NATIONAL OPEN UNIVERSITY OF NIGERIA

FACULTY OF HEALTH SCIENCES

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE

EHS213: GENERAL MICROBIOLOGY PRACTICALS

MANUAL

EHS 213: GENERAL MICROBIOLOGY PRACTICALS

B.SC. ENVIRONMENTAL HEALTH SCIENCE

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FACULTY OF HEALTH SCIENCES

(DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE)

Student Identification

Affix

Passport

Photograph

Name of Student: .....

Matriculation Number: .....

Level:.....Year of Entry:.....

Student Study Centre: .....

Session: .....

Group: ..... Date: .....

Name of Centre Director: ..... Signature: .....

Name of Lab. Coordinator: ..... Signature: .....

Name of HOD: ..... Signature: .....

PREFACE

This basic guide is designed to facilitate and promote the practical aspect of Microbiology in Environmental Health Science among the Environmental Health Students. Other target groups include students in medicine, medical laboratory science, nursing as well as other health professionals.

The guide is designed to be used in conjunction with basic environmental health Text. It includes an orientation to the recommended teaching practicals and the rationale for its use, a description of selected methods and guidelines for organizing a practical. The description of the methods should assist the students in adapting the exercises to meet their needs.

The Practical manual is basically on faecal, urine, blood, water, milk, food and air samples. The overall aim of this practical is to transfer functional knowledge, develop positive attitudes and horn-in the skills you need to effectively function in the team that ensures the delivery of wholesome Environmental Health Science management.

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MODULE 1

UNIT 1: INTRODUCTION TO LABORATORY AND LABORATORY EQUIPMENT

CONTENTS

1.0 INTRODUCTION

Diagnostic chemical/Health laboratory is an umbrella filed a number of different disciplines. The major disciple/department of clinical, medical, health and diagnostic laboratory science are

a) Chemical Pathology
b) Haematology
c) Histopology
d) Medical Microbiology

2.0 OBJECTIVES

Upon completion of studying this unit, you should be able to:

1. Define the meaning of Clinical/health laboratory
2. List different types of Clinical/health laboratories
3. Identify different types of Clinical/health laboratory equipment

3.0 MAIN BODY

3.1 What is a Laboratory?

A laboratory is a place equipped for experimental study in science or for testing and analysis. It provides opportunity for experimentation, observation, or practice in a field of study. There are different types of laboratory which includes clinical or medical laboratory, analytical and quality laboratory, biosafety laboratory, incubator laboratory, production laboratory, cleanroom laboratory, research and development laboratories etc. The laboratory helps students learn scientific concepts and to enhance students interest, motivation, practical skills and problem solving abilities. It is a facility that provides controlled conditions in which scientific or technological research, experimental and measurement may be performed. The major role of medical laboratory is to assist in the definition and treatment of diseases by performing qualitative, quantitative or screening test procedures or examination on materials derived from human body. The laboratory plays a vital role in the overall comprehensive health care system by:

i. Confirming provision diagnose of the clinician
ii. Ruling out a diagnose.
iii. Deleting disease
iv. Regulating therapy.

The laboratory equipments you will encounter in this course include: Microscopes, Slides, Pipette, cover slides, beakers, reagent bottles Bunsen burner, Colorimeter etc.

Glass wares in the laboratory include: Beaker, Conical flask, cylinder, Pipette**,** Petri dish**,** Aspirator, Autoclave, Biosafety cabinet**,** Colony counter, Laboratory centrifuge, Crucible**,** Hot air oven, Incubator, Retort stand, Spectrophotometer, Stirring rod and Thermometer.

4.0 CONCLUSION

The student should have learnt what a Clinical/health laboratory is and different type of laboratory equipment.

5.0 SUMMARY

A Clinical laboratory is a place equipped with tool and equipments for investigative procedures and for preparation of reagent. In this unit, you learnt about different laboratory equipment and glassware.

UNIT 2: LABORATORY SAFETY CONTENT

CONTENTS

1.0 INTRODUCTION

Laboratory safety involves the development of skills and responsibility and must be an integral part of every laboratory. Clinical/health investigations will be of little value without good field and laboratory work. These investigations are normally carried out through the active use of processes which involves laboratory or other hands-on activities.

2.0 OBJECTIVES

At the end of this lesson student should be able to know the common laboratory safety and precautions in the laboratory.

3.0 MAIN BODY

3.1 Safety in Clinical/health laboratory

The practice of clinical laboratory is associated with hazards and accidents. The laboratory worker is very much at risk of acquiring transmissible disease through contact with patients or handling of clinical specimen. Though accidents do occur in the best laboratories, a poorly designed and overcrowded lab has the increased possibility of hazards and accidents. It should be noted however, that bad laboratory practices are the major courses of laboratory accidents. It follows therefore, that the laboratory should be maintained by well trained, dedicated and meticulous staff. Every laboratory must have an appropriate code of safe laboratory practice. It is important that all accidents be promptly be reported to the safety officer or to the officer in charge of laboratory.

The hazard as accidents in the lab may come from:

Infection

Burns

Cuts and pricks

Hazard of toxic chemicals

Electric shock

3.2 Lab Safety Rules

Laboratory safety rules are guidelines designed to help keep you safe during analysis or examination. Some equipment and chemicals in a microbiology laboratory can cause serious harm. It is always wise to follow all laboratory safety rules. Don't forget, the most helpful safety rule is to use plain old common sense.

3.2.1 Be Prepared

That means you should read your lab manual to know exactly what you will be doing. Review your microbiology notes and relevant sections in your microbiology book before your lab begins. Make sure you understand all procedures and purposes, as this will help you understand the lab activities you will perform. It will also help you get your thoughts organized for when you have to write your lab report.

3.2.2 Be Neat

When working in a microbiology lab, make sure you keep your area neat and organized. If you happen to spill something, ask for assistance when cleaning it up. Also remember to clean your work area and wash your hands when you have finished.

3.2.3 Be Careful

An important microbiology lab safety rule is to be careful. You may be working with glass or sharp objects, so you don't have to handle them carelessly.

3.2.4 Wear Proper Clothing

Accidents do happen in a microbiology lab. Some chemicals have the potential to damage clothing. Laboratory coats are not status symbol but are meant to protect the wearer from chemical splashes and infectious material. Cotton is better material for a better lab coat than nylon as it has a greater absorptive capacity and is generally resistant to chemical splashes.

