 to 30 hooks. The scolex also has 4 suckers. The neck is long and slender. They may have 100 to 200 segments that are wider than they are long. The eggs generally measure between 30 to 47µm in diameter. Eggs are round to oval and contain a 6-hooked oncosphere. Polar filaments lie between the egg shell and the oncosphere for this organism. The genital pores are unilateral (one side of the segment). Each mature segment contains 3 testes.

Hymenolepis diminuta. This organism is referred as the rat tapeworm causing Hymenolepiasis. Morphologically, this organism resembles that of the *Hymenolepis nana.* The scolex of this organism has 4 suckers, a retractible rostellum that is unarmed. It grows to be about 20 to 60 cm or more in length.

It may even consists of up to 1000 proglottids that are approximately four-times as wide as they are long. Each proglottid contains 3 round testes, a bi-lobed ovary, a compact vitelline gland, a large uterus opening to a lateral genital pore. Eggs are round or slightly oval that is yellowish brown in color. Eggs are larger at 60 to 80µm in diameter than the eggs of the *Hymenolepis nana.* The egg contains an oncosphere with 6 hooklets.

Echinococcus granulosus. This organism is a minute tapeworm that inhabits the intestines of dogs and other Canidae. This organism is the cause of the Echinococcus disease or unilocular echinococcosis or otherwise referred as the Hydatid disease. The adult worm measures about 3 to 6mm long. It has a typical Taeniid scolex, a short neck, and only 3 proglottids.The scolex has a nonretractable rostellum bearing a double crown of 28 to 50 hooks and 4 suckers. The anteriormost segment is the immature, the middle segment is the mature and the terminal segment is the gravid. The gravid uterus is an irregular longitudinal sac. Eggs are similar to those of the *Taenia* egg.

Taenia saginata and *Taenia solium.* These 2 *Taenia spp.* are the common tapeworms affecting man. The *Taenia saginata* is also referred as the beef tapeworm or hookless tapeworm causing Taeniasis saginata. The adult worm measures 5 to 10m long to 25 m or more in certain cases. It attaches to the mucosa of intestines by means of their hold fast organ called a scolex. Their scolex is similar to the *Taenia solium* except for the absence of rostellum, spines or hooklets. There are more segments present in the *Taenia saginata* as compared to the *Taenia solium*. The lateral uterine branches of uterus in the gravid segments are 15 or more. Each gravid proglottid may contain up to 80,000 eggs. Its larval stage is the Cysticercus bovis.

The *Taenia solium* is also known as the pork tapeworm or hook tapeworm causing Taeniasis solium. This parasite is also responsible for the occurrence of human cysticercosis due to the ingestion of the Cysticercus cellulosae. The adult worm measures about 2 to 3m long. The scolex is globular in shape with 4 cup shaped suckers with rostellum armed with double row of hooklets. The neck is short measuring 5 to 10mm long. There are about 800 to 1000 proglottids and the common genital pore is marginal and the gravid uterus consists of a median stem with 5 to 13 lateral uterine branches on each side. The gravid segment may contain 30,000 to 50,000 eggs. Eggs are spherical, brown in color, measuring 31 to 56µm in diameter. There are 2 radially striated shells: outer shell that is thin and an inner shell that is brown, thick and striated. Inside the egg it contains an embryo or oncosphere with 6 hooklets. The larval stage or bladder worm known as the Cysticercus cellulosae is dense milky spot at 1 side where an invaginated scolex with hooks and suckers are found.

Activity 7
The Cestodes

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the stages of each specimens belonging to the Class Cestoidea and properly label all its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II.Fill in the table and compare the morphological characteristics of the *Taenia spp.*

Characteristic	*Taenia saginata*	*Taenia solium*
Size		
Scolex		
Mature proglottid – ovary		
Testes		
Gravid proglottid – uterine branches		
Larval stage		
Intermediate host		
Definitive host		
Infective Stage		
Disease		

III. What is Sparganosis?

IV. Draw and indicate the relative sizes of the eggs of trematodes and cestodes. Arrange them in order according to their sizes. (Student may use a separate paper)

V. Fill in the table with the appropriate sketches of the following specimens.

Structure	*Taenia solium*	*Taenia saginata*	*Diphyllobothrium latum*	*Hymenolepis nana*	*Hymenolepis diminuta*
Proglottid					
Scolex					
Structure	*Dipylidium caninum*	*Echinococcus granulosus*	Structure	*Taenia solium*	*Taenia saginata*
Proglottid			Larva		
Scolex			Egg		

10

Nematoda

This activity gives the students the opportunity to learn and explore the Phylum Nematoda – The Roundworms. The Nematodes typically are bilaterally symmetrical, elongated, tapered at both ends, possess a pseudocoel (false body cavity) derived from the embryonic blastocoels. They are widely distributed and they include terrestrial, freshwater, marine and parasitic forms. Members of this phylum are differentiated from other pseudocoelomate groups based on their possession of spicules and ventral excretory pore.

In this exercise, students will investigate the diversity of the Phylum Nematoda particularly those belonging to the Classes Secernentea or Rhabditia (Phasmidia) and Adenophorea or Enoplea (Aphasmidia).

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Nematoda phyla particularly those in the Classes Phasmidia and Aphasmidia.

Materials

Compound microscope

Specimen slides (*Ascaris lumbricoides, Capillaria spp., Trichuris trichiura, Trichinella spiralis, Strongyloides stercoralis, Ancylostoma spp., Necator americanus, Angiostrongylus cantonensis, Enterobius vermicularis,Trichostrongylus spp.Wuchereria bancrofti, Brugia malayi*)

Procedure

1. View the specimens of the nematoda phyla, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section 1. General Features

Roundworms are bilaterally symmetrical, elongated bodies covered with a flexible, nonliving cuticle, tapering at both ends, and possess a pseudocoel. The digestive system is complete for these organisms. The lumen of the pharynx is characteristically triradiate. The circular muscles of these organisms are lacking in the body wall and the longitudinal muscles are arranged in 4 groups separated by epidermal cords. The excretory system consists of lateral canals, ventral glands or both. Cilia are completely lacking except for some sensory endings of modified cilia. Most organisms are dioecious. Females are usually larger than the males and the males have tails that are curled. Some species are hermaphroditic, others are parthenogenetic. Most are oviparous, but some are ovoviviparous.

