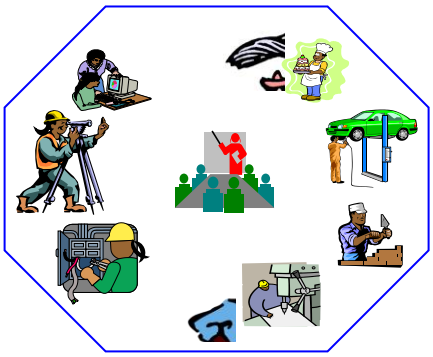

Animal Health Care Service

Level –III

Based on March 2018, Version 3

Occupational Standard

Module Title: - Applying Laboratory Techniques and Procedures

LG Code: AGR AHC3 M23 LO (1-4) LG (89-92)

TTLM Code: AGR AHC3 TTLM 0321V1

Adama, Ethiopia

June 2021

THE WORLD BANK
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East Africa Skills for Transformation and Regional Integration Project (EASTRIP)

Table of Contents

LG #89	LO #1- Follow OHS practices

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Maintaining personal hygiene and cleanliness standards.
- Applying appropriate safety precautions equipment and hazardous chemical materials.
- Using appropriate laboratory glassware and measuring equipment.
- Handling the specimens in a manner that minimize the spread of pathogens to animals and humans
- Following workplace procedures and work instructions for controlling risks accurately.

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Maintain personal hygiene and cleanliness standards of laboratory procedures.
- Apply appropriate safety precautions for use of laboratory equipment and hazardous chemical materials.
- Use appropriate laboratory glassware and measuring equipment.
- Handle the specimens in a manner that minimize the spread of pathogens to animals and humans
- Follow the workplace procedures and work instructions for controlling risks accurately.

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).

Information Sheet 1- Maintaining personal hygiene and cleanliness standards

1.1 Sanitation, personal hygiene and cleanliness

Sanitation is the act or process of making sanitary or the promotion of hygiene and prevention of disease by maintenance of sanitary condition as (by removal of sewage and trash) often used attributively a sanitation equipment's and sanitation worker. Sanitation is important for all, helping to maintain health and increase life-spans. Hygiene refers to behaviors that can improve cleanliness and lead to good health or is a series of practices performed to preserve health or conditions and practices that help to maintain health and prevent the spread of diseases. Personal hygiene refers to maintaining the body's cleanliness. Cleanliness is both the abstract state of being clean and free from germs, dirt, trash, or waste, and the habit of achieving and maintaining that state. Many people equate hygiene with 'cleanliness,' but hygiene is a broad term. It includes such personal habit choices as how frequently to take a shower or bath, wash hands, trim fingernails, and wash clothes. It also includes attention to keeping surfaces in the home and workplace, including bathroom facilities, clean and pathogen-free (Alp *et al.*, 2006).

Objective of sanitation

- Reducing the number of people practicing open waste disposed container (e. g. Do not open defecation area, tools that contain pus, blood, necrotic organ etc.).
- Increasing the number of people with access to basic sanitation facilities.
- Improving the affordability and availability of sanitation products and services.

- Increasing the amount of specimens waste effectively captured and treated on-site, or collected, transported, and treated offsite.
- Improving the ability of educational and health systems to manage sanitation and hygiene facilities in the laboratory institutions.
- Increasing the number of people with safely managed sanitation services.
- Increasing washing laboratory equipment with detergent (soap, Ajaksi, Omo, Barkina) at critical times.
- Increasing the rate of safe management of household drinking water and nutrition.

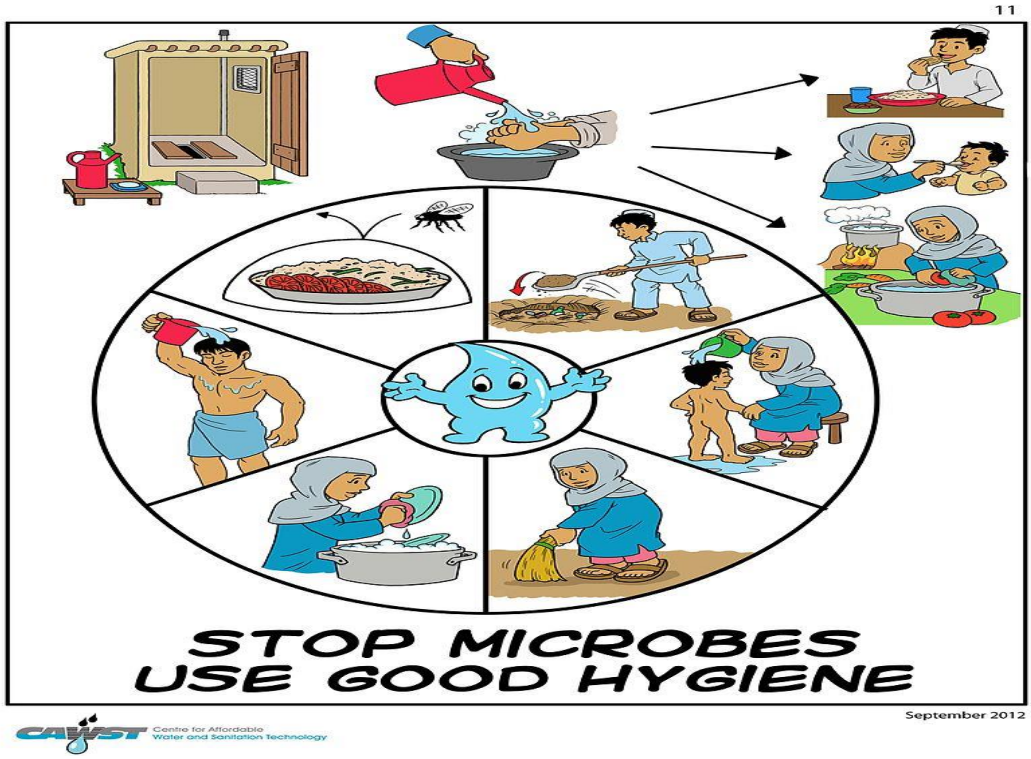


Figure 1: Hygiene that explanied by sanitation

Cleanliness is both the abstract state of being clean and free from germs, dirt, trash, or waste, and the habit of achieving and maintaining that state. Cleanliness is

often achieved through cleaning. Personal hygiene in the laboratory is directed mainly toward the prevention of occupational acquired disease or physical injury (Brown *et al.*, 2008). The following guidelines are standard operating procedures for personal hygiene and cleanliness:-

- Smoking, eating, drinking, storing food, beverages or tobacco, applying cosmetics or lip balm and handling contact lenses are not permitted in laboratories/ animal rooms.
- Food for human consumption should be stored only in refrigerators specifically designated for that purpose but don't store in laboratory refrigerators.
- Don't drink from tape water found in laboratory.
- Do not use laboratory equipment for food preparation and empty food containers for laboratory materials or samples.
- Shaving or brushing teeth is not permitted in laboratories.
- Shave beards to prevent airborne contamination and for adequate fit of a face or respirator mask.
- While working, Keep hands away from the mouth, nose, eyes, face and hair.
- Books and journals should be used only in clean areas if possible.
- Don't use personal handkerchiefs but disposable tissues should be available in .laboratory.

Figure 2: cleaning techniques to kept both animal and human sanitation.

Self-check 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Choose the best answer (4 points each)

1. _____ is the act or process of making sanitary or the promotion of hygiene and prevention of disease by maintenance of sanitary conditions.

   A. Hygiene B. Cleanliness C. Safety

2. _____ is both the abstract state of being clean and free from germs, dirt, trash, or waste, and the habit of achieving and maintaining that state.

   A/ Hygiene B. Cleanliness C. Safety

Test II: Short Answer Questions

1. Why personal hygiene maintenance in laboratory is important? (1 point)
2. Mention some of the standard guidelines that should be followed by laboratory workers to maintain their personal hygiene and cleanliness (3points).

You can ask your teacher for the copy of the correct answers.

Note: **Satisfactory rating - 4 points** **Unsatisfactory - below 4 points**

Answer Sheet

Score = _____

Rating: _____

Information Sheet - 2: Applying appropriate safety precautions for use of laboratory equipment and hazardous chemical material

2.1 Safety equipment and hazardous chemical material

Safety is the state of being "safe", the condition of being protected from harm or other non-desirable outcomes. Safety can also refer to the control of recognized hazards in order to achieve an acceptable level of risk. Working safely with hazardous chemicals requires proper use of laboratory equipment.

First of all, pay attention to what you are doing and what you are working with. Always wear safety equipment when working with dangerous chemicals. Recognize any accidents immediately. Keep food and drinks out of the laboratory work area. Always read labels of chemicals carefully. Never do unauthorized experiments. Never work alone in laboratory. Keep your lab space clean and organized. Do not leave an on-going experiment unattended. Always inform your instructor if you break a thermometer. Do not clean mercury yourself!!Never taste anything. Never pipette by mouth; use a bulb. Never use open flames in laboratory unless instructed by lab rule. Check your glassware for cracks and chips each time you use it. Cracks could cause the glassware to fail during use and cause serious injury to you or lab mates. Maintain unobstructed access to all exits, fire extinguishers, electrical panels, emergency showers, and eye washes. Do not use corridors for storage or work areas. Do not store heavy items above table height. Any overhead storage of supplies on top of cabinets should be limited 0to lightweight items only. Also, remember that a 36’diameter area around all fire sprinkler heads must be kept clear at all times. Areas containing lasers, biohazards, radioisotopes, and carcinogens should be posted accordingly. However, do not post areas unnecessarily and be sure that the labels are removed when the hazards are no longer present. Be careful when lifting heavy objects. Only shop staff may operate forklifts or cranes.

Clean your lab bench and equipment, and lock the door before you leave the laboratory. Never mix two unknown chemicals. Wipe spill of chemicals immediately. In case if contact with chemical, rinse it off immediately with lots of water. Never taste, smell or touch anything chemical. Coats, backpacks, etc., should not be left on the lab benches and stools. Always wash your hands before leaving lab. Learn where the safety and firstaid equipment is located (fire extinguishers, fire blankets, and eye-wash stations) Consider all chemicals as if hazardous. Only liquid be put in the lab sinks. Always pour acids into water. Never leave burners unattended. Label all materials clearly. Never pipette anything by mouth. Clean up your work area before leaving. Avoid working alone. Do not use flammable liquids near open flames (M+¬nard and Trant, 2020; Van Noorden, 2013).

- Treat every chemical as if it were hazardous. Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
- Never return chemicals to reagent bottles. Try for the correct amount and share any excess. Comply with fire regulations concerning storage quantities, types of approved containers and cabinets, proper labeling, etc. If uncertain about regulations, contact the building coordinator. Use volatile and flammable compounds only in a fume hood. Procedures that produce aerosols should be performed in a hood to prevent inhalation of hazardous material. Never allow a solvent to come in contact with your skin. Always use gloves. Never "smell" a solvent!! Read the label on the solvent bottle to identify its contents. Dispose of waste and broken glassware in proper containers. Clean up spills immediately. Do not store food in laboratories.
- Do not use any equipment unless you are trained and approved as a user by your supervisor. Never eat, drink, or smoke while working in the laboratory.

Shorts and sandals should not be worn in the lab at any time. If you have long hair or loose clothes, make sure it is tied back or confined.

- Turn off all ignition sources and lock the doors when leaving. Properly dispose wastes and used material in appropriate containers. Never return chemicals to reagent bottles.

1.2 Personal hygiene and General laboratory safety

Never eat, drink, or smoke while working in the laboratory. Read labels carefully. Do not use any equipment unless you are trained and approved as a user by your supervisor. Wear safety glasses or face shields when working with hazardous materials and/or equipment. Wear gloves when using any hazardous or toxic agent. Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should not be worn in the lab at any time. Shoes are required when working in the machine shops. If you have long hair or loose clothes, make sure it is tied back or confined. Keep the work area clear of all materials except those needed for your work. Coats should be hung in the hall or placed in a locker. Extra books, purses, etc. should be kept away from equipment, which requires air flow or ventilation to prevent overheating. Disposal - Students are responsible for the proper disposal of used material if any in appropriate containers. Equipment Failure - If a piece of equipment fails while being used, report it immediately to your lab assistant or tutor. Never try to fix the problem yourself because you could harm yourself and others. If leaving a lab unattended, turn off all ignition sources and lock the doors. Never pipette anything by mouth. Clean up your work area before leaving. Wash hands before leaving the lab and before eating (Baum *et al.*, 2004; Kapur and Zhang, 1995).

Figure 3: Safety for all thing icluding used equipment and you your self

2.2 Laboratory Safety Rules

- All materials and clothes other than those needed for the laboratory are to be kept away from the work area.
- A lab coat or other protective clothing must be worn during lab. The lab clothing is not to be worn outside of the laboratory.
- Clean the lab table before and after lab with the disinfectant solution provided
- Wash hands before leaving lab, even take all showers even done zoonotic hazard techniques is applied.
- Any item contaminated with bacteria or body fluids must be disposed of properly. Disposable items are to be placed in the BIOHAZARD container. Reusable items are to be placed in the designated area for autoclaving prior to cleaning. Sharps are to be disposed of in the appropriate container.
- Reusable items should have all tape and marks removed by the student before being autoclaved.
- Because organisms used in this class are potentially pathogenic, aseptic technique must be observed at all times. NO eating, drinking, application of cosmetics or smoking is allowed. Mouth pipetting is not allowed.
- Cuts and scratches must be covered with Band-Aids. Disposable gloves will be provided on request.

- Long hair should be tied back while in lab.
- All accidents, cuts, and any damaged glassware or equipment should be reported to the lab instructor immediately.
- Sterilization techniques will involve the use of Bacticinerators that are fire and burn hazards. Bacticinerators reach an internal temperature of 850^{o} C or 1500^{o} F. Keep all combustibles away from the Bacticinerators. Do not leave inoculating loops or needles propped in the Bacticinerator.
- Microscopes and other instruments are to be cared for as directed by the instructor.
- It is the responsibility of the student to know the location and use of all safety equipment in the lab (eyewash, fire extinguisher, etc.)
- Cultures may not be removed from the lab. Visitors are not allowed in the lab.
- Doors and windows are to be kept closed at all times.
- For the best lab experience, read labs before coming to class. Make notes as necessary. Wait for a laboratory introduction by the instructor before starting work.

I have read and understand the above rules and agree to follow them.

Signed____ Date____ Name (Please print) _____

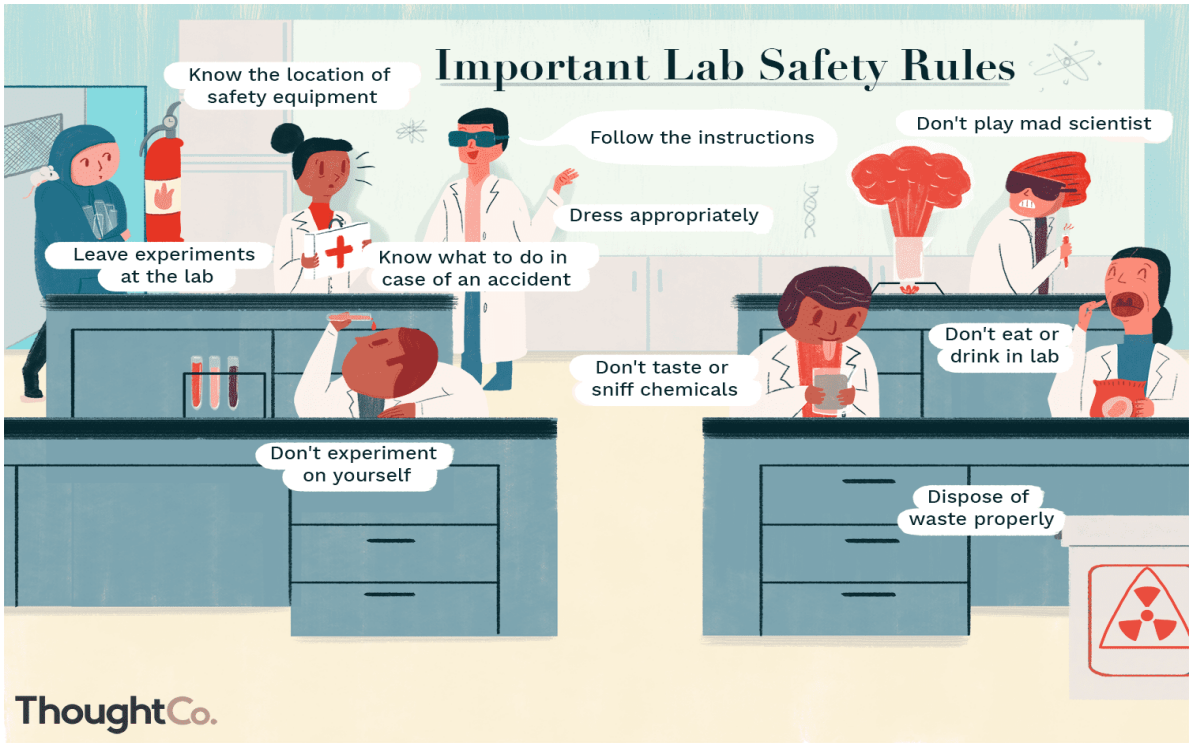


Figure 3: Safety Rules to maintain personal hygiene and cleanliness.