3.2.5 Be Cautious With Chemicals

The best way to remain safe when dealing with chemicals is to assume that any chemical you handle is dangerous and handled accordingly. Be sure you understand what type of chemicals you are using and how they should be properly handled. If any chemical comes in contact with your skin, wash immediately with water and inform your lab instructor. Wear protective eyewear when handling chemical

3.2.6 Wear Gloves

Hand gloves must be worn when handling corrosive substances such as strong acids or alkalis. Light weight disposable gloves should be worn during weighing and handling of chemicals to avoid the risk of absorption through the skin

3.2.7 Safety Equipment

Be sure you know where to find all safety equipment in the microbiology lab. This includes such items as the fire extinguisher, first aid kit, broken glass receptacles, and chemical waste containers. Also be sure you know where all the emergency exits are located and which exit route to take in case of an emergency. The following symbols represent some of the safety signs used in the laboratory:

Sign for combustible material

Sign for corrosive material

Sign for toxic chemicals

Sign for environmental hazard

Sign for eyewash station

Sign for live electricity

Open Flame Prohibited Sign

Sign for fire extinguisher

Sign for nonpotable water

Sign for explosive material

Sign for materials that can be recycled

Sign for Inflammable material

3.3 Precautions in the Microbiology Laboratory

There are several things in a microbiology lab that you must always avoid. Here are a few major laboratory do nots.

Do Not

- eat or drink in the lab
- taste any chemicals or substances you are working with use your pipette for pipetting substances
- handle broken glass with bare hands
- pour chemicals down the drain without permission
- operate lab equipment without permission
- perform your own experiments unless given permission
- leave any heated materials unattended
- place flammable substances near heat
- engage in childish antics such as horseplay or pranks

3.4 Have a Good Experience

Microbiology lab is an important aspect of any microbiology course. In order to have a good lab experience, make sure that you follow these lab safety rules and any instructions given to you by your lab instructor.

3.5 Laboratory Housekeeping

Housekeeping is important in any work area. A clean, well-maintained work area improves safety by preventing accidents and can enhance the overall efficiency of work performed. Keeping things clean and organized helps provide a safety. Avoid slipping hazards by cleaning up spilled liquids promptly and keeping the floor free of stirring rods, glass beads, stoppers, and other such items. Never block or even partially block the path to an exit or to safety equipment such as a fire extinguisher or safety shower.

Make sure that supplies and equipment on shelves provide sufficient clearance so that fire sprinkler heads operate correctly. There shall not be any storage within 18 inches of a sprinkler head.

3.5.1 Washing and drying of glassware in the laboratory

Clean glassware is essential in microbiology. The problem is that the tolerance for shmutz varies with the work you are doing, and sometimes a scientist does not know how important clean glassware is to an experiment until it has failed. When cleaning laboratory glassware, wear appropriate gloves that have been checked for tears or holes. Avoid accumulating too many articles in the cleanup area around the sink; space is usually limited, and piling up glassware leads to breakage. Do not clean food containers in a sink that is used for cleaning contaminated glassware.

Many fingers have been badly cut by broken glass from glassware that was intact when put into the sink water. Handle glassware carefully and watch out for broken glass at the bottom of the sink. A rubber or plastic mat in the sink will help minimize breakage.

3.5.2 General Information

The general laboratory rules and regulations must be closely followed in all practical schedules. Students must ensure that they wear their laboratory coat in all practical classes and must be very careful in the handling of specimens containing live micro-organisms and equipment while working in the laboratory. Students should ensure that they familiarize themselves in advance with microbial techniques and analysis to be carried out. Therefore, a hard cover note book/practical file(s) should be used for recording all laboratory records (result of laboratory analysis and text) be it positive or negative. Laboratory records should include the followings:

a. Title of the texts and date

b. Aim of the analysis/texts.

c. Materials and methods

d. Details of the methods used (procedure)

e. The results obtained

4.0 CONCLUSION

With the tips on laboratory safety and housekeeping student should have been equipped with what it takes to work safely and efficiently in a biochemical laboratory.

5.0 SUMMARY

- Laboratory safety means freedom from harm or accident when working in the laboratory
- Laboratory safety rule are guideline design to help keep one safe when experimenting.
- A clean well-maintain work area prevent accident and enhance the overall efficiency of work performed

UNIT 3: MICROSCOPY

CONTENTS

1.0 INTRODUCTION

A microscope is an instrument used to see objects that are too small to be seen with the naked eye. Microscopy is the science of investigating small objects and structures using such an instrument. A microscope is an instrument that makes an enlarged image of a small object, thus revealing details too small to be seen by the unaided eye. The compound binocular microscope is an indispensible piece of apparatus in all medical laboratories, and a theoretical knowledge of its working principle is essential. It is a precision instrument, and its efficient use requires same measure of skill in training. The magnification and clarity of the image depends upon the quality of its lenses, but definition is readily lost if the instrument is improperly used. Microscopes are made in two forms, monocular and binocular. Binocular microscopes are generally used. Microscopes can magnify images making it easier to observe the size, shape and motility of cells and microorganisms.

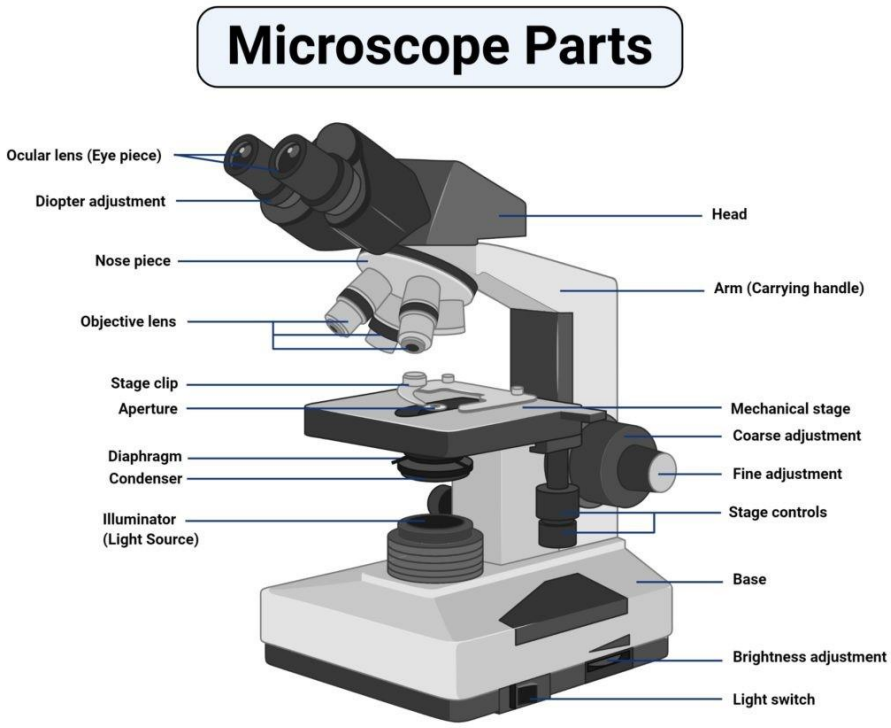


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

1.1 Parts of a Microscope

(a) Head: This is also known as the body, it carries the optical parts in the upper part of the microscope.