Section 2. Classification of Phylum Nematoda (Unsegmented round worms)

The nematodes comprise a large number of taxa that include free-living forms as well as both plant and animal parasites.

Class Enoplea (Adenophorea; Aphasmidia)

The Amphids are well developed except in parasitic forms. The caudal and hypodermal glands are common. The excretory system is formed of single, ventral, glandular cells or is entirely absent. Most are free-living, some are parasitic on plants and animals.

Order Trichurida

Organisms' anterior ends are more slender than their posterior ends. The lips and buccal capsule is absent or reduced. The esophagus is very slender, capillary-like tube, embedded in one or more rows of a large glandular cell. Male organisms have one or no spicules. Eggs have polar plugs except in the *Trichinella spp.*

Order Dioctophymatida

These are stout worms with esophageal glands that are highly developed with lips and buccal capsules that are reduced and are replaced by a muscular oral sucker in Soboliphymatidae. Esophagus is cylindrical. Male organisms have a bell-shaped muscular copulatory bursa without rays and possess a single spicule.

Order Muspiceida

These organisms have an alimentary tract that is reduced or vestigial. The males are unknown. These are parasitic for rodents, deer, bats, marsupials and crows.

Order Mermithida

These organisms' juvenile stages are parasitic to various invertebrates.

Class Rhabditea (Secernentea; Phasmidea)

The Amphids are ventrally coiled. There are 3 esophageal glands. The subventral glands open near the base of the esophageal corpus while the dorsal gland opens at or near the buccal cavity.

Order Rhabditida

These are minute worms commonly characterized with 6 small lips, muscular esophagus, buccal capsule small or absent, tail conical for both sexes, spicules are equal. Most of these organisms are free-living but some are parasitic to amphibians, reptiles, birds and mammals.

Order Drilonematida

These organisms have phasmids that are sucker-like in appearance. The cephalic hooks are present and are found to be parasitic in earthworms.

Order Rhigonematida

These organisms have complex cuticular modifications present at the base of their buccal cavity and the vagina is long and muscular.

Order Strongylida

These are long, slender worms with esophagus that are swollen posteriorly but lack a definite bulb. Male organisms have well developed copulatory bursa supported by sensory rays.

Order Ascaridida

These organisms have 3 prominent lips present with numerous caudal papillae. The esophagus is variable in structure. The lifecycle is variable as organisms may either take a direct or indirect lifecycle with a vertebrate or invertebrate intermediate hosts.

Order Oxyurida

The medium to small worms often have sharply pointed tails, esophagus with prominent posterior bulb with valve, males with single or no spicule and reduced number of caudal papillae. They are found to be parasitic in arthropods and vertebrates. They take a direct lifecycle.

Order Spirurida
These organisms have mouths that are surrounded by 6 lips, lips lost, or lateral pseudolabia present. They have a well developed buccal capsule and an esophagus divided into 2 glandular portions. They are found to be parasitic in all vertebrate classes.

Section 3. Nematodes

Trichinella spiralis. This organism is referred to cause trichinosis, trichiniasis or trichinelliasis. It is a parasite in man, hogs, rats and other omnivorous or carnivorous mammals. Larvae of this organism are oftentimes encysted in the muscles. Note how many worms are coiled in a cyst. The male worms measure 1.4 to 1.6 mm long. They possess a large copulatory pseudobursa and no copulatory spicule. The female worms measure twice the size of males and are tapered towards the anterior end.

Trichuris trichiura. This organism is referred as the whipworm responsible for causing trichuriasis or whipworm infection. Eggs appear looking like football-shaped or barrel-shaped with prominent transparent bipolar plugs at each end. Eggs measure 20 to 50mm in diameter and are brownish in color. The worm appears to be pinkish gray in color measuring 30 to 50mm long with males being smaller than the females. The female worms have a curved tail. The buccal cavity is small and is provided with a small spear. The esophagus is very long occupying 2/3 of the body length and consists of a thin-walled tube surrounded by large, unicellular glands (stichocytes).

Strongyloides stercoralis. This organism is the cause of strongyloidiasis or also known as the Cochin-China diarrhea. Parasitic and free-living males and females may exist. The free-living female worms are shorter but stouter than the parasitic female. They have a double bulbed muscular esophageal pharynx. The parasitic female worms are delicate filiform worms measuring an average of 2.2 mm long with the esophagus occupying 1/3 anterior part of the worm. These worms are parthenogenetic. The free-living male worms on the other hand are broadly fusiform, smaller than the free-living female worms with pointed tail that is curved ventrad. There are no parasitic male. Male worms develop from the filariform larva passed out to feces. It is the rhabditiform larva that is passed out in the feces and is characterized by a muscular elongated esophagus with a pyriform posterior bulb. A short buccal cavity and a relatively conspicuous genital primordium halfway down the midgut. The filariform larva is a long, delicate worm having a long esophagus occupying half the length of the larva and the tail is forked or notched. This is used to differentiate this filariform larva to that of the hookworm filariform larva.

The hookworm species in man are *Necator americanus, Ancylostoma duodenale, Ancylostoma braziliense, Ancylostoma caninum, Ancylostoma ceylanicum*. These hookworms are the cause of Ancylostomiasis, uncinariasis, necatoriasis, hookworm infection. The adult worms look like an odd piece of a thread measuring about 1cm long. They are white or light pinkish in color when they are alive. The female worms are slightly larger than the males. Male worms' posterior end is expanded to form its copulatory bursa. Eggs are oval in shape, thin shelled and are colorless and may contain 2 to 8 cells. Eggs of the *Necator* and *Ancylostoma* are morphologically impossible to differentiate. Observe the mouth capsule and the copulatory bursa of these worms, note their differences.

