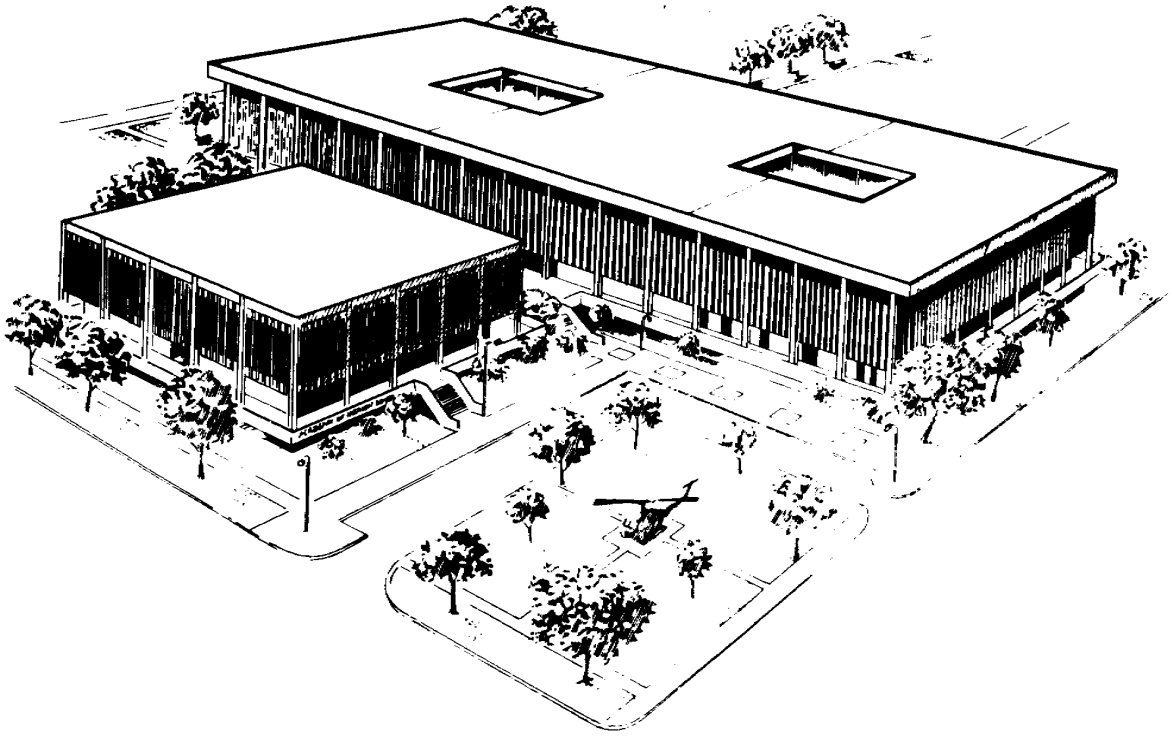
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6100



PARASITOLOGY I

SUBCOURSE MD0841 EDITION 100

DEVELOPMENT

This subcourse is approved for resident and correspondence course instruction. It reflects the current thought of the Academy of Health Sciences and conforms to printed Department of the Army doctrine as closely as currently possible. Development and progress render such doctrine continuously subject to change.

ADMINISTRATION

Students who desire credit hours for this correspondence subcourse must enroll in the subcourse. Application for enrollment should be made at the Internet website: http://www.atrrs.army.mil. You can access the course catalog in the upper right corner. Enter School Code 555 for medical correspondence courses. Copy down the course number and title. To apply for enrollment, return to the main ATRRS screen and scroll down the right side for ATRRS Channels. Click on SELF DEVELOPMENT to open the application; then follow the on-screen instructions.

For comments or questions regarding enrollment, student records, or examination shipments, contact the Nonresident Instruction Branch at DSN 471-5877, commercial (210) 221-5877, toll-free 1-800-344-2380; fax: 210-221-4012 or DSN 471-4012, e-mail accp@amedd.army.mil, or write to:

NONRESIDENT INSTRUCTION BRANCH
AMEDDC&S
ATTN: MCCS-HSN
2105 11TH STREET SUITE 4191
FORT SAM HOUSTON TX 78234-5064

Be sure your social security number is on all correspondence sent to the Academy of Health Sciences.

CLARIFICATION OF TERMINOLOGY

When used in this publication, words such as "he," "him," "his," and "men" 'are intended to include both the masculine and feminine genders, unless specifically stated otherwise or when obvious in context.

USE OF PROPRIETARY NAMES

The initial letters of the names of some products may be capitalized in this subcourse. Such names are proprietary names, that is, brand names or trademarks. Proprietary names have been used in this subcourse only to make it a more effective learning aid. The use of any name, proprietary or otherwise, should not be interpreted as endorsement, deprecation, or criticism of a product; nor should such use be considered to interpret the validity of proprietary rights in a name, whether it is registered or not.

TABLE OF CONTENTS

CORRESPONDENCE COURSE OF
THE U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL

SUBCOURSE MD0841

PARASITOLOGY I

INTRODUCTION

Parasitic infection can greatly interfere with a soldier's ability to complete his mission. The presence of parasites in a soldier's system can not only interfere with his ability to function, but also can make him susceptible to certain diseases. Since soldiers may serve in most areas of the world, you must be able to identify parasites that are found in the various parts of the globe.

In your job as a medical laboratory specialist, you will perform a variety of test procedures on samples taken from humans. Some of these samples will include feces and tissue scrapings used in the diagnosis and treatment of parasitic infection. Therefore, you must be knowledgeable in several areas of parasitology. The knowledge you will need is reflected in the two subcourses you are about to study. Subcourses MD0841, Parasitology I, and MD0842, Parasitology II, address areas of particular importance in parasitology.

Subcourse MD0841, Parasitology I, will give you a basic background in parasitology. An overview of parasitology; safety and quality control in the parasitology laboratory; taxonomy of parasites infecting humans; and microscopy are presented in this subcourse.

It is necessary for you to master the content of this subcourse before you proceed to the next subcourse. If you already have a parasitology background, use this subcourse as a refresher before starting your study of Subcourse MD0842.

Subcourse Components:

The subcourse instructional material consists of two lessons and an appendix as follows:

Lesson 1, Introduction to Parasitology
Lesson 2, Collecting, Preserving, and Processing Clinical Specimens.
Appendix, Glossary.

Here are some suggestions that may be helpful to you in completing this subcourse:

--Read and study each lesson carefully.

--Complete the subcourse lesson by lesson. After completing each lesson, work the exercises at the end of the lesson, marking your answers in this booklet.

--After completing each set of lesson exercises, compare your answers with those on the solution sheet that follows the exercises. If you have answered an exercise incorrectly, check the reference cited after the answer on the solution sheet to determine why your response was not the correct one.

Credit Awarded:

Upon successful completion of the examination for this subcourse, you will be awarded 8 credit hours.

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Branch at Fort Sam Houston, Texas.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

A listing of correspondence courses and subcourses available through the Nonresident Instruction Section is found in Chapter 4 of DA Pamphlet 350-59, Army Correspondence Course Program Catalog. The DA PAM is available at the following website: http://www.usapa.army.mil/pdffiles/p350-59.pdf.

LESSON ASSIGNMENT

LESSON 1	Introduction to Parasitology.
LESSON ASSIGNMENT	Paragraphs 1-1 through 1-33.
LESSON OBJECTIVES	After completing this lesson, you should be able to do the following:

1-1. Given a term pertaining to parasitology and a group of statements, select the statement that defines that term.

1-2. From a group of statements, select the statement that describes the pathogenic effects produced by a parasitic infection on a host.

1-3. From a group of statements, select the statement that describes how parasites can infect a host.

1-4. Given a group of statements, select the statement that describes the key factor that must be known in order to eradicate a particular parasite from an area.

1-5. From a group of statements, select the statement that describes a safety practice that should be followed in the laboratory.

1-6. Given the name of a type of material (e.g., specimen) and a group of statements, select the statement that describes how that material should be properly disposed.

1-7. Given the name of a particular category of chemical compound and a group of statements, select the statement that describes a safety consideration pertaining to that type of chemical substance.

1-8. From a group of statements, select the statement that describes a quality control consideration in the parasitology laboratory.

1-9. Given a group of statements and a taxonomic group, select the statement that best characterizes that group.

1-10. Given a group of statements pertaining to organism classification, select the statement that describes the use of genus and species classifications in reporting identified organisms.

1-11. Given the name of a part of a binocular microscope and a group of statements, select the statement that best describes that part or its use.

1-12. Given the multiplying power of the ocular lens and objective lens and a list of magnifications, select the total magnification of the given lens system.

1-13. From a group of statements, select the statement that best describes the proper care and/or maintenance required for a microscope or steroscope.

1-14. Given an unlabeled illustration of a microscope or stereoscope and a list of names of the parts of that piece of equipment, match the name of the given piece with its location on the instrument.

1-15. Given a group of statements related to the use of a binocular microscope, select a guideline for focusing a microscopic preparation.

1-16. Given a group of statements, select the means by that the amount of light entering the microscope can be controlled.

1-17. Given the name of a type of sample preparation and a group of statements, select the statement that describes how that specimen should be scanned for the identification of parasites.

SUGGESTION

After completing the assignment, complete the exercises of the lesson. These exercises will help you to achieve the lesson objectives.

LESSON 1

INTRODUCTION TO PARASITOLOGY

Section I. OVERVIEW OF PARASITOLOGY

1-1. INTRODUCTION

Today the third world countries are in constant fight for their survival against parasites. However, parasitic infections are not restricted to any particular geographical location and many researchers believe that the United States is heading toward an epidemic due to parasites. The size of parasites is varied, from minute microscopic forms to some measuring as much as 10 meters in length. Their life cycles can be as uncomplicated as simple cell division or so complex as to need two intermediate hosts to complete the required larval molting. Most parasites survive better in tropical weather, but there are some that have a predilection for cold climates.

1-2. HISTORY OF PARASITOLOGY

The history of the world is full of cases where parasitic diseases are mentioned. From the parasites found in the Egyptian mummies to various references used in the Bible, man has known and battled parasites since the beginning of recorded history.

a. **Ancient History.** The Ebers papyrus, written about 1600 BC, contains references to the presence of parasitic worms in man. The laws of the Mosaic code prohibiting the consumption of meat from unclean animals indicate that the early Israelites had knowledge about parasitic infections. There is also evidence of parasitic recognition among the Roman, Greek, Persian, and Phoenician scientists. Chinese doctors were aware of parasites as early as 300 BC.

b. **Modern History.** The liver fluke Fasciola hepatica was discovered by Jehan de Brie in 1379 from sheep. Anton Van Leewenhoek (1632-1723) described the morphology of the protozoan Giardia lamblia from his own stool. In 1880, Laveran demonstrated the intracellular parasites of malaria. These are but a few of the many discoveries made in the nineteenth and twentieth century that expanded the field of parasitology.

c. **Misconceptions.** Parasites were thought to be beneficial to humans during the seventeenth and eighteenth centuries. For example, many people believed that lice protected children from disease and leeches were used extensively for blood letting as a cure. For a time, intestinal parasites were thought to help in cleaning the tract of excess food and waste and until recently, the Chinese believed that powdered Ascaris was helpful for medicinal treatment of impotency. In France, the heads of tapeworms (scolices) were once used as weight control measures.

d. **Present Ideology.** Modern medicine has recognized the pathogenic effects of parasites and is searching for drugs useful in the treatment of parasitic infections. Prevention is the best method to attack the problem of parasites. Therefore, to prevent and to treat parasitic infections, the life cycles of the parasites must be known. To this end, much time and money have been invested in the eradication of vectors (carriers) that spread parasites. In fact, the fauna (animal life) and flora (plant life) of entire regions have been changed in an attempt to disrupt the reproduction of Schistosoma in some snails.

1-3. TYPES OF ORGANISM RELATIONSHIPS

a. **Normal Flora.** Normal flora consists of microorganisms that are normally and consistently found in or on the body in the absence of disease.

b. **Symbiosis.** This is the close association or living together of two organisms of different species; each party involved in this relationship is called a symbiont.

c. **Mutualism.** This is a type of symbiosis in which both organisms (host and parasite) benefit from the association.

d. **Commensalism.** This is also a type of symbiosis, but in this case, the parasite (commensal) is benefited and the host is neither benefited nor harmed by the relationship.

e. **Parasitism.** Parasitism is an obligatory relationship in which one organism, the parasite, is metabolically dependent on another organism, the host. The host may be harmed by such a relationship.

1-4. TERMS IMPORTANT IN PARASITOLOGY

a. **Facultative Parasite.** A parasite which normally has a free-living existence, but that will establish a parasitic relationship with a host only if the opportunity presents itself, is called a facultative parasite.

b. **Obligate Parasite.** This parasite cannot survive a free living state.

c. **Pathogenic Parasite.** The main concern of the medical parasitologist is the identification, treatment, and prevention of parasites that harm man, his crops, and his domestic animals.

d. **Ectoparasite.** This parasite lives on the outer surface of the host.

e. **Endoparasite.** This is a parasite that lives inside the host.

1-5. PARASITIC ATTRIBUTES THAT INFLUENCE DISEASE

a. **Virulence.** Virulence refers to the ability of a parasite to establish itself in a host, maintain that infection, and damage the body of the host. For one reason or another, one strain of a parasite may cause a more serious disease process than other strains of the same species.

b. **Parasitosis.** The more parasites infecting a host, the more severe will be the effect on that host.

c. **Life Span of the Parasite.** The longer the parasite lives in or on the host, the greater the damage that will be caused.

d. **Repeated Contact.** The more times that a host is parasitized by an organism, all other factors being equal, the worse the prognosis of the disease.

e. **Competition for Food.** Intestinal parasites deprive the host of the necessary nutrients required for survival.

f. **Mechanical Interference.** Some parasites accumulate (like in the intestines) in such great numbers that the normal flow of nutrients, waste, or fluid is obstructed. For example, the microfilariae of some nematodes obstruct the lymphatic system in elephantiasis. Their presence prevents lymphatic fluid from being circulated out of lymphatic tissue and the swelling associated with elephantiasis results.

g. **Toxic Effects.** By-products of metabolism and anatomic parts of the parasite can be very toxic to the host. In cases of massive infections with Trichinella spiralis, it is this toxicity that is lethal to the host.

h. **Tumor.** Tumor formation in the host is a common occurrence with parasites that invade or irritate the tissue of the host. Pathologists have suggested that repeated infections with Trichomonas vaginalis could lead to cervical cancer, and recently, some cases of malignant neoplasms have been reported from Egypt and attributed to Schistosoma infections. Some benign tumors are caused by the larval stage of the tapeworm Taenia solium in muscle tissue.

i. **Loss of Blood and Body Fluids.** The loss of blood and body fluids caused by parasites is significant. For example, it has been calculated that about 0.5 ml of blood per worm per day is lost by the host during a hookworm infection. Remember, these fluids contain nutrients and electrolytes needed by the host.

j. **Destruction of Host Tissue.** Parasites destroy the host's tissue structures by the presence and activity of the adults, by the migration of larval forms, and by ovipository migrations.

k. **Psychological Disorders.** The knowledge of parasites crawling inside the body can affect anxiety level of the host. But, the more detrimental psychological effects are those caused by the accumulation of chemicals in the system of the host.

1-6. HOST ATTRIBUTES THAT INFLUENCE RESISTANCE TO PATHOGENIC EFFECTS OF PARASITISM

a. **Fitness of the Host.** The nutritional and emotional state of the host can effect the degree of pathogenicity caused by the parasite.

b. **Host Age.** Both the very young and the very old are affected to a greater degree by a parasitic infection than are young adults.

c. **Specific Factors That Influence Resistance.**

(1) Immunity. Host resistance to parasitic infection is very similar to the resistance shown against bacteria. The immune system works by the formation of antibodies against a limitless amount of substances recognized as foreign antigens by the B lymphocytes. These blood cells, due to constant mutation, are much different from each other in such a manner that the system contains myriads of "coded" lymphocytes. When a parasite meets with one of these B lymphocytes that have a specific antibody against its antigen, the lymphocyte reproduces at a high rate to produce plasma cells. When these second generation antibodies encounter the antigens coating the parasites, a neutralization process takes place that kills the invaders. Immunity against parasites can be inherited or acquired. The acquired immunity may be natural or artificial, while the artificial can be active or passive.

(2) Complement system. This is a complex system of enzymes found in normal serum that aids the lysis (destruction) of the parasite during an antigen-antibody reaction. Complement is composed of nine components labeled C-1 through C-9. Component C-1 is further subdivided into $C\text{-}1_g$, $C\text{-}1_r$, and $C\text{-}1_s$. The system is activated by the immunoglobulins I_gM and I_gG. Complement also participates in other biological activities such as antibody- mediated immune lysis, phagocytosis, opsonization, and anaphylaxis.

(3) Interferon. Interferon is composed of a group of soluble small proteins produced by infected cells that inhibit multiplication of virus. The proteins are not virus specific, but they are cell specific in production and effects.

d. **Nonspecific Factors That Influence Resistance.** There are some barriers that affect parasitism. These barriers may be chemical, physical, or biological (the antagonistic effect of organisms already present at the site in question).

(1) Chemical barriers. The host combats the invasion of micro-organisms by the secretion of chemicals that are antimicrobial in nature. The acid pH of the stomach, skin, and vagina; the bile salts of the intestines; and the lysozymes of the eyes and saliva serve as deterents to the invasion of microorganisms.

(2) Physical barriers. The intact skin, mucus (sticky lining of the mucous membranes), nasal hairs, cilia of the respiratory epithelium, peristaltic movement, and normal microbial flora (occupying attachment sites) prevent the entrance of microorganisms.

(3) Microbial antagonism. Antiparasitic factors present in the serum and competition for nutrients from the normal flora also serve as obstacles for the possible pathogenic parasites.

1-7. EPIDEMIOLOGY

Epidemiology is the science that studies propagation and prevalence of human disease. It determines the frequency and distribution of a disease in a community. Epidemiologists, scientists who specialize in epidemiology, are always in search of specific causes of localized outbreaks of infection. Below are some terms commonly used in epidemiology.

a. **Endemic.** A disease is endemic when it is present in a community at all times but is clinically found in a few individuals and with low morbidity.

b. **Hyperendemic.** A hyperendemic disease is one that is present in a high frequency in a community.

c. **Sporadic.** A low frequency of disease that is not widely diffused is referred to as sporadic.

d. **Epidemic.** An epidemic occurs when a disease spreads rapidly through a community and affects a large number of individuals.

e. **Mode of Infection.** Some parasites can infect the host by direct contact, while others require a complex mechanism to enter the host.

(1) Congenital. Some parasites can be transmitted to the younger generation by the older generation. The transmission of malaria, toxoplasmosis and several other parasitic diseases may be accomplished by parasitic forms crossing the placental barrier from the mother's blood to the unborn fetus.

(2) Direct contact. Some parasites can complete their life cycle by being transmitted from an infected site to another site where there is no disease. Then they can parasitize either the new or the same host.

(3) Ingestion. Food and water are very important in the spread of parasitic diseases because most parasites inhabit the gastrointestinal tract. The infective form of the parasite (ova or larvae) may be present in the flesh of the intermediate host (plant or animal) or may be swimming free in contaminated water. The organism may gain access to food through contamination with night soil, water, by the hands of foodhandlers, by mechanical agents or by biological carriers such as insects. In some instances, the intermediate hosts, like small arthropods, are consumed with the food or water.

(4) Arthropod-borne. Members of the phylum Arthropoda serve as vectors (carriers) of parasitic diseases and bacterial and viral infections. The vector is an integral part of the life cycle of the parasite. In some instances the arthropods are intermediate hosts while in others they are the definitive host.

(5) Active penetration. In some parasitic infections, the infective form is a larval stage that has the capability of penetrating the host tissues.

(6) Transfusion. Certain blood and tissue parasites may be present in donor blood at the time of transfusion. Thus, these parasites may be introduced into the new host system and cause parasitic infection.

1-8. PREVENTIVE MEASURES

The individual, as well as the community, must get involved in the prevention of parasitic infections. Life cycles of parasites may be interrupted by eradication of the vectors. Education about hygiene, eating habits, and disposal of human and animal wastes can also be used in combating parasites. Treatment of diseased individuals not only relieves the suffering, but also prevents the spread of the disease.

1-9. THE EVOLUTION OF PARASITES

It is believed that at one time parasites were free living and that the loss of genetic information forced them to adapt to a parasitic existence over many years.

a. **Hybrid Vigor.** Some parasites replicate by asexual methods. This type of reproduction tends to concentrate detrimental genetic traits. On the other hand, sexual reproduction adds vitality to the species.

b. **Mutation.** Mutations, in the majority of cases, tend to be detrimental to the species, but occasionally the change may become beneficial by adding or subtracting a trait that tends to enhance the survival chances of the mutated species.

c. **Natural Selection.** "Survival of the fittest" is the expression used to describe the phenomena governing the extinction of some species and the survivability of others. In the evolutionary schema of life, natural selection plays an important role in the adaptation of organisms.

d. **Lag in Adaptation.** Some writers consider parasitism to be a deteriorated type of existence--a lag in adaptation. Others argue that the success of parasitic survival should be considered as progress.

Section II. SAFETY AND QUALITY CONTROL IN THE PARASITOLOGY LABORATORY

1-10. INTRODUCTION.

The fecal specimen may contain parasites and many potentially pathogenic bacteria and contagious viral agents. Therefore, the manner in which the specimens are handled and the means employed in the disposal of waste material are of the utmost importance in order to maintain good health for the laboratory personnel and the community. In addition to potential sources of infection, the parasitology laboratory has other dangers such as poisons, toxic chemicals, and flammable fluids. Some of the chemicals used in parasitology have the added potential danger of polluting the environment. Therefore, the disposal of these substances is governed by local, state, and Federal regulations. Every laboratory must have a written document (standing operating procedures (SOP)) indicating the methodology to be used in ensuring the workability of the equipment, the correct use of reagents, and the adequate and timely training of the technicians. Quality control ensures reliability, responsibility, and reproducibility of results in the laboratory.

1-11. INDIVIDUAL HEALTH HAZARDS

Because most of the parasites that affect man use the oral route as the mode of infection, care must be taken to avoid ingestion of infective organisms. Direct contact and inhalation can also transfer the parasites to hosts. Likewise, chemicals used in the laboratory can be dangerous if they are used unwisely. The guidelines below can make the laboratory a safer place to work.

a. **Eating and Drinking.** No food or drink should be allowed in the laboratory. Food and drink can become contaminated by samples and chemicals.

b. **Smoking.** Smoking should not be allowed in the laboratory. One, disease can be spread by smoking. Two, smoking can cause a fire or explosion.

c. **Handwashing.** You should frequently wash your hands while working in the laboratory. Frequent handwashes, especially when leaving the laboratory, can help prevent the spread of parasites.

d. **Caring for Uniforms.** When possible, uniforms and laboratory coats should be laundered at the working site. If that is not possible, special attention must be given to avoid contamination of other items in your household.

e. **Eye Safety.** An eye fountain or spray should be readily available in the event of an accidental splash with a specimen or chemicals.

f. **Obtaining Immunizations.** A complete series and periodic boosters for polio, typhus, and typhoid are recommended for parasitology workers.

1-12. DISPOSAL OF CONTAMINATED MATERIALS

All specimens should be considered to contain pathogenic organisms and treated as such. Properly labeled waste containers and written instructions (SOP) for the collection and disposition of the trash can waste must be carefully followed in the laboratory. Below are some considerations in this area.

a. **Slides.** A large container filled to one-third to one-half capacity with a disinfectant is convenient for the disposal of contaminated slides. When the container is too full for the slides to be covered with disinfectant, it should be autoclaved and emptied.

b. **Specimens.** The recommended method of destroying unwanted samples is incineration. Steam under pressure (autoclaving) is suggested if burning is not feasible.

c. **Small Items.** Applicator sticks, tongue depressors, pipettes, and other small items may be discarded in the same container as the slides.

d. **Spillage.** When a specimen is spilled, the spillage must be soaked with a disinfectant and allowed to stand for a time. Then the residue should be cleaned and discarded as contaminated trash.

1-13. HANDLING OF HAZARDOUS REAGENTS

All chemicals in the laboratory must be considered poisonous, flammable, corrosive, or any combination of the above. Distinct labels (such as "POISON" and "FLAMMABLE") must be affixed to the respective containers. Work with chemicals should be performed in a chemical fume hood. Some chemicals require special attention. Read about these below:

a. **Mercuric Compounds.** The half-life for the decomposition of mercury is very long; therefore, mercuric compounds cannot be disposed of as regular trash because they pollute the environment. The easiest and safest method for the disposal of mercury is to contract a local company that works with these substances. But if local disposal is necessary, a closed steel container is suitable for accumulation pending disposal.

b. **Corrosives.** Strong acids and bases are harmful because of skin burns and inhalation. They can also be the cause of corrosion of the laboratory equipment. A sandbox is required for the storage of these chemicals. Avoid storing strong acids and bases together.

c. **Flammables.** Some liquids and solids have a low flash point and react so violently to sudden changes in temperature or pressure that they are considered to be explosives. When using these materials, ensure that there is adequate ventilation and that there are no open flames in the area.

d. **Poisons.** Some substances can intoxicate to the extent of death, even when small amounts are ingested or inhaled. Pipetting by mouth should be avoided at all times. Poisons that are given to patients as fixative (PVA) must have a prominent red label marked "POISON."

e. **Carcinogens.** Certain reagents, such as xylene and formaldehyde, upon prolonged contact with the skin or mucous membranes, are suspect as the cause of cancerous processes.