(b) Base: The base acts as microscopes support. It carries the microscopes illuminators.

(c) Arms: This is the part connecting the base and to the head and the eye piece tube to the base of the microscope and it also used when carrying the microscope.

(d) Eyepiece: They are also known as the ocular. It is used to look through the microscope. It is found at the top of the microscope. Its standard magnification is 10x with an optical eyepiece having magnifications from 5x-30x.

(e) Eyepiece tubes: It is the eyepiece holder. It carries the eyepiece just above the objective lens.

(f) Objective lens: These are the major lenses used for specimen visualisation. They have magnification power of x40 – x100.

(g) Nose piece: this is also known as the revolving turret. It holds the objective lenses and it is moveable.

(h) Adjustment knobs: They are used to focus the microscope. There are two types of adjustment knobs, the fine adjustment knob and the coarse adjustment knob.

(i) Stage: This is the section on which the specimen is placed for viewing. It has a stage clip that holds the specimen slide in place.

(j) Aperture: This is a hole on the microscopic stage, through which the transmitted light from the source reaches the stage.

(k) Microscopic illuminator: This is the microscopes light source, located at the base. It is used instead of a mirror. It captures light from an external source of a low voltage of about x100.

(l) Condenser: These are lenses that are used to collect and focus light from the illuminator into the specimen. They are found under the stage next to the diaphragm of the microscope. They play a major role in ensuring clear sharp images are produced with a high magnification of x400 and above. The higher the magnification of the condenser, the more the image clarity.

(m) Diaphragm: It is also known as the iris. It is found under the stage of the microscope and its primary role is to control the amount of light that reaches the specimen.

(n) Condenser focus knob: This is the knob that moves the condenser up or down thus controlling the focus of light on the specimen.

(o) Abbe condenser: This is a condenser specially designed on high quality microscopes, which makes the condenser to be moveable and allow a very high magnification of about x400.

(p) The rack stop: it controls how far the stages should go preventing the objective lens from getting too close to the specimen which may damage the specimen. It is responsible for preventing the specimen slide from coming too far up to hit the objective lens.

2.0 OBJECTIVES

At the end of this lesson students should be able to learn about a microscope, how to use the microscope and also know the Do's and Don'ts of microscopy.

3.0 MAIN BODY

3.1USE OF MICROSCOPE

3.1.1 Materials Required

Microscope, slides, cover slide. Specimen, normal saline and oil immersion

3.1.2 Procedures

1. Plug the microscope to the main power source and switch on the microscope.

2. Place a slide with a mounted specimen on the stage of the microscope with the slide containing the specimen facing upwards.

3. Adjust the slide such that the area to be viewed is directly over the hole in the centre of the stage. Locate the specimen using low power objective lens.

4. Under x40 objective and while watching the objective lens and the stage from the slide, lower the body tube or raise the stage by means of the cord adjustment until the objective is very close to but not touching the slide.

5. Continue this exercise until you can clearly see the slide.

6. Then focus with the fine adjustment knob to obtain a sharp image.

7. For optimum light intensity, adjust the condenser and the iris diaphragm.

8. Adjust the slide to position the portion of the specimen to be examined exactly at the centre of the x10 objective field

9. Raised the body tube or lower the stage and slowly rotate the nosepiece until the oil-immersion objective hicks into position.

10. Put a drop of oil immersion on the portion of the slide directly under the objective. This is for stained slides.

11. Carefully lower the objective until it touches the oil, but not the slide, while watching from the slide.

12. Observe through the eyepiece and bring the specimen into focus with the fine adjustment.

13. Make drawing as appropriate

3.2 Do's and Don'ts of microscopy

1. Do cover the microscope when not in use

2. Do remove immersion oil from objective after use by wiping with lens tissue

3. Do clean the optics with lens paper before use

4. Don't rack objective downwards to focus object while looking down microscope

5. Don't attempt to dismantle the object

6. Don't use high powered objective when low power is sufficient

8. Don't lubricate microscope with any oil other than that provided for the purpose

9. Don't place wet preparation on the stage without wiping under surface of the slide

4.0 CONCLUSION

The student should have learnt how to use a microscope and also learn about the Do's and don'ts of microscopy.

5.0 SUMMARY

Microscopy is the use of a microscope to view and study microorganisms. In this study unit, you learn to use a microscope and you also learn the do's and don'ts of microscopy. The microscope can magnify images approximately 1,000x, making it relatively easy to observe the size, shape, and motility of cells and microorganisms.

MODULE 2

UNIT 1: FAECAL SAMPLES

CONTENTS

1.0 INTRODUCTION

Faecal samples are samples gotten from faeces and sent to the laboratory in a suitable container for examination. It is a specimen commonly used for the detection of intestinal parasites.

2.0 OBJECTIVES

Upon completion of studying this unit, the students should be able to;