Enterobius vermicularis. This worm is also referred as the pinworm, seatworm or the *Oxyuria vermicularis*. This worm is the cause of enterobiasis, oxyuriasis, and pinworm infection. The adults are whitish in color and look like a pin due to its cuticular extensions called "cephalic alae." Female worms are bigger than the male worms and are fusiform in shape. The tail of the male worm is curved. The slender esophagus is terminating in a prominent posterior bulb called "esophageal bulb." Both the

cephalic alae and the esophageal bulb are the important features in the identification of this species. Eggs appear like D-shaped. The eggs are colorless and transparent, thick and asymmetrical in shape. Eggs may contain a larva.

Ascaris lumbricoides. This whitish or pinkish worm is also referred as the intestinal roundworm causing Ascariasis, ascaris infection, roundworm infection. The adult males are slightly smaller than the female worms. The worm's body is smooth with finely striated cuticle. The male worms have 2 spicules. The terminal mouth of this worm is with 3 oval lips with sensory papillae not visible in the compound microscopy. Eggs measure 45 to 70µm long by 35 to 50µm wide. The outer portion of the egg is coarsely mammillated and the albuminous covering acts as an auxiliary barrier. In certain cases, this covering may be absent. The egg has thick, transparent, hyaline shell with relatively thick outer layer acting as its supporting structure. The egg has a delicate vitelline, lipoidal, inner membrane. Eggs may appear to be fertilized, unfertilized and or embryonated. Unfertilized eggs have a thinner chitinous layer and albuminous coat to those of the fertilized eggs. The content of the unfertilized eggs is made up of many refractable granules in various sizes. The content of the embryonated egg is made up of larva.

Filarial worms. The filarial worms are arthropod transmitted parasites and are inhabiting the circulatory and lymphatic systems, muscles, connective tissues and or serous cavities of vertebrates. The principal species are *Wuchereria bancrofti, Brugia malayi, Onchocerca volvulus, Loa loa, Dipetalonema perstans, Mansonella ozzardi.* The adult worms are whitish in color and are thread-like in appearance. There are 2 rings of small papillae on their heads. The female worms are longer than the males. The male worms have a curved tail with 2 copulatory spicules. The microfilaria of these worms measure 177 to 296µm long. A sheath with free endings is present and the anterior end is bluntly rounded and the posterior end is tapered to a point. A nerve ring with no nuclei at the anterior 1/5 of the body is present. In the laboratory, note the morphological differences of the *Wuchereria bancrofti* and the *Brugia malayi.* Take note of the worms' cephalic space, terminal nucleus, and nuclei.

Capillaria spp. These worms are the cause of intestinal, hepatic and pulmonary capillariasis. The eggs are pale yellow in color, have moderately thick striated shell with flattened bipolar plugs. Eggs are peanut-shaped in appearance. The atypical female worms produce eggs that are thin shelled without the flattened bipolar plugs and are multi-segmented or are embryonated. This is a small worm where the males are slightly smaller than the female worms. The male worm has a small caudal alae and a spineless spicule sheath. The esophagus of the female is about ½ as long as the body.

Trichostrongylus spp. This worm is the smallest as it seldom exceed 7 mm long. The organisms are colorless, lack cervical papillae and have a rudiment and unarmed buccal cavity. The eggs resemble those of hookworms but are usually larger.

Angiostrongylus cantonensis. This is a slender worm with simple mouth and no lips or buccal cavity. Males are slightly smaller than the female worms. The bursa is small and lack dorsal lobe. Spicules are long, slender and equal in length and form. Eggs are thin-shelled and are unembryonated when passed out to feces.

Activity 8
The Nematodes

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the specimens belonging to the Phylum Nematoda and properly label all its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II.Fill in the table and compare the morphological characteristics of the *Wuchereria bancrofti* and the *Brugia malayi*.

Characteristic	*Wuchereria bancrofti*	*Brugia malayi*
Size		
Cephalic space		
Nuclei		
Terminal nuclei		
Infective Stage		
Transmission Stage		
Diagnostic Stage		

III. Differentiate the terms hermaphroditic, parthenogenetic, oviparous, and ovoviviparous?

IV. Fill in the table by sketching the mouth capsules and the copulatory bursa of the following hookworm species.

Structure	*Necator americanus*	*Ancylostoma duodenale*	*Ancylostoma caninum*	*Ancylostoma ceylanicum*	*Ancylostoma braziliense*
Mouth capsule					
Copulatory bursa					

V. Complete the table indicating the morphological differences of the *Anyclostoma duodenale* and the *Necator americanus*.

Characteristics	*Ancylostoma duodenale*	*Necator americanus*
Size		
Shape		
Mouth		
Copulatory bursa		
Copulatory spicule		
Caudal spine		
Vulva position		

11

Arthropoda

This activity gives the students the opportunity to learn and explore the Phylum Arthropoda. The arthropods are found in almost every habitat in our environment. Many species are recognized to be beneficial for man but there are others that are parasitic. The Phylum Arthropoda is a diverse group embracing the chelicerates, crustaceans and the mandibulates.

In this exercise, students will investigate the diversity of the Phylum Arthropoda particularly those belonging to the Subphylum Chelicerata and Uniramia or Mandibulata.

Objectives: At the end of the activity, the student should be able to

1. Identify the distinguishing characteristics of the specimens
2. Differentiate each specimen of the Arthropoda phyla particularly those in the Subphylum Chelicerata, Crustacea, and Uniramia or Mandibulata

Materials

Compound microscope/Stereoscopic dissecting microscope
Specimen slides and specimen collections available

Procedure

1. View the specimens of the Arthropoda phyla, noting their key identifying features. Make a drawing of each specimen, labeling all of the characteristic structural features.