1-14. EQUIPMENT IN THE PARASITOLOGY LABORATORY

A small amount of work (cleaning and calibrating the equipment) will save a large amount of money and time by eliminating breakdowns and repeated tests. Careful use and maintenance of the equipment below will ensure a more smoothly working laboratory:

a. **Refrigerator.** The refrigerator should be kept clean and defrosted. A daily log of the refrigerator temperature should be maintained. Flammables should be stored only in explosion-proof refrigerators. No food or drinks should be placed in the parasitology refrigerator.

b. **Autoclave.** The autoclave should be cleaned after every use to prevent accumulation of various deposits. The temperature and pressure of the autoclave should be recorded for each load (the temperature chart can be used as a permanent record). A processing indicator (autoclave tape) should be affixed to each item and a viability test (spore-test) must be performed weekly. This information must be kept as a permanent record.

c. **Microbiologic Safety Hoods.** Cleaning, checking for sufficient airflow, and verifying of the condition of ultraviolet (UV) light should be performed at regular intervals on all hoods.

d. **Centrifuges.** Maintenance personnel should calibrate the centrifuge as required by your SOP. The centrifuge must be kept clean at all times.

1-15. STAINS AND REAGENTS

When you prepare solutions, follow meticulously the recommended instructions of the manufacturer or currently approved literature. Dark amber bottles with a tight cap should be used to prevent deterioration or evaporation of the substance in the bottle. The following information must be included on a reagent or stain label: the date that the bottle was opened if it was purchased, the complete name of the reagent, the date of manufacture, the expiration date, and the initials of the person who prepared it. The stock should be rotated to use the older, nonexpired reagents before the newer ones.

a. **Stains.** All stains must be checked periodically with known samples to ensure potency and correct color reaction. Checking the expiration dates of stains will help prevent erroneous results. When consecutive stains are used, carryover from one dish to another should be kept at a minimum. Do this by blotting the tray of slides on paper towels prior to immersing into the next reagent.

b. **Reagents.** Positive and negative controls should be performed with all reagents to ensure their adequacy. Refrigerate and store reagents according to instructions furnished by the manufacturers.

1-16. CONTINUING EDUCATION

Laboratory personnel must be kept abreast of new trends, ideas, and procedures in the field of parasitology.

a. **Inservice Seminars.** A set schedule of conferences within the laboratory should be established to update technicians. Members of the staff can present lectures on current changes and findings within the field of parasitology.

b. **Surveys.** Known samples can be used to test the reproducibility and proficiency of the laboratory in terms of performing certain procedures.

(1) Internal. The supervisor may initiate a survey by introducing a control specimen as a routine sample.

(2) External. Commercial surveys that verify the results obtained in the local laboratory with those obtained by other laboratories on a regional or national level are available from various groups and societies.

c. **Workshops, Seminars, and Symposia.** In order to maintain proficiency in the parasitology laboratory, personnel must be given access to training in current trends and methodology. This is best accomplished by attending workshops, seminars, and symposia offered at local, regional, or national conferences.

d. **References.** A modern library should be available to the laboratory staff. It should contain textbooks, periodicals, and newsletters.

Section III. TAXONOMY OF PARASITES INFECTING HUMANS

1-17. INTRODUCTION

All living organisms have been divided into groups with similar characteristics. These groups have been subdivided further until organisms that have identical traits are classified under the same genus and species. It is important that you be familiar with the principles of taxonomy pertaining to parasites.

1-18. DIVISIONS OF LIVING ORGANISMS

a. **Kingdom.** This is a large group of organisms with similar features. In the literature, various authors list from two to five kingdoms.

(1) Kingdom PLANTA. This kingdom contains all of the plants. There are no plants parasitic to man.

(2) Kingdom PROTISTA. Members of this kingdom are unicellular (one-celled) organisms. The kingdom is further divided into two subkingdoms.

(a) Subkingdom EUCARYOTA. Eucaryotes are characterized by a nuclear membrane separating the nucleus from the cytoplasm, DNA that is grouped into units called chromosomes, multiplication accomplished by mitosis, and energy produced in structures called mitochondria. Some examples are protozoans and fungi.

(b) Subkingdom PROCARYOTA. The procaryotes are characterized by no nuclear membrane (therefore, there is no organized nucleus); no chromosomes (the DNA is not separated but is a continuous strand); no mitosis (multiplication is accomplished by simple cell division); and no mitochondria (energy is produced at the mesosomes). Some examples are bacteria and bluegreen algae.

(3) Kingdom ANIMALIA. The higher animals, including man, are placed in this kingdom. There are many parasites that infect man in this kingdom.

b. **Phylum.** A phylum is a major division of a kingdom. There are four phyla (plural of phylum) that contain human parasites: PROTOZOO from the subkingdom EUCARYOTA, and phyla PLATYHELMINTHES, ASCHELMINTHES, and ACANTHOCEPHAHELMINTHES from the kingdom ANIMALIA.

c. **Class.** A phylum is divided into classes. The name of the class should end in "a." Some examples are Cestoda and Nematoda.

d. **Order.** Several orders may be contained within a class. This name ends in "ea." Some examples are Filaroidea, Pseudophyllidea.

e. **Family.** An order may be subdivided into families. The family's ending is "ae." Some examples are Heterophyidae and Endamoebidae.

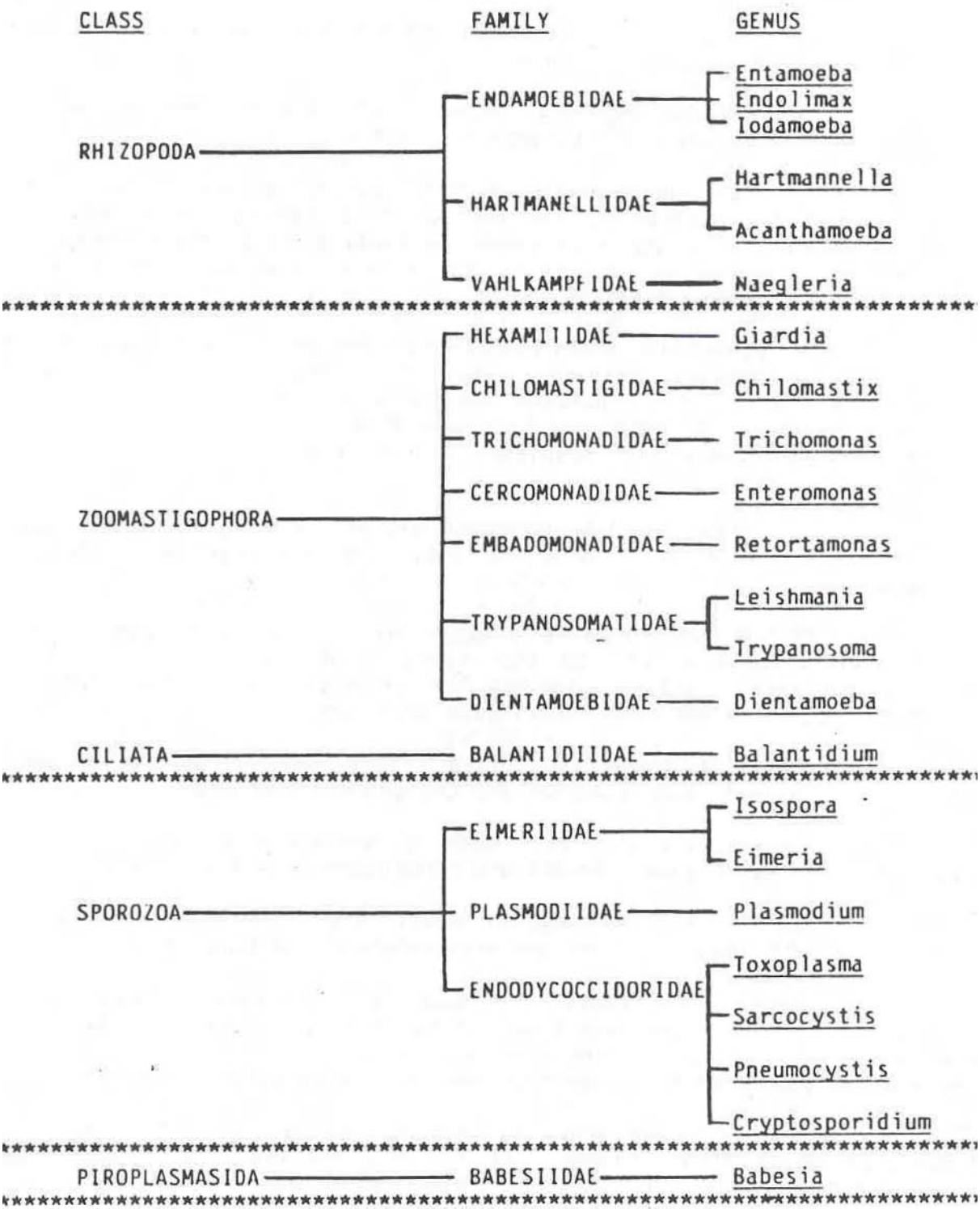
f. **Genus.** Each family is made up of various genera (plural of Genus). The genus name may have various endings. The first letter of the genus is capitalized and the name is underlined. The first letter may be used as an abbreviation. Some examples are Macracanthorhynchus and Entamoeba.

g. **Species.** Each genus is composed of species. The species' name may also have various endings. All letters are small case and the name is underlined. However, the species' name should never be abbreviated. Some examples are E. histolytica and M. hirudinaceus.

h. **Identification**. Parasitic organisms identified by laboratory procedures are reported by using the genus and species names. Some examples are Giardia lamblia and Enterobius vermicularis.

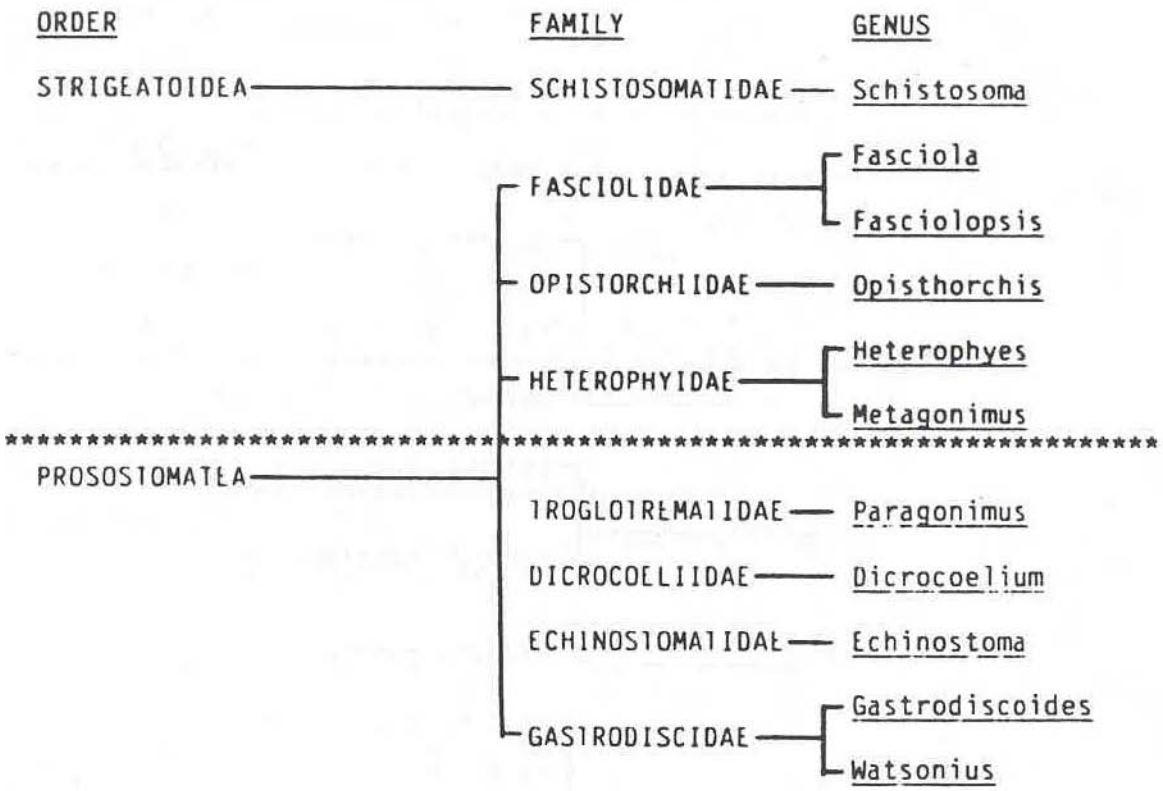
1-19. PHYLUM PROTOZOO

Class NEMATODA

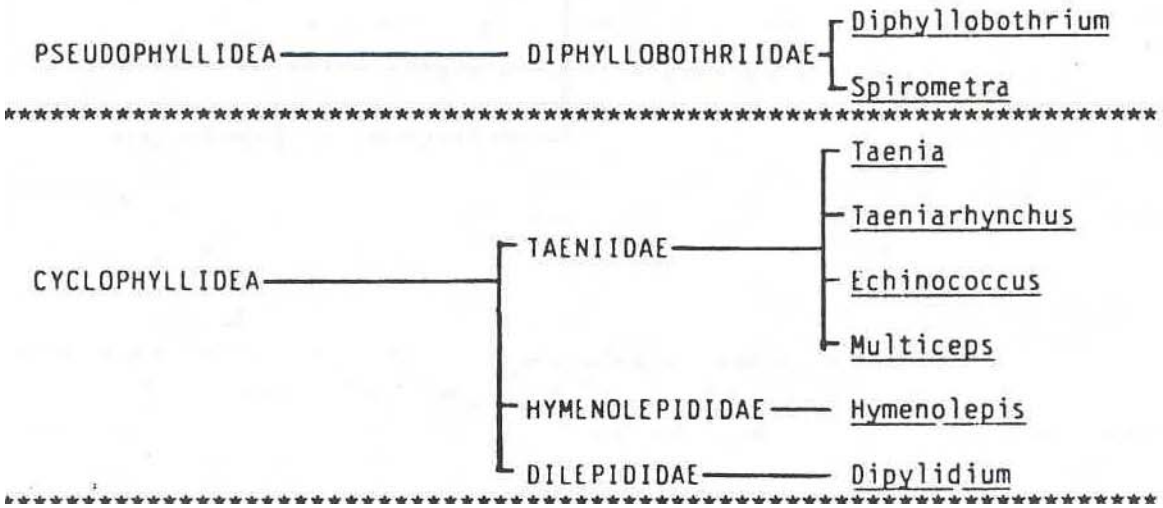


1-20. PHYLUM PLATYHELMINTHES

a. Class TREMATODA.



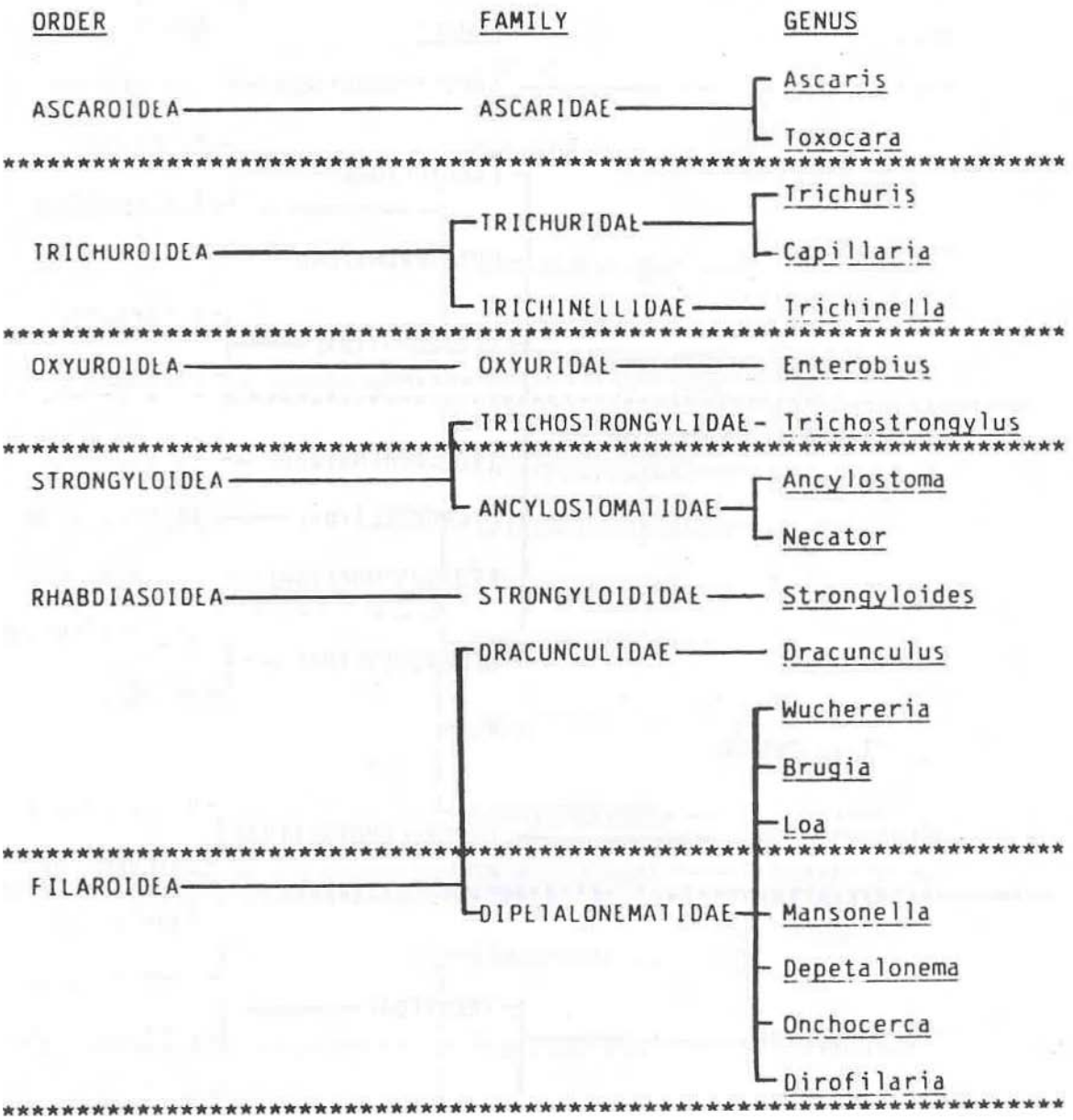
b. Class CESTODA.



c. Class TURBELLARIA. Free-living flat worms, Planaria species, etc.

1-21. PHYLUM ASCHELMINTHES

a. Class NEMATODA.



b. Classes ROTIFERA, GASTROTRICHA, KINORHYNCHA, PRIAPULIDA, and NEMATOMORPHA. Free-living roundworms.

1-22. PHYLUM ACANTHOCEPHAHELMINTHES

Class ACANTHOCEPHALA.

Genus Macracanthorhynchus.

Section IV. MICROSCOPY

1-23. INTRODUCTION

A modern binocular microscope is essential for the study of parasites. A compound microscope uses a combination of lenses (i.e., the objective and the ocular) to magnify the object. The objective lenses projects an enlarged primary image near the top of the tubular barrel. This aerial image is further magnified by the ocular lens, which projects it to the retina. The final image at the retina is called the virtual image. Through the compound microscope, this image is projected in a phase contrast system that allows for the differences that occur between light altered by the object and the unaltered (or background) light. The application of this principle (as provided by Kohler) gives a higher quality of resolution in observing cellular phenomena.

1-24. EQUIPMENT

A microscope (see figure 1-1) properly equipped with a lens system, an illumination system, a condenser, filters, a diaphragm, a prism, and a mechanical stage is suitable for diagnostic parasitology.

a. **The Lens System**. The lens system consists of the oculars (eye pieces) and the objectives.

(1) The oculars (eye pieces). In general, microscopes used in parasitology are provided with ten power (10X) wide field oculars.

(2) Objectives. Microscopes have a rotating nosepiece to which three or four objectives are attached. These objectives are of different magnifications, different working distances, and are distinguished by a color band.

OBJECTIVE	COLOR BAND	FOCAL DISTANCE	MAGNIFICATION
Scanner	Black	7.2 mm	4X
Low power	Green	4.3 mm	10X
High power	Yellow	0.7 mm	45X
Oil immersion	Red	0.1 mm	100X

b. **Magnification**. By multiplying the power of the ocular (10X) by the power of the objective (4X, 10X, 45X, or 100X), total magnification (40X, 100X, 450X, or 1000X) is obtained.

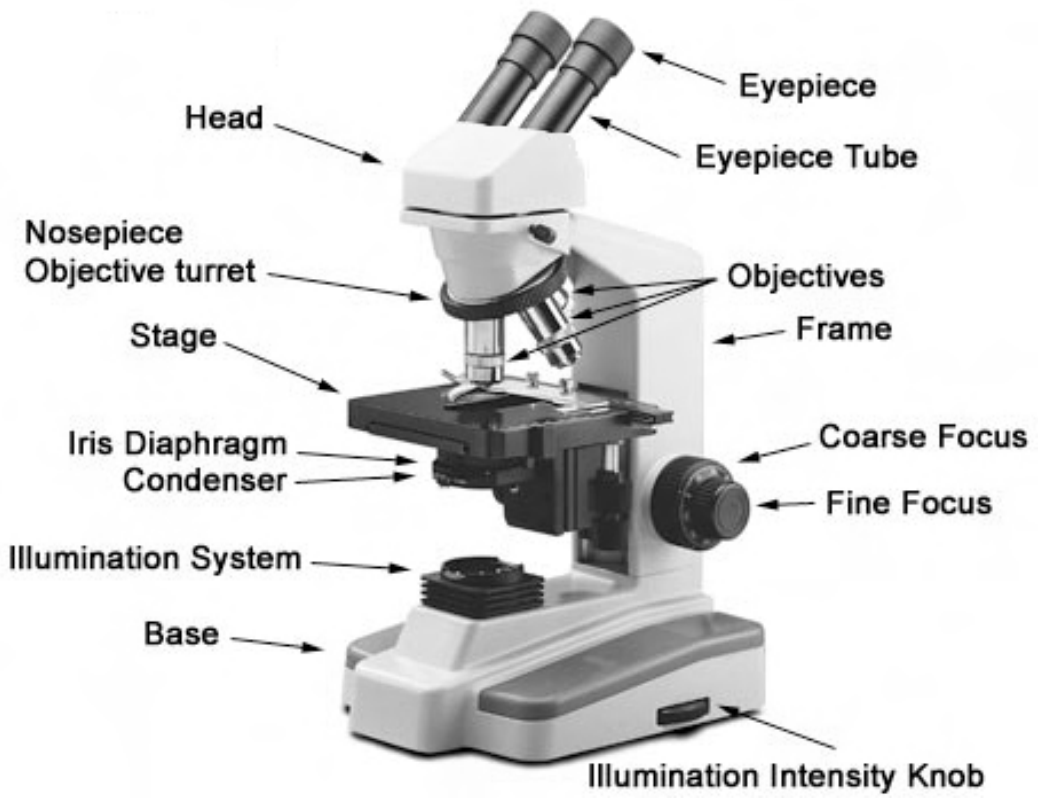


Figure 1-1. Typical binocular microscope.

c. **Illumination.** Correct illumination can be obtained from reflected (mirror) or direct (under the stage) light. The size of the opening of the condenser (iris diaphragm) and the position (high or low) control the amount of light entering the system. As the condenser is lowered, less light arrives at the ocular. When using a mirror, the flat surface should be employed for artificial light, whereas the concave side of the mirror is utilized in order to concentrate the scattered rays of natural light. Kohler developed a type of illumination (which bears his name) that achieves an even and optimum light irrespective of the light source. This type of critical illumination should be established prior to use of the microscope.

1-25. CARE OF THE MICROSCOPE

The microscope is a delicate precision instrument. Therefore, it must be handled with the utmost care.

1-26. USE OF THE MICROSCOPE

a. **Use**. Unauthorized persons should not be allowed to manipulate the microscope. Authorized personnel should receive training on microscope use on an as needed basis.

b. **Protection for the Microscope.** To prevent dust from settling on the lenses and mechanical parts, the microscope should be kept covered or stored in a closed cabinet when it is not being used.

c. **Transportation of the Microscope.** The microscope should always be carried with two hands--one on the handle and the other on the base. Two trips may be required when other equipment is used with the microscope. One trip may be necessary to carry the microscope and the other to carry the light source. The microscope should be kept upright because there is a danger that the oculars may fall and break if the microscope is tipped.

d. **Maintenance of the Microscope.** Cleanliness of the microscope is important for good performance. Loose dust or dirt on the optical surfaces should be blown off before attempting to wipe the lens. This prevents scratching of the lens. Chemical solvents should not be used to clean the lenses because these solvents will dissolve the cement that holds the lens in place. A good grade lens paper with a small amount of commercial lens cleaner or isopropyl alcohol may be used for the removal of residual oil that will cause loss of contrast and definition. Prevention of oil carryover to the dry lenses can be done by cleaning the oil immersion objective last. The body of the microscope can be cleaned with gauze and alcohol.

1-27. OPERATION OF THE BINOCULAR MICROSCOPE

The proper use of the microscope will result in excellent performance and the prevention of malfunction. The procedures below serve as guidelines for the proper use of this delicate and expensive instrument.

a. **Preparing the Microscope.**

STEP 1: Plug the microscope or transformer into the outlet.