2.3 Cleaning of splashes of chemicals

A chemical spill is considered to be minor only if:

- The person who spilled it is familiar with the chemical
- Knows the associated hazards
- Knows how to clean up the spill safely

The recommended steps for dealing with a minor spill include:

- Alert coworkers, then clean up spill.
- Follow procedures for disposal of materials used to clean up spill
- Absorb free liquids with an appropriate absorbent, as follows

- ✓ Caustic liquids—use polypropylene pads or diatomaceous earth
- ✓ Oxidizing acids—use diatomaceous earth
- ✓ Mineral acids—use baking soda or polypropylene pads
- ✓ Flammable liquids—use polypropylene pads
- Neutralize residues and decontaminate the area.

Anything beyond a minor spill and that requires help from outside of the laboratory group constitutes a major spill. Steps to deal with major spills include:

- Alerting coworkers
- Moving to a safe location and
- Calling authorities to report the situation

Self-Check – 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short answer

1. Define hygiene and cleanliness? (3pts)
2. Write the method cleaning of equipment? (4pts)
3. How we can keep our laboratory health? (2pts).

Note: **Satisfactory rating - 5 points** **Unsatisfactory - below 4 points**

You can ask your teacher for the copy of the correct answers

Answer sheet **Score = _____**

Rating: _____

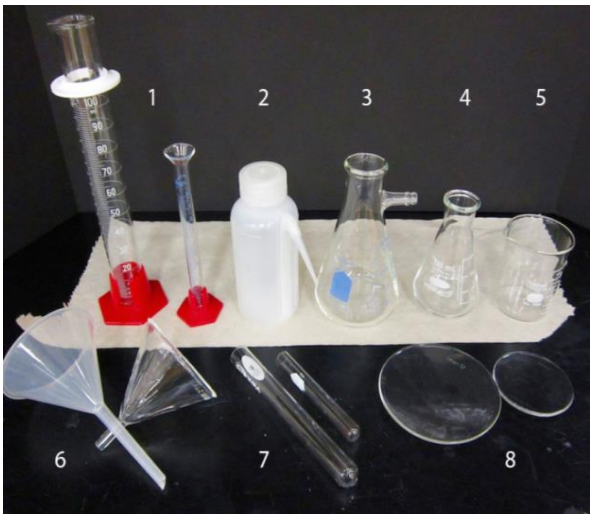
Information Sheet 3- Using laboratory glassware and measuring equipment

3.1 Use and Maintenance of Equipment and Glassware

Good equipment maintenance is essential for safe and efficient operations. Laboratory equipment should be regularly inspected, maintained, and serviced on schedules that are based on the manufacturer's recommendations, as well as the likelihood and hazards of equipment failure. Maintenance plans should ensure that any lockout procedures cannot be violated.

Carefully handle and store glassware to avoid damage. Discard or repair chipped or cracked items. Handle vacuum-jacketed glassware with extreme care to prevent implosions. Evacuated equipment such as Dewar flasks or vacuum desiccators should be taped, shielded, or coated. Only glassware designed for vacuum work should be used for that purpose.

Use tongs, a tweezer, or puncture-proof hand protection when picking up broken glass. Small pieces should be swept up with a brush into a dustpan. Glassblowing operations should not be attempted unless an area has been made safe for both fabrication and annealing. Protect your hands and body when performing forceful operations involving glassware. For instance, leather or Kevlar® gloves should be used when placing rubber tubing on glass hose connections. Cuts from forcing glass tubing into stoppers or plastic tubing are a common laboratory accident and are often serious. Constructing adaptors from glass tubing and rubber or cork stoppers is obsolete; instead, use fabricated, commercial adaptors made from plastic, metal, or other materials (Sullivan *et al.*, 2010; World Health Organization, 2020a).



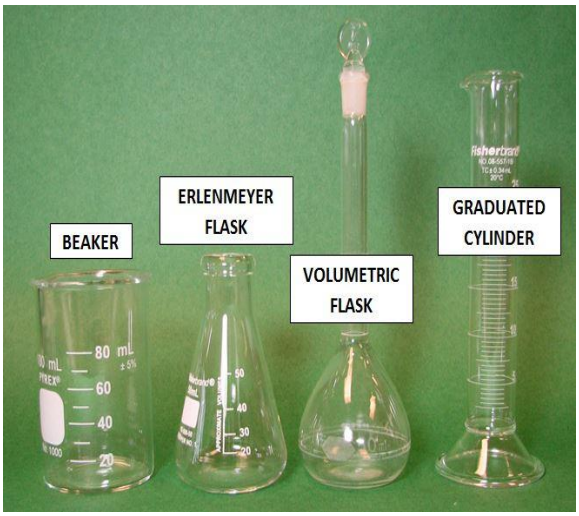


Figure 4: Glass ware types used in laboratory

Major Glass Ware Used In Laboratory

Cylinders, Wash bottle, Side arm, Flask, Beaker, Funnels, Test tubes, Watch glasses, Universal bottle, Slide and cover slip, Microscope are the major used glass ware. When used glass ware Always wash new glasses before first use, Hand wash glasses individually, do not soak with other glasses to reduce the chance of

mechanical shock and use a suitable detergent and warm water with a non-abrasive sponge or cloth.

1. Microscope

Introduction Microorganisms are too small to be seen with the naked eye so a microscope must be used to visualize these organisms. While a microscope is not difficult to use it does require some practice to develop the skills necessary to use the microscope to its maximum capabilities. Bacteria and other cellular microorganisms are measured in micrometers (mm) or 1 x 10-6 meters. Viruses are even smaller and are measured in nanometers (nm) or 1 x 10-9 m. When carrying a microscope always use both hands. One should be on the arm of the microscope and one should be under the base of the microscope. Discussion there are several types of microscopes but the only one used in this laboratory is the compound light or bright-field microscope. Individual microscopes will vary depending on the manufacturer but all microscopes have the same basic features.

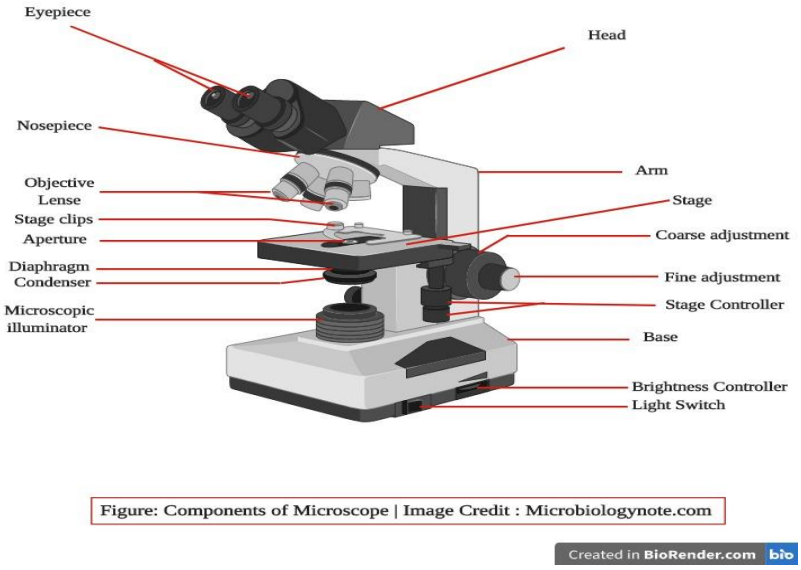


Figure 5: Microscope body part

2. Beakers

The beaker is one of the most common pieces of glassware in the laboratory. It is a simple cylindrical container used to hold solids and liquids with sizes ranging from very small (10 mL) to very large (4,000 mL). It has a lip for ease of pouring and decanting liquids. The graduations are approximate, but very useful when exact volumes are not needed.

3. Flasks

Flasks are designed so the contents can be swirled without spilling. They are also easily fitted with stoppers and often have the stopper size written directly on the flask. The rounded shape is better for applications that involve boiling.

4. Test Tubes

Test tubes are relatively small cylindrical vessels used to store, heat, and mix chemicals. While the test tube comes in specific sizes, it's typically used in qualitative observational procedures.

5. Pipettes

Volumetric pipettes are known for high precision, like volumetric flasks, but are used to dispense liquids, typically in the preparation of solutions in a volumetric flask. The pipette also has an etched mark denoting a precise volume, and the solution is drawn into the pipette using a pipette bulb, never by mouth.

6. Micropipettes

Micropipettes are a specialized class of volumetric pipettes used for very small volumes from 1 µl to 1,000 µL. The micropipette uses plastic disposable tips, but these can be re-used under appropriate situations. Most micropipettes have an adjustable range of volumes using separate withdraw and dispense actions on the

pipette body. The mechanism for adjusting, determining volume limits, and ejecting disposable tips varies by manufacturer.

7. **Burettes**

The burette is an analytical piece of glassware used to dispense variable (but precise) volumes of liquids. Commonly found in analytical chemistry, the burette is used in a variety of titration experiments.

8. **Funnels** (used for filtering and transferring)

Traditional funnels used for gravity filtration have a wide cone-shaped body, for adding and filtering solutions, and a long narrow stem, for delivery into a flask. Filter paper is folded into a cone shape, inserted into the funnel, and wetted with a solvent (typically water). The powder funnel has a wider stem designed for dispensing solids and viscous liquids. Filter paper is only used in conjunction with the filter funnel.

9. **Ceramics**

The ceramic Büchner funnel fits into the filter (Büchner) flask using a rubber cone or 1-hole rubber stopper. The funnel is typically made of ceramic with pin-sized holes in the flat bottom. Filter paper is placed on top of the holes and wetted with solvent (water) to prevent solids from getting under the filter paper.

10. **Crucible**

A crucible is made of ceramic and holds small amounts of chemicals during heating at high temperatures. Depending on the specific type, the crucible can withstand temperatures above 1,000 °C and is used in conjunction with a Bunsen burner or furnace. Common uses include heating a hydrated solid to remove water or combusting a compound to determine organic content.

11. Mortar and Pestle

While the mortar and pestle originated in chemistry (and alchemy) laboratories, it is more common in pharmacology, biology, and culinary applications. Made of ceramic or stone, materials are placed in the bowl-shaped mortar and ground and crushed using the pestle.

Washing and sterilization of glass and plastic wares, bottles, tubes, pipettes, slides

There are 6 major effective ways to ensure sterile lab equipment

A. Steam Sterilization

Autoclaving offers effective steam sterilization of a range of laboratory equipment at high temperature. The steam is typically heated between 121-134 ˚C and a pressure of 100 kPa. To ensure effective sterilization, the autoclave must allow the steam to penetrate the loaded samples.

B. Dry Heat Sterilization

Dry heat sterilization requires higher temperatures than steam sterilization, as the heat takes longer to penetrate and destroy the microbes. The hot air oven is a good alternative method for using samples such as powders that are not always suited to steam sterilization.

C. Other Heat Sterilization Methods

In addition to dry and steam sterilization methods, other heat sterilization techniques include flaming, incineration and boiling.

D. Sterile Filtration

Sterile filtration is an ideal method for sterilizing liquids that could otherwise be damaged by heat. Filtration is a rapid sterilization method; solution is passed over a filter with pore diameters that are too small to enable microbes to flow through.

E. Chemical Sterilization

Chemicals are also effective for sterilizing samples when heat is not suitable, such as sterilizing biological samples, plastics and electronic materials. Gas sterilizers expose the organisms to a high concentration of a reactive gas such as ozone, nitrogen dioxide and alkylating agents.

F. Radiation Sterilization

Electromagnetic radiation such as UV, X-ray and gamma rays are extremely effective at penetrating organisms and killing them. UV sterilization is effective for sterilizing small sample areas such as laminar hoods, whereas X-ray and gamma rays can be used to sterilize large samples during manufacturing (Rubbo and Gardner, 1965; Sinha *et al.*, 2020).

Self-Check – 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. When comparing glassware with plastic wares, plastic wares are?
   A. Cheaper and safer to use. B. Re-usable and autoclavable.
   C. Heavier, more costly D. easily broken

2. _____ is/are calibrated to deliver a constant volume of liquid

A. Micropipettes B/ Volumetric pipettes C/ Graduated pipettes D/ measuring pipettes

3. Glass ware not calibrated to hold a particular or exact volume, but rather are available for various volumes, depending on the use desired

A. Volumetric glass wares B. Non- volumetric glass wares
C. Semi-volumetric Glass wares D. Volumetric flasks

Note: **Satisfactory rating - 10 points** **Unsatisfactory - below 5 points**

You can ask your teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet 4- Handling the specimens in safe manner that minimize the spread of pathogens to animals and humans

4.1 Specimen Handling in Safe Manner

'**Specimen**': means the group of elements (sampling units) drawn from a population, on which tests are performed or parameters measured to provide surveillance information. A **sample** is an unbiased number of observations taken from animal. So the sample, in other words, is a portion, part, or fraction of the whole group, and acts as a subset of the population. Samples are used in a variety of settings where research is conducted. When handling specimens, universal safety precautions should always be employed. Appropriate protective clothing and personal protective items should be worn. No tissue, even fixed material, should ever be handled with bare hands. It must be kept in mind that noxious agents are present in the laboratory.

4.2 General consideration of specimen handling

Careful consideration must be given to the collection, containment, and storage of the specimens, including biosafety measures that must be in place to prevent contamination of the environment or exposure of other animals and humans to potentially infectious materials (Standard for managing biological risk in the veterinary laboratory and animal facilities). Prior to sampling, consideration must be given to the type of specimen(s) needed including the purpose of the testing and the test technologies to be used. The volume or quantity of specimen must be sufficient to perform initial testing, to perform any subsequent confirmatory testing and to provide sufficient residual specimen for referral or archival purposes (Granata, 2011; Larkin *et al.*, 2011).

General consideration for taking and handling sample are:-

- Restrain the sampled animal appropriately and identifies the types of sample taken.
- Use only aseptic sample collection material (those packed or sterilized).
- Use always PPE and rule of sample collection according to laboratory rules
- Sample should be taken from living or recently dead animals.
- Sample should be taken from the affected site(s) and as early as possible.
- Collect samples from clinical cases and in contact animals.
- Samples should be taken from the edge of the lesion and include some macroscopically normal tissues.
- Collect specimens as aseptically as possible.
- Collect specimens before the administration of any form of treatment.
- Gloves must always be worn when handling specimens.
- Fresh tissues are potentially infective and all specimens are placed in fixative as soon as possible.
- Retail packages taken as samples should not be opened or their contents moved to other sample containers.
- These samples should be directly labelled as final samples. Samples are packed and sealed according to the packing requirements.
- Care should be taken not to erase or conceal the information on the original packing.
- Sampling method of animal

The sampling method depends on**:**

- The purpose of sampling (Virology, bacteriology, mycology, serology, parasitology, immunology etc.)

- The type of sample (Blood, serum, feaces, mucous, retained fetal membrane, external parasite, skin scraping, milk, urine, rumen fluid, etc.)
- The amount and uniformity of the sample (e.g. blood 2ml, feaces 3 gm, synovial fluid 0.5 ml, 5 Pcs of eggs,).
- The site of taken, size, duration and life span of sample.