Section 1. General Features

Arthropods are organisms that have jointed appendages. The bodies of these organisms are constructed of an extended series of repeated segments called metamerism. The 3 arthropod subphyla to be studied in this activity will show several examples of appendage specializations. Please note of these characteristic in this activity. Organisms in this group have a 1) triploblastic development 2) true coelom 3) bilateral symmetry 4) cephalization and 5) possess all organ systems.

Section 2. Classification

Phylum Arthropoda

Subphylum Chelicerata

This organism's first pair of appendages are modified to form chelicerae. They have a pair of pedipalps and 4 pair of legs, no antennae, and no mandibles. Both the cephalothorax and abdomen are usually unsegmented.

These organisms include a diverse group including spiders, mites, ticks, scorpions and the horseshoe crab. All these organisms lack antennae and mandibles.

Examine the demonstration specimens and live specimens of the horseshoe crab, *Limulus polyphemus*, a member of the **Class Merostomata** and a so-called "living fossil" from the Ordovician Period. Locate the carapace with its dorsal compound eyes. Flip the specimen over and count the seven pairs of jointed legs. Locate the book gills and telson (caudal spine) on the abdomen.

Examine the preserved and live specimens of a typical member of the **Class Arachnida**, count the six pairs of legs; from front-to-back the **chelicera** (modified to form venomous fangs), the **pedipalps**, and four pairs of **walking legs**. Note the **multiple compound eyes** on the cephalothorax and the **spinnerets** at the tip of the abdomen.

Examine the preserved specimen of the scorpion, another terrestrial arachnid. Notice the second pair of legs, the pedipalps are modified into large claws. The caudal abdominal segments are modified into a tail tipped with a venomous stinger.

Examine the preserved specimen of the tick, *Boophilus spp.*, another terrestrial arachnid. Notice the tick does not exhibit any definite body regions. The abdomen and the thorax are strongly fused together. Head consists of only a pair of protruscible **chelicerae** and a ventrally located median **hypostome** with numerous teeth-like projections. Count the number of legs. Each leg has 6 segments namely the **coxa**, **trochanter**, **femur**, **genu**, **tibia**, and **tarsus**. The female tick is usually bean-like in shape.

Subphylum Crustacea

These organisms are also referred as the crustaceans. The organism has gills, the body is covered with a carapace, the exoskeleton has limy salts, pair of appendages in each body segment and are biramous (two-branched) and are modified depending on its function, two pairs of maxillae on the head, and a head with 2 pairs of antennae. These organisms include a diverse group. Some organisms include crayfishes or lobster, crabs, shrimp, copepod (*Cyclops*), barnacle, and water flea (*Daphnia.)*

Obtain a live specimen or preserved specimen of the crayfish *Procambarus* for examination. Observe that the body is divided into two regions, the cephalothorax and the abdomen. Locate the long antennae and the stalked eyes attached to the head. Most crustaceans have a shell-like chitinous **carapace** covering most of the cephalothorax. The point of the carapace extends anteriorly between the eyes and is called the rostrum. Note that the mouth is surrounded by a series of specially modified, serially arranged mouth parts. The pair closest to the mouth is the **mandibles**. Locate the large pincers or **chelae** on the anterior set of walking legs. Locate the abdominal legs or swimmerets. What do you think is their function? Locate the fan-like tail of the body called the **telson**. The anus is located ventrally at the base of the telson. Tap on the tail of the crayfish. It may exhibit a tail-flip, which is an extremely fast, stereotyped, escape maneuver.

Examine alive or preserved specimens of the *Portunus* crab. Examine the sex of the organism. The organism has 8 pairs of appendages. The first 3 pairs are modified as **maxillipeds.** The last 5 pairs are the **walking legs.** The first pair of legs is the **chelipeds.** The ends in claws and the last pair of legs are modified for swimming. If the crab morphology confuses you, try grabbing one and flip it over. You will find the abdomen folded up under the cephalothorax.

Examine a preserved specimen of the *Balanus*, barnacle. The barnacle is a sessile crustacean. The part of the barnacle that is attached is called the **basis.** There 6 calcareous **plates** surrounding the barnacle. The opening is covered by an operculum that consists of a movable pair of tergum and scutum. The **thorax** bears 6 pairs of feeding appendages called **cirri.** During feeding, the terga and scuda opens and the cirri unroll outside the opening.

Examine a mounted slide of a water flea, *Daphnia*. Water fleas are common in pond water. They are covered by a thin transparent carapace. The large two-branched second antennae are the chief organs of its locomotion. There are 5 pairs of small leaf-like swimmerets on its thorax. Their paired eyes are fused. The brood pouch in female is large and situated posterior to the abdomen.

Examine a mounted slide of a copepod. Copepods are found in fresh and in brackish water environments. The organism has a median eye near the base of its rostrum and possesses long antennae, which is modified in the male. The cephalothorax bears appendages and the abdomen has no appendages. The 6th thoracic segment in the female carries large, pendulous egg sacs while the last abdominal segment bears a pair of caudal projection covered with setae.

Examine a preserved or live specimen of a shrimp. Note that the organism has 11 pairs of similar appendages used for several functions like locomotion, respiration and egg carrying. The organism has no carapace. Note the dark eyes borne on its unsegmented stalks. Females carry their eggs in a ventral brood sac.

Subphylum Uniramia or Mandibulata

These organisms include insects and myriapods. They are considered as a large group of mandibulate arthropods breathing by means of their trachea. These organisms also have only one pair of antennae and only one-branched appendages (uniramous) or unbranched legs. The organism has well-developed mandibles surrounding the mouth.