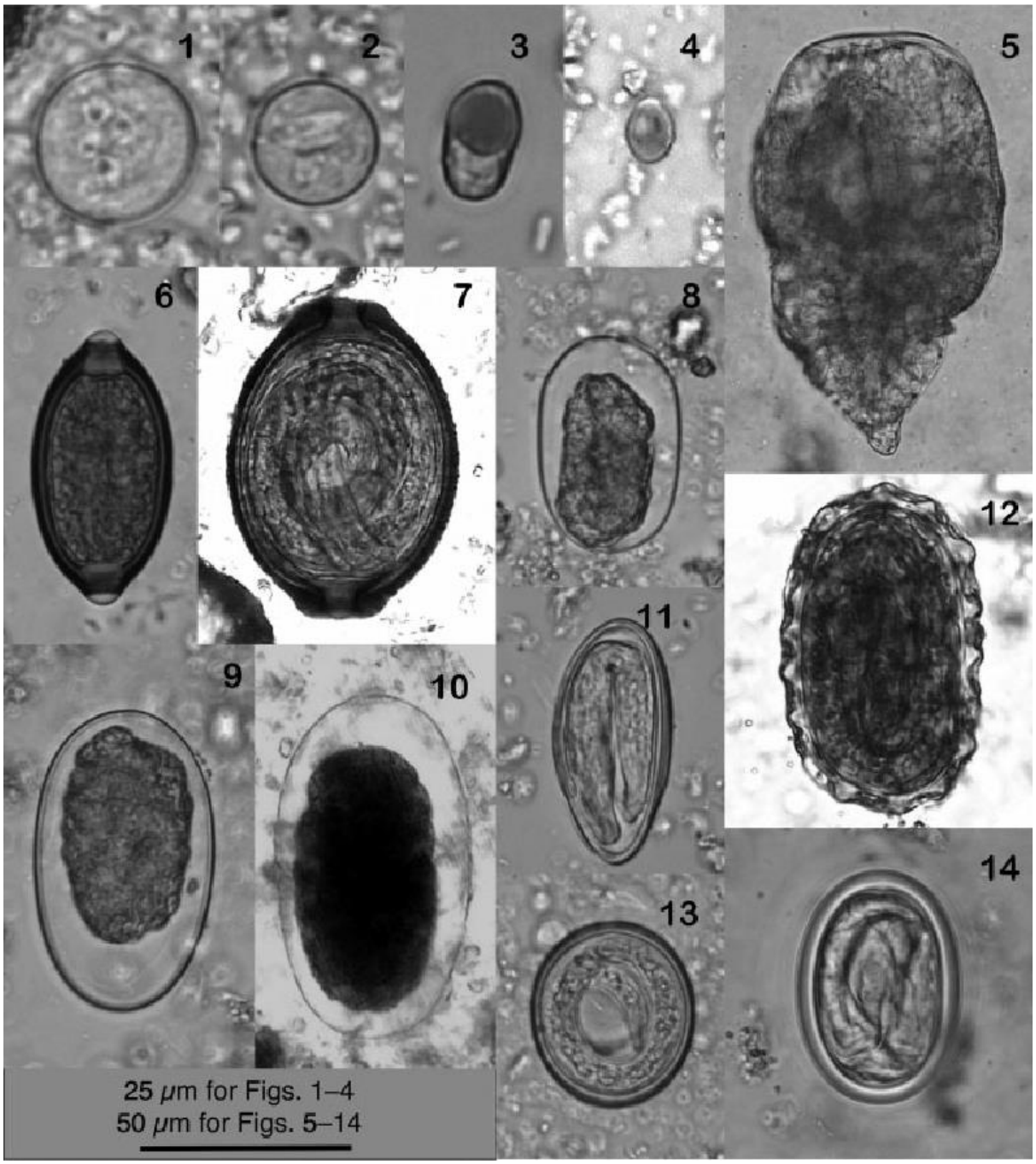
1. Collect faecal samples

2. Number faecal samples
3. Macroscopic and Microscopic examination of faecal samples
4. Technique/Method of preparing slides for microscopic examination

3.0 MAIN BODY

3.1 LABORATORY TECHNIQUES FOR SELECTED INTESTINAL PARASITES

The most commonly used specimen for the detection of intestinal parasites is faeces . Faecal specimens are examined for the presence of trophozoites and cysts of protozoa, eggs or ovae and larvae of helminths as shown below. Whole adult worms or segments of some worms may also be seen. Trophozoites , cysts, eggs or ovae and larvae can be seen only with the microscope , but the adult worms or segments of tapeworms can be seen with the naked eye.. Samples of faeces must be properly collected, processed and examined for the detection of parasites.



Protozoans and helminth eggs found from the feces of humans. 1. Entamoeba coli (cyst). 2. Entamoeba sp. (cyst). 3. Iodamoeba buetschlii (cyst). 4. Chilomastix mesnili (cyst). 5. Troglodytella abrassarti (trophozoite). 6. Trichuris sp. 7. Anatrichosoma sp. 8. Strongyloides fuelleborni 9. Strongylida sp. (small type), 10. Strongylida sp. (large type). 11. Enterobius anthropopitheci 12. Ascarididae gen. sp. 13. Subulura sp. 14. Protospirura muricola .

3.1.1 COLLECTION OF FAECES

A faecal specimen should be collected in a clean, wide-mouthed container with a tightly fitted lid. A waxed cardboard box with an overlapping lid or a plastic cup with a tight fitting lid is best. Tin boxes or glass jars may also be used, but containers such as match boxes are not suitable.

The specimen should be collected directly into the container and should not be contaminated with water that may contain free-living organisms, or with urine which can cause trophozoites to lose their motility or to undergo lysis. Sufficient quantity of the specimen should be obtained for satisfactory examination (about 4-5gm). The container should be labelled with patient's name or number; and date and time of collection.

While handling the faecal specimen, it must be remembered that it can contain potentially infectious micro-organisms. Loose or semi-formed specimens should be examined within 30 minutes to 1 hour of collection. Trophozoites of protozoa cannot survive for longer periods outside the body. The formed specimen must be examined on the same day of the collection. If these time limits cannot be met, a portion of the stool or faeces should be preserved by one of the methods, for example, 10% formal saline. The rest of the specimen should be refrigerated at 3-5^{o}C and stored in closed containers to prevent dessication. At these temperature protozoan cysts, helminthic eggs and larvae can survive for several days. Specimens of faeces should never be incubated or frozen before examination.

3.1.2 NUMBER OF SPECIMENS

The number of specimens required to detect intestinal parasites will depend on the quality of the specimen, the severity of infection and the accuracy of the examination performed. For a routine examination for parasites before treatment, examination of three consecutive specimens is recommended. However, examination of specimens collected on alternate days shows a higher percentage of positive findings. A maximum of six specimens, collected within six to ten days, may be required if intestinal amoebiasis or giardiasis is suspected. Sometimes, it may b e necessary for the patient to take a mild laxative before collecting the sample.

After a patient has received treatment for a parasitic infection, the faeces should be rechecked for absence of parasites. A gap of three to four weeks for protozoan infection, five to six weeks for taenia infection and three to four weeks for other helminths is recommended for rechecking.

Medications containing mineral oil, bismuth, antibiotics, antimalarials or other chemical substances may interfere with the detection of parasites in faeces. Therefore, examination of faeces must be postponed at least by one week after such therapeutic procedures have been completed.