Self-Check – 4	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. How we can handle sample? (3pts)
2. List the types of sample collection? (2pts)
3. What are the necessary criteria of sample collection? (4pts)

Note: **Satisfactory rating - 5 points** **Unsatisfactory - below 4 points**

You can ask your teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet 5- Following the workplace procedures and work instructions for controlling risks accurately

5.1 Risk control at workplace

Risk control measures are actions that are taken in response to a risk factor that has the potential to cause accident or harm in the workplace. The control measure can either designed to reduce the risk or eliminate them completely. As a first step eliminate risk from workplace, a laboratory biorisk assessment addressing biosafety and laboratory biosecurity issues, including any control or mitigation measures to be implemented, must be completed. The laboratory should consider all biorisk management measures needed to protect the integrity of the sample, as well as the health of the workers and environment, from the time the original sample is received through the long-term storage and ultimate use or destruction of the material(s).

The appropriate level of laboratory biosecurity, including controlled access to the archived samples and inventory records, is an important consideration for laboratories maintaining biological inventories and archives. The laboratory should also have a back-up plan for the transfer or destruction of potentially dangerous archived materials in the event of power failures or other compromises to the storage environment. National and international regulations and legislation, including requirements for permits and licenses to receive, maintain, work with, and distribute specific agents and tissues must be followed for all laboratory archives. Whereas **biosafety** aims at protecting public health and environment from accidental exposure to biological agents, **biosecurity** deals with the prevention of misuse through loss, theft, diversion or intentional release of pathogens, toxins and any other biological materials (Njoroge and Nichols, 2014; World Health Organization, 2020b).

Workplace is saved from both biohazard and non-biological hazard by:

1. use PPE
2. use hygienically laboratory reagent and chemical effectively
3. disposed laboratory waste safe manner
4. training technician lab rule and regulation

NOTE: Biosafety + Biosecurity = Biorisk management

Figure 6: Hazard and risk control lab log

5.2 Safe work practices and using personal protective equipment

Safe laboratory practice is based on understanding and respect, not fear; the regulations intended to help you work safely with chemical reagents. Before beginning an experiment, be sure you have this information at hand and that you understand it. Do not hesitate to consult or questions about any experiment or about the regulations. A biosafety cabinet also called a biological safety cabinet or microbiological safety cabinet is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with pathogens requiring a defined biosafety level.

Figure 7: biosafety cabinet

2. Personal protective equipment (PPE)

PPE is comprised of clothing or equipment that is used to isolate a worker from direct exposure to workplace hazards.

- It is used to provide worker health and safety

- PPE provide adequate protection if it is properly worn and appropriately used.

PPE examples include:

- Partial and full body protective garments (aprons, lab coats and coveralls)
- Headwear
- Face and eyewear (goggles and mask)
- Gloves
- Footwear (shoe covers/boots)
- Respirators (disposable, air purifying and air supplied)
- Hearing protectors (earplugs and earmuffs)

Guidance for the Selection and Use of Personal Protective Equipment

- Types of PPE Used in Healthcare Settings
- Gloves – protect hands
- Gowns/aprons – protect skin and/or clothing
- Masks and respirators– protect mouth/nose
- Respirators – protect respiratory tract from airborne infectious agents
- Goggles – protect eyes
- Face shields – protect face, mouth, nose, and eyes

Factors Influencing PPE Selection

- Type of exposure anticipated
- Splash/spray versus touch
- Durability and appropriateness for the task
- Fit

- Gloves

Purpose:

- To reduce the risk of staff acquiring infections from patients,
- To prevent staff from transmitting their skin flora to patients,
- To reduce contamination of the hands of staff by microorganisms that can be transmitted from one patient to another (cross-contamination)
- Glove material – vinyl, latex, nitrile, other, sterile or non-sterile, one or two pair, single use or reusable

Gloves should be worn when: there is a reasonable chance of hand contact with blood or other body fluids, mucous membranes, or non-intact skin. Before handling soiled instruments, contaminated waste items or touch contaminated surfaces.

- When disposing contaminated waste items
- Handling chemicals or disinfectants

Self-Check – 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What PPE? (2pts).
2. Write the major used PPE in lab? (3)

Note: **Satisfactory rating - 3 points** **Unsatisfactory - below 2 points**

You can ask your teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

LG #90	LO#2- Prepare Working Solutions

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics:

- Identifying relevant standard methods for solution preparation.
- Selecting and preparing materials and solvent of specified purity.
- Measuring and recording appropriate quantities of reagents for solution preparation.
- Preparing labels and log solution details in prepared in laboratory
- Labeling solutions are transferred to appropriate containers

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, **you will be able to**:

- Identify relevant standard methods for solution preparation.
- Select and prepare materials and solvent of specified purity.
- Measure and record appropriate quantities of reagents for solution preparation.
- Prepare labels and log solution details in prepared in laboratory
- Label solutions are transferred to appropriate containers

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets’’
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.

Information Sheet 1- Identifying relevant standard methods for solution preparation

1.1 Solution preparation

Solution is a homogeneous mixture of two or more substances. OR a mixture of substance dissolved in another so the properties are the same throughout. Solution: composed of a solute and the solvent.

- **Solute** is the dissolved substance, OR the substance found in small amount
- **Solvent** is a substance in which solutes dissolves to make the mixture or the substance that is present in the greatest amount.

  - ✓ **Water** is the Universal Solvent but there are many things it cannot dissolve. For example water and oil do not mix. We say oil is immiscible in water. Water is a good solvent due to its polarity.

- **Mixtures:** combinations of different substances where each substance retains its chemical properties.
- **Concentration**- amount of a substance dissolved in a given amount of solvent
- **Compound-** composed of two or more substances (elements) but in a ratio that cannot vary.

✓ E.g. Water; there are 8 grams of oxygen for each gram of hydrogen. It won't be water if that ratio changes.

1.2. Ways of preparing a solution

- Dissolution
- Dilution

A. Dissolution: Is the process by which a solute forms a solution in a solvent. Weighed amount of solid dissolved in a required solvent. The solute, in the case of solids, has its crystalline structure disintegrated as separate ions, atoms, and molecules form.

Factors affecting dissolution

Surface area: the larger the surface area, the faster it gets dissolved.

3. **Temperature:** as the temperature increases, it dissolves more quickly.
4. **Volume of solvent:** The higher the amount of solvent, the quicker the dissolution
5. **Solubility of the solid:** It depends how soluble the solute is to water.
6. **particle size:** the smaller particle size, the faster to dissolved
7. **PH** of the dissolving medium: neutral medium is best for dissolution
8. **Agitation:** produced by stirring or mixing a solution increases the rate of dissolution

✓ E.g. if there are 10 grams of salt (the solute) dissolved in 1 liter of water (the solvent), this solution has a certain salt concentration

B. Dilution of the solution

Dilution is a process by which the concentration or activity of a given solution is decreased by the addition of solvent. A dilution represents the ratio of concentrated or stock material of the total final volume of a solution. Dilution is made to prepare:

- A working solution from the stock
- Measurable concentration of a sample (for reporting the actual concentrations of bodyfluid constitutes)

- If the specimen at hand is less than a procedure calls for
- If the concentration of substances (analyte) is too high to be accurately measured.
- Whenever a solution is diluted, it is volume is increased and its concentration decreased, but the total amount of solute remains unchanged
- They are of two types of dilution. This is simple dilution and serial dilution (Hudlicky, 1980; Xu *et al.*, 2008).

Self-Check – 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

1. Which of the following is false about Solution?

   A/ is a homogeneous mixture B. is a heterogeneous mixture C. composed of a solute and the solvent D. is a substance in which solutes dissolves

2. Concentration of solution is the

   A. Quantity of solvent in solute B. Quantity of solute in given solvent

   C. Unite to measure concentration D. Volume of solvent in solution

3. Which of the following is true about dissolution of solute in solvent?

   A. The smaller the surface area, the faster it gets dissolved. B. Temperature decrease, it dissolves more quickly. C. The higher the amount of solute, the quicker the dissolution D. the smaller particle size, the faster to dissolved

4. Dilution is a process by which the concentration or activity of a given solution is increased by the addition of solute

   A. True B. false

Note: **Satisfactory rating - 2 points** **Unsatisfactory - below 2 points**

You can ask your teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet 2- Selecting and preparing materials and solvent of specified purity

2.1 Materials used to prepare solution

• Balance	• Labeling Materials	• Glass Rod
• Flask	• Reagent Bottles	• Glass Bead
• Measuring Cylinder	• Burette Stand	• Spatula
• Funnel	• Burette	• Scoop
• Desiccators	• Water Bath	• Pipettes
• Incubator	• Mortar	• Pestles

2.1.1 Laboratory glass wares and plastic wares

- ✓ Laboratory glassware and plastic wares are materials used in clinical laboratory for: measuring pipetting transferring Preparation of reagents Storage etc.
- ✓ Most of the routine laboratory wares used to be of glass, but recent advantage made in the use of plastic resin to manufacture a wide range of plastic ware having led to a gradual replacement of glass wares with durable plastic ware. The plastic ware used in the laboratory should be of high quality. Also cheaper and safer to use than glassware.
- ✓ The glass wares have the minor advantage of being re-usable and autoclavable. But heavier, more costly and easily broken. In fact, in this age of good awareness of the dangers posed by hepatitis and human immunodeficiency viruses (HIV), most of the plastic wares are disposable, thereby cutting down on the cost of cleaning.
- ✓ The plastic ware are fashioned and shaped exactly like the glass ware.

Types of Laboratory glass wares

A. Can be divided in to five main types according to their composion:-

1. Glass with high thermal resistance – borosilicate glass can resist about 500^{o}c and low alkaline contact.
2. **High silica glass-** contains 96% silicon; it is thermal endurable, chemically stable and electric resistance. Glass with high resistance to alkali- Boron free, used in strong alkali low thermal resistance
3. **Low actinic glass** – amber color to protect light
4. **Standard flint glass**- soda lime glass, poor resistance to increased temp. Contains free soda in its walls

B. Based on their use

1. volumetric wares
2. Semi-volumetric Glass wares
3. Non- volumetric glass wares.

a. **Volumetric wares:** Apparatus used for measurement of liquids Can be made either from glass or plastic. It includes:

- Volumetric flasks
- Graduated centrifuge tubes
- Graduated serological pipette
- Medicine dropper
- Burettes
- Micropipettes
- Diluting or thoma pipettes etc.

b. **Non- volumetric glass wares:** are not calibrated to hold a particular or exact volume, but rather are available for various volumes, depending on the use desired.
   - Erlenmeyer flask
   - Round bottom flask
   - Flat bottom flask
   - Beaker
   - Centrifuge tube
   - Test tube
   - Pasture pipette

c. **Semi-volumetric Glass wares:** are used for approximate measurement. It includes;
   - Graduated cylinder
   - Graduated specimen glass
   - Beakers
   - Conical flask
   - Medicine droppers with or without calibration mark
   - Graduated beaker with double beaks
   - Graduated glass

1.2.1. Pipettes

There are several types each having their own advantages and limitations. They are designated as class 'A' or 'B' according to their accuracy.

1. Class "A" pipettes are the most accurate and the tolerance limits are well defined that is, ± 0.01, ± 0.02 and ± 0.04 ml for 2, 25, and 50 ml pipettes respectively.

2. Class “B” pipettes: are less accurate but quite satisfactory for most general laboratory.

Read the volume at lower meniscus. Significant errors will result if the temperature of the liquid pipetted is widely different from the temperature of calibration. The usual temperature of calibration is 20^{o}C and this is marked on the pipette.

2.2.1.1. Micropipettes

There are several types each having their own advantages and limitations. They are designated as class ‘A’ or ‘B’ according to their accuracy.

1. **Class “A” pipettes** are the most accurate and the tolerance limits are well defined that is, ± 0.01, ± 0.02 and ± 0.04 ml for 2, 25, and 50 ml pipettes respectively.
2. **Class “B” pipettes:** are less accurate but quite satisfactory for most general laboratory.

Dilution preparation

Dilution is a process by which the concentration or activity of a given solution is decreased by the addition of solvent. A dilution represents the ratio of concentrated or stock material of the total final volume of a solution. Dilution is made to prepare:

- A working solution from the stock
- Measurable concentration of a sample (for reporting the actual concentrations of body- fluid constitutes)
- If the specimen at hand is less than a procedure calls for If the concentration of substances (analyte) is too high to be accurately measured.

- Whenever a solution is diluted, it is volume is increased and its concentration decreased, but the total amount of solute remains unchanged

Types of dilution

1. Simple dilution
2. Serial dilution

1. **Simple dilution:**

A general process of preparing less concentrated solutions from a solution of greater concentration, a unit volume of a liquid material of interest is combined with an appropriate volume of a solvent liquid to achieve the desired concentration. To dilute a solution means to add more solvent without the addition of more solute to bring a solution into the desired concentration. The resulting solution is thoroughly mixed so as to ensure that all parts of the solution are identical the ratio of concentrated or stock solution to the total volume equals the dilution factor

Dilution factor (df) = volume of stock

Total volume of solution

The df is inversely related to the concentration thus, the dilution factor increases as the concentration decreases.

- a 1:5 dilution (verbalize as "1 to 5" dilution) entails combining 1 unit volume of solute (the material to be diluted) + 4 unit volumes of the solvent medium
- hence, dilution factor could be: 1 + 4 = 5
- Mathematically this relationship can be shown in the equation:

D = Vs/Tv

Where:

• D= dilution

• Vs= volume of solute (sample)

• Tv= Final volume

• In the performance of dilution, the following equation is used to determine the volume (V2) needed to dilute a given volume (V1) of solution of a known concentration (C1) to the desired lesser concentration (C2).

C1xV1=C2V2

Likewise, this equation also is used to calculate the concentration of the diluted solution when a given solution is added to the starting solution. In making a simple dilution, the laboratory technician must decide on the total volume desired & amount of stock solution to use

Using Proportion

It is used when reagents are prepared by adding a specific amount of one solution to a specific amount of another solution.

- V =C/A+B
- Where: C – total volume of final reagent
- A – total parts of solution A
- B – total parts of solution B
- V – volume of each part

Example 1: a buffer made by adding two parts of ‗solution A' to five parts of solution B would be required to make 70 mL of the buffer.

- Formula: C/A+B V = 70mL required 2 parts of A + 5 part of B = 70 mL

7 part = 10 volume of one part

- A = 2 x 10 = 20 mL
- B = 5 x 10 = 50 mL

Example 2: a 100mg/mL N2 standard is diluted 1:10. then the concentration of the resulting solution is 100mg/mL x 1/10 = 10 mg/mL

Using C1V1 = C2V2

This formula is useful only if the units for concentration & volume are the same & if three of the four variables are known.

- Example1. What volume is needed to make 500ml of 0.1M solution of tris-buffer from a solution of 2 M tris-buffer?
- Example:2 To make 45 ml of 30% Solution from 70% solution C2 = 30% V1 = C2V2
- V2 = 45ml, C1 = 70%
- V1= 30 X 45 = 19.3 ml 70 %

Therefore, 19.3 ml of 70% solution must be diluted with 25.7 ml of distilled water to obtain 45ml of a 30% solution

Diluting body fluids/standards

- Example: To make 8ml of a 1 in 20 dilution of blood.
- C1xV1=C2V2 =>20xV1=1x8V1 (blood volume) =0.4

Therefore, to prepare 8 ml of a 1 in 20 dilution, add 0.4 ml of blood to 7.6 ml of the diluting fluid.

- To make 4ml of a 1 in 2 dilution of serum in physiological saline.
- C1xV1=C2V2 => 2xV1=1x4 =>V1 (serum volume) =0.4

To prepare 4ml of a 1 in 2 dilution, add 2ml of serum to 2 ml of physiological

saline. Calculating the dilution of a body fluid

Examples: Calculate the dilution of blood when using 50 micro liters (µ l) of blood and a 50 µ l of diluting fluid.