STEP 2: Turn the instrument on and adjust the amount of light with the variable intensity control.

STEP 3: Open the field diaphragm.

STEP 4: Place the neutral density filter on dim.

STEP 5: Open the iris diaphragm on the Abbey condenser.

STEP 6: Place a slide with a specimen on the stage.

STEP 7: Turn the low power objective (10X) into place.

NOTE: The microscope should always be adjusted on the low power objective before using a higher magnification.

b. **Focusing the Specimen.**

STEP 1: Move the swing-out lens (under the microscope) out of the light path (if the scope is so equipped).

STEP 2: Place the substage condenser in the extreme upper position.

STEP 3: Rotate the coarse focus adjustment counterclockwise to lower the nosepiece completely.

STEP 4: Use the fine focus knob to bring the specimen into sharp focus.

(a) Rotate the fine focus knob completely counterclockwise until it reaches its stop.

(b) Rotate the knob back four to five turns clockwise while looking through the oculars. The scope should be in focus.

c. **Adjusting the Interpupillary Distance.** Adjust the interpupillary distance by using the thumb wheel.

NOTE: Do not pull the oculars apart, this action may result in irreparable damage to the system.

d. **Focusing the Oculars.**

STEP 1: Focus the specimen while looking through the right ocular and adjusting with the fine focus knob.

STEP 2: Adjust the left ocular by turning the ocular focus knob.

1-28. PARFOCALITY

Most microscopes are parfocal; that is, once the object is in focus with one objective, the other objectives should be in focus with just a slight adjustment of the fine focus.

1-29. KOHLER ILLUMINATION

Kohler illumination may be obtained once the preceding adjustments of the microscope have been made. Since most of the confirmatory work in examining parasitological specimens is usually accomplished by the use of the high power objective, adjustment aimed at obtaining this type of illumination should be made using the high power objective.

STEP 1 Adjust the field diaphragm in the illuminator system so that light centers directly on the diaphragm leaves of the microscope condenser.

STEP 2: Ensure that the specimen is still in focus.

STEP 3: Focus the substage condenser until the leaves of the iris diaphragm come into focus.

STEP 4: Close the field diaphragm until no more than the field to be examined is illuminated.

STEP 5: Remove the right ocular and examine the back lens of the objective. The diaphragm of the condenser is now opened or closed until the light just fills the back lens of the objective. When this occurs, a thin silhouette of prismatic color may be seen at the edge of the diaphragm.

STEP 6: Replace the ocular and the illumination should now be correct.

1-30. LIGHT INTENSITY

a. One of the most important considerations in parasitology work is the application of light in the microscope. Without proper illumination and light intensity parasitic identification forms can easily be missed.

b. The light intensity should be increased or decreased by the use of the neutral density filter control. The iris diaphragm can be used if more or less light is required.

NOTE: Do not move the substage condenser (condenser focusing knob) to adjust the amount of light. This will destroy the critical illumination effect.

1-31. THE USE OF THE OIL IMMERSION OBJECTIVE

The oil immersion objective can be used to view extremely small objects. Follow these steps:

STEP 1: Adjust/focus the microscope on low power.

STEP 2: Rotate the nosepiece until no objective is in place.

STEP 3: Place a small drop of oil on the slide.

STEP 4: Rotate the nosepiece until the oil immersion objective is in place.

STEP 5: Focus the object carefully with the fine adjustment knob.

STEP 6: Adjust the light intensity by using the neutral density filter control. If more light is desired, open the iris diaphragm on the substage Abbey condenser.

1-32. SCANNING TECHNIQUES

The oil immersion objective should never be used with wet preparations such as formalin preparations, MIF slides, and iodine wet preparations. At least fifteen minutes should be dedicated to the examination of each slide. When in doubt about the identity of an organism, continue scanning until absolute identification is made.

a. **Wet Preparations.** DO NOT USE OIL. These preparations contain specimens in a suspended state. Therefore, oil will ruin the slide. These slides should be observed with the low and high dry objectives only. Scan the complete slide on low power for helminthes. In order to find protozoans, the high dry objective should be used. Identify all organisms with the high dry objective.

b. **Tissue Preparations.** Observe these slides on low or high power objectives. Do not use oil immersion on these slides.

c. **Permanent Stains.** Iron Hematoxylin, Trichrome, and Chlorazol Black E stains should be scanned and identified under the oil immersion objective.

d. **Malaria Smears.** Observe the thick portion of the smear first for the presence of organisms. Identify the parasites on the thin portion of the slide. Scan and identify malaria organisms under the oil immersion objective.

1-33. THE STEREOSCOPE

This instrument (see fig. 1-2) is utilized for the identification of large larvae, helminthes, and intermediate hosts that are too large for the binocular microscope. Follow these suggested steps in using the sterescope:

STEP 1: Adjust the interpupillary distance by grasping the ocular tubes, one in each hand, and adjust to desired length by gently pushing or pulling apart.

STEP 2: Focus the oculars in the same manner as for the binocular microscope.

STEP 3: Select the proper magnification using the zoom magnification knob.

STEP 4: Sharpen the image by using the focusing knob.

STEP 5: Clean and store in a manner similar to that used with the binocular microscope.

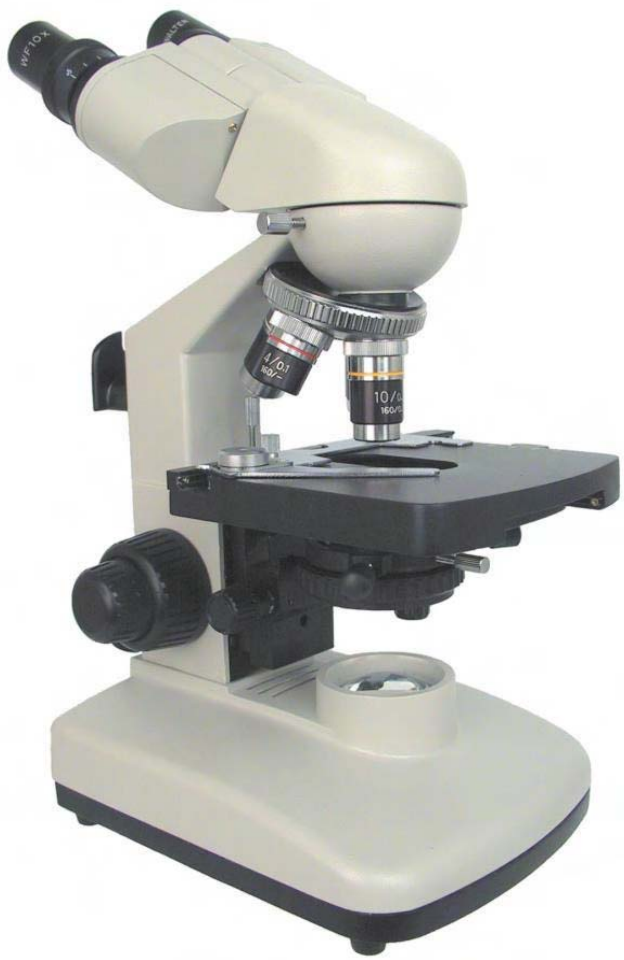


Figure 1-2. Typical stereoscope.

Continue with Exercises

EXERCISES, LESSON 1

INSTRUCTIONS. Answer the following exercises by marking the lettered response that best answers the question or best completes the incomplete statement.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Symbiosis is defined as:

   a. A state in which two members of the same species band together to form a group for hunting and protective reasons.

   b. The close association or living together of two organisms of different species.

   c. A type of commensalism in which a parasite lives off another organism (i.e., the host).

   d. A condition in which two organisms must live together in order to exist.

2. A pathogenic parasite is one that:

   a. Lives on the outer surface of the host organism.

   b. Can harm man, crops, and domestic animals.

   c. Lives on the inside of the host organism.

   d. Has a long life span in the host organism.

3. Select the statement that describes the pathogenic effect produced by a parasitic infection on a host.

   a. Toxins produced by parasites inhibit the production of vitamins in the body of the host.

   b. Some parasites are so numerous that their presence in the intestines can obstruct the flow of lymph in the lymphatic system.

   c. Some parasitic infections can cause a loss of body fluids and blood.

   d. All parasitic infections produce tumors in host tissues.

4. Select the statement(s) that describe how parasites can infect a host.

    a. They can be injected into the host by arthropods that harvest the infectious agent.

    b. They can enter the body through the active penetration of the host's skin by parasitic larvae.

    c. They can enter the body through the gastrointestinal tract.

    d. All the above.

5. Which of the following statements describes a safety practice that should be followed in the laboratory?

    a. Spilled specimens should be soaked with a disinfectant and allowed to stand before they are cleaned up and disposed as contaminated trash.

    b. All solutions used in the laboratory should be pipetted by mouth to ensure accurate measurement.

    c. Mercuric compounds should be disposed by flushing them down the toilet.

    d. Uniforms worn in the parasitology laboratory should be incinerated to prevent contamination of personnel outside the hospital.

6. Slides should be disposed by:

    a. Incinerating the slides.

    b. Placing the slides in a disinfectant solution prior to autoclaving them.

    c. Placing the slides in a metal container which is then given to a civilian firm for contracted destruction.

    d. Soaking the slides in a disinfectant solution prior to placing them in the trash.

7. Which of the following statements describe a safety consideration pertaining to mercuric compounds?

   a. Do not place these compounds in the regular trash because of their potential to pollute the environment.

   b. These compounds should be stored in a sandbox because of their potential to corrode and explode.

   c. Mercuric compounds should not be used around open flame.

   d. These substances are toxic even when small amounts are inhaled; therefore, they should be stored in an exhaust hood at all times.

8. Select the statement that describes a quality control consideration in the parasitology laboratory.

   a. Microbiologic safety hoods should be checked for sufficient air flow and operation of the ultraviolet light as required by your laboratory's SOP.

   b. Tests to ensure the proper functioning of the laboratory autoclave should be performed on a quarterly basis.

   c. Food and drinks should be placed in the parasitology refrigerator when it is empty.

   d. Centrifuges should be cleaned and calibrated by properly trained personnel on an annual basis.

9. Organisms in subkingdom Eucaryota are characterized by _____.

   a. DNA in a continuous strand.

   b. Energy production by mitosis.

   c. A membrane separating the nucleus from the cytoplasm.

   d. The lack of a nucleus.

10. You are using an ocular lens that has a power of 10X and an objective lens that has a power of 100X. What is the total magnification power of this lens system?

    a. 10X.

    b. 100X.

    c. 110X.

    d. 1000X.

11. When you carry a microscope you should carry it with two hands with:

    a. One hand on the handle and the other hand on the objective lens.

    b. One hand on the handle and the other hand on the auxillary light source.

    c. One hand on the handle and the other hand on the base.

    d. One hand on the handle and the other hand on the ocular lens.

12. Select the means by which the amount of light entering the microscope can be controlled.

    a. Manipulation of the coarse adjustment.

    b. Manipulation of the oil immersion objective.

    c. Changing the nose piece.

    d. Manipulation of the iris diaphragm.

13. How should you scan a wet preparation slide for protozoans?

    a. Use the oil immersion objective to identify the organisms after the slide has been scanned on the high dry objective.

    b. Scan the slide and identify the organisms using the low power objective.

    c. Scan the thick portion of the slide and identify the organisms on the thin portion of the slide.

    d. Scan the slide and identify the organisms with the high dry objective.

14. To report a parasitic organism you should include the name(s) of:

    a. Family and species

    b. Genus and species

    c. Species only

    d. Genus only

15. In focusing a microscopic specimen in a binocular microscope, it is important to:

    a. Place the substage condenser in the extreme lower position.

    b. Use the magnification recommended for identification of the suspected organism.

    c. Initially use the low power objective to focus the specimen.

    d. Check the focus before placing the specimen slide on the stage.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 1

1. b (para 1-3b)
2. b (para 1-4c)
3. c (para 1-5i)
4. d (para 1-7e)
5. a (para 1-12d)
6. b (para 1-12a)
7. a (para 1-13a)
8. a (para 1-14c)
9. c (para 1-18a(2)(a))
10. d (para 1-24b)
11. c (para 1-26c)
12. d (para 1-30)
13. d (para 1-32a)
14. b. (para 1-18h)
15. c. (para 1-27a-b)

End of Lesson 1

LESSON ASSIGNMENT

LESSON 2	Collecting, Preserving, and Processing Clinical Specimens.
LESSON ASSIGNMENT	Paragraph 2-1 through 2-21.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

2-1. Given a list of types of specimens, select the specimen(s) used in the laboratory to identify parasites.

2-2. Given the name of a parasitic disease and a list of names of serological tests, select the test(s) generally accepted as being useful and routinely used for the diagnosis of the given parasitic disease.

2-3. From a list of types of specimens, select the specimen(s) that can be used to determine the presence of blood parasites.

2-4. Given a description of a situation, select whether a venipuncture or a capillary puncture should be used to collect the required blood specimen.

2-5. Given a group of statements, select the statement that describes a step performed in either a venipuncture or capillary puncture to gain a blood sample.

2-6. From a list of names of anticoagulants, select the anticoagulant most often used in the collection of blood specimens for parasitic determinations.

2-7. Given the names of a group of chemical substances, select the chemical substance used as a preservative in parasitic specimens.

2-8. From a list of names of procedures, select the procedure that is the most satisfactory procedure for the definitive diagnosis of malaria, trypanosomes, and filariasis.

2-9. Given the name of a technique used to process a sample of blood and a list of statements, select the statement that describes the use of that technique.

2-10. Given the name of a technique used to process a sample of blood and a group of statements, select the statement that describes a step in that technique.

2-11. Given the name of a technique used to process a sample of blood and a list of statements, select the statement that describes the results of that technique.

2-12. Given the name of a technique for collection of gastrointestinal tract specimens and a group of statements, select the statement that describes proper application of that technique.

2-13. Given a characteristic to be observed during macrosopic examination of feces and a group of statements, select the statement that describes that characteristic.

2-14. Given a description of results from a stated test to determine occult blood in the feces and a group of interpretations, select the best interpretation of the results.

2-15. Given the name of a fixative solution and a list of fixative uses, select the statement that describes the use of that fixative.

2-16. Given a description of a specific parasitic characteristic and a list of techniques for direct microscopic examination of stool specimens, select the technique recommended for demonstration of that characteristic.

2-17. Given the name of a concentration technique for the detection of intestinal parasites and a group of statements, select the statement which best applies to the use of that technique.

2-18. Given the name of a permanent stain for protozoan organisms and a group of statements, select the statement that applies to the use of that stain.

2-19. Given the name of a medium used for the cultivation of parasites and a group of statements, select the statement that describes the preparation or use of that medium.

SUGGESTION

After completing the assignment, complete the exercises of the lesson. These exercises will help you to achieve the lesson objectives.

LESSON 2

COLLECTING, PRESERVING, AND PROCESSING CLINICAL SPECIMENS

Section I. OVERVIEW

2-1. INTRODUCTION

The precise laboratory diagnosis of parasitic infections is dependent on the manner in which the clinical specimens are handled. To obtain the maximum diagnostic information from clinical samples, the specimens must be processed in such a manner as to minimize deterioration and distortion. Today, the most reliable method of parasitological diagnosis is the direct examination of clinical samples. Indirect methods, such as serological and skin tests, are being introduced into the market at a rapid pace. These methods, while also reliable, are not as routinely employed as the already established direct observation procedures, using microscopic or macroscopic identification procedures. In some instances, examination is not possible within a short period of time. Therefore, preservation of the sample is imperative to retain the "in vivo" appearance of parasites. There are factors that affect the success in accurate laboratory diagnosis. One important factor is having an adequate number of well-trained personnel. Another factor is the availability of supplies and facilities. Perhaps the most important factor is the proper collection and satisfactory processing of clinical specimens.

2-2. TYPES OF SPECIMENS

The specimens usually obtained for laboratory identification of organisms parasitic to man are: blood, stool, exudates, aspirations, tissue biopsies, urine, sputum, and discharges from the vagina and urethra. Stages of parasites can also be identified from soil, water, insects, and samples from animal or vegetable sources.

2-3. PRESERVATIONS OF SPECIMENS

Clinical specimens must be preserved in order to maintain the integrity and features of parasites they may contain. The solution that preserves all kinds of specimens has not been developed. Therefore, you can choose from various methods and techniques depending on the sample you wish to preserve. Different clinical specimens require different methods of preservation (to be discussed at a later time).

2-4. PROCESSING

Many laboratory procedures have been developed to ease the identification of parasites. Every laboratory must have a standard operating procedure (SOP) that provides technicians with guidelines to follow in performing specific procedures. The more methods of examination available to the laboratory, the better the recovery of parasites. The choice among these methods must be dependent upon the sources available and must meet the local needs and conditions.

2-5. SEROLOGICAL DIAGNOSIS

The immunodiagnostic tests employed in diagnosis of parasitemia are, in general, modifications of commonly used procedures. These procedures are complement fixation, precipitation, hemagglutination, flocculation, and fluorescent antibody techniques. Various types of immunodiagnostic tests and the present status of their applicability in a variety of parasitic diseases are shown in table 2-1. Specimens collected for serological diagnosis of parasites are taken as for other types of serologic tests. The serum should never be inactivated.

DISEASE	INTRADERMAL TEST	COMPLEMENT FIXATION	PRECIPITATION	AGGLUTINATION	FLOCCULATION
Ascariasis	I	I	E		E
Trichinosis	A	A	A		A
Toxocariasis	I				I
Cysticercosis		A	A		A
Echinococcosis	A	A	E		A
Schistosomiasis	E	A	A	I	E
Clonorchiasis	E	E			
Paragonimiasis	E	A			
Filariasis	E	E			
Chagas Disease	I	A	I	I	
Leishmaniasis	A	E		A	
Toxoplasmosis	A	A		I	
Amebiasis		E	I		
Giardiasis				I	

DISEASE	HEMAGGLUTINATION	LATEX FIXATION	FLUORESCENT ANTIBODY	INDIRECT IMMUNO-FLOURENCENSE	ENZYME-LINKED IMMUNOSORBANT ASSAY (ELISA)
Ascariasis	E				A
Trichinosis	E				
Toxocariasis	I				A
Cysticercosis	A				
Echinococcosis	A				
Schistosomiasis	A	E		A	A
Clonorchiasis	E				
Paragonimiasis					
Filariasis	A				E
Chagas Disease			I	I	
Leishmaniasis					
Toxoplasmosis	E		I	A	E
Amebiasis	A		E		E
Giardiasis			I		E

A = Generally accepted, useful, routine diagnostic test.

E = Used for diagnosis but requires further evaluation for the routine use.

I = Under experimental investigation.

Table 2-1. Parasitic diseases and the appropriate serological tests available.

Section II. BLOOD SPECIMENS

2-6. INTRODUCTION

Proper collection and handling of specimens to be examined for blood parasites are important since inadequate or poor samples may lead to erroneous conclusions. Not all the organisms usually grouped as blood parasites are diagnosed from blood. In certain instances, spinal fluid, peritoneal fluid, aspirates, and biopsies of organs and tissues are also used to diagnose blood parasites.

2-7. COLLECTION OF SPECIMENS

The blood specimens used for clinical testing are obtained using a procedure called venipuncture. Venipuncture is a minor surgical procedure. Therefore, you must be aware of the precautions associated with the technique when you perform a venipuncture. Sometimes, the venipuncture technique is not practical. For example, the physical condition or age of the patient or the need for only a small volume of blood may make the venipuncture technique impractical. In these instances, a capillary puncture is indicated. No matter which method is chosen for blood specimen collection, you must attempt to reduce the trauma for the patient while you obtain an acceptable specimen for clinical testing.

2-8. THE VENIPUNCTURE

The venipuncture procedure is employed when more than a few drops of blood are needed for the performance of the required procedure. Blood collection can be achieved in two different ways: the use of a syringe and needle or by the use of a vacuum system. The chosen vein for phlebotomy must be large, easily accessible, and close enough to the surface to be seen and palpated with the finger. The most common site for venipuncture is the medial surface of the elbow joint--the antecubital area. The veins of the hand and foot are also used, but these areas are more painful when punctured and give discomfort to the patient. Also, they are not anchored as well as those in the antecubital area. **UNDER NO CIRCUMSTANCES SHOULD A TECHNICIAN WITHDRAW BLOOD FROM A SAGITTAL SINUS, JUGULAR VEIN, OR FEMORAL VEIN. THE LABORATORY TECHNICIAN SHOULD ALSO AVOID THE USE OF AN ARTERY.** The physician in charge of the patient should be consulted when difficulties are encountered in performing the phlebotomy procedure. Strict aseptic technique must be used for venipuncture. That is, care must be exercised to complete the procedure without contaminating the sample or introducing any foreign material into the patient's vein.

a. **Equipment.** Sterility of the appropriate equipment is required.

(1) Alcohol sponges. Gauze pads soaked in 70 percent Isopropyl alcohol are used to cleanse the site of the venipuncture. Commercially prepared pads can be purchased for this purpose.

(2) Tourniquet.

(3) Sterile syringes with needles or vacuum collecting apparatus.

(4) Sterile gauze pads (2 x 2 inches).

(5) Collecting tubes and labels.

b. **Procedure.** See the following steps and figure 2-1.

STEP 1: **Select the site for the venipuncture.**

a. Place the tourniquet on the upper arm using a quick release knot.

b. Palpate (touch) and view the area of choice.

c. Note the vein's orientation.

d. Release the tourniquet.

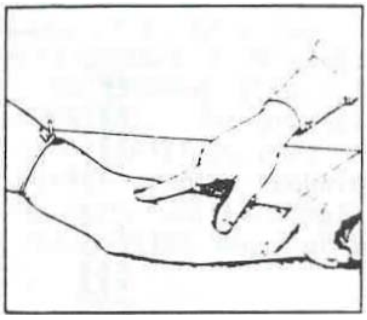


Figure 2-1a. Locate the vein.

STEP 2: **Cleanse the venipuncture site.**

Use a spiral or over-lapping technique.

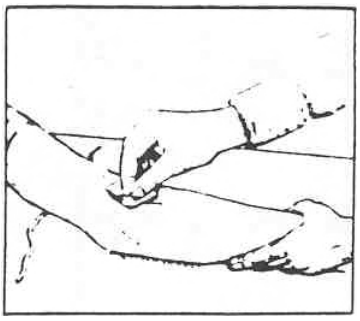


Figure 2-1b. Clean the puncture site.

STEP 3: **Perform the puncture.**

a. Replace the tourniquet.

b. Ask the patient to pump the hand three to four times and then to make a fist.

c. Anchor the vein by pulling the skin with the thumb of the left hand in order to stabilize it, since some veins roll aside at entry. This is especially true in elderly or debilitated patients.

d. Puncture the skin at a 30-degree angle while keeping the bevel of the needle in an upward position and parallel to the vein.

e. Thread the vein (place the needle in the vein) for a short distance.

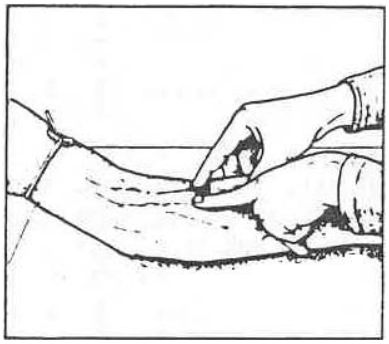


Figure 2-1c. Guide needle toward the vein.

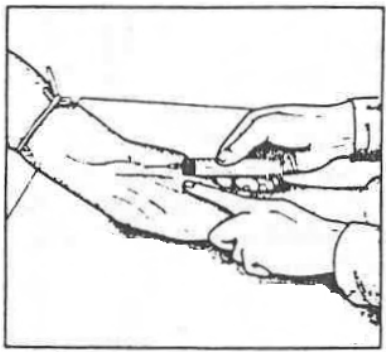


Figure 2-1d. Insert needle into the vein.

NOTE: The puncturing, entering, and threading of the vein should be performed in one motion.

STEP 4: **Collect the blood.**

a. Syringe method. Aspirate the blood by slowly retracting and slightly rotating the plunger until the required amount of blood is collected.

b. Vacutainer procedure. Insert the vacutainer tube into the holder until the stopper is even with the guide- line. The rear needle should puncture the stopper, but must not penetrate completely across the membrane (the vacuum will be lost if the needle penetrates). Once the needle has been threaded into the vein, push the test tube completely into the holder without jerking the holder or moving the needle. If more blood is needed, withdraw the test tube after it is full with blood and replace with another.

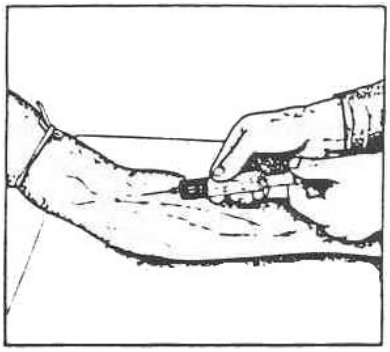


Figure 2-1e. Aspirate the blood.

STEP 5: **Release the tourniquet.**

Release the tourniquet and ask the patient to relax the fist.

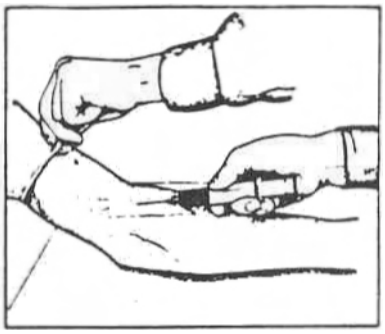


Figure 2-1f. Remove the tourniquet.