3.1.3 VISUAL OR MACROSCOPIC EXAMINATION OF FAECES

Before processing the specimen of faeces, it should be visually examined. Its colour, consistency and presence of blood, pus, mucus or parasites should be reported.

COLOUR: Normally, stool or faeces is brown in colour. Variation from this colour may occur under certain conditions. Reddish colour may be due to bleeding from the lower gastro-intestinal tract. Consumption of beet-root may also give a red colour to the stool. Black –tarry colour may be due to bleeding from the upper gastro-intestinal tract or due to consumption of iron.

Clay-coloured stool is seen in obstructive jaundice or after barium sulphate meal.

Green stool may result from the consumption of leafy vegetables such as spinach or sometimes due to oral antibiotic therapy.

CONSISTENCY: A normal stool specimen is formed or semi-formed in consistency. Loose or watery stool may be seen in diarrhoea. Trophozoites of intestinal protozoa are usually seen in loose or watery specimens while cysts are found in formed and semi-formed ones. Eggs of helminths may be found in any consistency. Large amounts of mushy, foul smelling, frothy specimens are seen in giardiasis and other conditions associated with malabsorption.

BLOOD: The presence of blood in or on the specimen must always be reported. Fresh, bright red blood is often from the lower gastro-intestinal tract whereas dark red colour may indicate bleeding from the upper gastro-intestinal tract. When very small amounts of blood are being passed in faeces, it may be said to contain occult (hidden) blood. Some of the causes for the presence of occult blood include iron deficiency anaemia, peptic ulcer or cancer of the gastro-intestinal tract.

3.1.4 MICROSCOPIC EXAMINATION (WET MOUNTS)

The easiest and the simplest technique for the direct microscopic examination of faeces is wet mount. Wet mounts can be prepared directly from the faecal material in saline and iodine. Other useful wet mounts are buffered methylene blue (BMB) and eosin. The saline wet mount is used for the preliminary microscopic examination of faeces to detect protozoan trophozoites and cysts; helminthic larvae and eggs or ovae. A characteristic motility of a parasite can be used for its identification. Excessive cellular exudate in the faeces in the form of pus (white blood cells) or blood (red blood cells), macrophsges or any other significant material such as charcot-leyden crystals can also be detected in wet mounts.

In the iodine wet mount, most cysts can usually be specifically identified because the iodine stains their nuclei and glycogen, if present. However, the parasites are not motile in iodine.

3.2 PREPARATION OF WET MOUNTS

REAGENTS:

(i) Saline, physiological (0.85%)

(ii) Lugol's iodine

Stock (5%) solution

Iodine	5g
Potassium iodide	10g
Distilled water	100ml

Dissolve the potassium iodide in about 30ml of water and add the iodine. Mix until dissolved and make up the volume with distilled water store in a brown bottle.

WORKING IODINE SOLUTION (1%)

Lugol's iodine, stock (5%) solution	5ml
Saline (0.85%)	20ml

Prepare fresh every two weeks

(iii) Buffered methylene blue (BMB) solution

Solution A

Glacial acetic acid	1.2ml
Distilled water	98.8ml

Solution B

Sodium acetate (CH_3COON_a)	1.6g
Or Sodium acetate, crystalline ($CH_3COON_a.3H_2O$)	2.6g
Distilled water	100ml

WORKING SOLUTION

Solution A	46.3ml
Solution B	3.7ml
Methylene blue powder	0.5g
Distilled water	50ml

3.2.1 TECHNIQUE/METHOD:

(i) Place a drop of saline in the centre of the left of the slide and place a drop of iodine solution in the centre of the right half of the slide.

(ii) With an applicator stick, pick up a small portion of faeces from an appropriate site and mix it with saline on the slide to form a uniform suspension. In the same way, prepare a suspension of faeces in the iodine on the slide.

(iii) Cover the drop of saline suspension with a cover slip by holding it at an angle and lowering it gently on to the slide to reduce formation of air bubbles. Cover the

iodine suspension with another cover slip, taking care not to mix with the saline suspension.

EXAMINATION

a. Focus on the wet mount using a lower power (x10) objective.
b. Regulate the light with the sub stage condenser, the diaphragm and the light source. Since most of the parasites are pale or colourless, too much or too little light may not be useful. Lowering the condenser and closing the diaphragm partially can give adequate light.
c. Examine the entire cover slip in a systemic order. Focus the objective on the top left hand corner and move slide slowly up and down or backwards and forwards. When any parasite or suspicious material is observed, change to the high-dry objective (x40). Increase the light by opening the sub stage diaphragm to observe the detailed morphology. Examine each microscope field carefully, focussing up and down, before moving to the next field.

3.3 SUPPLEMENTARY TECHNIQUES/METHODS

Other techniques have been developed to detect and identify intestinal parasites in addition to direct wet mounts. The two most commonly used techniques are:

a. Concentration methods for recovering cysts, eggs and larvae
b. Permanent staining techniques for identifying trophozoites and cysts of protozoa.

3.3.1 CONCENTRATION METHODS

It may be necessary to use concentration methods for the detection of faecal parasites for the following reasons:

(i) The parasites are not detected in the direct microscopic examination but the symptoms of intestinal parasitic infection still persist.
(ii) The eggs of parasites such as Taenia species or Schistosoma species are usually few in number, and therefore, may have been missed out in the direct wet mount.
(iii) After treatment, it is necessary to check if it has been effective
(iv) To investigate the prevalence and incidence of parasitic infection for epidemiological purposes.

There are two types of concentration methods, floatation techniques and sedimentation techniques. These methods employ the use of specific gravity to separate most of the faecal debris from the parasites.

NOTE: A floatation technique uses a liquid with high specific gravity for the separation of protozoan cysts and certain helminthic eggs and larvae from the faecal debris. The parasites remain in the surface film while the debris remains at the bottom. However, some helminth eggs, eg, operculated eggs or very dense eggs such as unfertilized eggs of Ascaris, may not float.

NOTE: This manual will only describe **Saturated Sodium chloride floatation technique /method.**

3.3.2 Saturated Sodium chloride floatation technique /method

REAGENT:

Saturated sodium of sodium chloride: Prepare a saturated solution of sodium chloride (NaCl) till some salt remains undissolved at the bottom of the container. This solution should have a specific gravity 1.2 .