Total volume of blood and diluting fluid 50 + 50 = 100 µ l

Sample: total 50:100 1 in 2 dilutions

Calculate the dilution of urine using 0.5 ml of urine and 8.5 ml of diluting fluid (physiological saline)

Total volume of urine and diluting fluid, 0.5 + 8.5 = 9.0 µ l => 0.5: 9 = 1 in 18 dilutions

Serial dilutions

A serial dilution may be defined as multiple progressive dilutions ranging from more concentrated solutions to less concentrated solutions. Simply a series of simple dilutions which amplifies the dilution factor quickly beginning with a small initial quantity of material (bacterial culture, a chemical, orange juice, etc.). The source of dilution material (solute) for each step comes from the diluted material of the previous dilution step.

- Final dilution factor (DF) = DF1 * DF2 * DF3 etc.
- It is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion.
- Used to accurately create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale.
- used to reduce the concentration of microscopic organisms or cells in a sample

- It is required for certain quantitative tests

Serial dilution is extremely useful when the volume of the concentrate &/or diluents is in short supply. Large dilutions may be difficult to make because of the amount of diluent that needs to be added.

- For example, a 1/1000 dilution may be difficult to create accurately even with 0.1mL of serum & 99.9 mL of diluent. A series of dilutions, also called serial dilutions, may be a better way to make the dilutions.
- The dilution fold of a system can be determined by the formula: 1 = volume transferred

Dilution fold total volume

- Volume transferred = is equal to the constant volume transferred to each successive tubes in the serial dilution system.
- Total volume = is equal to the volume being transferred plus the volume of diluents already in the tube.
    - ✓ E.g. 1. What is the dilution fold of the following serial dilution system consisting of five tubes?
- The following amount of diluents have been added to the tubes; 0.5 mL to tube 1 & 0.5 mL to tube 2 to 5. Next, 0.5 mL of patient serum is added to tube 1 and 0.5 mL is serialy transferred through tube 5.finally, 0.5 mL is discarded from tube 5.
    - ✓ 1/Y = 0.5/1.0
    - ✓ Y x 0.5 = 1
    - ✓ Y = 1/0.5 = 2
- E g. 2. it is often desirable to determine the dilution of a given tube (Y) in a serial dilutionsystem. This dilution can be calculated by Solution of tube 1 = dilution of Y x [1/dilutionfold]

- What is the dilution of tube 3 in the preceding example?

Y= ½ x (½) (Y-1)

= ½ x (½)2

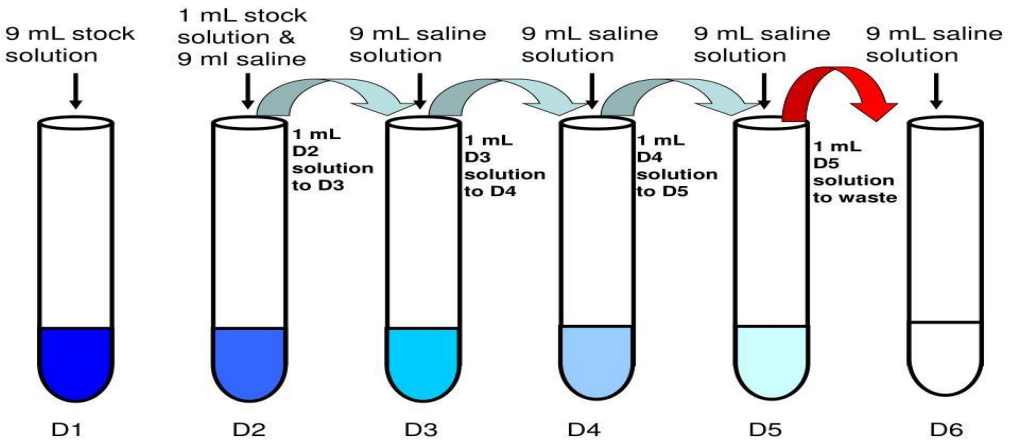
= ½ x ½ x ½

= 1/8 ;

The dilution of serum in the tube 3 is 1/8.

E.g. In a typical microbiology exercise the students perform a three step 1:100 serial dilution of a bacterial culture in the process of quantifying the number of viable bacteria in a culture. Each step uses a 1 ml total volume. The initial step combines 1 unit volume of bacterial culture (10 ul) with 99 unit volumes of broth (990 ul) = 1:100 dilution. In the second step, one unit volume of the 1:100 dilution is combined with 99 unit volumes of broth now yielding a total dilution of 1:100x100 = 1:10,000 dilution. Repeated again (the third step) the total dilution would be 1:100x10, 000 = 1:1,000,000 total dilution. The concentration of bacteria is now one million times less than in the original sample.

Serial Dilution



Self-Check – 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Choice the best answer

1. Which one of the following is the smallest size of needle? **(3pts)**
   A/ 16 guage B/ 10 guage C/ 21 guage D/ 25 guage
2. During examination of animal pain, consistency shape by touching with hand is called? **(2pts)**
   A/ Percussion B/ Palpation C/ percussion D/ Auscultation
3. Buffer solution is made by adding two parts of 'solution A' to three parts of solution B. which volume of solution A and solution B would be required to make 100 mL of the buffer respectively.
   A. 10, 90 B. 20, 80 C. 30, 70 D. 40, 60
4. 2. Calculate the dilution of urine using 0.5 ml of urine and 8.5 ml of diluting fluid (physiological saline)
   A. 1: 3 dilutions B. 1:9 dilutions C. 1:12 dilutions D. 1:18 dilutions

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet 3- Measuring and recording appropriate quantities of reagents for solution preparation

3.1 Prepare reagent solution

3.1.1. Reagent preparation

Distilled or deionised water should be used in making up all of these solutions.

Weigh balance

1. Consult manual for operating instructions
2. Keep balance and weights clean and dry to protect from corrosion
3. Changes in the surface of the balance parts will affect accuracy
4. Do not place material to be weighed directly on the pan-always-use a container or weighing paper
5. Subtract weigh paper from the combined weight of the container and material being weighed
6. To prevent contamination of stock materials do not return unused chemical to the stock bottle
7. Protect the balance from drafts of air
8. Air moving cross the pans will cause an inaccurate reading
9. Some balances have compartments which are to be closed during reading

Media for bacterial growth

Culture media gives artificial environment by simulating natural conditions necessary for growth of bacteria.

Types of media

- **Synthetic Media** & **Non-Synthetic Media**.

Types of Culture Media

- **General Purpose Media**: Support the growth of a large variety of microbes, particularly chemoheterotrophs. E.g. Nutrient agar (beef extract, peptone) and agar.
- **Differential Media**: often contain reagents or chemicals that produce differences in growth and allow differentiating between types of microbes. PH indicator dyes may also be used to distinguish between growth producing acidic, neutral or alkaline end products. E.g. MacConkey Agar is used to identify Enterobacteriacae (e.g. E.coli and Salmonella).
- **Selective Media**: Allow for the growth of only certain types of organisms. Due to addition of inhibitors or a specific carbon source, Enrichment culture techniques exploit the principle of survival of the fittest to favor the growth of certain organisms.

Ingredients commonly used in media

- Beef extract, Peptone, Yeast Extract, Agar: solidifying agent. Salt.

Self-Check – 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Define what dilution? (2pts).
2. How we dilute concentrate reagent to dilute one?(2).

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 4: Preparing labels and log reagent solution in details

4.1 Laboratory Reagent Labels

A log is a piece of a thick written or of the written of a tree that has been differentiate the security of reagents and chemical. These Laboratory Reagent Labels are perfect for documentation of reagent and buffer information. They provide a space for storage temperature notation. Wrap them around vials. Latex impregnated with a permanent acrylic adhesive. Each label includes space for the following information:-

- Solution Label (EF14813B): Solution, Concentration. Procedure, Preparation Date, Expiration Date, Prepared By, and Storage (Room or Refrigerator)
- Laboratory Reagent Label (EF14813B): Reagent, Conc., Prep. Date, Storage, and Tech.
- Key Features
  - ✓ Excellent for reagent and buffer documentation
  - ✓ Storage temperature can be noted on the labels
  - ✓ Wrap around vials

4.2 Example of format lab and log recorded are:

- Solution _____
- Concentration _____
- Procedure _____
- Preparation date _____
- Expiration date _____
- Prepare by _____
- Storage Room Refrig.
-

Self-Check – 4	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What is log means? (2pts).
2. write the major contents of log?(4pts)
3. Can we use reagent without log? If yes how? (2pts)

Note: **Satisfactory rating - 4 points** **Unsatisfactory - below 4 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 5: Transferring label solutions (reagents) to appropriate containers

9.1 Labeling of Reagents

Purpose of labeling: Workplace reagent labeling primarily serves two purposes, to:

- Identify the contents of the container
- Warn of hazards.

Reagent labeling is a complement to other sources of information such as the MSDS and other labeling requirements. It aims to assist with the safer use of a substance by identifying hazards likely to be associated with the use of the substance.

Responsibility: - Chemical suppliers and employers have the primary responsibility to ensure that in the workplace, hazardous substances are correctly labeled.

Employers must ensure that:

- Chemicals are appropriately and correctly labeled
- Libeling is not removed or modified
- Decanted substances are labeled
- There are prescribed measures for lost labels and unknown substances.

Workplace labels: Hazardous substances must be labeled to show

- contents
- significant hazards

- complementing other information (including recorded information such as directions for use, first aid and emergency procedures)
- Date opened.

Scope: Workplace labels are required for containers containing:

- Hazardous substances
- Drugs and poisons

And labeling is also required for:

- Decanted hazardous substances, not for immediate use
- Items (and substances) that can produce hazardous substances in use
- Containers not cleaned.

Lost Labels: -If the label is lost and the contents are unknown, the container should be:

- Marked CAUTION DO NOT USE: UNKNOWN SUBSTANCE
- Stored in isolation until the contents can be identified
- If contents cannot be identified, the contents should be suitably disposed of (with advice from relevant authorities).

Replacement of labels: A new label must be issued and placed on the container when:

- the substance changes (including new ingredients)
- new information becomes available that affects the information provided on the label (often instigated through a change of MSDS)
- A new expiry date (if used) is required.

Self-Check – 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Write the main concern when we transfer reagent from one container to another? (4pts)

Note: **Satisfactory rating - 2 points** **Unsatisfactory - below 2 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Operation Sheet 1– Make serial dilution and labeling each dilution

Objective: Serial dilution arrange

Material: Balance, aluminum sheet, Test tube, labeling marker, pipette, rack, Backer, O'clock, reagent,

To carry out successive 1/10 dilutions, 5 times

The step making serial dilution is indicated as follow: -

1. Set up seven clean tubes that hold about 20 mL as you will need to leave some room for mixing.
2. Carefully label each tube with the concentration and name of the reagent.
3. Leaving the first tube empty carefully pipette 9 mL of the diluent into each of the remaining five tubes Carefully pipette 10 mL of the solution to be diluted into the first tube.
4. Using a 1 mL pipette carefully transfer 1 mL of the starting solution into the second tube.
5. Discard the pipette and using a fresh pipette, mix the contents in the second tube by pipetting up and down ten times. You now have a solution that is 1/10 the strength of the starting solution in the second tube.
6. Using the same pipette carefully transfer 1 mL from the second tube to the third tube and repeat step.
7. You now have a 1/100 dilution of the starting solution in the third tube. Repeat steps 6 and 7 until you have finished the dilution series

LAP TEST	Performance Test

Name……. ID……..

Date…….

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within **2** hour. The project is expected from each student to do it.

Task-1: Perform successive 1/2 dilutions, 5 times

LG #91	LO-3: Check Existing Stock of Solutions

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics:

- Monitoring shelf life of working solutions according to laboratory procedures
- Replacing out-of-date or reject solutions according to laboratory procedures
- Conducting routine titrimetric analyses, if appropriate, to determine if solutions are fit for purpose.

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, **upon completion of this learning guide, you will be able to**:

- Monitor shelf life of working solutions according to laboratory procedures
- Replace out-of-date or reject solutions according to laboratory procedures
- Conduct routine titrimetric analyses, if appropriate, to determine if solutions are fit for purpose.

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets’’

7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.
10. If your performance is satisfactory proceed to the next learning guide.

Information Sheet – 1: Monitoring shelf life of working solutions

1.1 Check Solutions for Visual Deterioration and Expiry Date

Many chemicals which are fairly stable do not come with an expiry date. In such situations, some labs dispose unopened chemicals after 5 years even if the chemical is perfectly in working condition. A carefully prepared solution will only be viable for a certain period of time. No matter how the solution is prepared and stored, it will deteriorate over time. Deterioration can be caused by many factors. The following factors can reduce the quality of laboratory solutions.

- **Incorrect Storage** - temperature, light and cleanliness are all factors here
- **Chemical Contamination** - caused by sloppy procedures.
- **Microbial Contamination** - reagents may be autoclaved to avoid this
- **Chemical Instability** - unstable reagents may break down or react to form other chemicals.
- **Calculation Error** - not a cause of deterioration necessarily, but a significant quality concern.
- **Precipitation** - reagent components may precipitate out of solution and sometimes adhere strongly to the interior of the container (eg protein solutions) thus reducing the molarity of the solution.

Many of the factors mentioned previously can be controlled by 'shelf life'. If it takes six months for a reagent to deteriorate to a point where it is no longer usable, then putting a ***shelf life*** of three months on the container should solve the problem. This, of course, relies on the user checking solutions for visual deterioration and expiry dates. In microbiology laboratories it is second nature to check expiry dates and then to hold reagents, growth media etc up to the light and look for evidence of

microbial contamination. Always check expiry dates and check the solution visually for signs of deterioration.

1.2 Shelf Life of Laboratory Solutions

Chemical Shelf Life is the time period in which a specific chemical product retains the same properties and characteristics that it possessed at the time of packaging. Expiration Date is the amount of time that a chemical should remain in use after opening. Most laboratory reagents have a limited shelf life. In general, solutions should not be kept for more than one year. In a great many cases, solutions will deteriorate in less than a year.

Some solutions are chemically unstable. Over time the concentration of such solutions will change as the unstable reagents break down, or react, to form other chemicals. Sodium hydroxide solutions have a limited shelf life, because sodium hydroxide reacts with atmospheric carbon dioxide to form carbonates. Therefore, the concentration of a sodium hydroxide solution cannot be known with certainty, unless it has been freshly standardized. There may be some evaporation of solutions kept in bottles. The moisture that condenses in the upper portion of the bottle will dilute the dispensed solution as it is poured out. In all cases, solutions should be mixed thoroughly before use. Solutions should be routinely checked prior to use for signs of turbidity or deposits that may indicate microbial contamination or chemical degradation. Crystals around the neck of a storage bottle may indicate a change in concentration. When this occurs decisions need to be made to discard, treat or re-check the solutions.

Example of Guide to Recommended Chemical Reagent Shelf Life

Chemical reagent	Shelf life (years)	Chemical reagent	Shelf reagent
Acetic acid	2	Chromium trioxide	3
Ammonium hydroxide: 2 years	2	Citric acid: 6 months	6
Azobisisobutyronitrile	5	Diethyl ether	2
Butylated hydroxytoluene	3 years	Dimethylformamide: 2 years	2
Ceric ammonium nitrate	5	Dodecylbenzene sulphonic acid	6
Chloroform	2-3	Ethanol	2
Chromic acid	2	Formaldehyde	1
Formic acid	2	Hyamine	2
Hydrochloric acid	2	Isopropyl alcohol	3
Hydrofluoric acid	2	Nitric acid	1-3
Hydrogen peroxide	2-3	Perchloric acid	2
Imidazole: 6 years	6	Phosphoric acid	3
odine: 2 years	2	Potassium hydroxide	2
Potassium permanganate	2	Sodium chloride	2
Silver nitrate	2	Sodium hydroxide	2
Sulfuric acid	2	Tetrahydrofuran Tetramethylammonium hydroxide	2

1.3 Re-standardize or dispose of dated or deteriorated solutions

Some solutions may not need to be discarded. For example, at expiry date, a 0.1M solution of sodium hydroxide may appear as clear and as fresh as the day it was made. An alternative to disposal is to re standardize the reagent. How do you find out it is safe to use? Sometimes reagents are beyond help. In the previous activity, the reagent might now have a very low molarity. This may indicate that the reagent container was not airtight or that there has been contamination of the reagent, e. g. carbon dioxide in the atmosphere would react with the sodium hydroxide. In these situations and in analyses that are very reagent sensitive or significant, the best approach is to discard the reagent. Max, the Senior Technician, asks you to discard those stock solutions you determined were deteriorated. He suggests you follow the correct procedure for waste disposal contained in the OHS Manual under SOP.