STEP 6: **Remove the needle from the vein.**

Cover the site of phlebotomy with a sterile gauze pad (2 x 2 inches). While applying slight pressure to the pad, withdraw the needle from the vein in a quick motion.

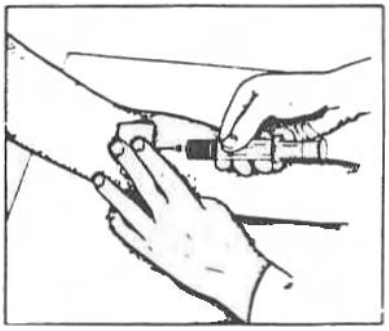


Figure 2-1g. Place a sterile gauze pad over the site and withdraw the needle.

STEP 7: **Complete the procedure.**

Instruct the patient to apply pressure to the venipuncture site while elevating the extended arm for several minutes until the bleeding stops.

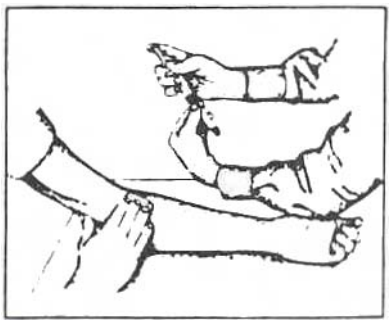


Figure 2-1h. Have the patient extend the arm and maintain light pressure on the site.

c. **Considerations and Precautions Associated with Venipuncture.**

(1) Behavioral problems may be encountered from some patients because of anxiety. Remember that the patient is not as familiar with the procedure as you are. The patient's apprehensions can be eased with a step-by-step explanation of what is going on. Always talk in a calm and assuring tone of voice. Do not forget to be tactful. Remember, treat the patient as you would want to be treated if you were at the other end of the needle.

(2) If problems are encountered entering the vein or if blood is emptying out of the vein into the tissue (hematoma), remove the tourniquet immediately. Then, withdraw the needle, apply pressure to the site, and elevate the arm.

(3) A frequent side effect of phlebotomy is fainting (syncope). When encountered with this situation, remove the tourniquet, withdraw the needle, apply pressure, and lay the patient down. Then elevate the patient's feet, loosen clothing, revive the patient with ammonia, and remove the patient from the view of other patients. Do not assume a position that may be construed as a threat to the awakening patient. Lastly, avoid comments that may be felt to be detrimental or insulting.

2-9. THE CAPILLARY PUNCTURE

When only a small amount of blood is required, a capillary puncture should be performed. This is the simplest means of obtaining blood, but remember, this is still a minor surgical procedure. Although there are several recommended anatomical sites for the procedure, the most often used is the lateral aspect of the palmar surface of the ring finger on the nondominant hand. This finger usually has the softest skin and puncturing

this site causes the least amount of discomfort to the patient. Other sites used for capillary puncture are the ear lobe (used when the finger is not suitable or when the patient is extremely frightened), the big toe, and the heel (when collecting from an infant).

a. **Equipment.** Again, this procedure is also minor surgery. Therefore, aseptic technique must be employed and all the appropriate equipment must be sterile.

(1) Alcohol sponges. Gauze pads soaked in 70 percent Isopropyl alcohol are used. Commercially prepared pads can be purchased for this purpose.

(2) Blood lancets (Hemolet^{R}). The most satisfactory instrument to use for capillary punctures is one that penetrates the skin to a depth of no more than four millimeters (three and four millimeters).

(3) Sterile gauze pads (2 x 2 inches).

(4) Slides and capillary tubes.

(5) Sterile silicone jelly (for a heel puncture).

b. **Procedure.** See the following steps and figure 2-2.

STEP 1: **Prepare the puncture site.**

Warm the area to assure good circulation of blood (38-40º C). Cleanse the area with an alcohol sponge.

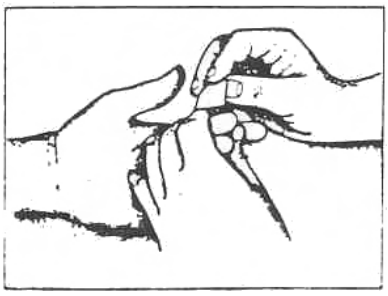


Figure 2-2a. Clean the puncture site.

STEP 2: **Perform the capillary puncture.** Puncture the skin with a quick firm stroke of the lancet. Depending on the selected area of the puncture, use the techniques below.

a. The finger puncture. Hold the patient's finger between your thumb and index finger while puncturing the finger and collecting the blood.

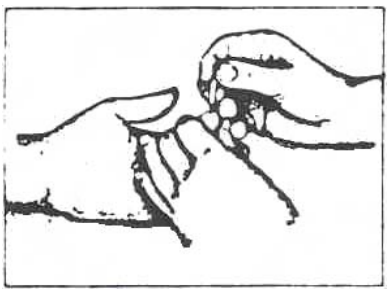


Figure 2-2b. Puncture the finger.

b. The ear puncture. Hold the patient's ear lobe between your thumb and index finger while puncturing the edge of the ear lobe.

Figure 2-2c. Puncture the ear.

c. The heel (or toe) puncture. Apply a thin film of sterile silicone jelly to the site of puncture--this facilitates the formation of well-rounded drops and helps to prevent clotting, especially when collecting over 0.5 ml. Puncture the plantar surface of the heel (or toe) and repuncture at a 90-degree angle forming an "X" wound.

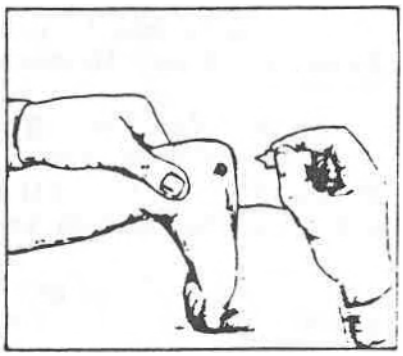


Figure 2-2d. Puncture the heel.

STEP 3: **Collect the Blood.**

Wipe away the first drop of blood. Place the collecting utensils into the drop of blood, do not touch the skin. After the collection of the sample is completed, have the patient hold a sterile gauze pad over the wound until the bleeding stops.

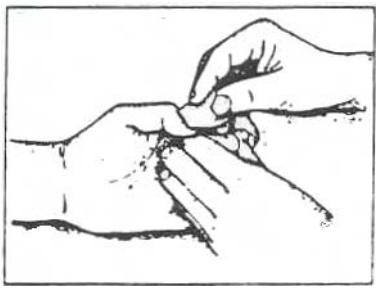


Figure 2-2e. Wipe away the first drop of blood.

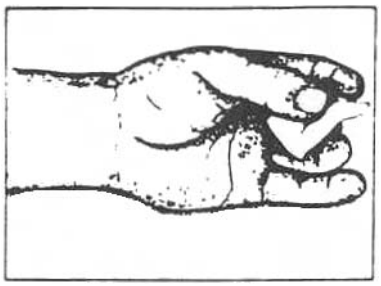


Figure 2-2f. Apply pressure to the site.

c. **Precautions.**

(1) Do not squeeze near the puncture area as this tends to shut off the blood supply and dilute the blood with interstitial fluids.

(2) At the time of puncturing the skin, it is advisable to have the patient looking away from the site, preventing a reflex pulling of the arm that may cause the operator to stick his/her own finger.

2-10. PRESERVATION OF BLOOD

Anticoagulants are used to prevent clotting. Smears that are stained within a short time after collection do not require fixation, but when a delay between collection and processing occurs, methanol is the recommended fixative for the thin smears. Thick smears should never be fixed. The anticoagulants routinely used in the laboratory do not distort parasitic organisms to a great extent, but, nevertheless, direct smears are preferred.

2-11. ANTICOAGULANTS

The commonly used anticoagulants are divided into two groups: calcium binders and antithrombins.

a. **Calcium Binders.** These anticoagulants prevent clotting by binding calcium and thus preventing the complete chemical reaction that produces fibrin. Ethylene-diamine-tetra-acetate (EDTA) is the most often used; others include sodium citrate, sodium fluoride, and oxalates.

b. **Antithrombin.** Heparin is an anticoagulant classified in this group. It works by interfering with the formation of intrinsic thromboplastin and the formation of thrombin.

c. **Capillary tubes.** Capillary tubes with a red ring are heparinized. Those with black or blue rings do not contain anticoagulants.

2-12. FIXATION AND STORAGE

The fixative used for blood smears is methanol. This alcohol prevents the humidity in the air from lysing the RBC's. Anticoagulated whole blood should be stored at refrigeration temperatures and not frozen. Freezing will cause hemolysis. Serum for serological diagnosis should be removed from the red cells and refrigerated or frozen.

2-13. PROCESSING THE BLOOD SMEAR

a. **Thin and Thick Blood Smears (Figures 2-3A and 2-3B).** The most satisfactory procedure for the definitive diagnosis of malaria, trypanosomiasis and filariasis is the use of thick and thin blood films on the same slide. This procedure serves as a convenient method for forwarding the specimen to another laboratory for examination or holding the specimen for examination at a later time. It is also a convenient method for performing field surveys on large numbers of individuals in endemic areas.

(1) Identification. Identify the slide by writing the patient's name or identifying number on the thickest portion of the thin smear with an ordinary pencil or a diamond scriber. A minimum of three thick-thin preparations should be prepared from each patient.

(2) Staining. Stain the slides within a day after preparation to get maximum staining qualities.

STEP 1: Use only chemically lean slides, free from grease.

STEP 2: Perform a finger puncture.

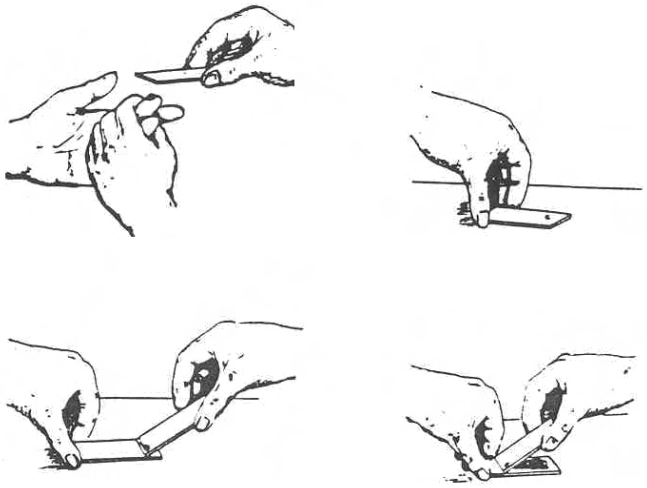
STEP 3: Two drops of blood may be placed on the same slide by touching the surface of the slide to the blood as it wells up from the puncture.

STEP 4: Immediately lay the slide down on a flat surface blood-side up. Smears should be prepared before the drops begin to dry around the edges. The thin smear should be made first. A small drop is best for the preparation of the thin smear, while a larger drop is required for the thick smear.

STEP 5: Hold a second slide, by the edges between thumb and finger, at a 30 degree angle to the surface of the first slide. Touch one end of the slide just ahead of the drop of blood.

STEP 6: Draw the top (second) slide back until it touches the blood. The blood will quickly spread across the surface of contact.

STEP 7: Holding the top slide at a 30 degree angle, push it smoothly and evenly away from the drop toward the opposite end of the bottom slide until the blood film "feathers" out. This should be accomplished rapidly, with one motion, before the blood spreads to the border of the slide.



Figures 2-3a. Preparation of thin blood smears.

STEP 8: The thick film is prepared by constantly stirring the larger drop of blood in a circular motion with a corner of the clean slide, spreading it to about the size of a dime. This aids in breaking up the fibrin strands (defibrination); allows the cells to readily release hemoglobin (dehemoglobination), and prevents the drop from floating away during the staining procedure. The thick film should be of such thickness that ordinary printing (newsprint) can barely be read through it.

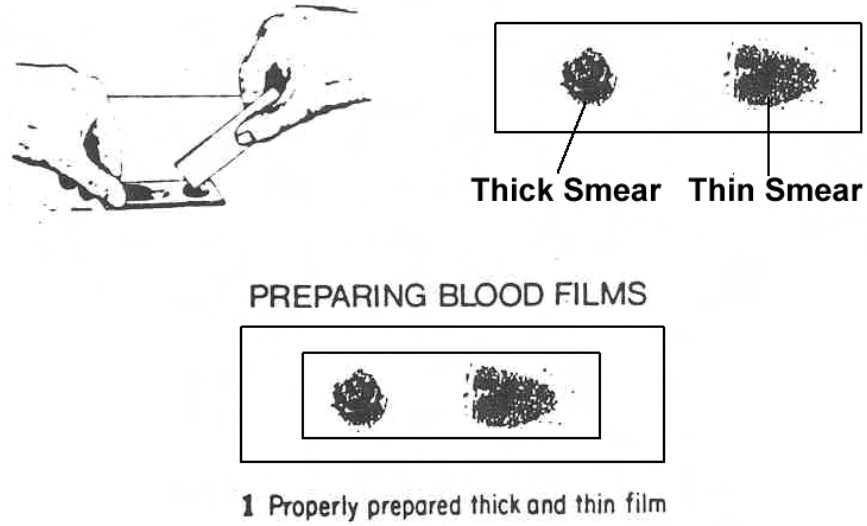


Figure 2-3b. Preparation of thick blood smears.

b. **Giemsa (Triton X-100) Technique for Staining Blood Films.** Giemsa stain, modified by the addition of polyethylene glycol monoisooctyl phenol ether (Triton X-100), is one of the best methods for demonstrating malaria, trypanosomes, and filarial parasites in blood films. Giemsa stains the organism deeply and such preparations resist fading for long periods. The addition of Triton X-100 reduces transfer of parasites from slide to slide during mass staining and enhances the parasite staining properties of the Giemsa stain.

(1) Reagents. Use the Azure B type Giemsa stain certified by the Commission for the Standardization of Biological Stain.

(a) Stock Giemsa Stain. Preparation of this stain requires the following ingredients:

Giemsa Powder CP..... 0.6 gm
Glycerine (Neutral) CP ($C_3H_5(OH)_3$)..... 50.0 ml
Methanol, Absolute (Acetone-Free) ..... 50.0 ml

NOTE: Use only chemically clean, dry glassware to prepare this stain.

STEP 1: Weight out 0.6 grams of Giemsa stain (dry powder) and place a small amount of stain in a dry mortar.

STEP 2: Measure out 50 milliliters of glycerin in a cylinder and add a small amount of glycerin to the mortar. Thoroughly grind the stain and glycerin together. Pour off into a flask.

STEP 3: Repeat the addition of stain and glycerin with grinding until all the stain has been mixed with glycerin. Rinse the mortar and pestle with the remaining glycerin and pour this into the flask.

STEP 4: Measure out 50 milliliters of absolute methyl alcohol. Pour some of this into the mortar and rinse the mortar and pestle with a portion of this. Pour the washings into a separate bottle and stopper tightly.

STEP 5: Place the glycerin-dye mixture in a 55ºto 60º C waterbath for six to eight hours and shake periodically.

STEP 6: Remove the container of glycerin-dye mixture from the waterbath. Cool and add the washing from the mortar and pestle and the remainder of the alcohol to the glycerin-dye mixture. Shake the resulting solution well and stopper the container tightly.

STEP 7: Filter and use the stain immediately if necessary. The stain can be filtered and used immediately. However, it is preferable to allow the stain to age two weeks before filtering and using it.

STEP 8: Store the stain in an amber bottle. Protect it from light.

(b) Stock buffers. These buffers may be kept in separate glass-stoppered bottles for a long time.

1 Acid Buffer (M/15) - Solution A.

Sodium phosphate, monobasic	9.5 gm
Distilled water, q.s. to	1,000.0 ml

2 Alkaline Buffer (M/15) - Solution B.

Sodium phosphate, dibasic, anhydrous	9.5 gm
Distilled water, q.s. to	1,000.0 ml

Dissolve dibasic sodium phosphate in a small quantity of the distilled water in a one liter volumetric flask. Add water to make one liter of solution.

(c) Working buffered water. Check the pH of the solution with an electric pH meter (pH 7 to 7.2).

Stock acid buffer (M/15) - Solution A	39.0 ml
Stock alkaline buffer (M/15) - Solution B	61.0 ml
Distilled Water	900.0 ml

(d) Preparation of Triton buffered water solutions. For thin blood films or combination thin/thick smears, add 1.0 milliliter of stock 10 percent aqueous solution of Triton X-100 to 1,000 milliliter of buffered water. The resulting concentration of Triton is 0.01 percent. Keep buffered water, a Triton-buffered water, in tightly capped amber bottles and check the pH of the buffered water before use.

(2) Procedure. See the following steps and figure 2-4.

STEP 1: Fix the thin film portion of the slide with absolute methanol for 3-5 seconds. Avoid getting alcohol or alcohol fumes on the thick film. Allow the thin film to air dry before proceeding.

STEP 2: Lake the thick smear by placing it in a small amount of water to which a few drops of methylene blue stain has been added. Dip the slide three to five times. Allow the slide to dry in a vertical position with the thick smear at the bottom.

STEP 3: Stain the whole slide in a 1:50 Giemsa/Triton-buffered water solution for 45 minutes.

STEP 4: Rinse the thin film by dipping three times in 0.01 percent Triton-buffered water, immerse the thick film an additional three to five minutes (a longer time may be required for slides which have been prepared longer).

STEP 5: Allow the slide to air-dry and examine it under the oil immersion objective of the microscope.

STEP 6: For the preservation of the slide, mount it using a suitable mounting medium.

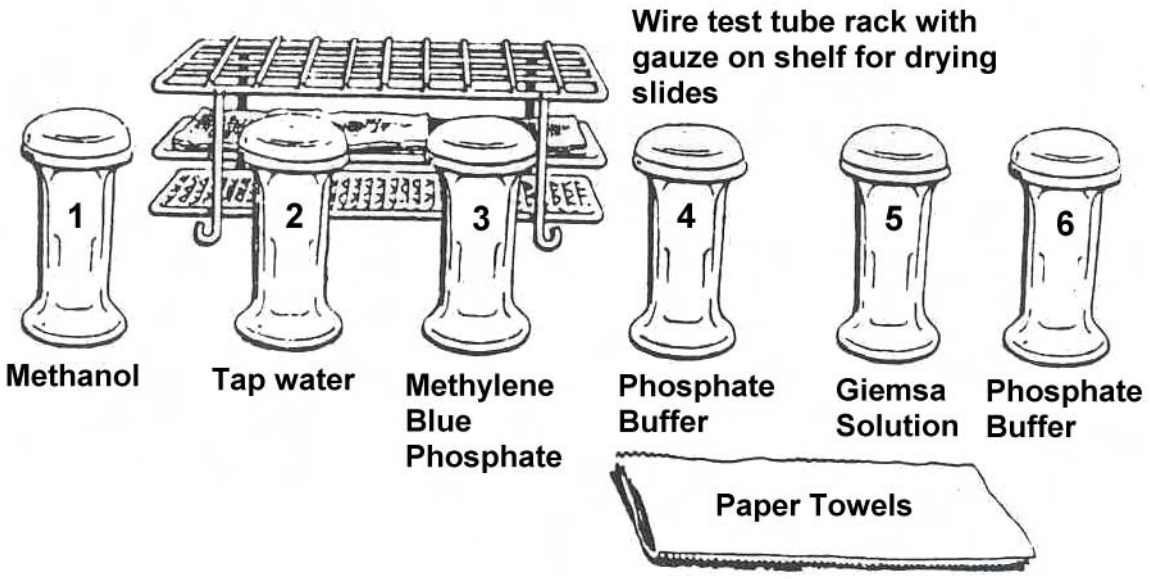


Figure 2-4. The Giemsa (Triton X-100) technique for staining blood films.

(3) Staining reactions.

(a) The nuclear portion of the malaria parasite stains magenta-red, while the cytoplasm stains light blue. The structural details of the organism are sharper and clearer with the Giemsa-Triton stain. The Schuffner's granules stain especially brilliant with this stain. The undulating membrane and flagella of trypanosomes are distinctly visible. In the microfilariae, the nuclear column is sharper and structures such as the inner body, the mouth, and the tail structures are more visible. The sheath does not stain well but it is visible.

(b) For staining thin blood films or combination thin/thick smears, Triton X-100 in 0.01 concentration is used. Higher concentrations will lyse the erythrocytes. For malarial thick smears, a concentration of 0.1 percent is preferred. For staining thick films for microfilariae, 0.5 percent is recommended.

c. **Field's Stain for Malarial Parasites.**

(1) Preparation of reagents.

(a) Solution A.

Methylene blue	0.80 gm
Azure B (American stain)	0.50 gm
Sodium phosphate, dibasic, anhydrous	5.00 gm
Potassium phosphate, monobasic, anhydrous	6.25 gm
Distilled water, q.s	500.00 ml

STEP 1: Dissolve a large portion of the phosphate salts in a 500 milliliter volumetric flask and add enough water to prepare 500 milliliters of solution.

STEP 2: Grind the remainder of the salts with the azure in a mortar and add this mixture to the solution, mix well, and allow to ripen for 24 hours. Filter and use the solution as needed.

(b) Solution B.

Eosin	1.00 gm
Sodium phosphate, dibasic, anhydrous	5.00 gm
Potassium phosphate, monobasic, anhydrous	6.25 gm
Distilled water, q.s	500.00 ml

STEP 1: Dissolve the phosphate salts in a portion of the water. Add the stain. Add enough water to prepare 500 milliliters of solution.

STEP 2: Allow the solution to ripen for 24 hours. Filter and use as needed.

(2) Procedure. Prepare a thick smear as for Giemsa stain.

STEP 1: Dip the smear two to three times into solution A.

STEP 2: Tap the slide on paper towels to remove excess stain. Immerse the slide immediately into distilled water. Dip three to four times.

STEP 3: Tap the slide on paper towels. Immerse the slide into solution B and dip two to three times.

STEP 4: Tap the slide on paper towels. Immerse the slide into distilled water and dip two to three times.

STEP 5: Place the slide vertically on a slide rack and allow the slide to air dry.

(3) Staining reaction. The malaria parasites are stained similar to staining with Giemsa (the red chromatin dots with light blue cytoplasm). However, there is usually substantial debris in the background. Thus, it is an excellent stain for mass production in an epidemic situation. Its use is limited to thick drop preparations. It is not recommended for thin films.

d. **Jaswant-Singh-Bhattacherji (JSB) Stain.** The JSB stain is a fairly rapid staining method for the detection of malarial parasites. This stain is superior to the Field's stain because the parasites stain clearer and both thick and thin smears can be stained. However, preparations fade quite rapidly. Therefore, this stain is not recommended when permanent slides are desired.

(1) Preparation of reagents.

(a) Solution A.

Medicinal Methylene blue	0.5 gm
Potassium dichromate	0.5 gm
Sulfuric acid (1.0 percent solution)	3.0 ml
Potassium hydroxide (1.0 percent solution)	10.0 ml
Distilled water	500.0 ml

STEP 1: Dissolve the methylene blue in the 500 milliliters of distilled water.

STEP 2: Add the sulfuric acid gradually while stirring. After mixing the solution well, add the potassium dichromate. A heavy amorphous purple precipitate of methylene blue/dichromate forms immediately.

STEP 3: Place the container with the precipitate in an autoclave for three hours at a temperature of 100-109º C and five pounds pressure until the purple color shifts to a deep blue. Allow the product to cool to room temperature. Add the potassium hydroxide slowly, drop by drop, while stirring.

STEP 4: Mix the solution well and age at room temperature for 48 hours. Filter and store in an amber-colored bottle.

(b) Solution B.

Eosin Y, water-soluble ..... 1.0 gm
Distilled water ..... 500.0 ml

STEP 1: Dissolve the Eosin Y in 500 milliliters of distilled water and shake vigorously.

STEP 2: Allow the solution to age for 48 hours and filter before use.

(2) Procedure.

STEP 1: Fix the thin smear and lake the thick preparation as done in the Giemsa stain procedure.

STEP 2: Immerse the whole slide in solution A for 30 seconds.

STEP 3: Tap the slide on towels to remove the excess stain. Dip the slide five times in a slightly acidified (pH 6.2-6.6) water solution.

STEP 4: Tap the slide on paper towels. Dip the slide twice into solution B.

STEP 5: Tap the slide on paper towels. Dip the slide five times in another jar with slightly acidified water.

STEP 6: Immerse the slide in solution A for 30 seconds.

STEP 7: Tap the slide on paper towels. Dip the slide five times into acidified water.

STEP 8: Allow the slide to air dry and examine it under the oil immersion objective.

e. **Knott's Technique for Examination of Microfilariae.** The laking of blood with two percent formalin solution followed by centrifugation will often reveal microfilariae when present only in small numbers. Staining of the blood smears clarifies the morphological detail of the microfilariae.

(1) Preparation of solutions.

(a) Ehrlich's Hematoxylin Stain.

1 Solution A.

Hematoxylin crystals ..... 1 gm
Absolute ethanol ..... 10 ml
Glacial acetic acid ..... 10 ml

NOTE: Mix the alcohol and acetic acid together. Slowly add the hematoxylin crystals while stirring until they are all dissolved.

2 Solution B.

Absolute ethanol	90 ml
Glycerin	100 ml

NOTE: Mix the alcohol and glycerin in a separate container from the one above.

STEP 1: Add solution A to solution B.

STEP 2: Dissolve 10 grams of aluminum potassium sulfate in 100 milliliters of distilled water with heat.