METHOD/TECHNIQUE

(i) Emulsify about 0.5g of faeces in 2 to 3ml of the saturated NaCl in a test tube having about 15mm internal diameter
(ii) Fill the tube with the NaCl solution. Mix well. Stand the tube in a vertical position in a rack on a flat surface.
(iii) Add more NaCl solution slowly, to fill the tube completely up to the brim.
(iv) Carefully place a clean cover slip on top of the tube so that it's under surface touches the solution.
(v) Leave undisturbed for 30-45 minutes for the cysts and eggs to float through the solution.
(vi) Carefully lift the cover slip, where cysts and eggs will be attached, by a straight pull upwards and place it on a slide face downwards. Examine microscopically as for the wet mounts for the eggs.

4.0 CONCLUSION

At the end of this study the students should have learnt how faecal samples are collected, numbered, macroscopic and microscopic examination, preparation of wet mounts and some other techniques.

5.0 SUMMARY

Faecal samples are samples used for the examination and detection of intestinal parasites. When specimen are collected and numbered, visual or macroscopic examination takes place first to observe the colour, consistency and also if there is presence of blood in the faecal sample. A microscopic examination is then carried out using wet mount technique to detect protozoan trophozoites, cysts and helminth larvae and egg or ova.

UNIT 2: URINE SAMPLE FOR Schistosoma haematobium

CONTENTS

1.0 INTRODUCTION

Urine is water containing the water-soluble waste products removed from the blood stream via the kidneys. Normal urine consists of approximately 95% water, the remainder being made up of urea, uric acid, sodium, potassium, chloride, calcium, phosphate. Microscopic examination of urine may yield useful information in many abnormal conditions. But for the purpose of this manual, only the detection of schistosoma will be discussed.

2.0 OBJECTIVES

The main objective of this study is the detection of *schistosoma haematobium* in urine and the sedimentation technique.

3.0 MAIN BODY

3.1 URINE SAMPLE FOR Schistosoma haematobium

Parasites which can be detected in Urine are:

(i) Eggs of Schistosoma haematobium
(ii) Microflariae of Wuchereria bancrofti
(iii) Trophozoites of Trichomonas vaginalis

If present in sufficient numbers, these parasites can be detected while examining centrifuged deposits of urine. In areas where schistosomiasis is endemic, the first indication of infection is haematuria which can be detected either by chemical test or microscopically. A heavy infection with Schistosoma may lead to gross haematuria which is seen visually. A milky urine may show microfilariae of Wuchereria bancrofti.

NOTE: FOR THE PURPOSE OF THIS MANUAL, detection of Schistosoma haematobium will be discussed

3.2 DETECTION OF Schistosoma

3.2.1 COLLECTION OF URINE: A special care is needed for the collection of urine for suspected schistosomiasis because the number of ova excreted in urine varies throughout the day. It is highest in the terminal portion of the urine between 10.00am to 2.00pm. The last few drops of urine contain the maximum number of eggs. Therefore, the specimen should be collected between these times and should be terminal urine at least 10ml in volume. Alternatively, a 24 hour specimen may be collected with formalin (1 ml per 100ml of urine) as a preservative for the eggs. If it is not possible to examine the fresh specimen within one hour of collection, this too, should be preserved with formalin using the same proportion. It is advisable to examine large volume of urine because the ova are very scanty and can be easily missed. If not preserved with formalin, the eggs may hatch to release miracidia.

3.2.2 EXAMINATION OF URINE FOR THE EGGS OF Schistosoma haematobium

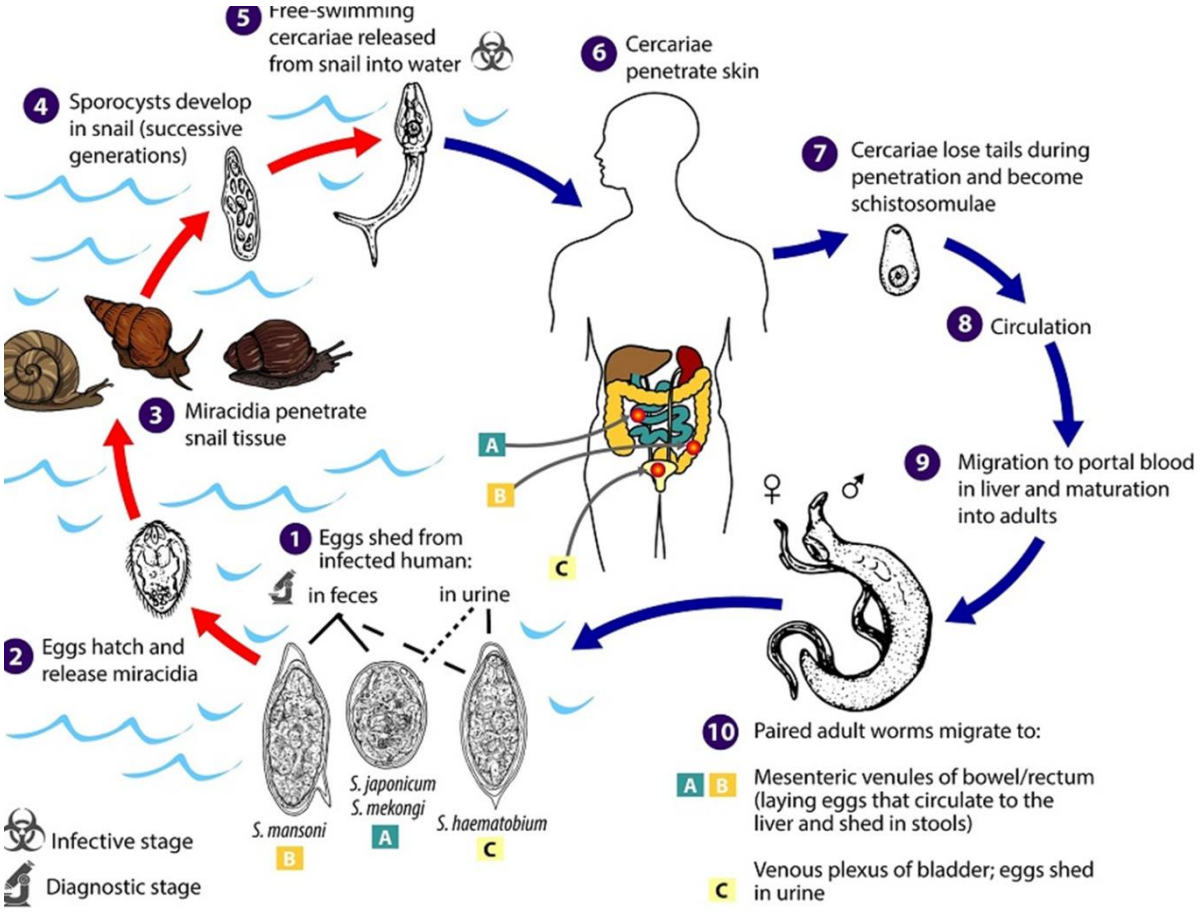
There are two methods in use for the detection of Schistosoma eggs in Urine.

