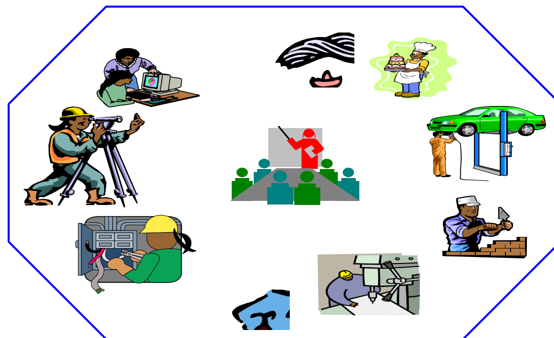
Animal Health Care Service

Level- IV

Based on March 2018, Version 3 Occupational Standards



Module Title: Conducting Basic Laboratory Techniques and Procedures

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LG # 54	LO # 1- Follow OHS practices and assist in work place hazard identification and risk control

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 according to OHS procedure
- Collecting specimens from domestic animals
- Handling specimens to minimise the spread of pathogens
- Recognising and reporting Hazards in the workplace
- Following workplace procedures and work instructions
- Recognizing and taking action to eliminate risks to self, bystanders, the public and animals

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 according to OHS procedure
- Collect specimens from domestic animals
- Handle specimens to minimise the spread of pathogens
- Recognize and reporting Hazards in the workplace
- Follow workplace procedures and work instructions
- Recognize and take action to eliminate risks to self, bystanders, the public and animals

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- Maintaining personal hygiene and cleanliness standards according to OHS procedure

1.1 Introduction

Hygiene generally refers to the set of practices associated with the preservation of health and healthy living. What is personal hygiene? Personal hygiene are practices performed by an individual to care for one's bodily health and well being through cleanliness. Many people equate hygiene with 'cleanliness' but hygiene is a broad term including personal habits choices as how frequently to bath, wash hands, trim fingernails and change clothing. Also includes keeping the environment clean and pathogen free. Personal hygiene is a concept that is commonly used in medical and public health practices. It is also widely practised at the individual level and at home. Personal hygiene is personal, as its name implies. In this regard, personal hygiene is defined as a condition promoting sanitary practices to the self. Generally, the practice of personal hygiene is employed to prevent or minimise the incidence and spread of communicable diseases.

Difference between cleanliness and hygiene is: the term cleanliness should not be used in place of hygiene. Cleaning in many cases is removing dirt, wastes or unwanted things from the surface of objects using detergents and necessary equipment. Hygiene practice focuses on the prevention of diseases through the use of cleaning as one of several inputs. Sanitation means the prevention of human contact with wastes, for hygienic purposes. It also means promoting health through the prevention of human contact with the hazards associated with the lack of healthy food, clean water and healthful housing, the control of vectors (living organisms that transmit diseases), and a clean environment. It focuses on management of waste produced by human activities.

1.2. Maintaining personal hygiene and cleanliness standards in laboratory

Laboratory is a place that provides facility and controlled conditions for carrying out scientific experiments and research. Personal hygiene in the laboratory is directed mainly towards the prevention of occupational acquired disease or physical injury. The following guidelines are standard operating procedures.

- Follow instruction and/or instructor
- Wear proper PPE
- Confine long hair
- Avoid wearing dangling jewelry in the lab.
- Do not eat, drink, smoke, chew gum, apply cosmetics, or take medications in the laboratory
- Do not use laboratory equipment for food preparation and empty food containers for laboratory materials or samples.
- Keep hands away from eye, nose, mouth, face , hair and open skin wound
- Clean all spills immediately and remove broken glassware
- Use books and journals only in clean area to prevent contamination
- Avoid excessive noise(example radio, TV and DVD player)
- Never taste any laboratory chemicals
- Know location of emergency equipment
- Label all containers with chemicals or solutions
- Dispose of waste chemicals in space provided
- Follow Material Safety Data Sheet (MSDS): that means information bulletin of a chemical and reagents that describes properties, healthy hazards, and routes of exposure, precautions for safe handling, emergency first aid and control measures.

Self-check 1	Written test

Name…………………………………………… ID………………………… Date…….

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

Short Answer Questions

1. Give the definition of personal hygiene (1 point)
2. Explain the difference between personal hygiene and cleanliness standards (2 points)
3. Mention some of the standard guidelines that should be followed by laboratory workers to maintain their personal hygiene and cleanliness (3points)
4. What is Material Safety Data Sheet (MSDS)? (2 points)

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

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 2- Collecting specimens from domestic animals

2.1 Introduction

The term “specimen” is very commonly used in the laboratory to indicate a sample taken from the human and animal body as well as from environment. The use of animals in research and teaching is a privilege granted to institutions, investigators, staff, and students that commit to meeting high ethical and regulatory standards. Uncontrolled adverse effects on animal wellbeing directly affect the validity of research results and the number of animals used to achieve a scientific objective. Therefore, an animal’s potential to experience pain and distress while it is being used for scientific purposes has ethical, scientific and practical implications. Concern about the wellbeing of animals used for scientific purposes, and the perception of the levels of pain and distress endured by such animals, have been translated into laws and regulations that seek to limit pain and distress in animals. Animals can inflict serious injuries to humans and to themselves as a result of improper handling.

- Handle animals gently. Do not make loud noises or sudden movements.
- Handle animals firmly. The animal will struggle more if it sees a chance to escape.
- Use an assistant whenever possible.
- Use restraint devices to assist when appropriate.
- Chemical restraint should be considered for any prolonged or potentially painful procedure.

Laboratory investigation of animal disease is critically dependent on the quality and appropriateness of the specimens collected for analysis. This chapter sets out the general standards involved in specimen collection, submission, and storage. Specimens must be collected using appropriate biosafety and containment measures in order to prevent contamination of the environment, animal handlers, and individuals doing the sampling as well as to prevent cross-contamination of the specimens themselves. Care should additionally be taken to avoid undue stress or injury to the animal and physical

danger to those handling the animal. Biological materials should be packaged to rigorously control for leakage, and then labeled.

2.2 Guiding Principles for Blood Sample Collection

Blood is collected from animals for a variety of scientific reasons. Whole blood samples may be collected for hematology, clinical chemistry, toxicology, direct examination for bacteria or parasites, PCR testing, immunological testing, or for culture for bacteria or viruses. Dependent on testing needs, whole blood, blood cells, and/or plasma samples can be obtained from whole blood collected into appropriate anticoagulants. In selecting the anticoagulant to be used the collector must be aware of the laboratory tests, including PCR-based diagnostics, clinical chemistry, and toxicology, which may be negatively affected by the presence of specific anticoagulants or preservatives.