1.4 Chemical Reagent Storage

Most chemical reagents should be stored in a cool, dry, dark place to maximize shelf life and prevent premature degradation. Room temperature is suitable for the majority of chemical reagents, though some may require specialized storage like refrigeration. To ensure safe and long-term storage, lab designers should be careful to select appropriate materials that won't corrode or react to regular chemical exposure. At One Pointe Solutions, we offer tons of storage options that can easily be customized or made modular to perfectly fit the needs of your busy facility. We recommend designed easy-to-reach storage within custom workbenches designed to house chemicals from the start. Our high-quality laboratory casework and storage systems come in a variety of configurations, from tall floor-to-ceiling cabinets to suspended cabinets that provide added storage under workbenches.

1.5 Recording quality monitoring

- Each reagent has been given a number.
- The reagents required and their numbers are indicated in the description of each technique.
- An alphabetical list of all the reagents used, with the numbers assigned to them, their composition, methods of preparation and storage requirements appears in the Annex at the end of the manual.

Self-Check – 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Choice the best answer

1. Many of the factors which causes deterioration of solutions can be controlled by 'shelf life'
   A. true B. false
2. The following factors can reduce the quality of laboratory solutions.
   A. Correct Storage B. careful procedures C. Microbial Contamination D. Stable reagents.
3. _____ is/are not necessarily a cause of deterioration of solution, but a significant Quality concern
   A. Precipitation B. Calculation Error C. Chemical Instability D. Incorrect Storage

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 2: replacing Out-of-date or reject solutions

2.1 Specified Pure Solvent

Pure solvent means absence of other substances or impurities. For example From Water and salt solution, Water is pure solvent while because of presence of salt it becomes impure. A solution can be defined as the homogenous mixture of two or more substances. So in a **solution**, the substance which gets dissolved is solute, whereas **solvent** is the substance in which the solute will dissolve.

2.2 Purity

Purity is one of the most useful external criteria for clustering quality evaluation, when prior knowledge on real class memberships is available. To compute purity, each cluster is labelled with the class which represents the majority of the points it contains. Then the accuracy of this assignment is measured by counting the number of correctly assignments divided by the number of points. Result precision is of utmost importance in laboratory testing. The choice of the right grade of reagent is essential for the application in hand, and it is also important to use reagents from the same source for high precision of results. With the provided information on reagent grading, you will be able to pick the correct reagents and ensure the quality of your testing.

Self-Check – 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. How we purify the any laboratory reagent? (4pts).

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 3: Conduct routine titrimetric analyses

3.1 Introduction

Titration is the quantitative addition of a solution of known concentration to a solution of unknown concentration until the reaction between them is complete to determine the concentration of the second solution. For example, it can be used to discover the amount of salt or sugar in a product or the concentration of vitamin C or E, which has an effect on product color. Routine titrimetric analyses are conducted, if appropriate, to determine if solutions are fit for purpose. The basic principle of the titration is the following: A solution - a so called titrant or standard solution - is added to sample to be analyzed. The titrant contains a known concentration of a chemical which reacts with the substance to be determined. The titrant is added by means of a burette.

Titrimetric Methods of Analysis

Titrimetric analysis consists in determining the number of moles of reagent (titrant), required to react quantitatively with the substance being determined. The titrant can be added (a) volumetrically, with a glass or automatic burette or with a low flow-rate pump or (b) coulometrically, with an electrochemical generation from a proper electrolyte. Titrimetric methods of analysis have the virtue of being like gravimetric methods, absolute in that the concentration of the substance in question is determined from the basic principles of chemistry, and no calibration curves are required.

- **Titration:** Volumetric determination of the amount of an acid or base by addition of a standard acid or base until neutralization has occurred
- **Analyte:** is solution whose concentration is to be determined.

- **Equivalent:** amount of the titrant just reacts with all analyte.
- **Indicator:** changes colour at end point of the titration.

To obtain valid results, it is important that measurements are precise and accurate. This can be achieved by using a standard procedure for carrying out a titration.

Method

1. Use a pipette and pipette filler to add 25 cm^3 of alkali solution to a clean conical flask.
2. Add a few drops of a suitable indicator and put the conical flask on a white tile.
3. Fill the burette with dilute acid. Flush the tap through to remove any air bubbles. Ensure the burette is vertical.
4. Slowly add the acid from the burette to the conical flask, swirling to mix. (The mixture may at first change colour, and then back again when swirled.)
5. Stop adding the acid when the end-point is reached (when the colour first permanently changes). Note the final volume reading.
6. Repeat steps 1 to 5 until three results are repeatable (in close agreement). Ideally these should lie within 0.10 cm^3 of each other.

Figure 8: Dilution of solution

Self-Check – 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Define what label? (2pts)
2. Write the elements of label? (2pts)

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

LG #80	LO-4: Apply Basic Laboratory Examination

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics:

- Caring out and processing samples
- Preparing smears of samples for subsequent staining to enable microscopic identification of germs
- Conducting direct microscopic faecal smear examination
- Conducting faecal sample concentration, staining and microscopic examination
- Conducting skin scraping samples are processed and gross/ microscopic examination
- Recording the results of the tests and feedback is given to the clients and/or target body

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, **upon completion of this learning guide, you will be able to**:

- Care out and processes samples
- Prepare smears of samples for subsequent staining to enable microscopic identification of germs
- Conduct direct microscopic faecal smear examination according to the laboratory procedure protocol and regulation
- Conduct faecal sample concentration, staining and microscopic examination
- Conduct skin scraping samples are processed and gross/ microscopic examination
- Record the results of the tests and feedback is given to the clients and/or target

body

Learning Instructions:

11. Read the specific objectives of this Learning Guide.

12. Follow the instructions described below.

13. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.

14. Accomplish the “Self-checks” which are placed following all information sheets.

15. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).

16. If your performance is satisfactory proceed to the next learning guide.

Information Sheet – 1: Caring out and processing samples

1.1 Sample Processing and collect

After collecting the samples from the any animal or people (for research, clinical analysis, and production test), the samples have to be processed before any test can be done. Sample processing is a process done to a sample to prepare it for testing. During collecting sample, the first concerned are collecting aseptically and handling hygienically. This is an important step due to the fact that the sample that was used wasn't rejected. The accuracy of the results depends upon the purity of the sample assessed.

1.2 Handling Laboratory Specimens

All most clinical specimens are considered potentially infectious and must be handled carefully to prevent contamination. Consequently, there is no need to use "Caution" labels on specimens from patients with known infections. The accuracy of the results depends on care in collecting and transporting the specimen to the lab. The quality of the results influences the diagnosis and treatment and therefore the clinical outcome. The risk of the health care worker being exposed to an infectious agent or contaminating the health care environment depends on maintaining continuous infection control practices.

1.3 Collecting specimens

1. Gather personal protective equipment –depending on symptoms and history of the patient:
   - **Gloves**: when handling any body fluids or risk of contaminating hands.

- **Masks/respirators**: if respiratory symptoms or initiating a cough from the patient with specimen collection, aerosolized excretions, risk of splash or spray (those zoonotic diseased patient).
- **Goggles:** if risk of splash or spray to eyes

2. Care should be taken when collecting and handling specimens to avoid contamination of the outside of the container.
3. Secure lids tightly to prevent leakage.
4. Place the specimen(s) into a plastic, zip-lock type bag. Requisition should be outside the pouch that the specimen is shipped in.
5. Hand hygiene must be performed following any direct contact with blood or body fluids, after the handling or transporting of laboratory specimens and after glove removal.

1.4 Handling specimens

1. Always wear gloves and any other indicated barrier protection when collecting and handling laboratory specimens.
2. Place each laboratory specimen in an appropriate leak-proof primary container (e.g. vacutainer tube, specimen cup, etc.). Care should be taken when collecting and handling specimens to avoid contamination of the outside of the container.
3. Insert the requisition slip(s) into the outside pocket of the bag.
4. Seal the bag before transporting it to the laboratory.
5. If specimens require refrigeration, they should be stored in a separate fridge from vaccines, medication and food items.

Collection, handling and transport sample processing

Sample collection equipment may include universal bottles, beakers, plastic bottles and tubes, vacutainer tubes, scalpels, scissors, forceps, petridishes, syringe and needles, stomach tubes, gloves, swabs, catheters. Equipment that main used sample process in laboratory may such as Refrigerators, freezers, preservatives, funnel, packaging materials, hand lens, baerman apparatus, trapping net, mortar and pestle.

Self-Check – 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What sampling means? (2pts)
2. How we can collect aseptic sample from patient? 3(pts)
3. What is sample processes means?(4pts)
4. List equipment that used during sample collection and handling? (4pts).

Note: **Satisfactory rating - 7 points** **Unsatisfactory - below 6 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 2: Preparing samples smear for subsequent staining to enable microscopic identification of germs.

2.1 Staining Microscopic Specimens

Smear means to spread specimen (Blood, pus, feacal etc.) over a surface slide to identify an agent or normal physiological detection. The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure. A smear can be prepared from a solid or broth medium.

A. To stain, smudge, or dirty by or as if by **smearing**.
B. Sully, besmirch specifically
C. To vilify especially by secretly and maliciously spreading grave charges and imputations.
D. To obliterate, obscure, blur, blend, wipe out, or defeat by or as if by **smearing**.

In clinical settings, light microscopes are the most commonly used microscopes. There are two basic types of preparation used to view specimens with a light microscope:

a. **Wet smear**
b. **Fixed** specimens.

A. Wet smear

The simplest technique, in which the specimen is placed on the slide in a drop of liquid. Some specimens, such as a drop of blood, are already in a liquid form and can be deposited on the slide using a dropper. Solid specimens, such as a skin

scraping, can be placed on the slide before adding a drop of liquid to prepare the wet mount. Sometimes the liquid used is simply water, but often stains are added to enhance contrast. Once the liquid has been added to the slide, a coverslip is placed on top and the specimen is ready for examination under the microscope.

B. Fixed Smear

The “fixing” of a sample refers to the process of attaching cells to a slide. Fixation is often achieved either by heating (heat fixing) or chemically treating the specimen. In addition to attaching the specimen to the slide, fixation also kills microorganisms in the specimen, stopping their movement and metabolism while preserving the integrity of their cellular components for observation. To heat-fix a sample, a thin layer of the specimen is spread on the slide (called a smear), and the slide is then briefly heated over a heat source. Chemical fixatives are often preferable to heat for tissue specimens. Chemical agents such as:

- Acetic acid, ethanol
- Methanol
- Formaldehyde (formalin) and
- Glutaraldehyde can denature proteins, stop biochemical reactions, and stabilize cell structures in tissue samples.

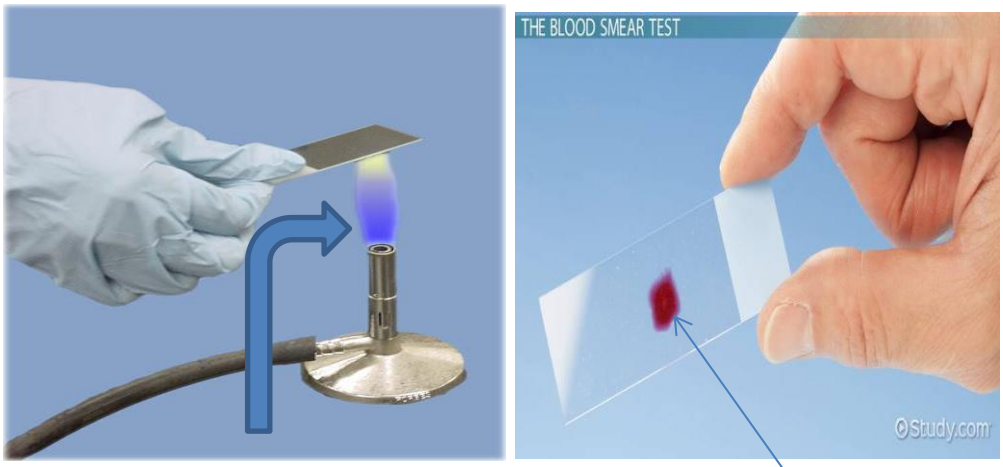


Figure 9: A= Heat- fixing staining smears B= Wet blood smear

In addition to fixation, staining is almost always applied to color certain features of a specimen before examining it under a light microscope. Stains, or dyes, contain salts made up of a positive ion and a negative ion. Depending on the type of dye, the positive or the negative ion may be the chromophore (the colored ion); the other, uncolored ion is called the counterion. If the chromophore is the positively charged ion, the stain is classified as a basic dye; if the negative ion is the chromophore, the stain is considered an acidic dye.

Dyes are selected for staining based on the chemical properties of the dye and the specimen being observed, which determine how the dye will interact with the specimen. In most cases, it is preferable to use a positive stain, a dye that will be absorbed by the cells or organisms being observed, adding color to objects of interest to make them stand out against the background. However, there are scenarios in which it is advantageous to use a negative stain, which is absorbed by the background but not by the cells or organisms in the specimen. Negative staining produces an outline or silhouette of the organisms against a colorful background.

Laboratory Procedure

General Instructions

A. Students work individually.
B. To sterilize an inoculating loop or needle insert the loop or needle into the Bacticinerator and observe it. It must glow red for three seconds to be sterilized. Loops and needles should never be propped in the Bacticinerator. The handles are aluminum and will melt. Also they conduct heat readily and can cause burns if the handles heat up. A hot loop or needle must cool slightly before touching a bacterial colony to prevent killing the cells.
C. To aseptically remove a lid from a bottle or tube, grasp the lid with the little finger of the dominant hand. Twist the bottle or tube to loosen and remove the lid. Do not put the lid on the table but keep it in your hand while removing material from the bottle or tube. Return the lid to the bottle or tube by turning the bottle or tube to tighten the lid.

Materials/Equipment: Clean glass slides**,** prepared cultures of Staphylococcus aureus and E. coli**,** Inoculating loop**,** Bacticinerator**,** Laboratory marker

Instructions

1. Glass slides should be relatively clean and grease free. Slides that do not appear clean may be washed in soap and water and dried with a paper towel. Label two slides across one end Staph. and two slides E. coli.
2. Work with one slide at a time. Sterilize an inoculating loop. Aseptically remove the lid from the water bottle and remove a loopful of water from the bottle. Return the lid to the bottle.
3. Tap the loopful of water onto the center of one of the labeled slides.
4. Sterilize the loop.

5. Obtain a slant culture of one of the organisms. Aseptically remove the lid. Insert
6. The sterile loop into the tube being careful not to touch the lip of the tube. Touch the loop to the surface of the agar. DO NOT scrape or dig into the agar. Remove the loop and return the lid to the tube.
7. Mix the material on the loop in the drop of water on the appropriately labeled slide. Spread the drop over the surface of the slide making a uniform preparation of bacteria and water. The thinner the smear the quicker it will dry.

7. Allow the smear to air dry.
8. Heat fixes the slide by passing it 10 times over the top of the Bacticinerator.
9. The slide is ready for staining. It may be stored until needed.
10. Repeat Steps 2-8 to make two smears of Staphylococcus aureus and two smears of E. coli. Store the slides in slide boxes for use in future lab exercises.

3.1 Examination of Samples Microscopically by Staining

Staining is a technique used to enhance contrast in samples, generally at the microscopic level. Stains and dyes are frequently used in histology (the study of tissue under the microscope) and in the veterinary fields of histopathology, hematology, and cytopathology that focus on the study and diagnoses of disease at a microscopic level. Stains may be used to define biological tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells), or organelles within individual cells.