Carefully examine preserved specimens of the centipedes (**Class Chilopoda**) and the millipede (**Class Diplopoda**). Note that these organisms have some of the major structural and ecological differences between these two classes. The *Scolopendra*, centipede has a body that consists of a **head** and a long **trunk.** The head consists of a pair of 18-segmented antennae on the anterior margin. There are 4 pairs of eyes located at the lateral side to the antennae. The mouthparts consist of a pair of mandibles surrounded by a pair of first maxilla functioning as a lower lip. A leg-like second maxilla overlies the first pair. Mouthparts are surrounded by a pair of **poison claws.** The claws are the appendages of the first trunk segment. A pair of appendages is borne out of each trunk segment. There are about 22 trunk appendages but when you count the segments, there will only be 21 because the first segment is hidden under the overlapping head. The *Thyropygus*, millipede has a body that also consists of a head and a long trunk. The head bears a pair of six-segmented antennae, pair of compound eyes, and strong mouthparts. The first trunk segment after the head is called the **collum.** The most anterior trunk segment bears only a

pair of appendages. Note that each segment bears 2 pairs of legs. This segment is referred as **diplo-segment.** The posterior segment called the **anal segment** does not have any appendages.

Examine a preserved specimen of a beetle. The beetle is a hard-bodied insect consisting of a head, thorax and abdomen. The head bears a pair of eyes, a pair of ventrally-directed antennae (that is close to the eyes) and the mouthparts. The beetle's thorax has 3 pairs of legs and 2 pairs of wings. The **prothorax** is free from the rest of the thoracic segments and is provided with a pair of legs. The **mesothorax** bears a pair of thickly chitinized wing called the **elytron**, which covers the metathorax and most of its abdomen. The **metathorax** bears the folded, membranous pair of wings used for flying. The functional metathoracic wings are tucked under the elytron.

Examine a preserved specimen of a *Orthetrum,* dragon fly. The dragonfly is a slender insect whose body is divided into 3 regions: head, thorax and abdomen. The head bears a pair of large eyes, a small pair of antennae and anterior mouthparts. The thorax is divided into an anterior prothorax bearing a pair of prothoracic legs, a middle mesothorax bearing a pair of wings and mesothoracic legs and the posterior metathorax bearing a pair of wings and metathoracic legs. Each leg of the dragonfly consists of 5 segments: **coxa, trochanter, femur, tibia** and **tarsus.** The tarsus bears a pair of terminal claws. The abdomen is long, narrow and divided to segments. The posterior part of abdomen bears the reproductive parts and the terminal anus.

Examine a mounted slide of a *Ctenocephalides,* flea. The flea's body is compressed laterally under a mounted slide. The body is divided into 3 regions: head, thorax and abdomen. The head bears pair of eyes and a row of eight, sharp, black genal teeth. The thorax bears 3 pairs of legs. The first thoracic segment bears a dark **pronotal comb**. Each abdominal segment bears a row of **bristles.**

Activity 9
The Arthropods

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Draw the specimens belonging to the Phylum Arthropoda and properly label all its distinguishing features. Follow the sample format. (Student may use separate paper)

1.
Scientific name:_____
Stage: _____

II.Fill in the table and compare the morphological characteristics particularly the type of front wing and type of back wing of the following insect order.

Order and example	Type of front wing	Type of back wing
Odonata, dragonfly		
Orthoptera, grasshopper		
Dictyoptera, Cockroach		
Isoptera, termite		
Hemiptera, true bug		
Homoptera, Aphid		
Coleoptera, Beetle		
Lepidoptera, butterfly		
Diptera, fly		
Siphonaptera, flea		
Hymenoptera, Bee		

III. Complete the table and differentiate the organisms according to their number of antennae, type of mouthparts, number of legs and place of their attachment and number of body parts and organization.

Organism	No. of Antennae	Type of mouthparts	No. of legs and Place of attachment	No. of body parts and organization
Spider				
Crab				
Millipede				
Centipede				
Mosquito				

IV. What is the function of a swimmeret?

V. Mosquitoes are important insect vectors. Three important Genus of mosquitoes are known to cause mosquito related diseases and these are the Genus *Culex, Aedes* and *Anopheles*. Differentiate the three Genus based on the morphological characteristics of their larva and adult forms?

Activity 10
Applied Entomology: Larval Mosquito Surveillance

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

This exercise is a simple larval mosquito surveillance method called the ovitrap technique. It is currently used as a method of mosquito control in urban Manila to prevent dengue outbreaks. The ovitrap technique is both a surveillance and economical control method. This exercise will only aim to collect larvae and identify them up to the genus level.

Materials:

- Commercially available mosquito larva catcher (ovitrap) or improvised ovitrap using:
  - Empty 150ml or 150g can (milk or canned good) painted all black (inside and out)
  - ~6"x1" wooden lawanit paddle or patpat (rough surface)
  - Clean water
- Clear plastic (used for ice)
- Rubber band
- Deep dish or microwaveable container
- Hot water
- Disposable Pasteur pipette or plastic dropper with opening cut wide
- Petri plate and stereomicroscope
- Vial with 10% formalin solution

Procedure:

1. Half-fill the ovitrap container or can with clean water (tap water allowed to settle overnight or distilled water) and place the lawanit diagonally (Fig. 12-1).

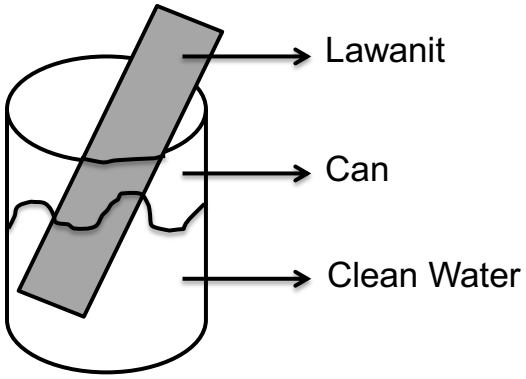


Figure 12-1. Ovitrap Set-up

2. Place the ovitrap in a low, dark corner of the school garden or outside one's house where it will not be disturbed by animals or children. Leave the set-up for at least 24 hours.