The choice of blood collection method should be selected in the most humane and efficient way so that any pain, discomfort, or distress is kept to a minimum while adequately fulfilling the needs of the study design. Factors to consider in choosing the method of blood collection include the size of the animal, the type of sample needed, the quantity of blood required, the frequency of sampling, use of anesthetics, the health status of the animal, and the experience of the personnel.

Manual restraint or sedation/general anaesthesia is required depending upon blood volume collection and species. General guidelines are as follows:

- Dog/Cat/Pig: manual restraint for small volumes, general anaesthesia for large volumes
- Ruminants and horses: manual restraint for small volumes, general anaesthesia for large volumes

Table 1: Blood sampling sites by species

Species	Recommended Sampling Location
Cat	Cephalic vein, jugular vein
Dog	Cephalic vein, jugular vein, saphenous vein
Pig	Jugular vein, cranial vena cava
Cattle	Tail vein, jugular vein
Sheep/Goat	Jugular vein
Horse	Jugular vein
Poultry	Wing vein

2.2.1 Procedures of blood sample collection from various species of domestic animals

Personal protective equipment (gloves, lab coat) must be worn at all times. Care must be taken to restrain animals properly and prevent injury, particularly needle sticks.

a. Dog and cat

Handling (restraining methods) during blood sample collection from jugular vein

a. Two people are needed for this procedure – one for restraint and raising the vein and the other for taking the blood sample.
b. Assemble all of the equipment. Attach appropriate sized syringe to a 21 gauge one inch needle. Manually restrain the dog and you can use bag cat.
c. If needed, clip hair around the sampling site with small clippers. Sterilize the area (skin or hair) by using a swab soaked in 70% alcohol.
d. Raise the jugular vein with one thumb by holding pressure across the jugular groove near the thoracic inlet, below the venipuncture site. This prevents blood flow and the jugular vein will enlarge and become turgid.
e. Remove cap from needle and position the needle with bevel facing upwards parallel to the skin overlying the jugular vein.

f. Insert the needle through the skin and into the vein at a 25° angle and retract the syringe plunger to let blood fill the syringe. Do not apply too much suction as this may collapse the vein and disrupt blood flow into the syringe.
g. If blood is not flowing into the syringe, the needle bevel may be lodged against the vessel wall. Rotate the needle slightly and reposition. Retract the syringe plunger again to let blood collect into the syringe.
h. When blood collection complete, release pressure on the vein and remove the needle.
i. Apply pressure with fingers immediately following removal of the needle until the blood flow stops from the puncture site (about 30 seconds).
j. Provide dog with a reward (e.g. food treat) and return dog to kennel.

B. Jugular Vein in cattle, horse, sheep and goat

a. Move animal into squeeze chute, and restrain properly. Restrain the sheep by straddling the animal, placing knees behind the shoulders of the animal and backing the animal into a wall to control the hindquarters. Restrain the horse, by having an assistant hold the horse by its halter. Do not tie the horse. Do not perform this procedure without an assistant holding the horse. Do not proceed with the venipuncture unless the horse is adequately restrained.
b. Identify the jugular vein by raising the vein and inspection and/or palpation. Clip the hair on the animal's neck at the area (2 inch x 2 inch) for needle insertion.
c. Clean the clipped area using 70% alcohol and swab.
d. Screw the vacutainer needle onto the vacutainer.
e. Insert the collection tube into the vacutainer, using care not to puncture the stopper. Hold the vacutainer and inserted collection tube in one hand.
f. Remove the needle cap and store safely.
g. Raise the jugular vein with one thumb by holding pressure across the base of the neck within the jugular groove. This prevents blood flow and the jugular vein will enlarge and become turgid.

h. Insert the needle (20 gauge) into the jugular vein in the upper third of the neck and at approximately a 45° angle. It is helpful to rest your wrist against the animal's neck.
i. Once the needle is through the skin, push the collection tube onto the needle within the vacutainer. Remember that vacuum will be lost if the needle is removed from the skin, therefore use care if re-directing the needle. If vacuum is lost, a new collection tube must be used.
j. Advance the needle slowly until blood starts flowing into the collection tube. Hold the needle in this position until the tube is filled. If more than one tube is required, remove the tube from the collection needle and insert a new tube without pulling the needle from the skin.
k. When collection is completed, remove the tube from the collection needle and then remove the needle from the skin. Lightly press a new swab with 70% alcohol on the site.
l. Discard the needle into a sharps container, using care to avoid needle-stick injury.
m. Label and process tubes as appropriate.

B. Faecal Sample Collection

Feaces can be collected freshly voided or preferably directly from the rectum/cloaca for tests such as culture for microorganisms or parasite examination.

- Faecal specimens should be kept chilled (e.g. refrigerated at 4°C or on ice) and tested as soon after collection as possible to minimise the negative impacts on test results caused by death of the targeted microorganism, bacterial overgrowth or hatching of parasite eggs.
- Double-packaging of faecal samples in screw cap or sealable containers that are subsequently contained within sealed plastic bags will help prevent cross-contamination of samples and associated packaging materials.

C. Skin Sample Collection

- In diseases producing vesicular lesions, collect, if possible, 2g of affected epithelial tissue as aseptically as possible and place it in 5 ml phosphate buffered glycerine or Tris-buffered tryptose broth virus transport medium at pH 7.6.
- Additionally, the vesicular fluid should be sampled where unruptured vesicles are present; if possible, vesicular fluid should be aspirated with a syringe andplaced in a separate sterile tube.
- Plucked hair or wool samples are useful for surface-feeding fungal infections (Eg. In Streptotrichosis).
- Deep skin scrapings, using the edge of a scalpel blade, are useful for in birds, feather tips can be taken for detection of viral antigen where Marek's disease is suspected.

D. Genital tract

- Samples may be taken by vaginal or preputial washing, or by the use of suitable swabs. The cervix or urethra may be sampled by swabbing.

E. Eye

- A sample from the conjunctiva can be taken by holding the palpebra apart and gently swabbing the surface.
- The swab is then put into transport medium.
- Scrapings may also be taken on to a microscope slide. The handles of metal-handled swabs are useful for this, to ensure that sufficient cells are removed for microscopic examination. Mucopurulent nasal and lacrimal discharges are rarely useful.