STEP 3: Cool the solution and add the alum solution to the hematoxylin-alcohol-acid-glycerin mixture. Stir constantly.

STEP 4: Expose the solution to light and air. Allow the solution to ripen for four to six weeks.

STEP 5: Ensure that the ripe solution has the color of port wine. The ripening process is hastened by standing the solution in a warm place and shaking it occasionally.

(b) Alcohol-ether mixture.

Ethyl alcohol, 95 percent	50 ml
Ether, anhydrous	50 ml

(c) Formalin solution, two percent.

Formaldehyde	2 ml
Distilled water	100 ml

(2) Procedure.

STEP 1: Draw one milliliter of blood by venipuncture and mix it with 10 milliliters of a two percent solution of formalin. Mix, store, and/or ship the sample to the laboratory. The formalin will lake the red blood cells.

STEP 2: Centrifuge the sample for five minutes at 1,000 revolutions per minute (rpm).

STEP 3: Pour off the supernatant without disturbing the sediment. With a capillary pipet, transfer a portion of the sediment to a clean slide.

STEP 4: Scan the whole preparation under low power and confirm findings under the high dry objective. If known or suspected microfilariae are found, proceed to fix and stain the remaining sediment.

STEP 5: Smear the remaining sediment over a slide. Let it air-dry and fix in alcohol-ether mixture for 10 minutes. Allow it to dry.

STEP 6: Cover the slide with Ehrlich's hematoxylin solution for 40 to 60 minutes. Follow this with a quick rinse (several dips) in a 0.05 percent hydrochloric acid solution. Wash it in gently running tap water until the blue color appears in the film. Air-dry the smear and examine it under low power on the microscope. Confirm the results under high dry objective.

(3) Staining reaction. With this stain, the nuclei will stand out sharply and the sheaths, if present, will be clearly visible. Alternate stains may be used (such as Giemsa's stain, methylene blue, or other hematoxylin procedures).

f. **Parasitized Cell Count.** For the determination of parasitemia in malaria, primarily two methods are used: a direct method and an indirect method. The more practical indirect method requires counting leucocytes (as numerical indicators) and parasitized red blood cells. The more accurate direct method requires counting the parasitized and non-parasitized red blood cells.

(1) Indirect method (leukocyte count).

(a) A leukocyte count is performed on the specimen.

(b) A thin film smear is then made and stained with Giemsa's stain.

(c) Parasitized red cells and the leukocytes are counted until at least 200 parasitized cells are counted.

(d) From the leukocyte count, calculations of the number of parasites per millimeter can be made.

(2) Direct method.

(a) A red blood cell count is performed on the specimen.

(b) A thin film smear is then made and stained with Giemsa's stain.

(c) A direct count is made of all the red cells in a given area.

(d) The ratio of infected cells to normal red cells is determined.

g. **Cultivation of Trypanosoma brucei.** Weinman's medium is the medium of choice for cultivating from both blood and spinal fluid.

(1) Preparation of media.

(a) Agar base preparation.

Nutrient agar, 1.5 percent, pH 7.3	31.0 gm
Agar	5.0 gm
Distilled water	1,000.0 ml

Dissolve the agar and nutrient agar in distilled water by heating. Distribute solution in 250 milliliter Erlenmeyer flasks. Plug the top of the flasks with gauze-covered rolled cotton. Autoclave the flasks at 12 pounds pressure for 30 minutes and store in refrigerator at 4-10.

(b) Blood collection and preparation.

Inactivated human plasma	200.0 ml
Saline washed human red cells	200.0 ml

STEP 1: Collect 450 milliliters of human blood in a plastic donor bag containing 50 milliliters of sodium citrate anticoagulant. The blood must be collected aseptically and with great care to the donor as well as to the container of blood. Outdated blood from blood banks may also be used, providing it still retains its sterility.

STEP 2: Refrigerate the blood at 4-10º C. After the plasma separates from the red cells by gravity, aseptically draw off the major portion of the plasma into a separate bag. Centrifuge the red cell pack. Draw off the supernatant plasma and add it to the first portion of plasma.

STEP 3: Inactivate the sample by heating it for 30 minutes in a 56º C waterbath.

STEP 4: Wash the red cells (as aseptically as possible) three times in three or more volumes of sterile 0.9 percent sodium chloride solution.

STEP 5: Reconstitute the whole blood by mixing equal volumes of inactivated plasma and washed red cells. Note the total volume.

(c) Final media preparation.

STEP 1: Melt sufficient quantities of nutrient agar base (previously prepared) so as to give an approximate three to one ratio of agar base to reconstituted blood (e.g., 250 milliliters of base to about 83 milliliters of blood).

STEP 2: Cool (or warm) the agar base to 45º C and add one part of reconstituted blood (warmed to 45º C) to three parts base. Quickly swirl until well mixed. Do not shake the mixture since this causes bubbles and partial hemolysis of the blood.

STEP 3: Aseptically dispense the still liquid media into 16 by 150 millimeter test tubes (5 milliliters of media per tube) and stopper the tubes with vaccine-type rubber stoppers. Place the tubes on a slanting rack at an angle that will result in the media's being in a long slant.

STEP 4: After the medium has set (which may require several days), incubate the tubes at 37º C and observe after 24 hours for sterility (lack of microbial growth). Store them in the refrigerator at 4-10º C until they are needed.

NOTE: 500 milliliters of blood should yield about 300 tubes of final medium. The medium, if kept under refrigeration at 4-10º C will remain satisfactory for about six months.

(2) Preparation of anticoagulant and innoculation of specimen.

(a) Anticoagulant preparation (PVSA).

Polyvinyl sulfuric acid, potassium salt ..... 0.5 gm
0.9 percent sodium chloride solution ..... 100.0 ml

STEP 1: Prepare a 0.5 percent solution of polyvinyl sulfuric acid in 0.9 percent sodium chloride solution.

STEP 2: Adjust the pH to 7.5 and sterilize by filtration through a sintered glass filter, "UF" porosity (pyrex), into sterile vaccine-stoppered test tubes. Refrigerate at 4-10º C until needed.

STEP 3: Use 0.1 ml. of anticoagulant for every milliliter of patient's blood.

(b) Venipuncture and inoculation.

STEP 1: Use a sterile one milliliter serological pipet to deliver 0.4 milliliter of anticoagulant into a sterile nine milliliter screwcap bottle.

STEP 2: Perform a venipuncture on the patient using a sterile syringe. Draw about four milliliters of blood and mix it thoroughly with the anticoagulant in the bottle.

STEP 3: Use a sterile five-milliliter syringe to withdraw the thoroughly mixed anticoagulated blood from the bottle and inoculate two tubes of medium (warmed to room temperature) with two milliliters each of the mixture.

STEP 4: For spinal fluid inoculation, inoculate approximately one milliliter of spinal fluid directly from its sterile container to the culture tube.

NOTE: It is important to mix the blood and anticoagulant solution thoroughly and to inoculate promptly, for coagulation is inhibited only temporarily and may take place within 15 minutes.

STEP 5. Add one milligram of dihydrostreptomycin sulfate in 0.2 milliliter of 0.9 percent sodium chloride solution or 4000 units of penicillin in 0.2 milliliter of 0.9 percent sodium chloride solution for two milliliters of blood. For spinal fluid, add 0.5 milligrams dihydrostreptomycin sulfate in 0.1 milliliters of saline or 2,000 units of penicillin in 0.1 milliliter of 0.9 percent sodium chloride solution for one milliliter of spinal fluid. Contamination with bacteria or fungi almost invariably inhibits trypanosomal multiplication and is the greatest single cause for failure of this procedure.

NOTE: Do not add any antifungal agents or mycotics because they may inhibit or kill trypanosomes.

STEP 6: Tilt the inoculated tubes back and forth several times to spread the blood or spinal fluid over the surface of the medium.

STEP 7: Incubate the tubes in darkness at 25º C. Cultures rarely become positive before five or after 30 days. To check for positives, withdraw samples from the tubes with sterilized Pasteur pipets (not with bacteriological loops). Aspirate fluid from the bottom of the tube and repeatedly wash over the surface of the slant to dislodge colonies. The colonies are colorless, translucent, round, with regular outline, and rarely exceed two millimeters in diameter. They are not completely distinctive on the medium. Therefore, do not identify them based on gross appearance alone.

STEP 8: Withdraw small amounts of fluid suspension. Make wet mounts or stained smears and search under the microscope for the characteristic forms. These are numerous and occur singly, or in dividing forms, or appear in large and small rosettes.

h. **Xenodiagnosis for Trypanosoma cruzi.** One method for diagnosing Chagas' disease consists of feeding noninfected laboratory-bred reduviid bugs (the insect vector) with the blood of the suspected person and demonstrating the cyclical development of the trypanosome in the insects' intestinal tract. This procedure is known as xenodiagnosis.

(1) The most important criteria for this technique is to select only clean noninfected reduviid bugs and rear them in the laboratory. Each generation must be properly protected from outside infection.

(2) The bug's fecal deposits are screened initially to determine the presence of T. cruzi.

(a) All newly collected insects to be used for xenodiagnosis are first placed singly in a specimen cup with gauze stretched over the top and filter paper covering the bottom.

(b) Collect insect fecal deposits as soon as possible after the first defecation. Mix the feces with a drop of 0.9 percent sodium chloride solution on a slide and examine it microscopically for metacyclic trypanosomal forms. Multiple checks (at least three) must be made over a period of time to ensure infection-free bugs. If bugs do not defecate, they must be given a blood meal from clean laboratory animals.

(c) All bugs found positive must either be destroyed or kept separated from those which are not infected.

(3) Patient testing.

STEP 1: Select bugs that have not had a blood meal for two weeks preceding this test.

STEP 2: Place three bugs, one each in three small ointment tin halves. Secure gauze over the top of each tin. The tins must have several small holes punched in the bottom.

STEP 3: Place the tins, gauze-side down on the suspected patient's forearm with tins clustered together. Wrap a strip of black cloth around the patient's arm covering the ointment tins and secure.

STEP 4: Instruct the patient to sit or lie very still for at least 30 minutes.

STEP 5: Check the bugs at the end of the allotted time to see if they have had a sufficient blood meal. That is, check to see if they have a distended abdomen and have ceased feeding.

STEP 6: Return the bugs to the lab and place each in a large gauze covered ointment tin. Each tin must have complete information written on it such as the patient's name, species of reduviid, and date the bug fed on patient. On the bottom of each tin, place a clean piece of filter paper. Change it daily thereafter.

STEP 7: Two weeks after feeding, collect the fecal deposits and inspect them for trypanosomes.

STEP 8: After at least three successive fecal inspections with negative results, remove the bug and dissect it under a stereoscope.

STEP 9: Carefully tear out the hindgut. Slice it longitudinally and mount it on a slide. Microscopically, search the entire hindgut carefully for presence of crithidial and metacyclic trypanosomal forms.

(4) It is necessary to keep in mind that xenodiagnosis gives a variable percentage of positivity and that not all reduviids fed on diseased persons become infected.

Section III. GASTROINTESTINAL TRACT SPECIMENS

2-14. INTRODUCTION

In humans, the site most frequently infected by parasites is the intestinal tract. Therefore, a great deal of emphasis must be placed on the collection, fixation, and examination of specimens from the gastrointestinal tract.

2-15. COLLECTION OF GASTROINTESTINAL TRACT SPECIMENS

Specimens should be collected and handled in such a manner that they arrive at the laboratory in good condition for the identification of protozoans and helminths. Frequently, intestinal parasites are diagnosed or ruled out based on examination of fecal specimens. However, perianal swabs, liver and duodenal aspirations, or duodenal contents may also be received for examination of intestinal parasites.

a. **Fresh Specimens.** Laboratory and nursing personnel are responsible for instructing the patient on the proper and correct procedure to be used in the collection of the stool samples. Water and urine will destroy the trophozoites and, when soil contaminates the stool, free living parasites may give a false positive result. Therefore, care must be taken to avoid contaminating the sample with water, urine, or soil.

(1) Number of samples. Recovery of intestinal parasites is enhanced by the collection of three or more specimens at intervals of two to three days apart. The time period between the collection of samples is necessary to compensate for the periodicity of the life cycles of the parasites.

(2) Specimen containers. The most suitable container for collection and transportation of stool specimens is one that has a wide leakproof cup, bottle, or carton that is clean and dry. This container should not be reused. It should be disposable.

(3) The label. Certain information should be affixed to the container to prevent the mixing of samples.

(a) Patient data. The name of the patient must be clearly written on the container. Other information which should be on the label includes the patient's identification or hospital number, the ward or clinic name, and the name of the attending physician.

(b) Collection data. The date and time of collection as well as the sequence number of the specimen should also be included on the label.

b. **Cathartic Induced Samples.** Some protozoans and helminthes are passed with more frequency in a loose stool. The use of purgatives (e.g., cathartic or laxatives) has been found to be beneficial in enhancing the recovery of intestinal parasites from constipated patients.

(1) Recommended cathartics. These purgatives are administered in saline solutions. Some examples of these cathartics are:

(a) Sodium sulfate.

(b) Buffered phosphosoda.

(c) Cascara sagrada.

(2) Unsatisfactory cathartics. There are some chemicals that interfere with the examination of the stool specimen. Thus, cathartics containing the following chemicals should be avoided:

(a) Castor oil.

(b) Mineral oil.

(c) Magnesia compounds.

c. **Perianal Samples.** Eggs of Enterobius vermicularis (and rarely Taenoid eggs) are deposited around the perianal folds and can be collected by adhering the eggs to the collecting apparatus.

(1) Cellulose-tape slide preparation.

STEP 1: Place a strip of "Scotch tape" about 3/4-inch wide and 3-1/2 inches long on one end of a tongue depressor, sticky side down (give the written instructions to the patient).

STEP 2: Just prior to collection, lift one end of the tape from the depressor and loop it over to expose the sticky surface.

STEP 3: Holding the depressor with the right hand, spread the buttocks with the left hand and press the sticky surface of the tape against several areas of the perianal region.

STEP 4: Either replace the tape on the tongue depressor for transportation to the laboratory or place the "Scotch tape" on a 25 by 75 millimeter glass slide, sticky side down.

STEP 5: Smooth the tape with cotton or gauze.

STEP 6: Examine the slide under the low power objective of the microscope and confirm findings under the high dry.

(2) Anal swab.

STEP 1: Prepare a paraffin/vaseline mixture in a 1:4 ratio and coat swabs with the warm mixture. Allow the swabs to cool.

STEP 2: Holding the swab with the right hand, spread the buttocks with the left hand and gently swab the perianal region with the coated swab.

STEP 3: Place the swab in a 13 by 100 tube to which two or three milliliters of Xylol has been added. Allow to stand for three to five minutes.

STEP 4: Twirl the swab in the Xylol, hold it against the sides of the test tube, and express the liquid; discard the swab.

STEP 5: Centrifuge the test tube at 2,000 rpms for two to four minutes.

STEP 6: Remove the supernatant by aspiration and transfer the sediment to a slide. Examine the specimen under the low power objective and identify any ova recovered under the high dry.

(3) Duodenal contents. Parasites that inhabit the small intestines are easily overlooked when examining a stool sample. Consequently, when Giardia lamblia or Strongyloides stercoralis are suspected, the duodenal content examination will be the test of choice. Duodenal intubation causes much discomfort to the patient; therefore, it is not a desirable procedure. In its place, the string capsule technique (described by Beal, et al, in 1970) offers an easy and almost painless method of examining duodenal contents. A gelatin capsule containing a line of 25 centimeters silicon thread covered with rubber and a 75-centimeter soft nylon yarn are used in this procedure. The patient swallows the capsule containing the string and a lead weight that helps the capsule to move to the duodenum (the end of the string that sticks out is held fast by taping it to the patient's neck). After three to five hours, the string is pulled out (the weight separates from the capsule and it is excreted), the portion of the string that has bile-stain contents is scraped off, placed on a slide, and examined for protozoan cysts and trophozoites or helminthes eggs and larvae.

(4) Sigmoidoscopic examinations. When amebiasis is suspected and stool samples are negative, material for examination may be collected by sigmoidoscopy. The procedure should be performed after a normal bowel movement or two to three hours after administration of a cathartic. The specimen is collected with a pipette rather than with a swab by aspirating material from any visible lesion and from the mucosa. Examination of sigmoidoscopic material should be done immediately, but fixation can be done with polyvinyl alcohol (PVA) after the wet preparation.

(5) Liver abscesses. Exudates aspirated from the liver may contain cysts and trophozoites of Entamoeba histolytica. The first portion of the specimen does not usually yield organisms, but the second (reddish) portion does. Aspirates should be examined microscopically with wet preparations as well as with permanent stains.

2-16. MACROSCOPIC EXAMINATION

Feces consist of food remnants, various products of digestion, nonpathogenic organisms, and possibly pathogenic organisms. Consistency, quantity, odor, color, and contents of a stool specimen depends on the health and diet of the individual. The direct observation of the fecal specimen may detect a pathogenic process that will not be detected by any other method.

a. **Consistency.** Normally, stool is soft and formed, retaining the bowel cast. Under abnormal conditions, other densities may be detected.

(1) Dry and hard. This is an indication of constipation and/or dehydration.

(2) Ribbon-like. Stool excreted in this manner may be found in spastic colitis, obstruction, or stricture of the rectum and anus.

(3) Mushy. Mushy stool is unformed (but not to the point where it flows readily when the container is tilted); however, the feces does not remain in the bowel cast. Stools of this sort include the "fluffy" stools which may be caused by trapped gas as in patients with sprue or from excessive carbohydrate fermentation.

(4) Semi-liquid. These stools are characterized as slowly flowing upon tilting the container. Included in this category are the stools found in diarrhea, dysentery, or following cathartics.

(5) Liquid. These are diarrheic stools that consist mainly of water. They readily flow upon tilting the box.

b. **Quantity.** A normal fecal daily output varies considerably, but usually is within the range of 100 to 200 grams for an average adult. The amount of feces depends on the type of food consumed and the water content of the stool. Vegetable diets considerably increase the quantity passed per day. Since the quantity of fecal specimens varies considerably and usually only random samples are sent to the parasitology section for examination, the quantity becomes an unimportant factor and is of no consequence.

c. **Odor**. Diet and degree of digestion are factors affecting the odor of a given sample. The normal offensive odor is due to indole, skatole, and butyric acid production. Stools from an individual who is on a meat diet have more of an odor than specimens collected from a person on a vegetable diet. An extremely foul odor is usually caused by the action of bacteria on undigested protein--such stools are normally alkaline in pH. A putrid odor is associated with lower bowel disorders. A rancid or sour smell is often associated with incomplete digestion, absorption of carbohydrates, gas fermentation, and the presence of fatty acids.

d. **Color**. All the components of the stool specimen contribute to its color. The normal light or dark brown color is due to the reduction of bilirubin and other bile constituents. Chocolate brown is usually due to excessive color contributed by the consumption of cocoa, chocolate, coffee, blackberries, and cherries. A black or tarry stool may be caused by medications that include iron, bismuth, and charcoal. A dark color may also be due to digested blood as part of the diet or from bleeding in the upper part of the tract. Red color may be due to excess amounts of tomatoes or beets in the diet; however, a bright red color may indicate bleeding in the lower intestinal tract. A gray color is usually due to the abundant presence of fats or to deficiency of bile components. A yellow color may be present due to unchanged bilirubin or to the consumption of milk, cornmeal, rhubarb, or certain medications. The specimen may be green due to unchanged biliverdin, the consumption of spinach, or the use of calomel.

e. **Mucus**. Mucus may normally be present in stool in small amounts. Mucus in an abnormal amount, readily visible as small white areas, is usually indicative of intestinal irritation. If the site of the intestinal irritation is located in the small intestine, the mucus is usually found on the outside surface of the specimen. This should be reported even if not requested.

f. **Leukocytes (Pus Cells)**. Few leukocytes are normally present in feces. In dysentery and in inflammatory or ulcerative conditions, the number of pus cells is increased considerably. The observation of these cells is aided by adding one or two drops of 10 percent acetic acid to a drop of the fecal emulsion. This makes the nuclei of the leukocytes more prominent. Reporting leukocytes is usually done by giving the average range of leukocytes per high power field.

g. **Gross Blood**. When blood is present in feces in gross amounts, the color of the stool specimen may be red, gray, or black. Some common causes of large amounts of blood in fecal specimens are nosebleeds, esophageal varices, ulcers, carcinoma of the gastrointestinal tract, and hemorrhoids. When blood is present in trace (occult) amounts, chemical tests are required for detection. Meat in an individual's diet may cause reactions in some of the chemical tests. Thus, the patient should be placed on a meat-free diet for two or three days before the test for reliable results.

h. **Adult Parasites.** Often helminths are observed in stool specimens. The most common is the roundworm, Ascaris lumbricoides, which is quite large. Some of the others such as the hookworm, Trichuris trichiura, and tapeworms are usually found only after comminution, straining, and searching the debris with a hand lens. Enterobius vermicularis adults are usually found on the surface of the specimen. There are constituents normally found in fecal specimens that resemble helminths, but are artifacts. Where sanitation is poor, fly larvae are frequently seen and mites are occasionally found in submitted samples.

i. **Pollen.** Pollen grains may frequently be confused with eggs of parasites and protozoan cysts. Varieties of pollen differ according to geographic location and quantities will vary with seasonal changes. When observed in fecal specimens, these pollen grains (artifacts) must be differentiated from the true parasitic objects.

2-17. OCCULT BLOOD IN FECES

Trace amounts of blood may be present in feces from gastrointestinal lesions or from the consumption of products containing blood. Testing for occult blood in suspected or unsuspected cases with internal bleeding assists in the diagnosis. Ideally, this test should be performed when the patient's diet is controlled since consumption of rare meat and other food may be the cause of positive reactions.

a. **Hemacult Tablets.**

(1) Procedure.

STEP 1: Smear a solid specimen of feces on filter paper, covering an area about the size of a nickel. Do not use an emulsion.

STEP 2: Place a blood detection tablet in the center of the smeared specimen.

STEP 3: Place one drop of distilled water on the tablet, wait five or 10 seconds, and place a second drop on the tablet so that it runs down the sides onto the filter paper.

STEP 4: Note the color change and then record results.

(2) Interpretation.

(a) Positive. The moistened area on the filter paper around the tablet turns blue within two minutes. The time of the appearance and the intensity of the color developed is in proportion to the amount of blood present. Disregard any color changes on the tablet or on the filter paper after two minutes.

(b) Negative. No blue color appears within two minutes on the moistened portion of the filter paper around the tablet.

(3) Precautions.

(a) Your hands, dropper, and working area must be clean and free of traces of blood.

(b) If there is any question concerning development of the blue color near the end of the two minute period, the test should be repeated on the same or subsequent stool specimen.

(c) Certain plants and bacteria contain reactive substances which may be present in a specimen in sufficient concentration to produce a false-positive reaction. When a false-positive reaction is suspected, testing of subsequent specimens should be performed.

b. **Guaiac.**

(1) Preparation of reagents.

(a) Saturated Guaiac solution.

Guaiac crystals..... 10 gm
Ethanol 95 %t..... 50 ml

Mix well and store. It is stable for three to five weeks.

(b) Hydrogen peroxide. Make a three percent solution by diluting the stock (30 percent) 1:10 with distilled water. Keep this solution in the refrigerator.

(c) Glacial acetic acid.

(2) Procedure.

STEP 1: Smear a solid specimen of pus on filter paper, covering an area about the size of a quarter. Do not emulsify.

STEP 2: Add two drops of the Guaiac solution.

STEP 3: Add two drops of glacial acetic acid.

STEP 4: Add two drops of three percent hydrogen peroxide.

(3) Interpretation.

(a) Immediate dark blue color indicates a 4+ reaction.

(b) Dark blue color after 30 seconds indicates a 3+ reaction.

(c) A blue color within three minutes indicates a 2+ reaction.

(d) A slow light blue color indicates a 1+ reaction.

(e) A negative reaction is indicated by the absence of any blue color within five minutes.

c. **Other Tests for Occult Blood.** There are various other products in the market that have satisfactory results.

2-18. PRESERVATION

Whenever specimens cannot be examined immediately after collection, a portion or the whole sample can be preserved for later examination. This procedure maintains parasites in their existing morphological form and structure. There is no perfect fixative and the method of preservation is usually determined by the particular organism suspected.

a. **Formalin.** While protozoan trophozoites are destroyed by formalin, cysts, ova, and larvae are well preserved. The stool specimens should be preserved within one hour after collection. A five percent formalin is used for protozoan cysts, for ova of Hymenolepis species, and for larvae. The 10 percent solution is used for the other organisms.

(1) Reagents.

Neutral Buffered Formalin	10%	5%
Formaldehyde	10.00 ml	5.00 ml
Distilled water	90.00 ml	95.00 ml
Sodium phosphate monobasic	0.40 gm	0.40 gm
Sodium phosphate, dibasic, anhydrous	0.65 gm	0.65 gm

Carefully mix the reagents and swirl vigorously. Store in an aspirator bottle or in a one liter glass-stoppered bottle.

(2) Procedure.

STEP 1: Mix a portion of feces with three times the amount of formalin.

STEP 2: Allow to settle for at least one hour and store in screw-cap bottles.

STEP 3: If shipment or prolonged storing is required, dip the top portion of the tightly fastened container, to include the cap, two or three times into a hot paraffin bath. This will prevent spillage and reduce the rate of evaporation.

(3) Notes.