A. Thin and thick smear

1. Prepare thin and thick blood smear

If you are using venous blood, blood smears should be prepared as soon as possible after collection (delay can result in changes in parasite morphology and staining characteristics). Thick smears are mainly used to detect infection and to estimate parasitemia. Thin smears allow the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms like schizonts and gametocytes. Thick smears

Thick smears consist of a thick layer of dehemoglobinized (lysed) red blood cells (RBCs). The blood elements (including parasites, if any) are more concentrated (app. 30×) than in an equal area of a thin smear. Thus, thick smears allow a more efficient detection of parasites (increased sensitivity). However, they do not permit an optimal review of parasite morphology. For example, they are often not adequate for species identification of malaria parasites: if the thick smear is positive for malaria parasites, the thin smear should be used for species identification.

Prepare smears

1. Place a small drop of blood in the center of the pre-cleaned, labeled slide.
2. Using the corner of another slide or an applicator stick spread the drop in a circular pattern until it is the size of a dime (1.5 cm2).
3. A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.
4. Lay the slides flat and allow the smears to dry thoroughly (protect from dust and insects!). Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made

with anticoagulated blood. At room temperature, drying can take several hours; 30 minutes is the minimum; in the latter case, handle the smear very delicately during staining. You can accelerate the drying by using a fan or hair dryer (uses cool setting). Protect thick smears from hot environments to prevent heat-fixing the smear.

5. Do not fix thick smears with methanol or heat. If there will be a delay in staining smears, dip the thick smear briefly in water to hemolyse the RBCs.

Step of Thin Blood Smear Prepare

1. Place a small drop of blood in the center of the pre-cleaned, labeled slide.
2. Take a small drop of blood sample.
3. Put it on the one end of a clean slide (If the slide is frosted placed the blood on the smooth glass near to the frosted end).
4. The slide should be hold firmly.
5. Take another clean slide placing on the first slide at 45o, behind on the drop of blood.
6. Move the angled slide along the first slide with a steady movement to forward direction. A quicker movement results a good smear.
7. Dry it in air.
8. Fix by methanol not less than 5 minutes or ethanol-ether (1:1) not less than 10 minutes, or ethanol not less than 15 minutes.
9. Allow to be dry the slide.
10. Stain with Giemsa solution for 30 minutes.
11. Wash with tap water.
12. Allow to be dry the slide.
13. Examine with oil immersion under high magnification power.
14. Result:
15. Positive ----------The parasite will be observed, if it is present.

16. Figure- Thin blood smears preparation

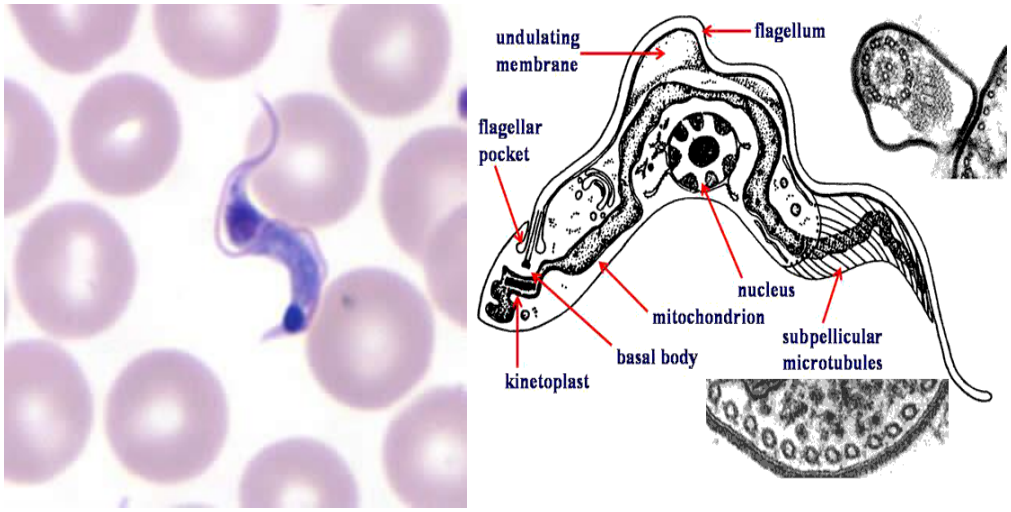


Figure 9: thin smear by giemsa staining

A. Direct blood films

Procedure

1. Place one drop of blood on a slide, add a droplet of physiological saline, mix and cover with a coverslip.
2. Examine directly under low power (10X) of a microscope for live microfilariae. Larvae can be immobilized by placing a drop of 10% formalin at the edge of the coverslip. This can also be used for detecting trypanosomes.

B. Indirect wet film

Drying and fixing of blood smears leads to some alteration of the morphology of intra erythrocytic parasites. The present technique overcomes the problem of biconcavity of erythrocytes so that intra erythrocytic parasites can be visualized because refraction of light induced by the biconcavity of bovine erthrocytes

makes it difficult to appreciate the morphology of intracellular, unstained parasites. In a hypotonic environment, an erythrocyte swells to its maximum and becomes a sphere and the parasites and associated structures become visible within the cells. Stained haemoglobin appears to mask intracellular inclusions associated with parasites (eg. *Theileria orientalis*), which are otherwise visible in the wet blood films.

Procedure

- A small drop of blood enough to give a single layer distribution of erythrocytes is taken on a glass slide and mixed with a smaller drop of water using a glass slide.
- A clean coverslip is gently placed over the preparation avoiding the formation of air bubbles.
- Excess blood is absorbed using a piece of blotting paper.
- The smear is then to be examined under oil immersion.
- Apart from ***Theileria***, ***Babesia*** and ***Anaplasma*** also can be detected in a live condition using this method.

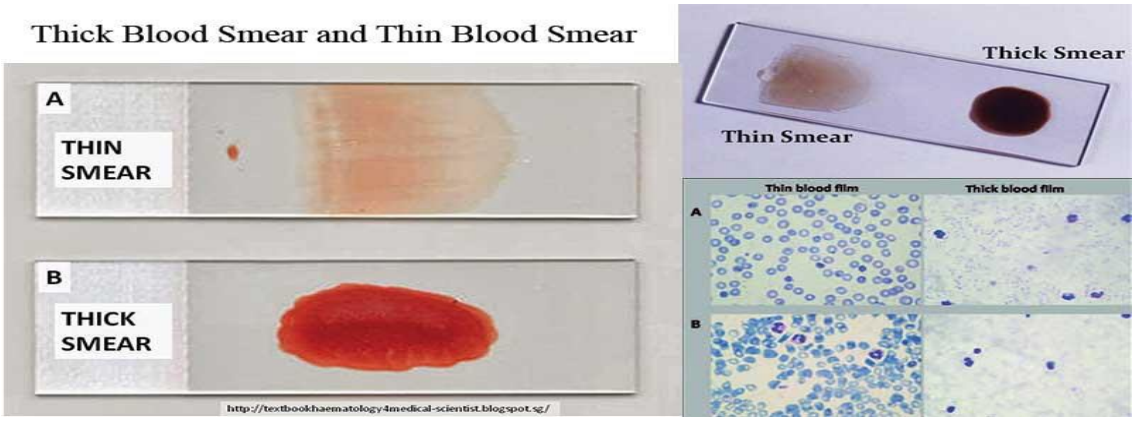


Figure 10: Differences between thin and thick

2. Gram staining

Gram staining differentiates the bacteria into Gram Positive and Gram Negative Bacteria, which helps in the classification and differentiations of microorganisms.

Principle of Gram Staining

When the bacteria is stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. The cell walls of gram positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cell causes this thick cell wall to dehydrate and shrink which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the Crystal Violet-Iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in colour.

Reagents Used in Gram Staining

- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counterstain

Procedure of Gram Staining

1. Take a clean, grease free slide.
2. Prepare the smear of suspension on the clean slide with a loopful of sample.
3. Air dry and heat fix
4. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.

5. Flood the gram's iodine for 1 minute and wash with water.
6. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
7. Add safranin for about 1 minute and wash with water.
8. Air dry, Blot dry and Observe under Microscope.

Interpretation

Gram Positive: **Blue/Purple Color**

Gram Negative: **Red Color**

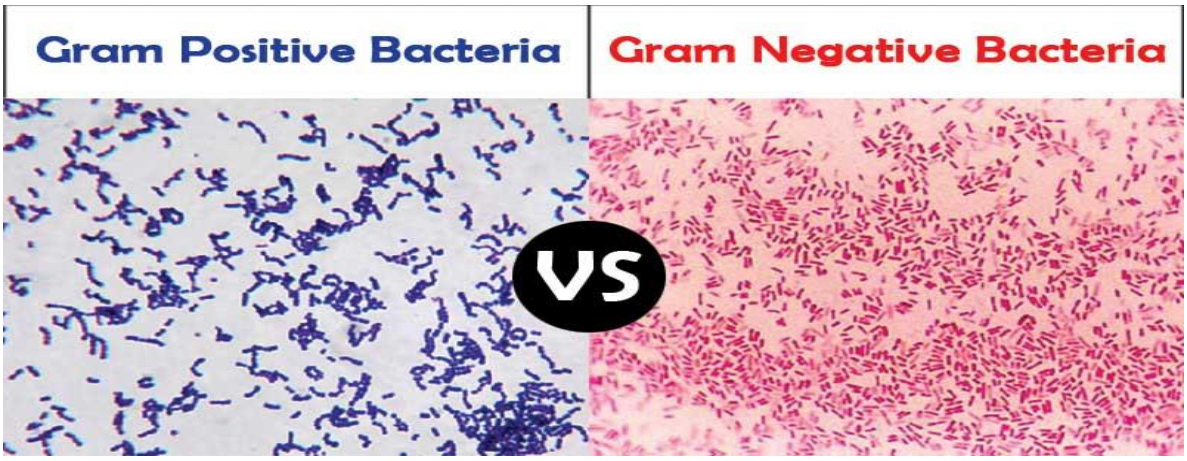


Figure 11: microscopic differentiation of gram positive and gram negative bacteria in lab.

3. Acid fast staining

It is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called *Ziehl-Neelsen staining* techniques. Neelsen in 1883 used Ziehl's carbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques were developed. The main aim of this staining is

to differentiate bacteria into acid fast group and non-acid fast groups. This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus *Mycobacterium,* are resistant and can only be visualized by acid-fast staining.

Principle of Acid-Fast Stain

When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appear blue while acid-fast cells retain the red color.

Table 2: Summary of acid fast and non- acid fast

Application of	Reagent	Cell colour	
		Acid fast	**Non-acid fast**
Primary dye	Carbol fuchsin	Red	Red
Decolorizer	Acid alcohol	Red	Colorless
Counter stain	Methylene blue	Red	Blue

Procedure of Acid-Fast Stain

1. Prepare bacterial smear on clean and grease free slide, using sterile technique.
2. Allow smear to air dry and then heat fix.
   - ✓ **Alcohol-fixation:** This is recommended when the smear has not been prepared from sodium hypochlorite (bleach) treated sputum and will not be stained immediately. M. tuberculosis is killed by bleach and during the staining process. Heat-fixation of untreated sputum will not kill M. tuberculosis whereas alcohol-fixation is bactericidal
3. Cover the smear with carbol fuchsin stain.
4. Heat the stain until vapour just begins to rise (i.e. about 60 C). Do not overheat. allow the heated stain to remain on the slide for 5 minutes.
   - ✓ **Heating the stain:** Great care must be taken when heating the carbol fuchsin especially if staining is carried out over a tray or other container in which highly fiammable chemicals have collected from previous staining. Only a small fiame should be applied under the slides using an ignited swab previously dampened with a few drops of acid alcohol or 70% v/v ethanol or methanol. Do not use a large ethanol soaked swab because this is a fire risk.
5. Wash off the stain with clean water.
   - ✓ *Note: When the tap water is not clean, wash the smear with filtered water or clean boiled rainwater.*
6. Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink.
   - ✓ *Caution: Acid alcohol is fiammable, therefore use it with care well away from an open fiame.*
7. Wash well with clean water.

8. Cover the smear with malachite green stain for 1–2 minutes, using the longer time when the smear is thin.
9. Wash off the stain with clean water.
10. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry (do not blot dry).
11. Examine the smear microscopically, using the 100 X oil immersion objective.

Interpretation of result

- **Acid fast: Bright red** to **intensive purple,** Red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded**.**
- **Non-acid fast: Blue color**

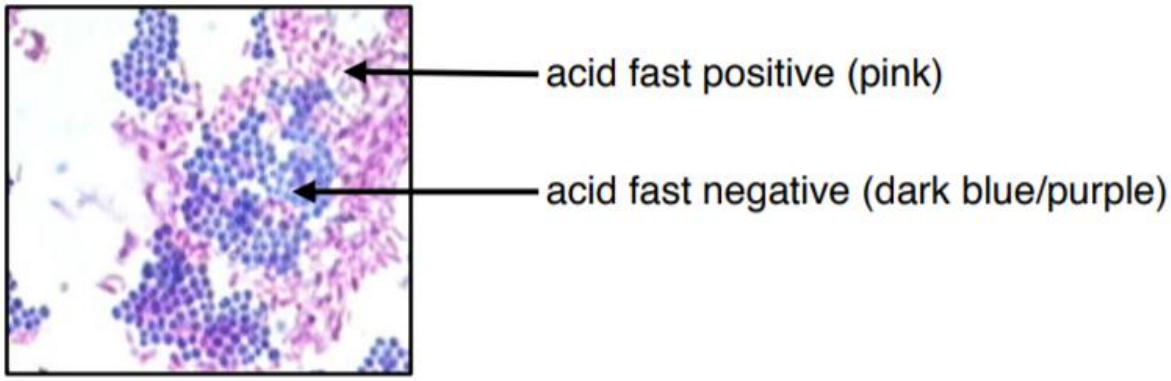


Figure 11: Acid fast and Non- acid fast techniques differentiation.

4. Capsule staining

The main purpose of capsule stain is to distinguish capsular material from the bacterial cell. A capsule is a gelatinous outer layer secreted by bacterial cell and that surrounds and adheres to the cell wall. Most capsules are composed of polysaccharides, but some are composed of polypeptides. The capsule differs from the slime layer that most bacterial cells produce in that it is a thick, detectable,

discrete layer outside the cell wall. The capsule stain employs an acidic stain and a basic stain to detect capsule production.

Principle of Capsule Staining

Capsules stain very poorly with reagents used in simple staining and a capsule stain can be, depending on the method, a misnomer because the capsule may or may not be stained. Negative staining methods contrast a translucent, darker colored, background with stained cells but an unstained capsule. The background is formed with india ink or nigrosin or congo red. India ink is difficult to obtain nowadays; however, nigrosin is easily acquired.

A positive capsule stain requires a mordant that precipitates the capsule. By counterstaining with dyes like crystal violet or methylene blue, bacterial cell wall takes up the dye. Capsules appear colourless with stained cells against dark background. Capsules are fragile and can be diminished, desiccated, distorted, or destroyed by heating. A drop of serum can be used during smearing to enhance the size of the capsule and make it more easily observed with a typical compound light microscope.

Reagents used for Capsule Staining

- Crystal Violet (1%)
  - ✓ Crystal Violet (85% dye content) = 1 gm
  - ✓ Distilled Water = 100 ml
- Nigrosin
  - ✓ Nigrosine, water soluble = 10 gm
  - ✓ Distilled Water = 100 ml

Procedure of Capsule Staining

1. Place a small drop of a **negative stain** (India Ink, Congo Red, Nigrosin, or Eosin) on the slide.
   - ✓ **Congo red** is easier to see, but it does not work well with some strains. **India ink** generally works, but it has tiny particles that display Brownian motion that must be differentiated from your bacteria.
   - ✓ **Nigrosin** may need to be kept very thin or diluted.
2. Using sterile technique, add a loopful of bacterial culture to slide, smearing it in the dye.
3. Use the other slide to drag the ink-cell mixture into a thin film along the first slide and let stand for 5-7 minutes.
4. Allow to air dry (do not heat fix).
5. Flood the smear with crystal violet stain (this will stain the cells but not the capsules) for about 1 minutes. Drain the crystal violet by tilting the slide at a 45 degree angle and let stain run off until it air dries.
6. Examine the smear microscopically (100X) for the presence of encapsulated cells as indicated by clear zones surrounding the cells.

Interpretation

- **Capsule:** Clear halos zone against dark background.
- **No Capsule:** No Clear halos zone.