F. Nasal discharge (saliva, tears)

- Samples may be taken with cotton or gauze, swabs, preferably on wire handles as wood is inflexible and may snap (break).
- It may be helpful if the swab is first moistened with transport medium.

- The swab should be allowed to remain in contact with the secretions for up to 1 minute, then placed in transport medium and sent to the laboratory without delay at 4°C.
- Long protected nasopharyngeal swabs should be used to collect samples for some suspected viral infections.

G. Milk

- Milk samples should be taken after cleansing and drying the tip of the teat, the use of antiseptics should be avoided.
- The initial stream of milk should be discarded and a tube filled with the next stream(s), a sample of bulk tank milk can be used for some tests.
- Milk for serological tests should not have been frozen, heated or subjected to violent shaking.
- If there is going to be a delay in submitting them to the laboratory, preservatives can be added to milk samples that are being collected for serological testing.
- Milk for bacterial examination can be kept at $+4^0$C.

H. Sample collection at post-mortem

- Samples of tissue from a variety of organs can be taken at post-mortem
- Animal health personnel should be trained in the correct procedures for post-mortem examination of the species of animals with which they work.
    - ✓ The equipment required will depend on the size and species of animal, but a knife, saw and cleaver will be required, and also scalpel, forceps and scissors, including scissors with a rounded tip on one blade, for opening intestines.
    - ✓ A plentiful supply of containers and tubes of transport media appropriate to the nature of the sample required should be available, along with labels and report forms.
    - ✓ Containers should be fully labelled with the date, tissue and animal identification. Special media may be required for transport of samples from the field.

  - ✓ The operator should wear protective clothing: overalls, washable apron, rubber gloves and rubber boots.
  - ✓ Additionally, if potential zoonotic diseases are being investigated, the post-mortem examination should be conducted in a biological safety cabinet; if this is not possible, an efficient face mask and eye protection should be worn.
  - ✓ If rabies or transmissible spongiform encephalopathies (TSEs) are suspected, it is usual to detach the animal's head.
- Tissues may be collected for microbiological culture, parasitology, biochemistry, histopathology and/or immunohistochemistry, and for detection of proteins or genome nucleic acids. In addition buccal, oropharyngeal or rectal (cloacal) swabs may be collected.
  - ✓ Each piece of tissue should be placed in a fully labelled separate plastic bag or sterile screw-capped jar.
  - ✓ Swabs should always be submitted in appropriate transport media.
  - ✓ Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents.
  - ✓ Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation.
  - ✓ The tissues may be sent to the laboratory dry or in bacterial or virus transport medium, depending on the type of specimen and the examinations required; swabs should be sent in transport medium.
  - ✓ After collection, the samples for microbiological examination should be refrigerated until shipped. If shipment cannot be made within 48 hours, the samples should be frozen; however, prolonged storage at –20°C may be detrimental to virus isolation.

2.3 Information to be sent with samples

It is essential that individual samples be clearly identified using appropriate methods.

- ✓ Marking instruments should be able to withstand the condition of use, i.e. being wet or frozen (use indelible marking pen).
- ✓ Information and case history should always accompany the samples to the laboratory, and should be placed in a plastic envelope on the outside of the shipping container.
- ✓ Name and address of owner/occupier and geolocation (latitude and longitude, if available) where disease occurred, with telephone and fax numbers.
- ✓ Diseases suspected and tests requested.
- ✓ The species, breed, sex, age and identity of the animals sampled.
- ✓ Date samples were collected and submitted.
- ✓ List of samples submitted with transport media used.

A complete history would be beneficial for the laboratory and should be included if possible.

Self check 2- Written test

Name…………………………………………… ID………………………… Date…….

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

Short Answer Questions

1. Write the purpose of specimens collection from domestic animals (2points)
2. Explain site of blood sample collection from cattle, sheep, horse and poultry (3 points)
3. Skin scrapping is collected for examination of ____________________(2 points)
4. Feacal sample is collected from_____________________(1 point)
5. Mention sterilization technique employed to clean equipments used for sample collection (2 points)

Note: **Satisfactory rating – ≥ 5 points** **Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 3- Handling specimens to minimise the spread of pathogens

3.1 Safe specimens handling

Veterinary diagnostic centers readily receive specimens that are submitted because they are suspect for a variety of diseases. The infectious nature of the specimens is usually unknown, but they have the potential to contain biological agents that may cause disease in animals and humans. Practices and procedures need to be in place that will minimize the risk of occupational exposure of employees to such pathogens. Unless suspected of containing a pathogen requiring a higher containment level, it is advisable that initial processing of all unknown specimens should be carried out with precautions.

Clinical specimens, including swabs, washes, and bodily fluids, should always be treated as if they contain infectious agents. Pathogenic microorganisms may gain access into the human and animal body if the body surface with cut or wounds is in direct contact with pathogenic microorganisms. All microorganisms and their culture therefore handled with caution. This is the result of a higher concentration of organism and the conduct of aerosol-generating procedures during routine identification of culture isolates. Manipulation of cultures is associated with increased risk of infection when compared with handling of primary clinical specimens.

3.2 Operating procedures to minimize transmission of pathogens to humans and animals

Hygienic measure should be emphasized while working with microorganisms. The following are operating procedures to minimize transmission of pathogens while working with biological specimens.

- Protective gloves and laboratory gowns should be worn if necessary.
- All wounds and cuts on body surface should be covered with sterile dressing before handling specimens.
- Always employ aseptic technique when working with microbial culture

- Before and after work, clean the bench surface with disinfectant as well as with hands
- All unwanted specimens should be disposed of properly after experiments.
- Carry and store culture of microorganisms in racks or baskets
- Do not leave cultures on the table or in unwanted areas when the laboratory session is completed
- Decontaminate work surface after spills and at the beginning and end of each laboratory periods.
- Autoclave containers before discarding
- Wash hands carefully with soap after any possible contamination and before leaving laboratories.
- Dry hands thoroughly after washing
- Develop the habit of keeping your hands away from your nose, face and eyes to avoid self inoculation
- Before and after injecting an animal, swab the site of injection with disinfectants
- Use rubber gloves and respiratory protection while cleaning

Self check 3- Written Test

Name………………………………………… ID……………………… Date…….