(a) Formalin is the most commonly employed reagent for fixing and preserving specimens. Formaldehyde solution is a near saturated solution of gas and water. Such a solution contains from 37 to 40 percent formaldehyde with nine volumes of water as 10 percent formalin. This 10 percent formalin dilution contains approximately 4.1 to 4.5 parts formaldehyde gas.

(b) Formalin preserved specimens stored for reference material should have the preservative changed every six months. The formalin volume should be maintained at all times.

b. **Polyvinyl Alcohol (PVA).** This is a mixture of fixative and water-soluble resin that is specifically used to fix and preserve trophozoites of intestinal amoebic organisms. These trophozoites are very fragile and will become distorted or disintegrate completely within a few hours after stool passage. This fixative will preserve trophozoites for long periods of time and will make it easier to identify them. PVA is primarily used for preserving fresh specimens to be shipped to central laboratories. Permanently stained films can be prepared from the preserved material. The solution serves as an adhesive as well as a preservative and prevents the loss of organisms during staining. This is advantageous when preparing smears from liquid specimens.

(1) Preparation of reagents. Polyvinyl Alcohol Fixative (PVA)

Modified Schaudinn's Fixative.

Glacial acetic acid	5.0 ml
Glycerol	1.5 ml
Schaudinn's fixative (two parts saturated aqueous mercury bichloride to one part 955 ethyl alcohol)	93.5 ml
Polyvinyl alcohol powder (PVA)	5.0 gm

(a) While constantly stirring at room temperature, slowly add the 50 grams of PVA to 1,000 milliliters of modified Schaudinn's fixative.

(b) While stirring, heat gently to about 75 degrees centigrade until the powder dissolves and the solution clears.

(c) Upon cooling, the solution should be clear. Solutions prepared from some lots of PVA may be slightly turbid and contain some precipitate. Unless these are excessive, the solution is satisfactory.

(2) Procedure.

(a) Polyvinyl alcohol (PVA) fixation-preservation in vials.

STEP 1: Stool specimens should be immediately submitted to the laboratory or fixed at the site of collection.

STEP 2: With applicator sticks, thoroughly mix in a labeled bottle one part (five grams) feces with three parts (15 grams) of PVA fixative. The fixative should be employed at room temperature or up to 50º C. This step should be performed immediately on receipt of the specimen.

STEP 3: Dip the screwcap and top portion of the bottle two or three times into a hot, melted paraffin bath to prevent leakage and evaporation upon storage.

(b) PVA fixation-preservation on microscope slides.

STEP 1: Stool specimens should be immediately submitted to the laboratory.

STEP 2: With an applicator stick, immediately mix one part of the specimen with three parts of PVA-fixative on a microscope slide. Three drops of mixed, bottled, PVA-fixed feces may also be spread onto a microscope slide. (The best yield of trophozoites or cysts is from the finely dispersed upper layer of the sediment).

STEP 3: Spread (do not smear) the mixture over one-third of the slide. To prevent later peeling, extend the specimen to the edges of the slide.

STEP 4: Allow the mixture to dry thoroughly (preferably overnight at 35º C). Dry films remain satisfactory for staining for several months.

(3) Notes.

(a) Commercially available vials and bottles are available.

(b) If the specimen has been stored for long periods in PVA and become jelled, it can be liquified by heating in a water bath. (Set at 37 to 50º C.)

(c) Specimens preserved in PVA may be maintained for record or for reference study material.

(d) When ordering polyvinyl alcohol powder, specify its use. All grades of PVA are not satisfactory for preserving fecal specimens.

(e) If dehydration occurs, add more fixative.

c. **Merthiolate-Iodine-Formaldehyde Fixative (MIF).** This solution can be used as a fixative, as a stain for wet preparations, and as a concentration procedure.

(1) Stock reagents.

(a) Merthiolate-Formaldehyde (MF)--Solution A.

Distilled water	250.0 ml
Formaldehyde (saturated)	25.0 ml
Tincture of merthiolate (Lilly 1:1,000)	200.0 ml
Glycerin	5.0 ml

Store in a stoppered brown glass bottle.

(b) Lugol's Iodine Solution (I)--Solution B.

Iodine crystals (powdered)	5.0 gm
Potassium iodide	10.0 gm
Distilled water	100.0 ml

Dissolve the potassium iodide in water. Add the iodine crystals slowly and shake until dissolved. Filter and store in a brown bottle. This solution is stable only for three weeks.

(2) Working reagent. Add 18.0 milliliters of solution A to 1.2 milliliters of solution B immediately prior to use. (For smaller or larger volumes, decrease or increase both components proportionately). Mixing the two solutions too far in advance causes a precipitate to form, thus reducing the staining properties.

(3) Procedure.

(a) Stool specimens should be immediately submitted to the laboratory or fixed at the collection site.

(b) With an applicator stick, thoroughly mix one part feces with 10 parts MIF in a labeled 23 milliliter screwcap bottle. This should be performed immediately on receipt of the specimen.

(c) Dip the screwcap and top portion of the bottle two or three times in a hot, melted paraffin bath. This will prevent leakage and reduce the rate of evaporation.

2-19. PROCESSING OF GASTROINTESTINAL SPECIMENS

There are many laboratory procedures used in the identification of human parasites. Each method has advantages and disadvantages--not one can be used for all occasions. There are some criteria that govern the choice of technique--the most common parasites encountered in the area, the amount of daily specimens, the resources available, the desirability of quantitative results, etc. Some of these procedures are suited to the most primitive conditions and others require the best equipped laboratories.

a. **Direct Smears.** The direct microscopic examination of fresh stools for the observation and examination of parasitic organisms should be performed on all samples. If this preparation does not show any organisms, concentration of the stool must be done prior to any other procedure.

(1) Saline solution. This preparation is particularly useful for the detection of motility and examination of all intestinal parasites. Chromatoidal bodies are very refractile and easily detected in saline direct smears. Place one drop (approximately 0.05 milliliter) of warm (37º C) physiological (0.85 percent) saline on one end of a 25 by 75 milliliter plain glass slide. Using an applicator stick, place a small portion of feces (approximately 0.1 milligram) in the saline drop and mix until the suspension becomes homogenous. The other end of the slide may be used for a stained direct preparation.

(2) Iodine. The iodine film is employed primarily to study the diagnostic morphological features of protozoan cysts. If the iodine solution is too strong, the fecal material becomes clumped and the refractile nature of the organisms is altered. However, a weak solution does not stain the organism well. Gram's iodine is too weak, while Lugol's iodine is considered too strong by some workers.

(a) Dobell and O'Connor's iodine.

Iodine (powdered crystals)	0.5 gm
Potassium iodine	1.0 gm
Distilled water	50.0 ml

Mix the potassium iodide in distilled water, add the iodine, and mix well. This concentration produces a "dark tea" color. Store in a stoppered brown glass bottle and filter before use. Formalin preserved specimens require a slightly darker solution. This solution is stable for 10 days.

(b) Lugol's iodine.

Iodine (powdered crystals) ..... 2.5 gm
Potassium iodide ..... 5.0 gm
Distilled water ..... 50.0 ml

Mix the potassium iodide in distilled water, add the iodine, and mix well. Store in stoppered brown glass bottles. Just before use, dilute 1:5 with distilled water. The stock solution is stable for three weeks; the working solution is stable for 10 days.

(c) Procedure. Place one drop (approximately 0.05 milliliter) of iodine solution on the other end of the slide used for the saline preparation. Using an applicator stick, place a small portion of feces (approximately 0.1 milligram) in the iodine drop and mix until the suspension becomes homogenous.

(3) Merthiolate-Iodine-Formaldehyde (MIF). Besides being used as a preservative, MIF is a suitable stain for wet smears. The reagents are prepared in a different concentration than the one described for the fixative.

(a) Stock reagents.

1 Merthiolate-formaldehyde (MF)--solution A.

Formaldehyde (saturated) ..... 15.0 ml
Tincture of merthiolate (Lilly 1:1,000)..... 75.0 ml

Store in a stoppered brown glass bottle.

2 Lugol's iodine solution (I)--solution A.

Iodine crystals (powdered)..... 5.0 gm
Potassium iodide ..... 10.0 gm
Distilled water ..... 100.0 ml

Dissolve the potassium iodide in water. Add the iodine crystals slowly and shake until dissolved. Filter and store in a brown bottle. This solution is only stable for three weeks.

(b) Working reagent. Add 9.0 milliliters of Solution A to 1.0 milliliter of Solution B immediately prior to use. For smaller or larger volumes, decrease or increase both components proportionately. Mixing the two solutions too far in advance causes a precipitate to form, thus reducing the staining capacity.

(c) Procedure. Mix one or two milligrams of feces with a drop of the MIF solution. Allow the mixture to stand for a few minutes so that the organisms stain well. Examine for the presence of diagnostic forms of intestinal parasites.

(4) Supravital Stain.

(a) Stock reagents.

1 Solution A (Eosin in isotonic saline).

Eosin Y, analyzed reagent ..... 0.5 gm
Physiological saline ..... 100.0 ml

Add the Eosin stain powder slowly to the saline while stirring and filter the solution after all the eosin has dissolved. Store in a screw cap bottle.

2 Solution B (Brilliant Cresyl Blue in isotonic saline).

Brilliant Cresyl Blue, analyzed reagent ..... 0.2 gm
Physiological saline ..... 100.0 ml

Mix filter and store as in solution A.

(b) Working reagent. Immediately before use, mix equal parts of Solutions A and B, but only in the quantity needed for that particular staining procedure.

(c) Procedure for feces.

STEP 1: Place one drop of the Eosin-Brilliant Cresyl Blue stain in the center of a 25 by 75 millimeter glass slide.

STEP 2: Use the end of a plain applicator stick to pick up a small quantity of feces from a passed stool. A portion taken from an area with mucus or flecks of blood is more likely to contain trophozoites.

STEP 3: Transfer the selected bit of fecal material to the drop of stain and mix until a thoroughly homogenous suspension is formed.

STEP 4: Cover the suspension with a 22 by 40 millimeter coverslip, avoiding formation of air bubbles and macroscopic debris, and place the slide on a warming apparatus at 37 for a minute before microscopic examination. This allows time for the supravital stain to effectively penetrate the organisms while the heat from the warming apparatus stimulates locomotion.

(d) Procedures for rectal scrapings.

STEP 1: If the material to be examined is obtained from a rectal proctoscopy, it must be rushed to the laboratory in a clean, 84 milliliter wide mouth, screw cap bottle that has been warmed to 37º C. Immediately upon laboratory receipt, place the specimen on a warming table and keep at 37º C until completion of examination.

STEP 2: Use a small disposable pipette to aspirate a small portion of the mucoid proctoscopic material.

STEP 3: Put two drops of stain on a clean slide and mix the fecal aspirate with the stain by repeatedly drawing up and expelling the suspension upon the slide.

STEP 4: After thoroughly mixing, leave a large drop on the slide and proceed as in step (4) for feces.

(5) Quensel's solution. Amoebic trophozoites are not easily identified using the saline preparation. Stains such as iodine are not suitable for the identification of this stage because it is completely distorted by the stains. Rapid demonstration of motile trophozoites on wet mount slide preparations can be achieved by the use of Quensel's solution. This preparation may be used on fresh or cultured specimens.

(a) Stock reagents.

1 Sudan III--solution A.

Sudan III powder..... 1.6 gm
Ethanol, 80% ..... 100.0 ml

Mix the stain with the alcohol and shake well. Allow the mixture to stand overnight to ensure that the solution is saturated. If at the end of 24 hours the stain has completely dissolved, add more Sudan III powder until saturation. Filter and store in a stoppered brown glass bottle.

2 Methylene blue--solution B.

Methylene blue powder (medicinal) ..... 3.5 gm
Distilled water ..... 100.0 ml

Mix the powder with water and shake well. Allow the mixture to stand overnight to ensure that the solution is saturated. If at the end of 24 hours the stain has completely dissolved, add more Methylene blue powder until saturation. Filter and store in a stoppered brown glass bottle.

3 Cadmium chloride--solution C.

Cadmium chloride (c.p.)..... 10.0 gm
Distilled water ..... 100.0 ml

Mix the powder with the water and shake well. Store in a stoppered brown glass bottle.

(b) Working solution.

Stock Sudan III (Solution A) ..... 20.0 ml
Stock Methylene Blue (Solution B)..... 30.0 ml
Stock Cadmium chloride (Solution C) ..... 50.0 ml

STEP 1: Mix solution A with solution B.

STEP 2: Add solution C to the above mixture.

STEP 3: Gently shake for 15 to 20 minutes until a flocculent precipitate develops and the fluid becomes almost colorless.

STEP 4: Filter and remove all excess liquid from the precipitate by placing the filter paper with the precipitate upon several layers of dry filter paper. Leave overnight.

STEP 5: Transfer precipitate to a fresh filter paper and pour it over 25 to 30 milliliters of distilled water to wash the precipitate.

STEP 6: Dissolve the washed precipitate in 250 milliliters of distilled water.

STEP 7: Filter in a few days if fine crystals of cadmium chloride precipitate out.

STEP 8: Store in a stoppered brown glass bottle.

(c) Procedure.

STEP 1: Apply a large drop of Quensel's solution to the center of a 25 by 75 millimeter slide.

STEP 2: Use an applicator stick to pick up a small amount of fecal sample and thoroughly comminute with the drop of Quensel's.

STEP 3: Apply a 22 by 40 millimeter coverslip to the suspension and seal the edges with a melted 1:1 vaseline/paraffin mixture.

STEP 4: Let stand for 10 to 15 minutes and scan with the low power of the microscope for trophozoites and identify under high dry (some authors recommend that the identification be done with the oil immersion objective).

(d) Interpretation. Trophozoite cytoplasm is stained pale blue while the nuclei takes on a darker blue color. Living cysts, ciliates, and flagellates do not stain. Nuclei of Dientamoeba fragilis trophozoites do not stain well.

(6) Buffered Methylene Blue. The Buffered Methylene Blue stain has been used successfully for staining protozoan trophozoites in wet preparations.

(a) Stock reagents.

1 0.2M acetic acid--solution A.

Glacial acetic acid ..... 11.55 ml
Distilled water ..... QS to 1,000.00 ml

In a 1,000 milliliter volumetric flask, add the glacial acetic acid to about 500 milliliter of distilled water and mix well and store in a stoppered glass bottle.

2 0.2M sodium acetate--solution B.

Sodium acetate (anhydrous $NaC_2H_3O_2$) ..... 16.4 gm
Distilled water ..... QS to 1,000.0 ml

NOTE: The hydrated salt ($NaC_2H_3O_23H_2O$) can be substituted, but with a quantity of 27.2 grams.

In a 1,000 milliliter volumetric flask, add the salt to about 500 milliliter of distilled water. Mix until dissolved and QS to the 1,000 milliliter mark with distilled water. Mix well and store in a stoppered brown glass bottle.

3 Methylene Blue powder.

(b) Working solution.

Solution A ..... 46.3 ml
Solution B ..... 3.7 ml
Methylene Blue powder ..... 3.0 gm

Mix the ingredients well. This solution should be prepared just prior to use for optimum results.

(c) Procedure. Using an applicator stick, mix a small amount of fecal material with a drop of Buffered Methylene on a slide. Mix well, coverslip, seal with a 1:1 vaseline/paraffin mixture. Examine for intestinal parasites.

b. **Concentration Techniques.** Intestinal parasites are readily found in direct smears when present in large quantities. However, in most cases, concentration is required for the detection of these parasites. Concentration procedures, when properly performed, are more reliable by ensuring a higher recovery ratio for protozoans as well as for helminths.

(1) Zinc sulfate. This is a simple and efficient method for the recovery of ova, larvae, and protozoan cysts. This method is recommended for mass examination of roundworm eggs and protozoan cysts. The procedure is not suitable for the recovery of eggs from Ascaris, Trichuris, trematodes, and pseudophyllids. Another drawback of the procedure is that it is not convenient when working with fatty stools.

(a) Reagent--zinc sulfate solution, specific gravity 1.18 to 1.20. Add 331 grams of zinc sulfate (USP) to 1,000 milliliters of warm tap water. Check the specific gravity with a hydrometer and adjust if needed by adding zinc sulfate or water.

(b) Procedure for concentration.

STEP 1: Place 0.3 to 0.5 grams (1/3 to 1/2 milliliters if liquid) of feces in a disposable container and emulsify with 1.0 milliliters of tap water.

STEP 2: Add 8.0 milliliters of tap water, mix well, and strain through two layers of wet gauze into a 15 milliliter centrifuge tube.

STEP 3: Centrifuge for one minute at 2,500 rpm.

STEP 4: Decant supernatant and add one milliliter of zinc sulfate solution. Mix well.

STEP 5: Fill the tube to the 13 or 14 milliliter mark with zinc sulfate.

STEP 6: Centrifuge for one minute at 2,500 rpm.

STEP 7: Carefully and without shaking or spilling, place the tube in a rack vertically and away from any vibrations caused by machinery.

(c) Wire loop method of removing the concentrate (preferred method).

STEP 1: Prepare a bacteriological loop so it is at a 90-degree angle to the wire.

STEP 2: Gently slide the loop just under the surface of the liquid and remove three to four loopsfull of the concentrate.

STEP 3: Mix with a drop of the desired stain and examine for parasites.

(d) Coverslip method of removing the concentrate (alternate method).

STEP 1: Carefully raise the fluid level of the tube until a positive miniscus is obtained (adding the solution down the side of the test tube prevents remixing of the surface concentrate.

STEP 2: Place a coverslip on top of the tube touching the meniscus and allow to stand undisturbed for 10 to 15 minutes.

STEP 3: With a quick motion, remove the coverslip and place it on a prepared slide to which one drop of the desired stain has been added.

STEP 4: Examine for parasites.

(2) Brine flotation. This procedure is easy to perform and it is used for the recovery of ova other than operculate and Schistosoma. Trophozoites are destroyed and cysts are distorted beyond recognition. The method is recommended for the screening of hookworms in a less than suitable environment.

(a) Reagent. Make a saturated solution of sodium chloride (table salt suffices) in boiling tap water. Allow to cool and check the specific gravity to make sure that it is at least 1:20. If the specific gravity is too low, reboil the solution and add more salt. Filter and store in a cool place.

(b) Procedure.

STEP 1: Make a 1:1 mixture of feces and the brine solution in a disposable container.

STEP 2: Transfer to a 20 milliliter round test tube (about one inch in diameter) and fill the tube to the brim with salt solution.

STEP 3: Collect the concentrate in the same manner as the zinc sulfate method.

STEP 4: Observe microscopically for parasites.

(3) Formalin-Ethyl Acetate (Ether) Concentration. This procedure is used for the routine recovery of protozoan cysts, helminth larvae, and ova (including operculate and Schistosoma eggs). The eggs of Hymenolepis nana and the cysts of Giardia lamblia and Iodamoeba butschlii do not show a good recovery ratio. Recently this procedure has been made comparatively safe with the replacement of ethyl acetate for ether. However, ethyl acetate is also a flammable and explosive reagent. Therefore, caution must be exercised when using this reagent.

(a) Reagents.

1 Physiological saline.

2 Neutral buffered 10

Formaldehyde (USP)	100.0 ml
Distilled water	900.0 ml
Sodium phosphate, monobasic	4.0 gm
Sodium phosphate dibasic	6.5 gm

3 Ethyl acetate.

(b) Procedure.

STEP 1: Mix a portion of stool about the size of a walnut with about 25 milliliters of physiological saline (0.85 percent). The ratio of stool to saline should be controlled to yield approximately one milliliter of sediment.

NOTE: Tap water may be substituted for saline to destroy Blastocystis hominis.

STEP 2: Strain about 15 milliliters of the suspension through wet gauze held by a small funnel into a 15 milliliter centrifuge tube.

STEP 3: Centrifuge at 2,500 rpm for two minutes and decant the supernatant. There should be from one to two milliliters of sediment. If the sediment is too much, resuspend and discard until the desired amount of sediment is acquired. On the other hand, if the amount of sediment is not enough, strain more suspended specimen.

STEP 4: Resuspend in saline or water, centrifuge, and decant. This step may be repeated until the supernatant is partially clear.

STEP 5: After the last decanting, resuspend the sediment in 10 milliliters of neutral buffered formalin, mix, and let stand for 15 minutes.

STEP 6: Add three milliliters of ethyl acetate, stopper, invert the tube, and shake vigorously until thoroughly mixed (about 30 seconds). Release the stopper with care to avoid spraying the area or self with the specimen.

STEP 7: Centrifuge for two minutes at 2,500 rpm. Four layers will result in the tube after centrifugation. From top to bottom, the layers are: ethyl acetate, debris plug, formalin solution, and the sediment containing the parasites (if present).

STEP 8: Free the debris plug with an applicator stick and carefully decant the top three layers, leaving the sediment undisturbed. Use a cotton swab to clean the remnants of ethyl acetate from inside of the tube.

STEP 9: Prepare saline and stained wet preparations and examine for parasites.

(4) Merthiolate-Iodine-Formaldehyde Concentration (MIFC). The MIFC combines fixing, concentration, and staining all in one procedure. Some authors claim that this procedure is of little value for identification of amoebic trophozoites. Nevertheless, it is a comprehensive technique and it is widely used.

(a) Stock reagents.

1 Merthiolate-formaldehyde (MF)--Solution A.

Distilled water	250.0 ml
Formaldehyde (saturated)	25.0 ml
Tincture of merthiolate (Lilly 1:1,000)	200.0 ml
Glycerin	5.0 ml

Store in a stoppered brown glass bottle. This solution is stable for months.

2 Lugol's iodine solution (I)--Solution B.

Iodine crystals (powdered)	5.0 gm
Potassium iodide	10.0 gm
Distilled water	100.0 ml

Dissolve the potassium iodide in water. Add the iodine crystals slowly and shake until dissolved. Filter and store in a brown bottle. This solution is only stable for three weeks.

(b) Working reagent. Add 18.0 milliliters of Solution A to 1.2 milliliters of Solution B immediately prior to use. For smaller or larger volumes, decrease or increase both components proportionately. Mixing the two solutions too far in advance causes a precipitate to form, thus reducing the staining component.

(c) Procedure. If the specimen has not been previously fixed in MIF, mix one portion with 10 portions of MIF solution in a disposable container.

STEP 1: Suspend the fecal material and strain through two layers of wet gauze into a 15 milliliter centrifuge tube.

STEP 2: If needed, add MIF to make 12 milliliters.

STEP 3: Add three milliliters of ethyl acetate, stopper, invert the tube, and shake vigorously until thoroughly mixed (about 30 seconds). Release the stopper with care to avoid spraying the area or self with the specimen.

STEP 4: Centrifuge for two minutes at 2,500 rpm. Four layers will result in the tube after centrifugation. From top to bottom, the layers are: ethyl acetate, debris plug, MIF, and the sediment containing the parasites (if present).

STEP 5: Free the debris plug with an applicator stick and carefully decant the top three layers, leaving the sediment undisturbed. Use a cotton swab to clean the remnants of ethyl acetate from inside of the tube.

STEP 6: Remix the sediment with an applicator stick, transfer a drop to a clean slide, and observe microscopically for parasites.

c. **Permanent Stains.** Because of the difficulty in recognizing protozoan organisms in wet preparations, a parasitology laboratory should have a procedure for permanent stain. In fact, no trophozoite should be reported without first being positively identified in a stained preparation under the oil immersion objective. Another advantage of permanent mounts is that they can be kept as a permanent record and/or for training.

(1) Iron hematoxylin stain. The phosphotungstic/iron hematoxylin stain for staining protozoans in fecal smears, proposed by Tompkins and Miller, is one of the best techniques for demonstrating nuclear details and cytoplasmic inclusions. It is a short procedure and does not have to be adjusted with microscopic observation as does Heidenheim's method.

(a) Reagents.

1 Iodine alcohol.

a Stock solution. Dissolve sufficient iodine crystals in 70 percent ethanol to make a dark brown concentrated solution. This solution is stable for two to three months.

b Working solution--solution A. Dilute the stock solution with enough 70 percent ethanol to make it the color of strong tea. This solution should be made just prior to use.

2 Mordant--solution B.

Ferric Ammonium Sulfate (violet crystals) ... 2.0 gm
Distilled water ..... 50.0 ml

Break the crystals into small pieces and mix with the water until dissolved.

3 Hematoxylin stain.

a Stock solution. Dissolve 10.0 grams of hematoxylin powder in 100 milliliters of 95 percent ethanol. Store in the presence of light for six weeks or longer (aging). Filter prior to use.

b Working solution--solution C. Dilute five milliliters of the stock solution with 95 milliliters of distilled water. Prepare this solution daily.

4. Phosphotungstic acid--solution D.

Phosphotungstic acid.....2.0 gm
Distilled water.....100.0 ml

5. Ethanol 70% with lithium carbonate.

a Stock solution. Dissolve sufficient lithium carbonate in 100 milliliters of distilled water to make a saturated solution. Mix well and save in a brown stoppered glass bottle.

b Working solution--solution E. Add six to 10 drops of stock solution for every 100 milliliters of 70 percent ethanol used. Prepare this solution just prior to use.

(b) Procedure.

STEP 1: Prepare at least five smears from each fresh specimen by streaking the feces on a clean slide with an applicator stick. Make the smear thin, but do not leave clear areas on the slide. The optimum smear should be about one inch long.

STEP 2: Fix the wet smears in Schaudinn's fixative for one to 24 hours. Do not allow the smear to dry. The fixation time may be shortened to five minutes if the fixative is heated to 50.

STEP 3: Set up a series of jars suitable for staining with the required reagents.

STEP 4: Transfer the slides singly or in racks from dish to dish.