3. Take the lawanit and place inside a clear plastic. Fill the plastic with the contents of the ovitrap and add additional clean water until the lawanit is fully immersed. Secure the plastic with rubber bands. Wait for at least 48 hours until mosquito larvae (kiti-kiti or wrigglers) that are ~1/2 inch in length are observed.
4. Transfer the contents of the clear plastic in a deep dish or microwaveable container. At this point, if mosquito larvae are still viable, add some hot water to kill the larvae. Using a disposable Pasteur pipette or plastic dropper (either tip should be cut to widen the opening), aspirate the larvae and transfer onto a petri plate by batches.
5. Identify the larvae by observing the posterior region, specifically the breathing tube. The ovitrap will mostly collect *Aedes* sp. and occasionally *Culex* sp. Calculate the ovitrap index for each genus:

$$ovitrap\ index\ (larvae\ per\ ovitrap) = \frac{\#\ of\ larvae\ collected}{total\ \#\ of\ larvae\ collected\ in\ 1\ ovitrap}$$

6. You can preserve the larvae in a vial with 10% formalin solution for submission to your laboratory instructor. Label with your last names and date of collection (removal from plastic bag)

Results:

Mosquito Genus	Number Collected	Ovitrap Index (larvae/ovitrap)
TOTAL		

Labeled Image/ Drawing of Collected Mosquito Larvae (one per box):

Answer the following questions:

1. Why is the ovitrap both a method of mosquito larvae surveillance and mosquito control?

2. Why is it important to wait until the larvae are about ½ inch in length before identifying them?

12

Laboratory Techniques

This activity gives the students the opportunity to learn and perform the different laboratory techniques in Parasitology. Proper performance of the laboratory techniques will enable the student to properly diagnose or identify the organism of concern based on the given specimen.

In this exercise, students will perform different laboratory techniques in detecting intestinal parasites.

Objectives: At the end of the activity, the student should be able to

1. Collect the appropriate specimen
2. Perform the laboratory techniques
3. Identify the parasites

Section 1. Collection of Fecal Specimen

The collection of specimen is the most important step in every laboratory procedure. The kind of specimen will also help the examiner to determine whether a specimen is negative or positive for any parasitic organisms. Fecal specimens should be collected in a clean, wide-mouthed container. Patients should be instructed not to contaminate the container and not to overfill the container with their fecal specimen. Patients should be warned of contaminating the fecal specimen with water from the toilet or that of urine.

Section 2. Fresh fecal specimens

Fecal specimens that are obtained fresh and are unpreserved must be examined immediately in the laboratory. Fresh fecal specimens should be examined macroscopically and microscopically. In direct wet mounts of the fecal specimens, they may be prepared with or without saline depending on the consistency of the fecal specimen and an iodine stain can be used to examine the specimen microscopically. When dealing with protozoa, a permanent stain may be used.

Section 3. Preserved Specimen

If specimen cannot be immediately processed in the laboratory, there are several preservatives that can be used. Some prefer to use preservatives like merthiolate-iodine–formalin solution (MIF), 10% formalin and polyvinyl alcohol (PVA) solution. The MIF solution can be used to preserve specimens that contain cysts and trophozoites of protozoa. The MIF solution can also be used as a stain for the preparation of direct wet mounts as it contains iodine. The 10% formalin solution on the other hand can preserve the existing morphological form and structure of the organisms as well as the debris in the fecal specimen. This preservative is suitable for concentration techniques to enhance the recovery of protozoa cysts, helminth eggs and larva. Formalinized specimens can be examined directly as wet mounts, with or without iodine. The polyvinyl alcohol fixative is a mixture of a water soluble polymer and the modified

Schaudinn's fixative. The PVA is an ideal fixative for fecal specimens containing trophozoites and cysts that are supposed to be processed immediately or after several months.

Section 4. Gross Macroscopic Examination of Fecal Specimen

Prior to the microscopic examination of fecal specimen, the fecal specimen needs to be examined macroscopically. Fecal specimens are examined for their **quantity, consistency, odor,** and **color.** In terms of quantity, fecal specimens submitted in the laboratory should be enough for the parasitic examination. In terms of consistency, stools are classified as formed, semi-formed or watery. Normally stools are soft and formed. In certain cases, stools may be 1) dry and hard 2) ribbon-like 3) semi-formed and 4) mushy or watery. In terms of color, the stool has a normal color of light yellow to almost black color. The color of the stools passed out is influenced by the consumption of food and medicines and by other substances like blood, undigested fats and many others. In terms of odor, stools may exude a sour or even a putrid odor.

Section 5. Stool processing techniques

A. Direct Fecal Smear (DFS)

Materials and Reagents:

Applicator sticks or matches
Glass slides and coverslips

Saline – 0.85%	**Iodine
0.85g NaCl	Iodine crystals 5g
100ml distilled water	Potassium iodide 10g
	100ml distilled water

1. Label the slide with the patient's name or identification number on the left hand of the slide.
2. Using one slide, place a drop of *saline in the center of the left half of the slide and place a drop of **iodine in the center of the right half of the slide. (may also be prepared in separate slides). NOTE: Iodine kills organism present hence no motility will be detected.
3. Take a small amount of fecal sample (about the size of a match head ~2mg) and add it to the drop of saline; add similar portion to the drop of iodine.
4. Using different sticks, mix the saline-stool and iodine-stool preparations to form suspensions.
5. Cover each suspension with coverslip.
6. Examine under LPO, HPO and OIO if necessary.

Alternative:

1. Prepare and examine the saline mount.
2. Add iodine at the side of the cover slip, and let it diffuse into the saline-stool suspension.
3. Examine under LPO, HPO and OIO if necessary.