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

Short Answer Questions

1. Define specimen (2 points)
2. Write hygienic measure that should be emphasized while working with microorganisms (4 points)
3. Explain clinical specimens that should always be treated as infectious agents (2points)
4. Describe route of transmission of pathogenic microorganisms to human and animals (2 points)
5. Discuss Practices and procedures need to be implement to minimize transmission of pathogens to public, animals and environment (2 points)

***Note:* Satisfactory rating – ≥ 6 points** **Unsatisfactory - below 6 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 4- Recognizing and reporting hazards in the workplace

4.1 Laboratory hazards and risks

There are many definitions for hazard but the most common definition when talking about workplace health and safety is “A hazard is any source of potential damage, harm or adverse health effects on something or someone.”

"Occupational health and safety standard - Hazard identification and elimination and risk assessment and control" uses the following terms:

- **Harm** – physical injury or damage to health.
- **Hazard** – a potential source of harm to a worker.

Laboratory work should be carried out with a minimum of risk to the health of the staff working in laboratory. This requires careful consideration of the risks involved in a particular procedure, followed by appropriate measures to minimise the risk of human disease. This concerned exclusively with risks from infectious agents, but physical and chemical injuries in laboratories must also be prevented. Risks from infection are reduced by good laboratory techniques and secured facilities which aid in the containment of pathogens. It is important to understand that containment of pathogens can be used for preventing disease in humans and animals.

4.2 Types of basic laboratory hazards

a. Biological Hazards

The use of bacteria, viruses, blood, tissue and/or bodily fluids in the lab can lead to potential biological hazards. These materials can all carry disease or hazardous allergens which could put the lab team at risk. The effects of the diseases and allergens can be immediate or take significant time to manifest, demonstrating the importance that all members of the lab team are given sufficient protection, even if the dangers are not yet known. The main biological hazards in veterinary laboratory include aerosols,

zoonoses, and laboratory-associated infections. Aerosols refer to both bio-aerosols and aerosols originating from laboratory animals.

Aerosols: Aerosols originating from mice or rats may carry rodent allergens, and thus cause harm to staff through inhalation, skin contact, and eye contact, among others. Bio-aerosols are another type of biohazard. Bio-aerosols are classified as airborne particles that are living (bacteria, viruses, and fungi) or that originate from living organisms. Bio-aerosols are ubiquitous, highly variable, complex, and natural or man-made in origin.

Zoonoses: are another important source of laboratory-acquired infections. Zoonotic infections, such as cases of infection by Brucella spp. These incidents indicate that zoonoses are an important biological hazard during animal experiments that can lead to serious infections in human.

Safety Practices for Specific Biohazardous Materials

- Use laboratory facilities appropriate to the required biosafety level.
- Use appropriate containment equipment, such as biological safety cabinets.
- Prevent or minimize the creation of aerosols.
- Limit use of needles, syringes, and other sharps to avoid unnecessary exposure
- Ensure proper biohazard disposal and decontamination.

b. Physical Hazards

Laboratory work involves many manipulations that are potentially dangerous such as:

- Handling glassware and work with needles or other sharp instruments. There must be special facilities for the proper disposal of needles and other sharps.
- Laboratory staff should be protected from the risk of receiving a burn from hot solids or liquids.
- Autoclaves must be fitted with safety devices to prevent accidental opening of doors when under pressure and be regularly serviced and tested.

- Heat-protective gloves must be provided.
- Extreme cold can also be a risk, e.g. when working with liquid nitrogen; splashes on exposed skin can be very damaging. Gloves should be worn that provide insulation from cold and that are also waterproof to prevent penetration of the liquid nitrogen.
- Face masks and boots should also be worn when working with liquid nitrogen.
- Irradiation is a serious health risk that may be present due to the use of X-ray machines or use of gamma-emitters or other sources.
- Equipment must be regularly serviced and tested. All use of radioactive material must be meticulously recorded.
- All staff must be provided with a radiation monitoring device and have annual health checks.

C. Chemical Hazards

A wide range of chemicals is in use in veterinary laboratories. These are;

- Many of which may be toxic or mutagenic and some may be carcinogenic. It should be remembered that it is the dose that makes the poison.
- Vapours are especially hazardous and some chemicals can be absorbed by penetration of intact skin.
- Procedures sufficient to protect pregnant laboratory workers should be followed at all times.
- A list of hazardous chemicals must be maintained, and a file record kept of chemicals to which individual staff members could be exposed.
- Chemicals must be correctly stored in appropriate containers and at the correct temperature. Those that are very flammable must be kept in a fireproof chemical store.
- A record must be maintained of the purchase and use of hazardous chemicals.
- Disposal of some chemicals is subject to official regulation.

4.3 Laboratory record and/ documentation

Departments and/or laboratory supervisors must maintain safety training records for all laboratory personnel.

- Proper recording of data and studies is critical to the success of the laboratory.
- The over-riding principle is that the test should be performed as written in the SOP (Standard Operating Procedures). The SOP should be written to reflect how the test is actually performed, and the laboratory notebook should provide a record of all critical details needed to confirm the integrity of the data.
- At a minimum, the laboratory write-up should include the following:
  - ✓ Date
  - ✓ Material tested
  - ✓ Laboratories' name
  - ✓ Procedure number
  - ✓ Document test results
  - ✓ Deviations (if any)
  - ✓ Documented parameters (equipment used, microbial stock cultures used, media lots used)
  - ✓ Management/Second review signature
- Where appropriate, logbooks or forms should be available and supportive of the laboratory notebook records.
- Equipment temperatures (water baths, incubators, autoclaves) should be recorded and traceable.
- Changes in the data should be crossed off with a single line and initialed.
- Original data should not be erased or covered over.
- Test results should include the original plate counts, allowing a reviewer to recreate the calculations used to derive the final test results.
- Methods for data analysis should be detailed in cited Standard Operating procedures.
- All laboratory records should be archived and protected against catastrophic loss.

- A formal record retention and retrieval program should be in place.

Self check 4- Written test

Name... ID............................ Date.......