NOTE: To prevent transfer of a previous solution, it is necessary to drain the rack or slides on paper towels prior to proceeding to the next reagent.

	REAGENT	SOLUTION	TIME (MIN)
STEP 1:	Working Iodine Alcohol	A	5
STEP 2:	Ethanol, 50%		3
STEP 3:	Tap Water		3
STEP 4:	Mordant	B	3-5
STEP 5:	Tap Water		1
STEP 6:	Working Hematoxylin Stain	C	1
STEP 7:	Tap Water		1
STEP 8:	Phosphotungstic Acid	D	2-4
STEP 9:	Running Tap Water		5
STEP 10:	Ethanol, 705 with Lithium Carbonate	E	3
STEP 11:	Ethanol, 95%		5
STEP 12:	Xylol		3

(c) Mount using a suitable mounting medium.

(2) Trichrome. The Gomori-Wheatley procedure is a very rapid and useful method for the identification of amoebic organisms. This is a multi- color stain that requires no differentiation step. Fresh PVA-fixed smears require increased timing and the fixation step is omitted.

(a) Reagents.

1 Schaudinn's fixative--solution A. Same as above.

2 Working iodine alcohol--solution B. Same as above.

3 Stain.

Chromothrope 2R ..... 0.6 gm
Light Green SF ..... 0.15 gm
Fast Green..... 0.15 gm
Phosphotungstic Acid ..... 0.7 gm
Glacial Acetic Acid ..... 1.0 ml
Distilled Water..... 100.0 ml

Mix the stains with the acetic acid and allow to stand at room temperature for 30 minutes. Add the water and mix vigorously.

4 Acidified 90% ethanol.

Ethanol, 90% 99.55ml
Glacial acetic acid..... 0.45 ml

Add the acid to the alcohol. Do not use 95 percent ethanol.

(b) Procedure.

NOTE: Prepare smears and staining tray as done for the iron hematoxylin procedure.

NOTE: Transfer the slides or racks from dish to dish. Remember that the slides or racks must be drained on paper towels between steps.

	REAGENT	SOLUTION	PVA-FIXED	TIME FRESH
STEP 1:	Schaudinn's	A		60*
STEP 2:	Working Iodine Alcohol		10-20	1
STEP 3:	Ethanol, 70%		3-5	1
STEP 4:	Ethanol, 70%		3-5	1
STEP 5:	Trichrome Stain		6-10	2-8
STEP 6:	Acidified Ethanol	One Dip **	One Dip**	
STEP 7:	Ethanol, 95%		1-3 Dips	One Dip
STEP 8:	Ethanol, 95%		5	Two Dips
STEP 9:	Absolute Ethanol		5	5
STEP 10:	Carbol-Xylene		5	5
STEP 11:	Xylene		5	5

* This step could be shortened to five minutes if the stain is heated to 50º C.

** This is a very critical step in the procedure. Destaining should be restricted to no more than 20 seconds. The amount of time that the slide is in contact with the acid alcohol includes the time in between dishes. For better results, this step should be carried out for one slide at a time. Mount using a suitable medium.

(3) Chlorazol Black E. Gleason and Healy modified the technique introduced by Kohn in 1960. This stain, in which fixation and staining is done in a single step, has shown good results in the clinical laboratory. The procedure is used for fresh specimens and is not suitable for PVA-fixed smears. The stock stain is stable indefinitely and the staining time must be determined for each "batch" of stain.

(a) Reagents.

1 Stock stain solutions.

a. Diluent.

Ethanol 90% ..... 170.0 ml
Methanol (absolute) ..... 160.0 ml
Acetic Acid (glacial) ..... 20.0 ml
Liquid phenol ..... 20.0 ml
Phosphotungstic acid, 1% ..... 12.0 ml
Distilled water ..... 618.0 ml

Mix the reagents well, remembering to always add acid to water, not water to acid.

b. Stock stain.

Chlorazol Black E Stain ..... 5.0 gm
Basic solution ..... 1,000.0 ml

Grind the dye in a mortar and add small amounts of the diluent until a smooth paste is formed. Continue to add more diluent while mixing until a homogenous mixture is obtained. Add the remaining diluent. Store in a stoppered brown glass bottle and allow to "ripen" for four to six weeks. Filter before use.

2 Working dilutions and times for staining.

	STAIN	DILUENT	TIME
DILUTION A	Undiluted		2-3 hours
DILUTION B	20.0 ml	20.0 ml	2-4 hours (24)
DILUTION C	30.0 ml	15.0 ml	2-4 hours
DILUTION D	15.0 ml	30.0 ml	2-24 hours
DILUTION E	10.0 ml	30.0 ml	4-24 hours

Stain two smears with each dilution and determine the optimum solution, keeping in mind that more than one solution may be suitable. Practice has shown that solution C is good for two hours and solution D stains well for four to 24 hours.

(b) Procedure.

1 Prepare fresh smears. Do not use a fixed specimen.

2 Transfer the racks or slides from solution to solution, remembering to drain them on paper towels between steps.

	REAGENT	TIME
STEP 1:	Stain Dilution	2 hours (24)
STEP 2:	Ethanol, 95%	2-3 dips
STEP 3:	Ethanol, Absolute	3-5 dips
STEP 4:	Carbol-Xylol	3-5 minutes
STEP 5:	Xylol	3-5 minutes

3 Mount with a suitable medium.

2-20. STAIN REACTIONS

	SALINE	IODINE	MIF	IRON	TRICHROME*	CBE
CYTOPLASM	colorless	yellow to tan	pink	gray	blue-green to gray	green-black
CYST WALL	refractile	dark brown refractile	pink to red refractile	colorless (halo)	colorless	green-black
NUCLEI	refractile	dark brown refractile	dark red refractile	black	dark red	green-black
CHROMATOID BODIES	refractile	dark brown refractile	pink to red refractile	gray to black	red	gray-black
GLYCOGEN VACUOLES	colorless	mahogany brown	colorless	colorless	colorless	color-less

	SALINE	IODINE	MIF	IRON	TRICHROME*	CBE
FLAGELLA & AXONEMES	colorless	colorless	dark red	black	red	gray-black
EGGS AND LARVAE	natural color	tan to dark brown	pink to dark red	gray to black	red	gray-black

* Small cyst nuclei may stain blue with trichrome.

2-21. CULTIVATION OF PARASITES

The cultivation of parasites has proven to be a beneficial technique in the study of human parasitology. The time, effort, and money spent in these techniques have prevented the methodology from being utilized in the routine medical parasitology laboratory. Teaching institutions have a constant need for study material, and therefore, have more of a need for the procedures enumerated below.

a. **Boeck and Drbohlav's Medium (modified).** This is a semi-solid egg-based medium that requires an overlay with Locke's solution. It is used for most intestinal protozoans (excluded: Balantidium coli and Giardia lamblia).

(1) Reagents.

(a) Locke's solution (modified).

Sodium chloride (NaCl) ..... 8.0 gm
Calcium chloride ($CaCl_2 \cdot 2H_20$) ..... 0.2 gm
Potassium chloride (KCl)..... 0.2 gm
Magnesium chloride ($MgCl_2 \cdot 6H_20$) ..... 0.01 gm
Disodium hydrogen phosphate (Na_2HPO_4) ..... 2.0 gm
Sodium bicarbonate ($NaHCO_3$) ..... 0.4 gm
Potassium dihydrogen phosphate (KH_2PO_4) ..... 0.3 gm
Distilled water..... 1,000.0 ml

1 Add the salts in the order listed to 500 milliliters of distilled water in a 2,000 milliliter Erlenmeyer flask and mix well. Slowly add the remaining 500 milliliters of water and mix until all the salts are dissolved.

2 Boil the solution for 10 minutes (a white precipitate should be formed).

3 Allow to cool at room temperature, filter, and autoclave for 15 minutes at 121º C (15 pounds).

(b) Egg slants.

Fresh eggs (medium-large)..... 4 ea
Locke's solution..... 50 ml

1 Emulsify the mixture in a large Erlenmeyer flask containing glass beads or in a blender.

2 Filter through a double layer of gauze and dispense into 16 by 125 millimeter screw cap test tubes in seven to eight milliliter aliquots.

3 Place the tubes in slant racks and inspissate or autoclave (with all exhaust valves closed) for 15 minutes at 15 pounds of pressure until the media gels.

NOTE: The pressure must be allowed to escape from the autoclave at a very low rate. A sudden drop of pressure causes bubbles and unsettles the media.

4 Allow slants to cool and overlay with two or three milliliters of sterile Locke's solution under aseptic technique.

5 Incubate overnight at 35º C and check for sterility. Discard all tubes that show a turbidity in the overlay layer of liquid.

6 Store in the refrigerator. Discard the unused media after a month of storage.

b. **Egg Yolk-Infusion**: (Balamuth, 1946; modified). The liquid Egg Yolk-Infusion medium has liver added and it is excellent for the cultivation of lumen protozoans. This medium has the added advantage of slow deterioration when stored for up to two months.

(1) Reagents.

(a) Liver Extract Solution.

1 Dissolve 5.0 gm of Dry Extract of Liver (Wilson's) in 1000 ml of distilled water and heat to boiling.

2 Filter through filter paper and sterilize at 121 for 15 minutes.

(b) Buffer solutions.

1 Stock solutions (1M).

2 Dihydrogen Phosphate solution (SOLUTION A)--dissolve 13.6 gm of KH_2PO_4 in 100 ml of distilled water and store in a dark glass bottle.

3 Dibasic Phosphate Solution (SOLUTION B)--dissolve 87.1 gm of K_2HPO_4 in 500 ml of distilled water and store in a dark glass bottle.

4 Working Solution (M/15 buffer)--mix 7.0 ml of solution "A" with 43.0 ml of solution "B" and add to 700 ml of distilled water. This solution should be prepared just prior to use.

(2) Preparation.

(a) Into 250 ml of Normal Saline, crumble the yolks of 8 hard boiled eggs and mix with a beater, mixer, or blender; or add 144 gm of dehydrated egg yolk to 144 ml of distilled water and 500 ml of Normal Saline, and mix with a beater, mixer, or blender.

(b) Heat in a double boiler for 20 minutes, and add 60 ml of distilled water (compensation for evaporation).

(c) Filter: for the fresh egg solution use a Buchner funnel with reduced pressure and two layers of #2 Whatman filter paper; for the dehydrated egg solution use a muslin bag and squeeze gently (this solution is too pulpy to filter through a Buchner funnel).

(d) Q.S. to 500 ml with Normal Saline.

(e) Sterilize at 121, for 15 minutes.

(f) Refrigerate fresh egg solution until it reaches a temperature of 8º C. Refrigerate the dehydrated egg solution for 24 hours, until a yellow-colored sediment is formed.

(g) Filter through a Buchner funnel with reduced pressure and two layers of #2 Whatman filter paper.

(h) Add an equal amount of the Working Buffer Solution (M/15 Buffer) to complete the egg infusion, and measure the total amount.

(i) Add 0.5 ml of liver extract for every 100 ml of infusion.

(j) Dispense into 16 x 125 mm screw cap test tubes in 7 to 8 ml aliquots, and sterilize for 15 minutes at 121º C.

(k) Store in the refrigerator.

(3) Inoculation, Incubation and Processing--use the same procedure as for Boeck and Drbohlav's Medium.

c. **Liver Infusion Solid Medium**: (Cleveland and Collier, Cleveland and Sanders, 1930). Entamoeba histolytica grows well in this medium while the other intestinal protozoans do not. Therefore, it is useful as a selective medium and frequently used in metabolic research laboratories.

d. Charcoal: (McQuay, 1956). This is a modified mycobacterial medium which is excellent for the isolation and storage of amoebic organisms (e.g., Entamoeba hartmanii, Dientamoeba fragilis, and to some extent Entamoeba histolytica). The charcoal medium can be prepared as semi-solid slanted medium or in a liquid form.

Continue with Exercises

EXERCISES, LESSON 2

INSTRUCTIONS. Answer the following exercises by marking the lettered response that best answers the question or best completes the incomplete statement or by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Which of the specimens below are used in the laboratory to identify parasites?

   a. Blood.

   b. Stool.

   c. Urine.

   d. All the above.

2. Select the serological test which is generally accepted as being useful for the diagnosis of Chaga's disease.

   a. Complement fixation.

   b. Flocculation.

   c. Latex fixation.

   d. Enzyme-Linked-Immunosorbant Assay (ELISA).

3. Select the serological test(s) which is/are generally accepted as being useful for the diagnosis of Schistosomiasis.

   a. Indirect Immunoflourescence.

   b. Enzyme-Linked-Immunosorbant Assay (ELISA).

   c. Hemagglutination.

   d. All the above.

4. Which of the specimens below can be used in the diagnosis of blood parasites?

    a. Spinal fluid.

    b. Peritoneal fluid.

    c. Tissue biopsies.

    d. All the above.

5. You wish to obtain approximately 10 milliliters of blood from a 30-year-old male in order to diagnose a potential parasitic infection. Which technique would you use to gather this blood sample?

    a. Venipuncture.

    b. Capillary puncture.

    c. Xenodiagnosis.

    d. Venous cutdown.

6. In performing a capillary puncture, you should:

    a. Squeeze the skin near the puncture site in order to increase the output of blood from the puncture.

    b. Apply a thin film of silicone jelly to the tip of the finger in order to facilitate the formation of distinct drops of blood.

    c. Touch the skin at the puncture site with the collection utensil in order to increase the volume of blood sample obtained.

    d. Cleanse the desired puncture site with an alcohol sponge before puncturing the site.

7. Which of the following is the most satisfactory procedure for the definitive diagnosis of malaria, trypanosomiasis and filariasis?

   a. Thick and thin blood film on the same slide.

   b. Xenodiagnosis.

   c. The Jaswant-Singh-Bhattacherji stain.

   d. The indirect cell-count method.

8. The Jaswawant-Singh-Bhattacherji stain is a fairly rapid staining method for the detection of:

   a. Malaria, trypanosomiasis, and filariasis.

   b. Trypanosoma brucei.

   c. Malaria.

   d. Chagas' disease.

9. Knott's technique for examination of microfilariae is useful when:

   a. The patient has had a microfilarial infection for a long period.

   b. The patient is very young or elderly.

   c. There are only small numbers of microfilariae present in the blood.

   d. There are only certain types of microfilariae present in the patient's blood.

10. In performing the Knott's technique for examination of microfilariae, the solution you prepare is supposed to be the color of port wine. You can hasten the ripening of this solution by:

    a. Placing it in a refrigerator for three to six weeks before using it.

    b. Placing five to six drops of a two percent formaldehyde solution in it.

    c. Letting the solution stand in a warm place and by shaking it occasionally.

    d. Autoclaving the solution for three hours at a temperature of 100 to 109º C.

11. In performing xenodiagnosis for Trypanosoma cruzi, you should:

    a. Ensure that all the bugs in the laboratory are fed with a blood meal every other day.

    b. Initially screen the bugs' fecal deposits in order to determine they are free from T. cruzi.

    c. Dissect the bugs only after you have confirmed the presence of T. cruzi in three successive fecal deposits.

    d. Place the bugs in a 0.5 percent formaldehyde solution immediately after they have been allowed to feed on the patient's blood.

12. In using the Field's stain for malarial parasites, you should:

    a. Prepare the various required solutions and dip a thin blood smear into them as per stated directions.

    b. Prepare at least two liters of each of the required solutions before beginning the staining process.

    c. Place the slide horizontally on a slide rack in order to maximize the staining of the malarial parasites.

    d. Prepare a thick blood smear to be stained.

13. Certain information should be affixed to the label of a stool specimen to prevent mixing. This information includes:

    a. The patient's name.

    b. The patient's identification number.

    c. The name of the attending physician.

    d. The date and time of the stool collection.

    e. All the above.

14. Which of the following are cathartics recommended to enhance the recovery of parasites from constipated patients?

    a. Castor oil.

    b. Magnesium hydroxide.

    c. Buffered phosphosoda.

    d. All the above.

15. The cellulose-tape slide preparation method is used to obtain:

    a. Aspirations from the patient's respiratory tract.

    b. Samples from the patient's duodenal area.

    c. Samples around the perianal folds for determination of Enterobius vermicularis infestation.

    d. Samples for determination of trophozoites around the perianal area.

16. When testing for occult blood in feces, the physician should control:

    a. The amount of exercise the patient takes since straining can cause a loss of blood through the gastrointestinal system.

    b. The diet of the patient since the ingestion of rare meat may cause positive results.

    c. The fluid intake of the patient since too little fluid intake can cause concentration of the blood.

    d. The weight of the patient since too rapid a weight loss can cause nutritional problems in patients who have a slow loss of blood through the gastrointestinal tract.

17. Polyvinyl alcohol (PVA) is a mixture used to:

    a. Fix and preserve the operculum on flukes and tapeworms for purposes of identification.

    b. Fix and preserve trophozoites of intestinal amoebic organisms.

    c. Fix and preserve ova of human cestodes.

    d. Fix and preserve for identification adult forms of Macracanthorrhynchus hirudinaceous.

18. Quensel's solution is used to demonstrate:

    a. Motile trophozoites on wet mount slide preparations.

    b. Ova of Echinococcus multilocularis.

    c. Cercaria in cases of suspected trematode infestation.

    d. Cilia in the class Ciliophora or Ciliata.

19. A positive Hemacult test for occult blood is represented by a:

    a. Yellow color on the filter paper.

    b. Blue color around the tablet within two minutes.

    c. Reddish color which persists for 5 minutes.

    d. Green color on the filter paper

20. Which of the following situations is considered as a possible disadvantage in the application of the Formalin-Ethyl-Acetate concentration technique?

    a. Operculated eggs and those of Schistosoma are not easily recovered.

    b. Helminth larvae cannot be concentrated.

    c. Caution must be exercised when using ethyl acetate.

    d. All of the above.

21. In the Phosphotungstic/Iron Hematoxylin method, once the optimum smear is made, the next step is the Schaudinn's solution. What is the most important concern at this time?

    a. Smears should be permitted to dry thoroughly before proceeding.

    b. Smears cannot be allowed to stay in the Schaudinn's solution but a few seconds.

    c. Smears should not be allowed to dry.

    d. Fixation is enhanced if smears are refrigerated.

22. What is the color of the glycogen vacuoles of amebic organisms as observed in iodine preparations?

    a. Colorless.

    b. Black.

    c. Blue-green.

    d. Mahogany brown.

23. The advantage of the trichrome staining technique as modified by Gomori and Wheatley is that:

    a. It is a rapid technique.

    b. The stain can be left overnight.

    c. Organic matter is eliminated.

    d. It also concentrates the specimen.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 2

1. d. (para 2-2)
2. a. (table 2-1)
3. d. (table 2-1)
4. d. (para 2-6)
5. a. (para 2-8)
6. d. (para 2-9b, step 1)
7. a. (para 2-13a)
8. c. (para 2-13d
9. c. (para 2-13e)
10. c. (para 2-13e(1)(a), step 4)
11. b. (para 2-13h(2))
12. d. (para 2-13c(3))
13. e. (para 2-15a(3))
14. c. (para 2-15b)
15. c. (para 2-15c(1))
16. b. (para 2-17)
17. b. (para 2-18b)
18. a. (para 2-19a(5))
19. b. (para 2-17a(2)(a))
20. c. (para 2-19b(3)(b) step 3)
21. c. (para 2-19c,(1)(b) step 2)
22. d. (para 2-20)
23. a. (para 2-19c(2))

End of Lesson 2

APPENDIX

GLOSSARY

A

ABOPERCULAR	Refers to the position opposite the operculum.
ACANTHOR	First larval stage of the acanthocephala which hatches from the egg.
ACETABULUM	The ventral sucker of a fluke, used as a hold-fast organelle.
ACTIVE TRANSPORT	In the acquisition of nutrients, molecules are accumulated against a concentration gradient and transported' across the cell membrane by a carrier in an energy-yielding metabolic reaction, used by certain protozoan cells.
ALAE	Wing like structures often seen as cuticular expansions on adult helminths.
ALGID MALARIA	A pernicious condition seen with P. falciparum where the gastrointestinal tract and other abdominal viscera are overwhelmed by enormous numbers of parasites causing marked congestion.
ALTERNATION OF GENERATIONS	The alternate occurrence of asexual and sexual reproduction in the life cycle of an organism.
AMASTIGOTE	The nonflagellated form of organisms belonging to the Irypanosomatidae also called a Leishman-Donovan (L-0) body.
ANTERIOR STATION	The development of a protozoan in the anterior portion of the intestine or salivary glands of its insect host.
APICAL COMPLEX	A combination of structures, polar rings; conoids, micronemes; micropores, subpellicular tubules, and rhoptries, etc. seen in certain developmental stages of the Apicomplexa.
APPLIQUE	An early form of the trophozoite, most often seen in P. falciparu , which appears as a delicate layer of cytoplasm with a protruding dot of chromatin on the margin of an erythrocyte. Also called accole' form.

ASEXUAL REPRODUCTION	Reproduction without the fusion of sexual cells, as by fission or budding.
ASYNCHRONOUS	The irregular occurrence of events that normally occur at distinct intervals of time. Spoken of malarial fevers which occur at irregular intervals.
AUTOINFECTION	Process of reinfection in which the patient is his own best source of infection from a source already present in the body.
AXONEME	The intracellular portion (or core) of a flagellum or cilium.
AXOSTYLE	The central tube-like support structure of some flagellates.

B

BASAL BODY	A small centriole at the base of a flagellum or cilium from which the axoneme arises. May also be termed a kinetosome or blepharoplast.
BIFURCATED	Divided into two; forked.
BILATERAL SYMMETRY	Having two sides with equal corresponding appearance.
BILHARZIA	A name formerly given to the flukes of the genus Schistosoma.
BINARY FISSION	The splitting of the nucleus and then of the cytoplasm of a cell into two equal parts.
BIOPSY	The removal and examination of a portion of tissue usually for diagnosis.
BIOTIC POTENTIAL	The reproductive capabilities of a species.
BIPOLAR	Having two poles; usually with structures at both poles.
BLACKWATER FEVER	A condition of P.falciparum malaria characterized by massive lysis of erythrocytes followed by high levels of free hemoglobin in the blood and urine (darkening the urine), often with renal insufficiency.
BLEPHAROPLAST	A centriole from which an axoneme arises; also called a basal body or kinetosome.

BOTHRIUM	A groove-like sucking organelle used for attachment on the scolex of a pseudophyllidean tapeworm.
BRADYZOITE	A small slowly developing stage within zoitocysts of the genera Sarcocystis and Toxoplasma.
BROOD CAPSULE	A small cyst that develops along the inner laminated membrane of a unilocular hydatid cyst containing several protoscolices, seen in the genus Echinococcus.
BUCCAL CAVITY	A small cuticular lined space in most nematodes between the mouth and esophagus.

C

CATHARSIS	A cleansing or purgation of the intestines.
CAUDAL	A position toward the tail or posterior region.
CECA	The blind sac-like digestive organ extending posteriorly from the esophagus as seen in the class Trematoda.
CEPHALIC ALA[	Cuticular wing-like expansions located near the neck or head region of certain nematodes.
CERCARIA	The final free-swimming larval stage of a trematode parasite produced by asexual reproduction in the intermediate host, consisting of a body and tail.
CESTOIDEA	The name given to the class of the Platyhelminthes which includes the tapeworm; also called Cestoda.
CHAGAS' DISEASE	A disease of humans and other mammals caused by the flagellate Trypanosoma cruzi.
CHARCOT-LEYDEN CRYSTALS	Double rhomboid crystalline structures which terminate in needle-like points at each end. They are found wherever osinophilic leukocytes are being fragmented and are ndicative of intestinal bleeding.
CHITIN	A white insoluble high molecular weight polysaccharide hought to be one of the principal constituents in formation of the mid-shell layers of the eggs of certain nematodes.
CHOANDMASTIGOTE	A form of the Trypanosomatidae with the flagellum emerging from a collar-like process as in Crithidia.

CHROMATIN	The dark staining portion of a cell nucleus which carries the genetic components of the cell and displays characteristics useful in species identification.
CHROMATOIDAL BARS	Masses of RNA, visible as dark staining bodies with light microscopy, in young cysts of the amoeba.
CILIA	Short hair-like organelles of locomotion composed of a surrounding sheath and a central axoneme seen in the class Ciliophora or Ciliata.
CIRRUS	A copulatory organ of the cestodes and trematodes usually opening into a common genital atrium.
COMMENSALISM	A kind of symbiosis in which one organism, the commensal, is benefited and the other, the host, is neither benefited nor harmed by the relationship.
COMPACT NUCLEI	Nuclei in which there are no clear areas of nucleoplasm and chromatin matter is distributed throughout the nucleus as opposed to being peripheral.
CONJUGATION	A sexual union of one organism with another in which nuclear material is exchanged.
CONOID	A truncated cone of spiral fibri.Is located within the polar rings of the Coccidia.
CONTRACTILE VACUOLES	Vacuoles which are involved in the osmoregulation of certain protozoan cells by pumping accumulated water out of the cell.
COPEPOD	A minute usually freshwater crustacean which serves as an intermediate host in the life cycle of Diphyllobothrium and Dracunculus.
COPULATORY BURSA	A tripartite appendage at the posterior end of the male of certain nematodes.
CORACIDIUM	A larva with a ciliated epithelium that develops in the egg of certain pseudophyllidean cestodes.
COSTA	The thickened base of an undulating membrane of some flagellate protozoa.