Concentrated wet mounts

This procedure enables the concentration of fecal materials to facilitate the detection of cysts and eggs when these are present in too small numbers to be detected easily by direct wet mounts. The use of concentration techniques increases the yield of eggs and cysts but the technique destroys the motile forms. There are two different types of concentration techniques: sedimentation and flotation. In sedimentation concentration technique, parasites are allowed to settle while in the flotation concentration technique, parasites particularly eggs and cysts are forced to float to the top of the suspension.

B. Formalin-ether (ethyl acetate) concentration technique (FECT)

Materials and Reagents:

Centrifuge, centrifuge tubes
Dispensing bottles
Applicator sticks
Small beaker – 50, 100ml
Glass slides and coverslips
Disposable pipettes with rubber bulbs
Rubber stopper for centrifuge tubes
Test tube rack
10% formalin
Ether or ethyl acetate, (if unavailable, use gasoline) NOTE: Ether is highly volatile and may explode quickly if there is an open flame or spark nearby. Store open cans or bottles on an open shelf in the coolest part of the laboratory. Do not put opened containers of ether in a refrigator as fumes can escape, build up and may cause an explosion when the door is opened.
0.85% saline
1% iodine

1. Place 1-1.5g feces to 10ml of 10% formalin. If the specimen is already preserved, stir the stool-formalin mixture.
2. Prepare a two-layer surgical gauze (~400μm mesh sieve).
3. Strain the stool-formalin mixture through the gauze directly into a centrifuge tube. Discard the gauze.
4. Add 10% formalin to the strained mixture to bring the total volume to 10ml.
5. Centrifuge for 10mins at 500 x g. The amount of sediment obtained should be approximately 0.5 to 1ml.
6. Decant the supernatant fluid and resuspend the sediment at the bottom of the tube in 10% formalin, until the tube is half filled.
7. Add 3ml of ether (ethyl acetate or gasoline) and shake the suspension vigorously for 10 seconds by putting a rubber stopper in the tube.
8. Remove the stopper directing it away from your face. Centrifuge for 10 min at 500 x g.
9. Remove the tubes from the centrifuge. Four layers should result: (1) top layer of ether (ethyl acetate or gasoline), (2) fecal debris, (c) formalin layer (d) sediment containing the parasite at the bottom of the tube.
10. Loosen the plug of debris with an applicator stick by a spiral movement and decant the supernatant fluid. Residual fluid from the walls of the tube will flow back onto the sediment.
11. Mix the fluid with sediment. Add a drop of saline if the sediment is still solid, or if fluid is insufficient to suspend the sediment.
12. Obtain a drop of suspension and place on a slide, add coverslip and start scanning with 10x objective; an iodine-stained preparation can also be made.

*Zinc Sulfate Flotation Technique (Faust et al., 1938; Faust, Beaver and Jung, 1968)

1. Prepare zinc sulfate solution of 1.18 specific gravity (add 330g of zinc sulfate crystals in 1 L distilled water)
2. In a 15mL centrifuge tube, emulsify 1g of feces with 3-5 mL of distilled water
3. Pour through 2 layers of gauze held in a small funnel inserted into another 15mL tube
4. Centrifuge for 1 minute at 2000 rpm

5. Pour supernatant in discard container
6. Add approximately 2 mL of water. Break up the sediment and fill tube with water.
7. Centrifuge and repeat the washings until supernatant is fairly clear
8. Add 3-5mL zinc sulfate solution and mix thoroughly with the sediment deposit using an applicator stick. After mixing, add more zinc sulfate solution to the level about ¼ inch of the rim of the tube
9. Carefully add more zinc sulfate solution down one side of the tube with a Pasteur pipette, until a slightly convex meniscus is formed above the rim
10. Touch the surface of the meniscus with a cover slip immediately, which is then immediately placed, liquid side down on a glass slide containing a drop of Lugol's iodine stain OR use a wire loop (5 to 6mm in diameter) immediately and remove a few loopfuls of a film to a glass slide. Add Lugol's iodine stain and cover
11. Immediately examine the slide

C. Kato-Katz Technique

Kato Katz is an egg counting procedure that aids in determining the intensity of worm infection. It also serves as a tool for assessment of the efficacy of antihelminthic drug. Schistosomiasis and infection of soil-transmitted helminthes such as *Ascaris*, *Trichuris*, and hookworm can be evaluated.

The technique is specific for fresh, formed and preferably drier stool sample. Drier stool samples yield higher egg counts than moist ones. Glycerine is used as a clearing solution. Malachite green is used to provide color to the cellophane so that a better contrast between the eggs and the background is achieved. *Ascaris* and *Trichuris* eggs will remain visible and recognizable for many months. Hookworm eggs clear rapidly and can only be seen within 30 to 60 minutes after preparation.

Materials and Reagents:

Wooden applicator stick

Screen, nylon or plastic (60-105mesh)

Any of the following template:

1.5 mm thick template with 6mm diameter hole (41.7mg of feces)

1mm thick template with 9mm diameter hole (50mg of feces)

0.5mm thick template with 6.5mm diameter hole (20mg of feces)

Plastic spatula

Glass slides

Hydrophilic Cellophane (strips 25x35 mm)

Flat bottom jar with lid

Forceps

Tissue

Newspaper

Glycerol - Malachite green or glycerol-methylene blue solution (1ml of 3% aqueous malachite green or 3% methylene blue added to 100ml glycerol and 100 ml distilled water, then mixed well) The solution made is poured onto cellophane strips in a jar and **left for at least 24 hours prior to use**. **Malachite green can be eliminated if green cellophane is used.**