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

Part 1: Choose the correct answers from the given alternatives (2 points each**)**

1. The main biological hazards in veterinary laboratory include _________.
   a. aerosols b. zoonoses c. laboratory-associated infections d. all
2. Bio-hazardous materials present in the laboratory may be minimized by using one of the following equipments
   a. microscope b. centrifuge c. biosafety cabinate d. refrigerator
3. ______ is any source of potential damage, harm or adverse health effects on something or someone
   a. risk b. hazards c. harm d. zoonoses

Part 2: Short answer questions

1. Explain potential physical and chemical hazards that a person working in the laboratory may encounter (5 points)
2. Define standard operating procedures (SOP) 3 Points

***Note:* Satisfactory rating – ≥ 7 points Unsatisfactory - below 7 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 5- Following workplace procedures and work instructions

5.1 Introduction

Safe work practices are generally written methods that define how tasks are performed while minimizing risks to people, equipment, materials, environment, and processes. Safe Work Procedures are documented procedures for performing tasks. The purpose of workplace safety procedures and instructions is to reduce the risk to health and safety in the workplace and reduce the likelihood of an injury by ensuring that employees know how to work safely when carrying out the tasks involved in their jobs. Safe work procedures may also be called safe work method statements.

5.2 Types of workplace safety procedures and instructions

Types of workplace safety procedures and instructions include the following

- Handling chemicals**:** these involve procedures on how to handle chemicals in workplace where chemicals are used.
- Lifting and moving objects**:** are procedures that pertain to how objects are to be lifted and moved safely and without strain to the person or worker.
- Slips, trips and falls: are procedures that pertain to safety procedures that should be in place to prevent slips, trips and fall accidents in the workplace.
- Housekeeping**:** are procedures that pertain to how housekeeping activities should be done while keeping in mind safety, health and well-being of workers in a facility or workplace

5.3 Component of workplace safety procedures and instructions

The following steps should be followed to ensure a sound safe workplace safety

Observe the task/activities: it is important to observe the task/activity being performed the preferred way to ensure safest method is documented.

1. Review associated legislative requirements**:** Some task/activities are governed by legislative requirements. These must be considered when developing a safe work procedure to ensure any legal requirements are included.
2. Record the sequence of basic job steps: write down the steps that make up the task/activity.
3. Record potential hazards of each step: Next to each step identify what may have potential to cause injury or disease
4. Identify ways of eliminating and controlling the hazards**:** list the measures that need to be put in place to eliminate or control any likely risk.
5. Test the procedure: Observe staff/student following the safe work procedure
6. Obtain approval**:** Before the safe work procedure can be used it must be approved by each approver nominated.
7. Monitor and review: Make sure the activity is supervised to ensure the documented process is being followed.

Self check 5- Written Exam

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

Part 1: Short answer questions

1. Give the definition of safe work practices (2 points)
2. Discuss types of workplace safety procedures and instructions(4 points)

Part 2: choose the correct answers (2 points each)

1. A type of workplace safety procedures and instructions which involve procedures on how to handle chemicals in workplace is;
   a. Handling chemicals b. Lifting and moving objects
   c. Slips, trips and falls d. Housekeeping
2. One of the following is not general laboratory safety procedures and instructions
   a. wearing PPE in the laboratory
   b. labeling of chemical substances used in the laboratory
   c. eating and drinking in the laboratory
   d. avoiding running in the laboratory
3. Which of the following is true regarding steps that should be followed to ensure a sound safe workplace safety
   a. Review associated legislative requirements
   b. Record the sequence of basic job steps
   c. Record potential hazards of each step
   d. all

Note: **Satisfactory rating – ≥ 6 points Unsatisfactory - below 6 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 6- Recognizing and taking action to eliminate risks to self, bystanders, the public and animals

6.1 Recognizing risks to self, bystanders, the public and animals

Laboratory activities including pathogen research, diagnostic tool development, pharmaceutical and vaccine development, and identification and characterization of etiological agents are critical to most successful control initiatives. While many of these activities clearly benefit animal health, the handling, isolation, storage, and disposal of infectious pathogens pose inherent safety and security risks to the laboratory, the staff, the community, the environment, and even the world. As a result, laboratory biosafety and biosecurity systems must be an integral part of any laboratory working with and handling dangerous microorganisms to prevent accidental and/or intentional release. Laboratory accidents and unintentional release of pathogens from veterinary laboratories can infect human and animal populations.

Although animal health and research laboratories handle primarily animal pathogens, many are zoonotic. Consequently, these agents pose significant risks to laboratory staff and the surrounding human and animal populations. Moreover, improperly inactivated laboratory waste containing pathogens, infected research animals, and/or contaminated laboratory staff and their possessions can contaminate the environment and infect surrounding communities and/or livestock. Many types of research, diagnostic and pharmaceutical laboratories isolate, amplify, and retain dangerous pathogens to conduct research, diagnose disease, and establish efficacious therapies. Consequently, these pathogens are vulnerable to theft and potential misuse, and must be protected through implementation of laboratory biosecurity programs.

6.2 Laboratory Biorisk Management Systems

Biorisk management (BRM) is a system of processes and procedures used to reduce safety and security risks associated with the handling, storage, and disposal of biological agents and toxins in laboratories.

Laboratory biosafety systems consist of;

- Engineering controls: Engineering controls are physical changes to work stations; use of specialized equipment, materials, or production facilities; or any other relevant aspect of the work environment that reduces the risk of accidental or intentional release. Examples of engineered biosafety controls include biosafety cabinets, chemical fume hoods, changes to the physical features of the facility that allow for better ventilation and air-flow, barrier walls and shields, and separation of incompatible activities; equipment and equipment maintenance, calibration and certification.
- Standard work practices
- Use of personal protective equipment: When used correctly, PPE is a biosafety mitigation control that provides protection to individual workers from the most hazardous pathogens and chemicals. PPE is considered the least effective control strategy, as it protects only the person wearing it.

Biosecurity systems consist of:

- physical security
- personnel security
- information security
- transportation security, and
- Material control and accountability

6.3. Other methods of minimising risks to self, others and animals againist microbial pathogens

a. Hand-washing

Good hygiene practices, such as the correct hand-washing technique and washing hands at appropriate times in the animal contact area, will decrease the risk of disease. Always wash hands with soap and running water:

- After touching animals, their enclosures or food containers.
- Any part of the animal or its surrounds can be contaminated

- After being licked or bitten by animals
- After having contact with soil, urine or faeces in an animal contact area.
- Always wash hands before eating, drinking or smoking.
- Always alert about the behavoir of animal

b. Avoiding activities with a higher risk

While visiting animals do not:

- Touch mouth with hands, or lick fingers
- Eat food intended for animals eat inside the animal contact area (although there can be exceptions to this where the operator implements control measures to mitigate zoonotic disease risk in a visitor eating area)
- Leaving open wounds uncovered
- Wipe hands on clothing, if avoidable
- Use dummies, spill-proof cups or baby bottles in the animal contact areas
- Return dummies or toys that have fallen on the ground or been in contact with animals to children until they have been washed with soap and water.