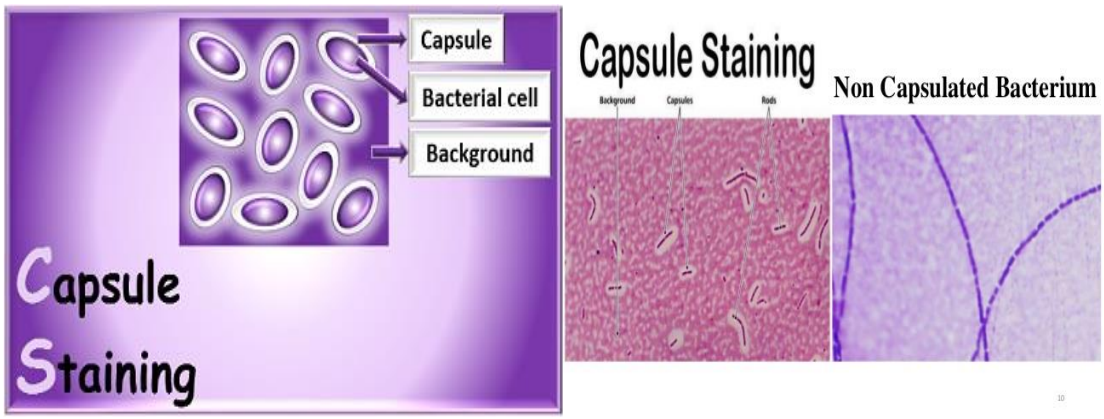


Figure 12: Capsule differentation by capsule staining

5. Endospore staining

The main purpose of endospore staining is to differentiate bacterial spores from other vegetative cells and to differentiate spore formers from non-spore formers.

Principle of Endospore Staining

Bacterial endospores are metabolically inactive, highly resistant structures produced by some bacteria as a defensive strategy against unfavorable environmental conditions. The bacteria can remain in this suspended state until conditions become favorable and they can germinate and return to their vegetative state. In the Schaeffer-Fulton`s method, a primary stain-malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolorized with water.

Safranin is then applied to counterstain any cells which have been decolorized. At the end of the staining process, vegetative cells will be pink, and endospores will be dark green. Spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell. Spore shape may also be of diagnostic use. Spores may be spherical or elliptical.

Reagents used for Endospore Staining

1. Primary Stain: Malachite green (0.5%wt/vol) aqueous solution
   - 0.5 gm of malachite green
   - 100 ml of distilled water
2. Decolorizing agent
   - Tap water or Distilled water
3. Counter stain
   - Stock solution (2.5% wt/vol) alcoholic solution)
   - 2.5 gm of safranin
   - 100 ml of 95% ethanol

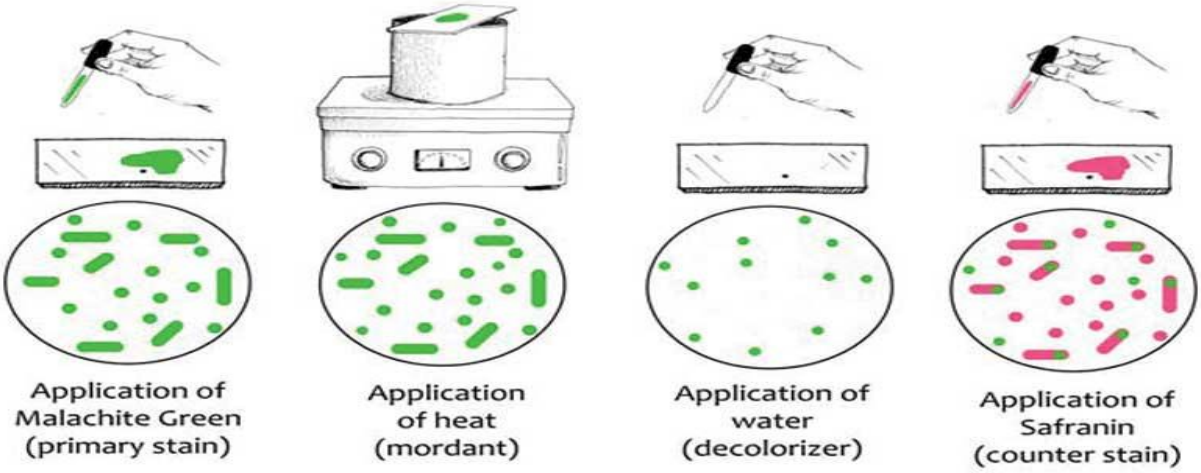
Procedure of Endospore Staining

1. Take a clean grease free slide and make smear using sterile technique.
2. Air dry and heat fix the organism on a glass slide and cover with a square of blotting paper or toweling cut to fit the slide.
3. Saturate the blotting paper with malachite green stain solution and steam for 5 minutes, keeping the paper moist and adding more dye as required. Alternatively, the slide may be steamed over a container of boiling water.
4. Wash the slide in tap water.
5. Counterstain with 0.5% safranin for 30 seconds. Wash with tap water; blot dry.
6. Examine the slide under microscope for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink.

Interpretation

- **Endospores:** Endospores are bright green**.**

- **Vegetative Cells:** Vegetative cells are brownish red to pink.



Self-Check – 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Write the chemical reagent the used to done endospore analysis? (3pts)
2. What staining means? (4pts)

Note: **Satisfactory rating - 4 points** **Unsatisfactory - below 4 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 3: Conduct direct microscopic faecal smear examination

3.1 Direct fecal smear examination

Direct fecal smear technique is the simplest and easiest technique to facilitate detection of intestinal parasites that infected subjects pass in their feces. The presence of intestinal protozoa (trophozoites or cysts) or helminth eggs can be observed directly with a light microscope. A small amount of fresh feces is mixed with either saline (to detect the protozoa motility) or lugol/iodine solution (to reveal the parasite structure).

Materials and samples: Wooden applicator sticks/Swab stick**,** Object glass**,** Cover-slip**,** Pen/marker for indelible labeling**,** Isotonic saline solution (0.85%; 8.5g/l)**,** Lugol's iodine (1% solution)**,** Pipettes**,** Microscopic slide**,** Light microscope

Procedure

1. Place a drop of saline on the centre of thr half of slide and a drop of lugol's solution on the centre of the right half of the slide on a microscope.
2. With a wooden applicator stick or match, pick up a small portion of the feacal specimen (Size of match head) and mix with a drop of saline to form suspension.
3. Similarly, pick up a small portion of the feacal specimen and mix with Lugol's solution to form suspension.
4. Place a coverslip over the smear.
5. The faecal material should not be left in a lump in the centre of the coverslip but evenly spread so that the microscope illumination can shine through.

Note: Faece may contain hazardous pathogens (bacteria, viruses etc.). Appropriate hygiene and safety procedures should be employed. Local health and safety regulations should be observed.

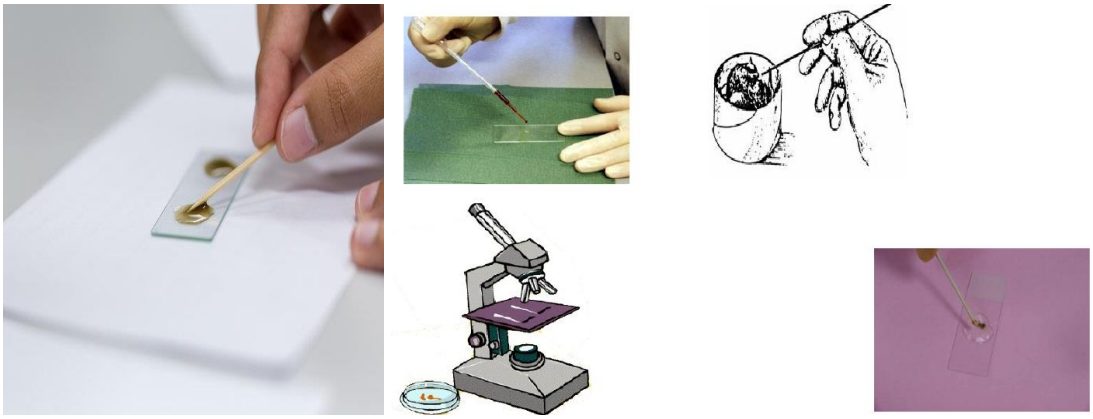


Figure 12: Feacal smear techniques

Self-Check – 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What is staining means? (8pts)
2. Write the equipment that basic in staining? (4pts)

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 4: Conduct faecal sample concentration, staining and gross and microscopic examination

4.1 Gross examination of feaces

The faeces are by far the most important excretion to be used in aid for diagnosis of internal parasitism. The examination of faeces will provide evidence or in some cause an accurate identification of most of the parasite that inhabits the GIT tract as well as certain parasite inhabiting respiratory tract. The advantage of examination of fecal sample is simple and less costly, and its disadvantage is certain parasites produce similar eggs; oocysts cannot be identified to the spps level limitation of fecal examination in diagnosis of helminth parasites such as the demonstration of parasite eggs or larvae in feces provide evidence the animal is infected but does not indicate degree of infection. For parasitological examination objective lenses magnification power of 4x, 10x, 40x are most often used, The faecal examination can be of two types:

1. Macroscopic examination
2. Microscopic examination

In macroscopic examination: faecal sample is taken and put on Petri dish, and can be examined by naked eye. The visual identification of the sample consists on the form, consistency, color and presence of blood or mucus. It is also possible to see different worms, immature stages of trematodes, tape worm segments and nematodes. When the animals have abnormal consistency in faeces you can determine the pathology. In cases where faeces are hard, you can make sure that the animal has constipation which you should research for the reason. On the other hand, if the faeces are soft you can determine the animal has diarrhea. In both cases the disease may be originated from parasites, bacterial infection or

associated with alimentary problem. The color of the faeces is generally dark depending on the alimentation. Even though, the color of the faeces can change if the animal has any pathology, and it can also produce some bad smell. Abnormal constituents in faeces like blood, mucus and epithelial tissues reveal different intestinal disease conditions.

- **Gross parasite:** spontaneously discharge tape worms and nematodes can be recognized by direct microscope inspection of the faeces, or naked eye. Probably the most common are proglottids of tape worms, entire round worm or even larval arthropods. Their morphology, shape, size and movement may aid identification.
- **Consistency:** the condition of faeces; that is weather the faeces are soft, watery (diarrhea) very hard or constipation should be noted. This description will vary with animal species.
- **Color:** light gray color faeces-indicates excusive fat in the faeces is a sign of poor intestinal absorption. Unusual fecal colors should always be reported
- **Blood:** may indicate sever parasitism as well as other intestinal diseases
- **Mucus:** may be associated with intestinal parasitism or some metabolic disease
- **Age of the faeces:** if the faeces appear old and dry this should be noted. An aged samples, parasitic eggs may be embryonated or larvated, Oocyst may have sporulated and pseudo parasite may be present.

Microscopic examinations: includes

1. Qualitative examination.
2. Quantitative examination

Qualitative examination is based on direct smear and concentration methods; on the other hand the Quantitative examination is based on obtaining accurate information regarding the severity of infection.

Qualitative Examination

It is used to know wheatear an animal is infected or not or presence or absence of infection. Common qualitative fecal examination techniques include: Direct smear, Sedimentation and Flotation techniques, which are used for qualitative fecal examination techniques.

4.2 Microscopic examination of fecal staining

The microscopic examination of the feacal specimen, normally called the ova and parasite examination, consists of three separate techniques: the direct wet smear, the concentration (sedimentation and flotation), and the permanent stained smear (Barmean techniques and permanent stained).

Feacal concentration procedure separate parasites from fecal debris and increase the chances of detecting parasitic organisms when these are in small numbers. They are divided into flotation techniques and sedimentation techniques.

1. **Floatation Techniques**

This techniques is effectively used for demonstration of Cestodes (except Diphillobothrium) and nematode eggs, using some flotation solutions, the most commonly used are NaCl, Sodium Nitrate, Magnesium Sulphate or Sugar sol. Most parasitic stages float efficiently at a specific gravity of 1.2 to 1.3.

Principle: this method is based on the determination of worm eggs, mixing the faeces with a solution with a specific gravity higher than that of the eggs than can

be present in the faeces; the eggs float congregated at the surface of the suspension.

Materials:- Microscope, Centrifuge, Balance, Hand gloves, Petri dish or sample container, Slide, Sieve, Cover slip, Mortar and pestle, Beaker, Centrifuge tube, Glass rod or wire or plastic loop, Teaspoon.

Procedure

1. Take approximately 3 gm of fecal sample
2. If the feces is pelleted grind it using pistle and mortar.
3. Add saturated floatation fluid (30-50 ml) and mix thoroughly.
4. Pour the fecal suspension through a tea strainer (sieve) to remove large fecal debris (you can use double layers of gauze).
5. Pour the suspension into a test tube until a convex meniscus is formed.
6. Cover with a cover slip/slide. Avoid trapping air bubbles.
7. Allow it to stand 10-15 minutes
8. The cover slip is removed, by lifting it vertically with a deliberate movement and placed on a microscope slide or one drop is taken using a loop from the supernatant of the centrifuge and spread over the slid.
9. Examine under microscope

Result: egg of nematode or cestode or oocyst.

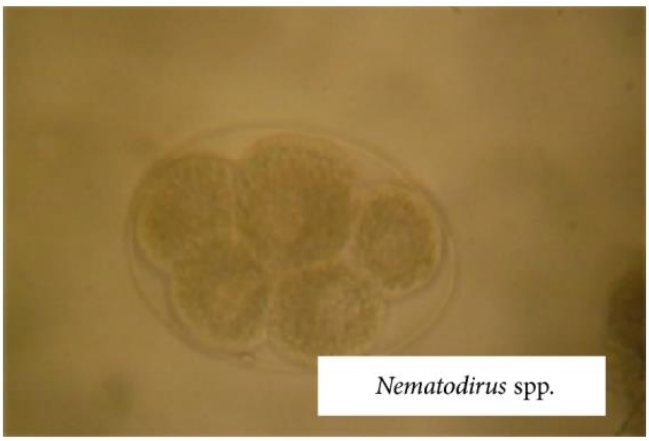


Figure 13: eggs of nematodes identified

Flotation by centrifugation

Procedure:

1. Take 3-5g of faecal sample or a tea spoon.
2. Dissolve the faecal sample with 15ml of the flotation solution.
3. Sieve the faecal suspension through a tea strainer.
4. Add it in to a centrifuge tube
5. Centrifuge with 1500rpm for 3-5 minute.
6. Smear a small drop from the supernatant on the slide.
7. Cover with cover slip.
8. Examine under microscope at low power objectives.

Result interpretation

- Positive = presence of eggs will be observed
- Negative = no eggs

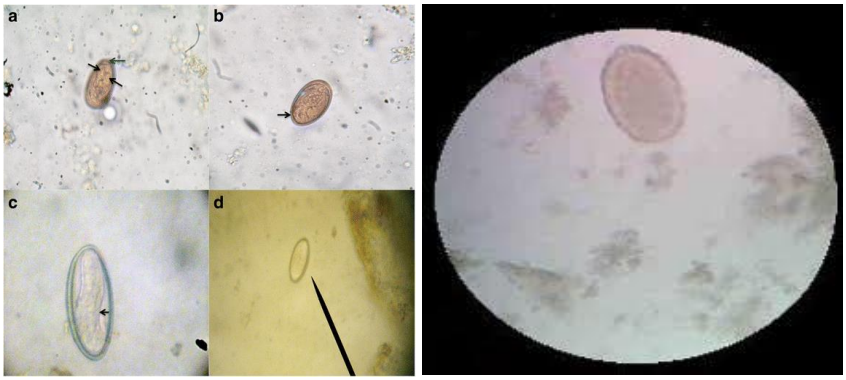


Figure 13: eggs of nematodes collected from bovine feacal by floatation

Note: It would be necessary to revise at least 3 fields for accurate results and to conclude that the result is positive. For a negative diagnosis instead it is required to revise the whole preparation.

2. Sedimentation techniques

Sedimentation techniques use solutions of lower specific gravity than the parasitic organisms, thus concentrating the latter in the sediment. Sedimentation techniques are recommended for general diagnostic laboratories because they are easier to perform and less prone to technical errors.