(i) The sedimentation technique is less sensitive, but cheaper and simpler to perform.
(ii) The filtration technique is used when quanttitive information is needed, eg in a public health laboratory.

NOTE: ONLY SEDIMENTATION TECHNIQUE IS DISCUSSED IN THIS MANUAL

3.3 SEDIMENTATION TECHNIQUE/ METHOD

(i) Collect a 24 hour or terminal urine sample as explained above, and check for haematuria.
(ii) Shake the urine well and allow it to sediment for 1 hour.
(iii) At the end of 1 hour, carefully withdraw the supernatant without disturbing the sediment.
(iv) Transfer the sediment to a centrifuge tube and centrifuge at 500g for 5 minutes
(v) Discard the supernatant and examine the sediment as a wet preparation, using the 10x objective to search for the ova of Schistosoma haematobium.



Schistosoma eggs are eliminated with feces or urine, depending on species 1. Under appropriate conditions the eggs hatch and release miracidia 2, which swim and penetrate specific snail intermediate hosts 3.

4.0 CONCLUSION

At the end of this study the students should have learnt about the constituents of urine, how urine samples are collected and how urine samples are examined microscopically for detection of *schistosoma haematobium*

5.0 SUMMARY

In this unit the study was restricted to the microscopic examination of urine to detect *schistosoma haematobium*

UNIT 3: BLOOD SAMPLE FOR PLASMODIUM SPECIE DETECTION

CONTENTS

1.0 INTRODUCTION

Human malaria caused by protozoa of the genus Plasmodium. Four species are involved namely *plasmodium vivax, plasmodium falciparum, plasmodium malariae*, and *plasmodium ovale.* In human host, the infectious form injected by the mosquito, are carried by the blood stream to the liver, where they infect liver cells. In the cells, the parasites enlarges and subdivides, producing thousands of merozoites which are released into the blood stream.

2.0 OBJECTIVES

The objective of this study is about blood sample collection, preparation of blood film and methods of staining.

3.0 MAIN BODY

3.1 BLOOD SAMPLE FOR PLASMODIUM SPECIE DETECTION

The parasites which can be detected in blood are:

(i) Plasmodia
(ii) Trypanosomes
(iii) Leishmania
(iv) Filarial worms

Some parasites such as microfilariae and trypanosomes can be detected in the direct wet mount of fresh blood by their characteristic shape and motility. However, specific identification of the parasite requires a permanent stain. For permanent staining, two types of blood films can be prepared. Thick films allow a larger volume of blood to be examined, thus making it easier to detect light infections with fewer parasites, while species identification is difficult. Thin films are necessary to see the morphological characteristics of the parasites and to identify them.

3.2 COLLECTION OF SPECIMENS FOR BLOOD FILMS:

3.2.1 CAUTION: Careful attention to safety technique is necessary at the time of collection of blood samples and preparation of blood films. A number of parasitological, bacterial and viral diseases can be transmitted through blood.

Blood films should be prepared before the commencement of any treatment. It is preferable to prepare blood films with fresh blood without anti-coagulant. If it is not possible, blood anti-coagulanted with EDTA (10mg/5ml blood) should be used; and blood films should be prepared as soon as possible, preferably, within one hour of collection.

3.3 PREPARATION OF BLOOD FILMS

For accurate examination of blood films, it is necessary to use absolutely clean, grease-free slides. Well washed slides cleaned with 70% alcohol are recommended.

3.3.1 THICK BLOOD FILMS

To make a thick film, place two or three small drops of fresh blood without anticoagulant on a clean slide. With a corner of another slide, mix the drops in a circular motion over an area about two cm in diameter. Continue mixing for about 30 seconds to prevent formation of fibrin strands that may obscure the parasites after staining. Allow the film to dry in air at room temperature. Before staining, the thick films are laked to lyse the red blood cells and to remove haemoglobin so that the parasites can be easily detected. To lake the films, they are either placed in buffer solution before staining or placed directly into an aqueous stain like Giemsa stain.

3.3.2 THIN BLOOD FILMS

In thin films, the number of parasites is much less than in the thick films, but it permits specific identification of parasites.

The thin film is prepared in exactly the same way as the one used for a peripheral blood smear examination. Allow the thin blood film to air-dry. The need for fixation before staining depends on the type of stain used.

3.4 STAINING BLOOD FILMS

Blood films should be stained as soon as possible as delay may result in stain retention.

Romanowsky stains such as Giemsa, Leishman or Field, can be used for staining of parasites in blood film. Leishman's stain has the fixative combined with the staining solution, so that fixation and staining both occur at the same time. Therefore, the thick film must be laked before staining by Leishman stain. In Giemsa and Field stains, on the other hand, the fixative and the stain are separate. Thus the thin film must be fixed in methanol before staining.

After staining, the smears should be air-dried.

3.5 GIEMSA STAINING TECHNIQUE/METHOD

Giemsa stain is a Romanowsky stain that requires dilution in buffered water or buffered saline before use. The stain is available commercially either as a concentrated stock solution or in a powdered form.

3.5.1 GIEMSA STAIN (stock solution)

Giemsa stain powder	0.6g
Methanol, absolute (acetone-free)	50ml
Glycerol	50ml

3.5.2 GIEMSA STAIN (working solution)

The stock should be diluted 1:10 with buffer for thin films and 1:50 for thick films. Phosphate buffer used for the dilution of the stain should be neutral or slightly alkaline

(pH7.0 to 7.2). If the pH of the Tap water in the laboratory is satisfactory, it may be used for the entire staining procedure, evening for the final rinsing.