Self check 6- Written test

Name…………………………………………… ID……………………….. Date…….

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

Part 1: Short answer questions

1. Discuss the mechanisms of reducing risks to self, other person, animals and environment against pathogenic microorganisms (4 points)
2. Write the difference between biosafety and biosecurity (2 points)

Part 2: Choose the correct answers from the given alternatives (2 points each)

1. Biosecurity systems consist of:
   a. physical security
   b. personnel security
   c. transportation security
   d. all
2. Which of the following activities should be conducted after touching or visting animals to reduce risks to self and bystanders
   a. avoid leaving open wounds uncovered
   b. avoid wipe hands on clothing
   c. avoid touch mouth with hands
   d. none

***Note:* Satisfactory rating – ≥ 5 points Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 7- Providing Safety training

7.1 Laboratory Safety Training

Training means helping people to learn how to do something, telling people what they should or should not do, or simply giving them information. Training isn't just about formal 'classroom' courses. A person who works in a laboratory must receive training to become informed about potential hazards in the laboratory. Principal investigators (PI) and/or laboratory supervisors are responsible for ensuring that all personnel are properly trained before they begin work in a laboratory and that they receive additional training when new hazards or procedures are introduced.

Laboratory work should be carried out with a minimum of risk to the health of the staff working in laboratory. This requires careful consideration of the risks involved in a particular procedure, followed by appropriate measures to minimise the risk of human disease. This concerned exclusively with risks from infectious agents, but physical and chemical injuries in microbiology laboratories must also be prevented. Risks from infection are reduced by good laboratory techniques and secured facilities which aid in the containment of pathogens. It is important to understand that containment of pathogens can be used for preventing disease in humans and animals. Often the same methods of containment are used for both preventing laboratory-acquired infection in humans and for preventing escape of pathogens that could cause an outbreak of animal disease. Although the methods, techniques and facilities required may be the same, the list of pathogens and categorization into levels of risk will differ depending on whether it is human or animal disease control that is the primary objective. Existing national and international reference laboratories have considerable experience in the operation of safe working practices and provision of appropriate facilities.

7.2 Laboratory Specific Safety Training

In addition to general Environmental Health and Safety (EH&S) training, all laboratory personnel, including students, must receive laboratory-specific training on the following topics:

- Emergency management
- location and content of the Laboratory Safety Manual
- physical, chemical, biological and radiation hazards in the work area, including signs and symptoms of exposure and allowable exposure limits, using applicable safety data sheets (SDS) content
- location of references describing hazards and safety practices associated with laboratory materials
- protective measures necessary to avoid exposure or injury, as specified in the laboratory's Standard Operating Procedures
- procedures for responding to laboratory emergencies (chemical spill(s), fire, severe weather, etc.) as outlined in the laboratory's Emergency Action Plan
- methods to detect the presence of contamination or the release of chemical, biological and radiological materials
- procedures for obtaining medical care in the event of exposure/ injury
- proper waste management and disposal procedures
- proper record keeping

Refresher Training: If an employee exhibits lack of knowledge, or if work conditions and tasks change, retraining is required.

Self check 7- Written test

Name…………………………………………… ID………………………… Date…….

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

Short answer questions

1. Define safety training (2 points)
2. Describe laboratory specific safety training (4 points)
3. Explain refresher training (2 points)
4. Discuss material safety data sheet (2 points)
5. Risks from infection are reduced by good laboratory techniques and secured facilities which aid in the containment of pathogens (2 points).

   a. true b. false

***Note:* Satisfactory rating – ≥ 5 points Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Operation sheet 1- Procedure for blood sample collection

Procedure for blood sample collection from jugular vein in ruminant animals

1. Restrain animals properly according to the technique applied for the species of sampled animals
2. Clip the hair on the animal's neck at the area (2 inch x 2 inch) for needle insertion
3. Clean the clipped area using 70% alcohol and swab
4. Identify the jugular vein by raising the vein and inspection and/or palpation.
5. Screw the vacutainer needle onto the vacutainer.
6. Insert the collection tube into the vacutainer tube. Hold the vacutainer and inserted collection tube in one hand.
7. Remove the needle cap and store safely.
8. Raise the jugular vein with one thumb by holding pressure across the base of the neck within the jugular groove.
9. Insert the needle into the jugular vein in the upper third of the neck and at approximately a 45° angle.
10. Once the needle is through the skin, push the collection tube onto the needle within the vacutainer
11. Hold the needle in this position until the tube is filled
12. When collection is completed, remove the tube from the collection needle and then remove the needle from the skin. Lightly press a new swab with 70% alcohol on the site.
13. Discard the needle into a sharps container, using care to avoid needle-stick injury.
14. Label and process tubes as appropriate.

Operation sheet 2- Procedure for milk sample collection

Steps for milk sample collection

1. Restrain the animals by using casting rope or within a crush and use hobble to avoid kicking
2. Clean and dry the udder and tip of teat with clean towel, the use of antiseptics should be avoided.
3. Discard initial one-two stream of milk
4. Fill the tube with the next stream(s), a sample of bulk tank milk can be used for some tests.
5. Milk for serological tests should not have been frozen, heated or subjected to violent shaking. If there is going to be a delay in submitting them to the laboratory, preservatives can be added to milk samples that are being collected for serological testing.
6. Store milk for bacterial examination at $+4^0$C.

LAP Test - Demonstration Test

Name……………………………… ID……………………………..
Date……………………………………

Time started: ________________________ Time finished: ________________

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks 1 and 2 within 10 minutes and task 3 within 15 minutes. The project is expected from each student to do it.