1. On a newspaper, place a small mound of feces and carefully press the screen on top so that the feces are sieved through the screen.
2. Using a spatula, collect the sieved feces.
3. Place the template on the center of glass slide.
4. Fill the hole of the template with the sieved feces. Remove excess feces from the edge of the hole
5. Remove the template carefully from the slide.
6. Cover the fecal material with the pre-soaked cellophane. The cellophane must be very wet with glycerol if the feces are dry and less so if the feces are soft.
7. Invert the glass slide and press the fecal sample against another glass slide. The fecal material must be spread evenly between the slide and the cellophane. It should be possible to read newspaper print through the smear after clarification.
8. Remove the slide by sliding it sideways to avoid separating or lifting off the cellophane. Keep the slide in dry place for about 1 hour.
9. Examine the smear in a systematic manner. The eggs of different species of helminth may be counted. Multiply the appropriate number to obtain the number of eggs per gram of feces:
   if using template with 6mm hole – x 24
   if using template with 9mm hole – x 20
   if using template with 6.5mm hole – x 50

D. Kato Thick Smear

Kato Thick is a simplified Kato Katz technique. It is relatively simpler and more economical. It is used for mass stool examination of helminths. The technique is specific for fresh and preferably drier stool. Thick shelled eggs are very visible (*Ascaris* and *Trichuris*), but not thin shelled eggs like that of hookworm.

Materials and Reagents:
Wooden applicator stick
Plastic spatula
Glass slides
Hydrophilic Cellophane (strips 25x35 mm)
Flat bottom jar with lid
Forceps
Tissue
Newspaper
Glycerol - Malachite green or glycerol-methylene blue solution (1ml of 3% aqueous malachite green or 3% methylene blue added to 100ml glycerol and 100 ml distilled water and then mixed well) The solution made is poured onto cellophane strips in a jar and left for at least 24 hours prior to use. Malachite green can be eliminated if green cellophane is used.

1. Place about 50 to 60mg (size of 2mongo beans) of stool on the glass slide.
2. Cover the slide with cellophane.
3. Invert the glass slide and press the fecal sample against another glass slide. The fecal material must be spread evenly between the slide and the cellophane.
4. Allow 20 to 30 minutes to clear.
5. Examine the smear under the microscope.

E. Cellulose tape or Scotch tape swab method: (Graham, 1941)

The method involves perianal sampling using cellulose tape. To prevent contamination, a wider tape (~1 inch wide) is used. The sticky side of the tape is applied on the skin and then placed on a glass slide. The slide bearing the tape is examined under the microscope for the presence of eggs or adult *Enterobius*.

Materials
Scotch tape
Glass slide
Tongue depressor
Cotton or gauze

1. Apply a strip of transparent cellulose tape or scotch tape 2 ½ to 3 inches in length on the upper side, beginning at one end, of a tongue blade. A small portion of the end on the opposite portion of the tongue blade should be folded on itself.
2. Hold slide against tongue depressor one inch from end and lift long portion of tape from slide
3. Loop tape over end of depressor to expose gummed surface
4. Hold tape and slide against tongue depressor
5. Press sticky surface of tape on the perianal folds. Note: DO NOT insert blade into rectum
6. Replace tape on slide
7. Smooth tape with cotton or gauze
8. Examine slide immediately.

Section 6. Blood Specimens

Protozoan as well as helminthic (filariae) parasites may be obtained from blood sample. Blood preparation and examination entail several techniques that must be followed properly to obtain accurate results. Clean and grease-free glass slides must always be used for blood films.

Section 7. Preparation and Staining of Blood Films

Materials and Reagents:
Disposable lancets
Glass slides
Methanol (thin smear)
10% Giemsa stain
Couplin jars
Distilled water

F. Thin Blood Films

Red blood cells appear discretely separated in thin smear. Parasitized RBCs roll to the edges and are carried to the tail of the film. This portion is also examined for malaria parasite. Using fixative in thin films does not lyse red blood cells, therefore the parasites appear inside the RBC. Thin smears are utilized for species identification of malaria parasites.

1. Place a small drop of blood to one end of a glass silde.
2. Allow the blood to run along the back edge of the spreader (another slide placed 45º to the first slide) and push it forward evenly and quickly without reaching the end of the slide.
3. The starting point of the spreader must appear thick and the other end as feathery.

4. Air-dry the slide. Cover the film if examination is to be done at a later time.
5. Fix the film in methanol for at least 30 minutes.
6. Stain the blood film with 10% *Giemsa for 45mins.
   *Dilute stain with neutral distilled water (one drop of stain to each ml water) prior to use.
7. Gently wash in distilled water to remove excess stain. Place the slide upright and at an angle while drying.
8. Examine the thin blood film.

G. Thick Blood Film

Red blood cells are piled on top of each other, giving a thick and irregular appearance. Thick smears are not fixated to allow dehemoglobinization of RBCs. Parasites become concentrated with thick films hence are most useful in qualitative screening for malaria parasites. It is also useful for counting parasites especially when monitoring treatment.

1. Place 2-3 drops of blood to the center of a glass slide.
2. Mix the blood rapidly and spread over an area of about ~2cm in diameter using the corner of another slide.
3. Air-dry the slide. Cover the film if examination is to be done at a later time.
4. Immerse in distilled water for 20mins, and allow to dry again.
5. Stain the blood film with 10% *Giemsa for 45mins.
6. Gently wash in distilled water to remove excess stain. Place the slide upright and at an angle while drying.
7. Examine the thick blood film.

Activity 11
Laboratory Techniques

Student's Name _____Date Performed _____

Instructor's Name _____ Date Submitted _____

I. Indicate the macroscopic exam of the fecal specimen:

A. Consistency _____

B. Color _____

C. Others _____

II. Indicate the result of the microscopic examination of your specimen by completing the table below. (Separate papers may be attached).

Technique	Stool source	Stool type	Parasite/s observed (+ or -; attach picture of the prepared slide whether + or -)	Comments about the prepared slide (eg. Too thick fecal debris. etc)
DFS				
FECT				
Kato-Katz				
KatoThick				
Perianal Swab				
Thin Blood Smear				
Thick Blood Smear				

III. Why is it necessary that when collecting the fecal specimen, it should not be contaminated with urine? With water?

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