Principle: This method is based in the determination of trematode eggs, mixing the feces with tap water specific gravity lower than that of the eggs than can be present in the feces; the eggs sediment and congregate at the bottom of the suspension.

M**aterials**:- Microscope, Centrifuge, Balance, Hand gloves, Petri dish or sample container, Slide, Sieve, Cover slip, Mortar and pestle, Beaker, Centrifuge tube, Glass rod or wire or plastic loop, Teaspoon.

Procedure:

1. Take 3-5g of faecal sample or 5-6 faecal balls or a teaspoon
2. Comminute the faecal sample with pestle and mortar
3. Emulsify with water (30-45ml)
4. Sieve through strainer
5. Pour the suspension in to a test tube
6. Allow to stand the test tube for 20 minutes
7. Centrifuge at 1500 rpm for 3 minutes
8. Decant the supernatant
9. Repeat the above procedure until the supernatant is clear
10. Take a small drop and put on the slide

Result interpretation

- Positive = Presence of eggs will be observed
- Negative = no eggs

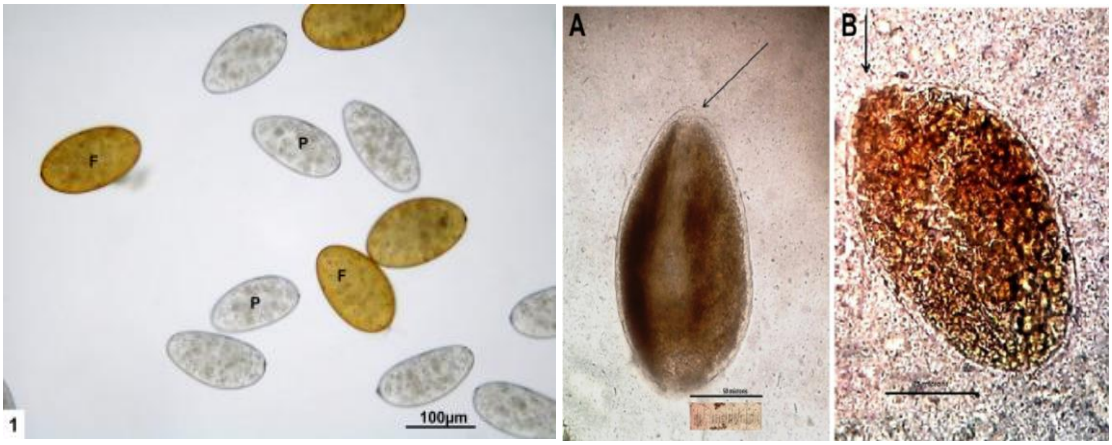


Figure 14: fasciola identified from sheep by sedimentation techniques

Note: It would be necessary to revise at least 3 fields for accurate results and to conclude that the result is positive. For a negative diagnosis instead it is required to revise the whole preparation.

3. Barman Techniques

Barman examination is used to isolate larvae from fecal samples and is used mostly to diagnosis lung worm infections. It is used to recover the larvae of nematodes from feces, soils or animal tissues.

Principle: The barman technique is used to isolate lung warm larvae from fecal samples and infective larvae from fecal cultures. It is based on the active migration of larvae from feces suspended in water their subsequent collection and identification.

Procedure

1. Take 3-5g of faecal sample or a tea spoon.
2. Wrap the faeces between two layers of gauze.
3. Put into a glass funnel or into a conical sedimentation glass.
4. Add warm water (25^0C - 40^0C) until the faeces are submerged that permit to cover the sample.
5. Keep it to stand for 12-16 hours or overnight.
6. Take the sediment with a pipette and put it on the Petri dish.
7. Examine under microscope at low magnification.

Result interpretation

- Positive: larval presence will be observed
- Negative: no larva.

Figure 15: larvae and egg of lung worm gained from dog by barmean techniques

Note: It would be necessary to revise at least 3 fields for accurate results and to conclude that the result is positive. For a negative diagnosis instead it is required to revise the whole preparation.

4. McMaster technique

It is used to determine the number of eggs present per gram of feces /EPG/. The McMaster slide consist of two glass or plastic slides join together between the mark areas of the upper and bottom slide two chambers of 0.15 volume each are formed. The chambers are filled with a suspension of feces in the flotation. The nematode and cestode eggs float to immediately below the upper glass of the chamber where they can be readily counted under the microscope, while most fecal debris sinks to the bottom.

Material: - Sensitive Balance, Flask, Beaker, stir, Sieve, Funnel, Tea strainer, cheesecloth, Measuring cylinder, Stirring device, Pasteur pipettes and rubber teats, McMaster counting chamber, Compound microscope.

Procedure

5. Mix directly 4 gm of feces with 56 ml of flotation fluid in beaker 1 with stirring device.
6. Filter the fecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
7. Take a sub-sample with a Pasteur pipette and fill both sides of the Mc Master slide.
8. Allow the counting chamber to stand for 5 minutes is important as it allows time for eggs to float in the chamber.
9. Examine the sub-sample of the filtrate under a microscope at 10 x 10 magnifications.
10. Count all eggs and coccidia oocytes within the engraved area of both chambers.
11. The number of eggs per gram of feces can be calculated as follows:
12. Add the egg counts of the two chambers together.
13. Multiply the total by 50.
14. This gives the e.p.g. of feces.
15. Example: 13 eggs seen in chamber 1 and 16 eggs seen in chamber 2 = (13 + 16) x 50 = 1450 e.p.g.
16. OR count the total no. of eggs in one chamber and multiply by 100 to get the e.p.g of feces.

6. Permanent stained smear

It is generally recognized that stained fecal films are the single most productive means of feacal examination for intestinal protozoa. The permanent stained smear facilitates detection and identification of cysts and trophozoites and affords a permanent record of the protozoa encountered. Small protozoa, missed by wet

mount examinations (of either unconcentrated or concentrated samples) are often seen on the stained smear. The Wheatley Trichrome technique for fecal specimens is a modification of Gomori's original staining procedure for tissue. It is a rapid, simple procedure, which produces uniformly well-stained smears of the intestinal protozoa, animal cells, yeast, and artifact material.

Material: - Ethanol, slide, cover slip, Acitic acid, Microscope, Oil immersion

Procedure:

1. For PVA smears, place the slide in 70% ethanol plus iodine for 10 minutes. For other fixatives, follow the manufacturer's instructions. Omit the iodine step for preservatives that do not contain mercuric chloride.
2. Place slide in 70% Ethanol for 5 minutes.
3. Place in second 70% Ethanol for 3 minutes
4. Place in Trichrome stain for 10 minutes.
5. De-stain in 90% ethanol plus acetic acid for 1 to 3 seconds.
6. Rinse several times in 100% ethanol.
7. Place in two changes of 100% ethanol for 3 minutes each.
8. Place in two changes of xylene or xylene substitute for 10 minutes.
9. Mount with coverslip using mounting medium (e.g., permount).
10. Examine the smear microscopically utilizing the 100× objective. Examine at least 200 to 300 oil immersion fields.

Self-Check – 4	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Write the types of feacal examination macroscopically? (2pts)
2. What are differences between floatation and sedimentation? (3pts)

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 5: Conduct skin scraping samples are processed and gross/ microscopic examination

5.1 Conduct microscopic examination of skin scraping

A skin scraping is a collected sample of skin cells that are evaluated under a microscope. Skin scraping tests are typically performed to aid in diagnosing certain skin inflammations, fungal infections, and skin cancer, along with being an effective way of determining the presence of mites. This technique is commonly used to identify mites such as Sarcoptes, Demodex, and Cheyletiella.

Material: glass slides**,** sterile scalpel**,** mineral oil**,** sterile screwcap cup, 4 oz. (must be large enough to hold the glass slides)**,** applicator sticks**,** scotch tape

Skin Scraping Technique:

1. Place a drop of mineral oil on a sterile scalpel blade. Mites will adhere to the oil and skin scales will mix with the oil. The refractivity differences will be greater between the mite and the oil.
2. Allow some of the oil to flow onto the papule.
3. Scrape vigorously six or seven times to remove the top of the papule. (There should be tiny flecks of blood in the oil.)
4. Transfer the oil and scraped material to a glass slide (an applicator stick can be used).
5. Add 1 or 2 extra drops of mineral oil to the slide and stir the mixture. Any large clumps can be crushed to expose hidden mites.
6. Place another slide on top of the slide with the material and tape the 2 slides together at each end to prevent them from coming apart.

7. Place in a sterile cup large enough to hold the slides so that the lid can be screwed on firmly.
8. Label the container with the appropriate patient information.
9. Fill out a requisition and request: skin scraping to r/o scabies.
10. Submit to laboratory for examination.

Figure 17: Skin scraping

Self-Check – 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Write the chemical and equipment that used in skin scraping? (4pts)
2. what is skin scraping means?(2pts)

Note: **Satisfactory rating - 14 points** **Unsatisfactory - below 12 points**

You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____

Rating: _____

Information Sheet – 6: Recording the results of the tests and feedback is given to the clients and/or target body

6.1 Record and report the client's results

The main purpose of the world health record is to provide policymakers, donor agencies, international organizations and others with the information they need to help them make appropriate health policy and funding decisions.

6.2 Clinical and client records

Clinical and client records should include

- details laboratory examination
- treatment administered, procedures undertaken
- medication prescribed and/or supplied
- the results of any diagnostic or laboratory tests (including, for example, radiograph, ultrasound or electrocardiogram images or scans)
- Provisional or confirmed diagnoses, and advice given to the client owner (whether over the telephone or in person).
- They should also include outline plans for future treatment or investigations, details of proposed follow-up care or advice, notes of telephone conversations, fee estimates or quotations, consents given or withheld, contact details and any recommendations or discussion about referral or re-direction.
- The utmost care is essential in writing records or recording a client's personal details to ensure that they are clear, legible, accurate and appropriately detailed.

Clinical and client records should be objective and factual, and veterinary surgeons and veterinary nurses should avoid making personal observations or assumptions about a client's motivation, financial circumstances or other matters. Ideally, client financial information and any other personal or sensitive information should be recorded separately from clinical records. Explicit consent may be required in order to record and use certain personal or special category data (previously known as sensitive personal data) about a client, such as any special needs of the client or other health information. It may be permissible to mark the client record to indicate that the client is aggressive, violent etc, without client consent, on the basis that an employer has a legitimate interest to record such information so as to afford protection to their employees.

Tips on Writing a Report on Health Care Quality for clients

Information on animal health care quality is complicated, so it's crucial to present this information as simply and clearly as possible. This section suggests ways to make the language in your printed or Web-based quality report cards easier for your intended audience to understand and use.

- Why good writing
- write that's easy for your audience to understand
- Be concise and well organized
- make it easy to skim
- use devices that engage your reader
- Make the report culturally appropriate
- Be cautious about using readability formulas
- test the report with your audience

Self-Check – 6	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Write the method of report to client's the laboratory result? (4pts).
2. Write the main concerned when report the lab result to clients? (2pts).
3. List the main elements of client result report? (2pts).

Note: **Satisfactory rating - 4 points** **Unsatisfactory - below 4 points**

You can ask you teacher for the copy of the correct answers.

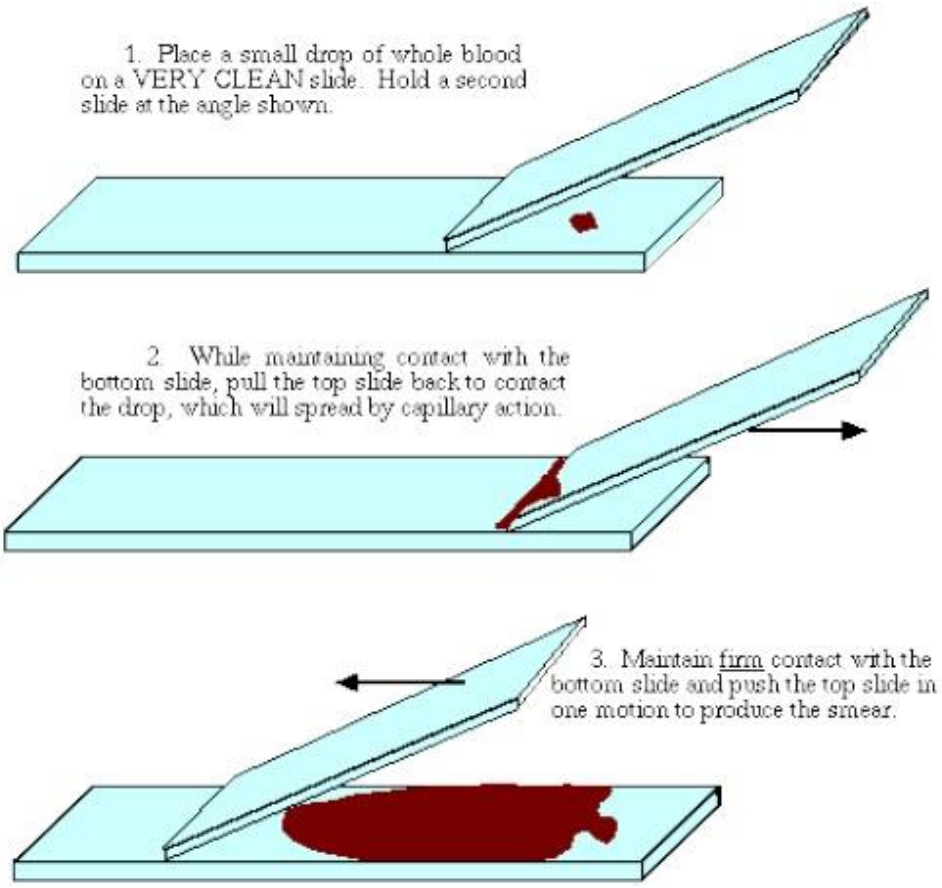
Answer Sheet

Score = _____

Rating: _____

Operation Sheet 1- Prepare blood smear

1. Place clean glass slide on a flat surface. Add one small drop of blood to one end.
2. Take another clean slide, and holding at an angle of about 45 degree, touch the blood with one end of the slide so the blood runs along the edge of the slide by capillary action. Push carefully along the length of the first slide to produce a thin smear of blood.
3. Make 2 smears, allow to air dry, and label clearly. Once dried place in the provided slide transport containers.



Operation Sheet 2- Gram Staining

Purpose: To differentiate gram positive and gram negative

Step:

1. Cover the smear with crystal violet for 1 min.
2. Wash with clean water and drain
3. Cover the smear with Grams Iodine (mordant) for 1 min.

4. Wash with clean water and drain
5. Decolorized the smear for 15 seconds with 95% ethanol, acetone or acetone-alcohol.
6. Wash immediately with clean water
7. Counter the stain the smear with dilute carbol fuchsine for 1 min.
8. Wash with clean water.
9. Allow to dry and examining under immersion oil.

Operation Sheet 3- Sedimentation techniques

Purpose: Isolate larvae or egg of parasite from feacal sample.

Steps:

1. Take 3-5g of faecal sample or 5-6 faecal balls or a teaspoon
2. Comminute the faecal sample with pestle and mortar
3. Emulsify with water (30-45ml)
4. Sieve through strainer
5. Pour the suspension in to a test tube
6. Allow to stand the test tube for 20 minutes
7. Centrifuge at 1500 rpm for 3 minutes
8. Decant the supernatant
9. Repeat the above procedure until the supernatant is clear
10. Take a small drop and put on the slide

LAP TEST	Performance Test

Name…… ID……

Date……

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within **5** hour. The project is expected from each student to do it.

Task-1: Perform Prepare blood smear

Task-2: Perform gram staining

Task -3: perform Sedimentation

AKNOWLEDGEMENT

We wish to extend thanks and appreciation to the many representatives of TVET instructors and respective industry experts who donated their time and expertise to the development of this Teaching, Training and Learning Materials (TTLM).

We would like also to express our appreciation to the TVET instructors and respective industry experts of Oromia Regional State TVET Bureau, Holeta Poly Technic College and the World Bank who made the development of this Teaching, Training and Learning Materials (TTLM) with required standards and quality possible.

This Teaching, Training and Learning Materials (TTLM) was developed on June 2021 at Adama, PAN-AFRICA Hotel.

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