Tools and equipments required for blood sample collection: Casting rope, scissor, scalpel handle and blade, soap, water, alcohol, vacutainer tube and needle, disposable syringe, test tube, cotton and glove

Materials used for milk sample collection: casting rope, hobble, clean water, test tubes, icebox, ice bag and glove

Task 1: Prepare tool and equipment used to collect blood sample for Heamoparasite examination

Task 2: Carryout blood sample collection from sheep

Task 3: Collect milk sample appropriately

LG #55	LO 2- Prepare material and equipment and process sample for test

Instruction sheet

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

- Testing equipment and materials set up according to organisational guidelines
- Performing pre-use and safety checks of materials and equipments
- Identifying and reporting faulty or unsafe equipment to appropriate personnel
- Checking calibration status of equipment and any out of calibration items
- Recording and comparing sample description with specification, and any discrepancies
- Preparing samples accordance with appropriate standard methods
- Identifying, preparing and measuring samples to the standard taste

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:

- Test equipment and materials set up according to organisational guidelines
- Perform pre-use and safety checks of materials and equipments
- Identify and report faulty or unsafe equipment to appropriate personnel
- Check calibration status of equipment and any out of calibration items
- Record and compare sample description with specification, and any discrepancies
- Prepare samples accordance with appropriate standard methods
- Identify, prepare and measure samples to the standard taste

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 the next “information sheets
7. If your performance is unsatisfactory, see your trainer for further instructions

Information sheet 1- Setting up test equipment and materials

1.1 Tools, Equipments and Materials

Any physical item that is used to achieve a goal but is not consumed during this process can be defined as a tool. Informally speaking, it can also be used to describe a specific procedure with a specific purpose as well. Tools are often also referred to as machine, apparatus, implements, instruments or utensils. Tools can perform a variety of functions. There can be specific tools designated for specific purposes whereas most tools can serve a combination of uses. Equipment is a set of tools that are designated for a specific task. This could be a small set of functional items in a finished product.

1.2 Test laboratory equipments and materials set up

a. Autoclaves

Autoclaves, also known as 'steam-under-pressure sterilizers', are pressure vessels that fall under the definition of pressure equipment. They are usually located in biological science laboratories and are used for sterilization of media and equipment required for the culture of microorganisms, and/or the decontamination of discarded cultures, or biological, clinical or waste materials. Pressure equipment also includes pressure piping and boilers (e.g. water-tube, electric and hot water heaters, fired pressure cookers etc).
Almost all pressure equipment is hazardous, i.e. has the potential to cause harm, injury or illness, or damage to plant, property and the environment. Therefore all work involving the use of high pressure equipment is also potentially hazardous due to the risks associated with the generation of high levels of pressure and high temperatures during operation. Serious accidents may occur if this type of plant or equipment is not designed, constructed, operated and maintained. Safety requirements for the use of high pressure equipment are specified as follows:

- Only materials and equipment designed to withstand high pressures shall be used in its manufacture

- If equipment includes a boiler (e.g. steam autoclaves), then the boiler shall have fitted an appropriate safety valve, water level alarm and fusible plug (in conjunction with a temperature gauge)
- Safety valves and other methods of pressure release, and remote methods of power cut-off, shall be sited so that their operation cannot injure people or damage equipment
- Safety valves incorporating a means of manual release shall be operated regularly, to ensure correct operation. They shall not be adjusted by unauthorized persons and, where provision is made for locking, shall be kept locked
- persons using an autoclave shall be appropriately trained, and understand the need to ensure that proper conditions for load sterilization are selected and produced in the chamber
- Operators must be provided with and use appropriate personal protective equipment
- personal protective equipment should include the use of heat insulating gloves of sufficient length when loading and unloading the autoclave, and the use of a face shield to protect the face from steam that may be present, especially when unloading
- wire racks or metal shelving should be provided in the vicinity of the autoclave for the cooling of autoclaved loads
- trolleys should be provided for the loading and unloading of large or heavy loads with sufficient space to allow for their movement
- sufficient penetration time should be allowed for all parts of the load to reach the appropriate temperature i.e. 15 minutes at 121°C or 4 minutes at 134°C
- during normal operation, the autoclave cycle should always be completed before opening the door
- prior to removal of the load, the autoclave door should be partly opened and sufficient time allowed for the load to cool down before handling

- extreme care should be taken when removing containers of fluids from the autoclave ensure the required amount of cool-down time has elapsed before removing
- Flammable materials, including samples containing solvents, must not be autoclaved
- Hand washing facilities, safety showers and eyewash facilities should be provided and be easily accessible
- A logbook for recording details of the load and cycle should be maintained, and chart records kept for regular checking by staff and qualified service personnel, so that the autoclave is maintained within the calibration specifications

b. Biosafety Cabinets

Biological safety (biosafety) cabinets are a type of special containment equipment designed to protect laboratory personnel and others from the risk of exposure to biological hazards and contamination posed by the generation of aerosols that may be produced during common laboratory operations. Surveys on the causes of laboratory-acquired infections indicate that 80% of laboratory infections result from exposure to aerosols that are produced by common procedures such as pipetting, blending and homogenizing. Therefore the objectives for the control of these hazards are to minimize the potential for exposure of personnel to these hazards and to prevent the liberation of micro-organisms or biohazardous material from the laboratory into the environment. The control of such hazards is known as 'containment', meaning that they are kept within specified limits, and primary containment is provided by the use of good microbiological techniques and by the use of appropriate safety equipment. The biosafety cabinet is the principal device for the containment of aerosols and as such forms the primary barrier of control.

Location of the cabinet

- away from doorways, passageways, air diffusers etc which could influence cabinet airflows
- limit personnel traffic, which can generate air movements and cause loss of barrier containment and reduce level of product protection, by using warning signs or partitioning
- make sure all windows and doors are closed during cabinet operation; o consider the location of cabinets away from fume cupboards which generate similar inward airflows
- location should permit the exhaust of fumigant gases to the outside atmosphere
- location should permit space for cleaning behind cabinet
- sweeping with brooms and the use of vacuum cleaners shall not be allowed as it produces airborne dust that can increase contamination of work in the laboratory
- all cleaning should be done outside of normal working hours or when laboratory is not in use

Figure 1: Biosafety Cabinet

c. Centrifuges

There are many types of centrifuges in use e.g. microfuges, medium and high speed centrifuges. Due to the nature of their function and operation, centrifuges can present a hazard to the user, to other laboratory staff, to the experimental work and to the laboratory environment. Unbalanced loads, rotor failure, or tube or bucket breakage can cause high speed ejection and scattering of infectious or hazardous material.

Safety Requirements: The following requirements apply to the safe use of centrifuges:

- Training: Persons required to operate centrifuges must receive adequate training in the correct use of the centrifuge including the necessity for precise rotor balancing, correct use of centrifuge tubes and cleanliness/decontamination of the centrifuge
- Vibration: Medium and high speed bench top centrifuges must be securely anchored to prevent movement caused by vibration
- Excessive Speed relative to the mass being centrifuged must not be used

- Location: The centrifuge should be located where vibration will not cause additional hazards, such as glassware or equipment to fall from shelves
- Centrifuge rotors and tubes must be inspected before use. Any tubes showing damage must be discarded and damaged rotors replaced
- Logbooks of usage must be kept for medium and high speed centrifuges to ensure timely maintenance and safety inspection of the rotors
- The manufacturer's instructions should always be followed, and a preventative inspection and maintenance program should be implemented.

d. Refrigeration

Refrigeration used in a laboratory should be purpose designed, built and dedicated to ensure that any specimens and other materials can be safely stored and maintained at the desirable temperature. The following general safety requirements apply to all refrigeration used within laboratories.