3.6 TECHNIQUE /METHOD FOR THIN FILMS

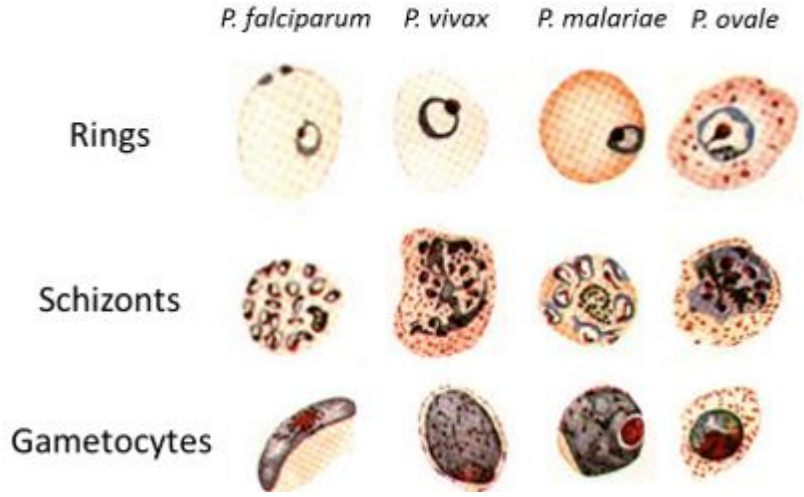
(i) Fix the thin blood film in absolute methanol for 1 minute. If both the thick and the thin films are on the same slide, carefully dip only the thin smear in methanol. Allow the smear to dry in air.
(ii) Prepare an appropriate dilution of the stock Giemsa stain using the phosphate buffer or tap water.
(iii) At the end of the staining period, gently flush the stain off the slide with water. Do not tip off the stain before washing, as this will leave stain deposits over the smear.
(iv) Dip the slide briefly in the buffer or rinse under gently running tap water.
(v) Wipe the under-surface of the slide to remove excess stain
(vi) Allow it to air-dry in a vertical position.

3.7 TECHNIQUE/METHOD FOR THICK FILM

The procedure to be followed for thick films is the same as that for thin films except that the thick film should not be fixed in methanol, but directly stained in diluted Giemsa.

3.8 RESULTS: Malarial parasites have dark red chromatins, blue cytoplasm, red Schuffner's dots and red-mauve Maurer's dots.

Differentiation of Malaria Parasites



4.0 CONCLUSION

At the end of this study the students should have learnt how to prepare thick and thin blood films. They will also learn the techniques/methods of staining films.

5.0 SUMMARY

This unit was restricted to plasmodium specie, collection of specimen, preparation of thick and thin film blood film, and also staining techniques for blood films.

UNIT 4: BACTERIAL EXAMINATION OF WATER

CONTENTS

1.0 INTRODUCTION

Large cities generally obtain their drinking water from surface water such as lakes or rivers. The quality of the surface water is also affected by the characteristics of the watershed, the land over which water flows into the river or lake. Rivers are also contaminated with faeces of animals and humans that inhabit the watershed. Water from various sources seeps through the soil and can lead to groundwater contamination.

2.0 OBJECTIVE

The objective of this study is to detect contamination of water with pathogenic bacteria.

3.0 MAIN BODY

3.1 BACTERIAL EXAMINATION OF WATER

Drinking water must be free from harmful microorganisms that can cause serious ill health. Supplies of drinking water may be contaminated with sewage being allowed to seep into wells or bore-holes, or faecal matter from man and animals being passed into rivers, streams or pools. Such contaminations may cause diseases like typhoid fever, cholera, bacterial dysentery, amoebiasis and helminthiasis.

The aim of bacteriological examination of water is to detect whether contamination with pathogenic bacteria has occurred or not. It is impracticable to directly attempt detection of the presence of numerous water- borne pathogens, some of which may only be present intermittently. The bacteriologist, instead, test for the presence of indicator bacteria which are the common intestinal commensals such as Coliforms, particularly Escherichia coli; Streptococcus faecalis (Enterococcus faecalis), and Clostridium perfringens. These organisms are excreted in large numbers by man and animals. Their presence in water indicates that:

(i) Faecal matter has entered the water supply
(ii) The faecal organisms have not been killed or removed during purification and treatment.
(iii) There is the possibility of contamination with intestinal pathogens..

Bacteriological examination of water is generally performed by the following tests:

(i) Presumptive coliform count
(ii) Tests for faecal streptococci and Clostridium perfringens.
(iii) Plate count.

3.2 COLLECTION OF WATER SAMPLES

Water samples for bacteriological examination should be collected in sterile containers. Accidental contamination during collection and transportation to the laboratory should be avoided. Methods of collecting water samples vary according to the source of the water. It is recommended to use sterilised glass bottles with ground glass stoppers or screw-caps for sampling.

The following precautions should be observed for collection of water samples;

(i) The sample should be an adequate representation of the water to be examined
(ii) To avoid contamination during collection of samples, flame the mouths of taps and hydrants and allow water to run for 3-5 minutes to waste before being collected into the bottle. When collecting samples from rivers or lakes, open the bottle and push the neck downwards about 30cm below the water surface with its mouth facing the current and ensure that water entering the bottle has not been in contact with the hand.

3.3 PRESUMPTIVE COLIFORM COUNT

There are two methods used to detect and count bacteria in water:

(i) Multiple tube method
(ii) Membrane filtration method

3.3.1 MULTIPLE TUBE METHOD FOR FAECAL COLIFORMS

Principle: measured volumes of neat and diluted water are added to a series of tubes containing a liquid indicator growth medium. A characteristic colour change in any tube indicates the presence of indicator bacteria in the sample. The most probable number (MPN) of indicator organisms in the sample depends on the number and distribution of positive and negative reactions.

3.3.2 REQUIRED

(i) Sample of water
(ii) Sterile test tubes
(iii) Ringer solution
(iv) 1ml, 10ml and 50ml pipettes
(v) MacConkey broth containing bromocresol purple indicator (double strength), with inverted Durham tube
(vi) MacConkey broth containing bromocresol purple indicator (single strength), with inverted Durham tube.

3.3.3 METHOD

(i) Mix the sample of water thoroughly by inverting the bottle 25-30 times.
(ii) Aseptically inoculate the tubes as follows:
1 x 50ml of broth +50ml of water
5 x 10ml of broth + 10ml of water
5 x 5ml of broth + 1 ml of water (in case of untreated water)
(iii) Mix the contents of each tube
(iv) Incubate all the tubes at 37^{o}C for 18-24 hours with tubes loosely capped.
(v) All tubes showing acid (colour change to yellow) and gas(bubble in the Durham's tube) are regarded as presumptive positive. Reincubate negatives for a further 24 hours.

4.0CONCLUSION

At the end of this study the students should have learnt how water is being contaminated, how to collect water samples and multiple tube method of faecal examination of coliforms.

5.0 SUMMARY

This unit talked about water contamination, bacterial examination of water, collection of water sample and the method for multiple tube examination of faecal coliforms.