CUTANEOUS LARVAL MIGRANS	A skin condition caused by hookworm larvae of cats, dogs and other animals that are unable to mature in man, also known as creeping eruption.
CUTICLE	A noncellular body covering secreted by an underlying hypodermis of the nematodes which is shed four times during maturation.
CYCLOPHYLLIDEA	An order of the cestodes characterized by four sucking discs on the scolex.
CYST	A protective form of certain protozoan organisms which serves as a means of transmission to a new host.
CYSTICERCOID	A cestode larva with a well-formed scolex and tail which develops from the hatched oncosphere of several Cyclophyllidea.
CYSTICERCOSIS	The condition of being infected with the cysticercus larva of Taenia solium.
CYSTICERCUS LARVA	A larval form of a cyclophyllidean tapeworm, consisting of a single scolex enclosed in a fluid filled bladder.
CYTOPLASM	The protoplasm of a cell, other than that of the cell nucleus.
CYTOSTOMAL GROOVE	A depression near the anterior end of certain flagellates.
CYTOSTOME	The mouth-like aperture of certain protozoa, the cell mouth.
CYTOPYGE	An opening in the cell membrane of many ciliates which permits the expulsion of indigestable material.

D

DEFINITIVE HOST	That which harbors the adult or sexually reproducing stages- of a parasite.
DIDELPHIC	Possessing a double uterus; as in many nematodes.
DIFFUSION	The component of nutrition in which molecules are passed through the permeable cell membrane where the concentration of that nutrient is lower in-side the cell than outside.

DIGENETIC	Having two types of reproduction, one sexual occurring in the definitive host and the other asexual (larval stages) occurring in the intermediate host.
DIOECIOUS	Having separate sexes; reproductive systems and sex organs in separate sexually defined individuals.
DIRECT LIFE CYCLE	Where there is no intermediate host required in the transmission of a parasite from one host to another of the same species.
DISTOME	A fluke with two suckers, one oral and one ventral.
DIURNAL	Occurring during the day.
DORSAL	Denoting a position at the back or more toward the back plane.

E

ECDYSIS	Molting or shedding of the inexpansible cuticle allowing growth of the organism to greater dimensions.
ECTOPARASITE	A parasite that lives on the outer surface of its host.
ECTOPIC	Located in a position or site other than the normal.
ECTOPLASM	The outer clear zone of cytoplasm on the immediate margin of the cell.
EDEMA	The presence of abnormally large amounts of tissue fluid, or lymph, in the intracellular spaces, causing localized swelling of the affected area.
EMBRYOPHORE	A gelatinous layer formed around the embryo after the first shell is diminished.
ENDEMIC DISEASE	A disease of low incidence that is always present in a designated region.
ENDOCYTOSIS	The ingestion of a parasitic form by a phagocytic host cell allowing the parasite to become established in its host.
ENDOPARASITE	A parasite that lives inside its host.

ENDOPLASM	The inner granular zone of cytoplasm which comprises the greater mass of the cell.
ENDOPLASMIC RETICULUM	A protoplasmic network of ultramicroscopic filaments in the endoplasm of the cell.
EPIDEMIC	A disease of high incidence which attacks many people in a region at the same time.
EPIMASTIGOTE	A form of the Trypanosomatidae with a flagellum attached to a short undulating membrane extending forward from a kinetoplast which is just anterior to the nucleus.
EPIZOOTIC	A high incidence of infection among animals other than human.
ERYTHROCYTIC PHASE	That stage in the life cycle of the malarial parasite where merozoites leave the liver cells to penetrate erythrocytes and become established in the blood.
ESPUNDIA	A disease caused by Leishmania braziliensis.
EUCARYOTE	A cell containing a membrane-bound nucleus.
EXFLAGELLATION	The rapid formation of microgametes from a microgametocyte of the Plasmodia, usually taking place in the gut of the mosquito.
EXOERYTHROCYTIC PHASE	A stage in the life of the malarial parasite in which the organism becomes established in the hepatic cells and continues subsequent liver cycles.

F

FACULTATIVE PARASITE	A free living organism that will establish a parasitic relationship with the host only if the opportunity presents itself.
FEBRILE PAROXYSM	Sudden recurrence or intensification of fever as with malaria.
FEULGEN REACTION	A microchemical reaction demonstrating the presence of the specific type of nucleic acid found in chromatin.

FIBRILS	Minute fibers or filaments that are the axilar components that comprise the flagella and may also be seen as cell inclusions.
FILARIFORM LARVA	A thread-like larva, the penetrating infective form of hookworm spp.and Strongyloides stercoralis.
FLAGELLUM	A mobile, whip-like, filamentous appendage originating in the cell wall or outer layers of cytoplasm; an organ of locomotion of the Zoomastigophora.
FLAME CELL	The functional unit of the excretory system of the Platyhelminthes; a single cell with a tuft of cilia that extends into a delicate collecting tubule.
FLUKE	A leaf-like flatworm; common name for the class Trematoda.

G

GAMETOCYTE	The sexual stage of the malarial parasite in the blood of the vertebrate host which may produce gametes when ingested by the mosquito host. It may be a male (microgametocyte) or female (macrogametocyte).
GEL STATE	The consistency of the ectoplasm (plasmagel) which helps to give structural rigidity to the protozoan cell.
GENITAL ATRIUM	An orifice in the body wall of certain platyhelminthes into which both male and female genital ducts open.
GENITAL PRIMORDIUM	Primitive cells seen in rhabditiform larva which give rise to the sex organs.
GEOGRAPHIC DISTRIBUTION	That area or region of the world where a given organism is known to exist.
GLYCOGEN VACUOLE	A large food storage vacuole found in the cysts of certain protozoans of the class Rhizopoda.
GOLGI BODY	A type of ultramicroscopic cytoplasmic inclusion seen in the protozoan cell. Thought to be responsible for concentrating and channeling certain intracellular materials; synthesis of mucopolysaccharides, and the storage of lipids and proteins.
GONOPORE	A small opening in the body of a platyhelminth which permits the products of the reproductive systems to escape.

GONOTYL	A muscular sucker often with spines which surrounds the genital atrium of-a digenetic trematode.
GRAVID	Pregnant or filled with eggs.
GROUND ITCH	An irritation of the cutaneous tissues caused by bacteria introduced by wandering hookworm larvae.
GUBERNACULUM	A process formed in the cloaca of certain nematode adult males that guides the exsertion of spicules during copulation.
GYNOCOPHORAL CANAL	A ventral longitudinal groove on the males of the genus Schistosoma where the female resides after mating.

H

HABITAT	The natural site in the host where a parasite resides.
HELMINTH	A general term applied to the various species of worms or wormlike parasites.
HEMOLYMPH	The blood-like nutrient fluid in the body cavity of certain invertebrate animals.
HEMOZOIN PIGMENT	The pigment formed through the metabolism of hemoglobin. It is the waste product of the malarial parasite within the red blood cell.
HEPATOSPLENOMEGALY	Enlargement of the liver and spleen.
HETEROXENOUS	A term applied to parasitic organisms which require more than one host in order to complete their life cycle.
HEXACANTHONCOSPHERE	The six-hooked larva of certain species of tapeworm which is the form that hatches from the egg.
HOLOZOIC	A typical nutritional aspect of many parasitic protozoans which feed by ingesting entire organisms or particles thereof.
HOST	An organism which harbors another organism (parasite).
HOST SPECIFICITY	The degree to which a parasite is capable of maturing in more than one host species.
HYALINE	Glassy and transparent, or approximating that state.

HYDATIC	The larval cyst of cestodes of the genus Echinococcus which may contain many budding or free protoscolices and brood capsules.
HYPERPARASITISM	The condition where an organism is the parasite of another parasite.

I

IMMUNOGENIC	A substance stimulating the production of antibody and thereby conferring immunity.
INCIDENTAL PARASITE	A parasite that has become established in a host other than its natural host. Also called an accidental parasite.
INDIRECT LIFECYCLE	A cycle in which a parasite requires a period of transition t hrough a host of a different species (an intermediate host) before transmission back to the original host species.
INFECTIVE FORM	The stage in the life cycle of a parasite capable of infecting the host.
INTERMEDIATE HOST	That which harbors the immature, larval, or the asexually reproducing forms of the parasite.

J

JUVENILE	The young or immature stage of a helminth prior to becoming an adult.

K

KALA-AZAR	A disease (visceral leishmaniasis) caused by Leishmania donovani. Also called Dum-dum fever.
KARYOSOME	One of the chromatin masses at or near the center of the nucleus which tends to stain more intensely than other nuclear components.
KINETOPLAST	A conspicuous part of a mitochandrion in a trypanosome usually near the kinetosome and associated with the motor activities of the cell.
KINETOSOME	A centriole from which an axoneme arises; also called a basal body or blepharoplast.

KUPFFER CELLS	Phagocytic cells of the reticulo-endothelial system which line the sinusoids of the liver.

L

LARVA	An immature stage in the life cycle of an animal in which it is unlike the parent.
LEISHMAN-DONOVAN BODY	An amastigote of the Trypanosomatidae which is non-flagellated. Also called an L-0 body.
LOBOPODIA	The type of pseudopodia seen in most of the amoeba which is broad cylindrical (finger-shaped) with a rounded tip.
LYMPHADENITIS	Inflammation of lymph nodes.
LYSOSOME	One of the ultra microscopic cytoplasmic inclusions of a cell, containing various enzymes, mainly hydrolytic, which may be responsible for autolysis of the protozoan cell and initiate the break-down of phagocytized materials.

M

MACROGAMETOCYTE	The female differentiated form of the malarial parasite and many sporozoans which, when ingested by the mosquito taking its blood meal or situated in the optimal host environment, becomes a macrogamete.
MAURER'S CLEFTS	Irregular blotches on the surface of an erythrocyte infected with Plasmodium falciparum which stain reddish with Giemsa's stain.
MEHLIS'S GLANDS	Unicellular mucous or serous glands surrounding the ootype of a flatworm, which lubricate the newly formed eggs.
MEIOSIS	The maturation process of gametes (sexually differentiated cells) consisting of chromosome conjugation and two cell divisions in which the diploid chromosome number becomes reduced to the haploid.
MEROZOITE	A subdivision of the schizont stage of the malarial parasite consisting of nuclear chromatin and cytoplasm. It is released from the host cell and attacks new tissue cells or erythrocytes.

METACERCARIA	The encysted stage of a monoecious trematode found in the tissue of the second intermediate host in the life cycle which may be the infective stage to man or other animals.
METACYCLIC	The stage in the life cycle of a parasite that is infective to its definitive host.
METACYST	The cystic stage of a parasite that is infective to its host.
METAZOA	A former division of the animal kingdom which includes all multi-cellular animals.
MICROFILARIA	The first-stage larva of the filariid nematodes in the blood or tissue fluids of man or other definitive hosts.
MICROGAMETOCYTE	The male differentiated form of the malarial parasite which, when ingested by the mosquito, becomes a microgamete.
MICRONEMES	Slender, elongated bodies of the apical complex that join a duct system with the rhoptries, opening at the anterior end of a sporazoite or merozoite.
MICROMUCLEUS	In the ciliates, the smaller of the two types of nuclei; which carries the genetic components of the cell.
MICROPYLE	A minute pore in the cell membrane of certain protozoa (Plasmodium; Eimeria).
MICROTRICHES	Minute projections of the tegument of a cestode.
MICROTUBULES	Ultra microscopic structures just beneath the cell membrane of certain protozoa which contribute to the supportive resistance, preventing deformity of the cell body
MIRACIDIUM	The free-swimming larva which emerges from the egg of a trematode as it hatches, and which penetrates (or is ingested by) the snail host.
MITOCHONDRIA	Ultramicroscopic cytoplasmic inclusions which are the sites of intracellular aerobic metabolism (respiration) of protozoan and many other cells. They bear the enzymes of oxidative phosphorylation and the tricarboxylic acid cycle.

MITOSIS	A type of indirect cell division as in asexual reproduction in which replication of the chromosomes and regular distribution of the daughter chromosomes to the daughter nuclei are carried through a complex of four stages in which the two daughter nuclei ultimately receive identical compliments of the number of chromosomes as in the parent cell.
MODE OF INFECTION	The means by which a parasite enters the host.
MONOECIOUS	Hermaphroditic; having male and female reproductive systems in a single individual.
MONOXENOUS	Living within a single host throughout the entire life cycle of the parasite.
MONOZOIC	A tapeworm whose strobila consists of a single unit.
MULTILOCULAR CYST	The larval form seen in Echinococcus multilocularis which exhibits extensive exogenous budding in abnormal hosts, with infiltration. Also called an alveolar hydatid.
MUTUALISM	A type of symbiosis in which both organisms benefit from the association.
MYIASIS	Infection by fly maggots.

N

NECK	The area behind the scolex of a tapeworm which is composed of germinative tissues from which proglottids begin to form.
NEMATODA	The name given to the class of the true roundworms.
NERVE RINGS	A concentration of nerve elements around the esophageal region of the nematode body.
NIDUS	A specific locality where a given disease exists; usually resulting from ideal ecological factors.
NOCTURNAL PERIODICITY	Exhibiting a frequency of occurrences at night, such as certain filariid larvae found in greater numbers between 10 P.M. and 2 A.M.

NUCLEAR MEMBRANE	The thin outer layer which encloses the nucleoplasm of the nucleus.
NUCLEOPLASM	The protoplasm which makes up the mass of the nucleus.
NUCLEUS	A spheroid body within a cell characterized by its dense structure and functioning in a control capacity in reproduction and other metabolic activities.

O

OBLIGATE PARASITE	An organism that is metabolically dependent on the host.
OOCYST	The cystic form in the Apicomplexa which is the immediate result of sporogony and will ultimately contain sporozoites.
OOKINETE	The motile, elongated zygote in the life cycle of the Plasmodium spp.
OOTYPE	An expansion of the uterus of a flatworm which is surrounded by the Mehlis's glands and into which the oviduct and seminal receptacle unite allowing fertilization to take place.
OPERCULUM	A lid-like structure on the egg-shell of certain flukes and tapeworms through which the larva escapes.
OPISTHOMASTIGOTE	A form of the Trypanosomatidae with the kinetoplast at the anterior end; with the flagellum passing through a long reservoir to emerge at the anterior end; without an undulating membrane.
OVA	A general term for helminth eggs.

P

PAPILLAE	Referring generally to the sensory organs (cephalic and caudal) extending through the cuticle of the nematodes that also have taxonomic significance.
PARABASAL B0DY	in certain flagellates, a Golgi body located near the basal body (kinetosome) which is connected to the basal body by a filament or fibril.

PARASITE	An organism which lives on or within another organism (the host), benefits from the relationship, but contributes nothing beneficial to the host, and may prove to be harmful to the host.
PARASITISM	An obligatory relationship in which one organism (the parasite) is metabolically dependent on another organism (the host); the host may be harmed by the relationship.
PARATENIC HOST	A host in which the parasite lives a portion of its life cycle but does not undergo any development.
PARTHENOGENESIS	Reproduction by development of an egg without it being fertilized by the presence of the male reproductive system.
PATHOGENESIS	The production and development of a disease condition.
PELLICLE	Ultramicroscopic structures comprising the outer membrane and structures immediately beneath the outer membrane.
PELTA	A structure that is an extension of the axostyle which extends to the extreme anterior end where it forms a support for the periflagellar lip.
PHAGOCYTOSIS	The active engulfment of a particle by a cell.
PHORESIS	A facultative, symbiotic relationship where the symbiont (phoront) is mechanically carried about by its host.
PINOCYTOSIS	"Cell drinking"; a means of acquisition of fluid nutrients where minute invaginations are formed in the cell permitting the nutrients to flow in; then the opening is closed to form a vacuole containing the nutrients.
PLEROCERCOID LARVA	The infective larval stage of pseudophyllidean tapeworms; found in the tissues usually of certain fish and amphibians.
POLYZOIC	A tapeworm with a strobila containing more than one proglottid.
POSTERIOR STATION	The development of a protozoan in the posterior portion of the intestine or hind gut of its arthropod host.

PRECYST	A stage at the onset of encystment of the protozoan organism where the trophozoite expels any undigested nutrients it holds and takes on a spherical shape.
PROBOSCIS	A variably shaped hollow, fluid-filled structure at the anterior end of an acanthoceohalan body. it has a thin muscular wall, is retractable, and is armed with recurved, scierotized books.
PROCERCOID	A larval stage of the pseudophyllidean tapeworms which develops from a coracidium after it enters the first intermediate host.
PROGLOTTID	One complete segment (reproductive unit) of the strobe] of a tapeworm.
PROKARYOTE	A cell (such as bacterium) in which the chromosomes are not contained within a membrane-bound nucleus.
PROMASTIGOTE	A form of Trypanosomatidae which has the kinetoplast and free flagellum anterior to the nucleus at the extreme anterior end.
PROTOSCOLEX	The juvenile scolex that buds from the laminated membranes of a coenurus or hydatid cyst of the genus Echinococcus.
PROTOZOAN	A single-celled (unicellular) or acellular organism of the Kingdom Protista which is composed of one or more membrane-bound -nuclei surrounded by cytoplasm and is contained within a limiting cell membrane.
PSEUDOCOEL	The interior fluid-filled body cavity enclosed only by the body wall; not contained within a peritonea] lining.
PSEUDOCYST	A pocket of protozoan organisms within a host cell or contained by a proliferation of host cells but not surrounded by a cyst wall of parasite origin.
PSEUDOPHYLLIDEA	An order of the class Cestoidea (tapeworms) characterized by an almond-shaped scolex with a pair of sucking grooves (bothiria).
PSEUDOPOD	A temporary extension of the ectoplasm of an amoeba; used for locomotion and for capture of food.

Q

QUARTAN MALARIA	Malaria fevers which recur approximately every 72 hours. Caused by Plasmodium malariae.
QUOTIDIAN MALARIA	A characteristic of overlapping malarial infections in which the fevers recur every 24 hours.

R

RECRUDESCENCE	The recurrence of symptoms after a temporary abatement of some days or a few weeks.
REDIA	A larval stage of the digenetic trematodes developed by asexual reproduction in the moluscan host; formed within the sporocyst larva or mother redia.
RELAPSE	The recurrence of a disease process after an apparent cessation of a few months or years.
RESERVOIR HOST	An animal which replaces man as a host in the life cycle of a parasite.
RETICULOENDOTHELIAL SYSTEM	The fixed tissue, macrophages, reticular connective tissue, and the epithelium lining the blood vascular system.
RHABDITIFORM LARVA	A small, rod-shaped, feeding and noninfective stage of the nematodes which develops within the egg and upon hatching may further develop in moist, warm sail.
RHOPTRIES	Elongated.electron-dense structures extending anteriorly within the polar rings of the Apicomplexa.
ROMANA'S SIGN	A symptom associated with infections of Trypanosoma cruzi in which there is edema of the eye orbit and swelling of adjacent lymph nodes.
ROSTELLUM	The raised area extending beyond the anterior portion of the scolex of certain tapeworms which may or may not be retractable.
ROUNDWORM	The common name for organisms belonging to the class Nematoda.

S

SAPROZOIC NUTRITION	Assimilation of nutrients through the cell membrane by diffusion.
SCHIZOGONY	The asexual cycle of the class Telosporea (Sporozoa); as with the asexual cycle of the malarial parasite in the red blood cells and liver cells of man.
SCHIZONT	A cell undergoing schizogony, in which nuclear divisions have occurred but maturity does not occur until morozoites are formed.
SCHUFFNERS DOTS	stippling that occurs on the membrane of erythrocytes invaded by parasites of Plasmodium vivax and P. ovale
SCLEROTIN	An insoluble highly resistant protein substance which in its stabilized form is associated with the egg shells of certain helminths.
SCOLEX	The head region of a tapeworm; where the organs of attachment are located.
SEXUAL REPRODUCTION	A reductional division in meiosis causing a change in chromosomes from diploid to haploid with a subsequent union of two cells to restore diploidy through the union of gametes (macrogametes and microgametes).
SLIME BALL	A mass of mucous-covered cercariae of trematodes of the genus Dicrocoelium which is released by infected terrestrial snails.
SOL STATE	Referring to the colloidal fluid state (plasmasol) of the endoplasm within the protozoan cell.
SPARGANOSIS	An infection with the migrating plerocercoid larva of an unidentified species of the Pseudophyllidea.
SPIRAL GROOVE	A furrow or indentation coursing diagonally along the mid-body surface of certain flagellates which may be responsible for their twirling motility.
SPOROBLAST	One of the divisions of the first developmental stages within the Oocyst of a coccidian; which will ultimately develop into a sporocyst containing sporozoites.

SPOROCYST	A developmental stage of a sporozoan within an oocyst giving rise to sporozoites. Also one of the asexual stages of development, which multiplies the larval forms within the moluscan host, in the life cycle of certain trematodes.
SPOROGONY	The sexual cycle of the Telosporidea (Sporozoa); multiple fission of a zygote which ultimately results in the production of sporozoites; in malaria, that cycle occurring in the mosquito host.
SPOROZOITE	One of the spindle-shaped cells resulting from sparogony; the form of the malaria] parasite which is infective to humans.
SPURIOUS	Referring to an organism or disease that occurs in an area where it is not actually endemic.
STICHOCYTE	A large rectangular cell which secretes into the esophogeal lumen of nematodes of the family Trichuridae.
STIEDA BODY	A plug in the inner wall of one end of the oocyst of certain coccidians.
STROBILA	The entire body of a tapeworm which is composed of chains of proglottids.
SWIMMERS ITCH	A skin irritation or rash caused by the cercariae of nonhuman or bird schistosomes which penetrate the skin but cannot develop in an unsuitable host.
SYLVATIC	Occurring or existing in the wild or in wooded areas.
SYMBIOSIS	The close association or living together of two organisms of different species; each of the organisms being known as a svmbiont.

T

TACHYZOITE	A minute, crescent-shaped form of Toxoplasma which resembles a merozoite and rapidly divides within host tissue cells causing cell destruction.
TAPEWORM	A parasitic. intestinal worm of the class Cestoda which is flattened, having a tapelike form and composed of segments.

TEGUMENT	The syncytial, or occasionally ciliated, cellular epithelium covering the body of the Platyheiminthes.
TERTIAN MALARIA	A malarial infection where the fevers recur every 48 hours. Caused by Plasmodium vivax, P.ovale and P. falciparum.
TRANSPORT HOST	A paratenic host; where the parasite lives a portion of its life cycle but does not undergo further development.
TREMATODA	The name give to the class of flatworms consisting of the flukes.
TROPHOZOITE	The active, vegetative, feeding, and motile stage of a protozoan organism.
TRYPANOSOME	An organism belonging to the genus Trypanosoma.
TRYPOMASTIGOTE	A form of the Trypanosomatidae which has the kinetoplast located at the extreme posterior end well behind the nucleus. The flagellum, attached to an undulating membrane, extends anteriorly terminating in a free flagellum.

U

UNDULATING MEMBRANE	A fin-like ridge of the cell membrane along the surface of the cell; with a flagellum imbedded along the edge; used for cell motility by certain flagellates.
UNILOCULAR CYST	A larval form seen in the genus Echinococcus which exhibits an inner budding of brood capsules with numerous protoscolices bound by a laminated membrane.
UNIT MEMBRANE	The three-layered membrane that covers the protozoan cell; a central lipid layer sandwiched between two protein layers.
URBANIC DISEASE	A disease that is prevalent in the areas where people live; peculiar to the human environment.

V

VACUOLE	Any of a variety of spaces or cavities in the cytoplasm of the protozoan cell which may contain food, glycogen or may be functional in purpose (osmoregulatory).
VECTOR	A carrier, usually an arthropod, which transmits an infective agent from one host to another.

VENTRAL GROOVE	A crevice on the underside of certain flagellates; it may contain flagella and may contribute to the ability of the organism to adhere to the host cell.
VERMINOUS INTOXICATION	A condition of systemic poisoning arising from the absorption of metabolites produced by heiminthic parasites.
VESICULAR NUCLEI	Nuclei of the protozoa which are characterized by a clear nucleoplasm in which one or more karyosomal bodies are seen; bound by a nuclear membrane with occasional distribution of chromatin along the nuclear membrane.
VIABLE	In a living state.
VISCERAL LARVAL MIGRANS	A condition caused by the larval forms of Taxocara or Ascaris spp.which hatch when fully embryonated eggs are ingested by an improper host (humans); the larvae migrate randomly throughout the viscera seeking to complete their life cycle.
VITELLARIA	Glands of the female reproductive system in the Platyhelminthes which secrete products which assist in the formation of the eggs; primarily for shell material.
VOLUTIN GRANULES	Metachromatic granules having a marked affinity for basic dyes; seen in the cytoplasm of certain protozoans.
VULVA	The external female copulatory structure of the nematodes; some are equipped with sensory papillae.

W

WINTERBOTTOM'S SIGN	An enlargement of the posterior cervical lymph nodes; a symptom seen in African trypanosomiasis.

X

XENODIAGNOSIS	A diagnostic means employing disease-free, laboratory-bred bugs which are allowed to feed on the patient. finding the disease forms in the feces of the bug after feeding on the patient is diagnostic; used in diagnosis of latent infections with Trypanosoma cruzi.

Y

Z

ZOITOCYST	A cyst stage in the development of the Sarcocystidae which contains numerous bradyzoites and is formed in the tissues of the host.
ZOONOSIS	A disease of animals that is capable of being transmitted to humans.
ZYGOTE	A fertilized cell, in sexual reproduction, formed by the fusion of the microgamete and macrogamete.

End of Appendix