- Laboratory refrigerators and freezers must not be used to store food or beverages intended for human consumption.
- flammable liquids requiring refrigeration must be stored in an approved 'explosion proof' or 'spark proof' refrigerator
- where a domestic refrigerator is installed in a laboratory a warning sign is to be displayed on the door indicating that 'Flammable liquids or food must not be stored in this refrigerator
- cold rooms must have door fittings that enable the doors to be opened from the inside
- a preventative inspection and maintenance program should be implemented

e. Microscope

Microscope is an instrument which helps in viewing of objects not visible by naked eye. It uses lenses to magnify the objects so that it can be seen through the naked eye. They are one of the most important diagnostic tools when examine the tissue samples.

Microscope should handle with care and put on table free from any vibration behind.

Clean microscope with its standard cleaning agents before and after use.

Self check 1- Written test

Name…………………………………………… ID……………………….. Date…….

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

Short answer questions

1. Explain safety requirements apply to the safe use of centrifuges (2 points)
2. _________ is categorized under pressure equipment used for steam sterilization of biohazardious materials (3 points)
3. Biological safety (biosafety) cabinets are a type of special containment equipment designed to protect laboratory personnel and others from the risk of exposure to biological hazards and contamination posed by the generation of aerosols that may be produced during common laboratory operations(1 point)

   a. true b. false
4. ______ is used to ensure that any specimens and other materials can be safely stored and maintained at the desirable temperature in the laboratory (1 point)
5. Mention safety requirements for the use of high pressure equipment used in the laboratory (3 points)

***Note:* Satisfactory rating – ≥ 5 points Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 2- Performing pre-use and safety checks of materials and equipments

2.1 Safety checks of laboratory materials and equipments

All equipment used in the laboratory must function properly and safely. To ensure this, laboratories must maintain equipment according to a manufacturer's specifications or established guidelines, and perform routine inspections for common problems: corrosion, damaged electrical cords, excessive contamination, leaks, worn parts, and ensure that alarms, guards, interlocks, or other safety devices have not been disconnected or disabled.

Working and non-working laboratory equipment must be free of contamination and inspected by environmental health and safety prior to disposal. Biosafety cabinets must be decontaminated by certified technician before disposal.

2.2 Safety checklists for laboratory materials and equipments

- All warning symbols are fixed, legible and clearly visible on equipment
- Correct warning labels are attached to the equipment according to the class of laser in use
- A supply of clearly labelled disinfectants for decontamination purposes is available
- Cylinders, pipes and valves are protected from mechanical damage
- Refrigerators are suitably labeled (e.g. no food, no drink, no flammables, biohazard)
- PPE is available and in good condition
- All power leads, outlets and switches are in good condition (not broken)
- Material safety data sheets (MSDS) available for all chemicals used in lab
- Chemicals stored away from heat and direct sunlight

Self check 2- Written test

Name…………………………………………… ID……………………….. Date…….

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

Short answer questions

1. Discuss Safety checklists for laboratory materials and equipments use (5 points)
2. Explain the requirements of pre-use safety checks of laboratory equipments (3 points)

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

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 3- Identifying and reporting faulty or unsafe equipment

3.1 Faulty or unsafe laboratory equipment

Equipment that has been subjected to overloading or mis-handling gives suspect results or has been shown to be defective or outside specified limits shall be taken out of service. It shall be isolated and/or clearly labeled out of service (Do Not Use) to prevent use until repaired and shown by calibration or test to perform correctly. Problems with equipment may present in many ways. The operator may notice subtle changes such as drift in quality control or calibrator values, or obvious flaws in equipment function. Sometimes, the equipment fails to operate. It is important to teach operators to troubleshoot equipment problems in order to quickly get the equipment functioning and resume testing as rapidly as possible. Manufacturers frequently provide a flowchart that can help determine the source of problems. If problems cannot be identified and corrected in-house, attempt to find a way to continue testing until the equipment can be repaired. Some ways to achieve this are as follow.

- Arrange to have access to backup instruments.
- Ask the manufacturer to provide a replacement instrument during repairs
- Do not use faulty equipment! Seek help from the manufacturer or other technical expert. Place a note on the equipment so all staff is aware that it is not in use
- Manufacturers may provide service and repair of equipment that is purchased from them. Be sure to set up a procedure for scheduling service that must be periodically performed by the manufacturer. When instruments need repair, remember that some warranties require that repairs be handled only by the manufacturer.

Equipment that has been mishandled, gives suspect results, is defective, or gives suspect results shall be taken out of service and clearly marked as being out of service until it has been repaired & recalibrated. The laboratory shall determine if there has been an effect on previous test results and perform corrective action.

After equipment has been installed, the following details need to be addressed before putting the equipment into service:

- assign responsibility for performing the maintenance and operation programs
- develop a system for recording the use of parts and supplies
- implement a written plan for calibration, performance verification, and proper operation of the equipment
- establish a scheduled maintenance program that includes daily, weekly, and monthly maintenance tasks
- Provide training for all operators; only personnel who have been trained specifically to properly use the equipment should be authorized as operators.

3.2 Retiring and disposing of faulty or unsafe equipment

It is very important to have a policy and procedure for retiring older laboratory equipment. This will usually occur when it is clear that the instrument is not functioning and is not repairable, or when it is outmoded and should be replaced with new equipment. Once a piece of equipment is fully retired and it has been determined that it has no further use, it should be disposed of in an appropriate manner. This last step is often neglected in laboratories and old equipment accumulates, taking up valuable space and sometimes creating a hazard.

When disposing of equipment, salvage any usable parts, particularly if the equipment is being replaced with another similar one. Then consider any potential biohazards and follow all safety disposal procedures.

Self check 3- Written test

Name………………………………………… ID……………………… Date……

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

Short answer questions

1. Describe the influences of faulty or unsafe laboratory equipments on the laboratory results (3 points)
2. Discuss the process of disposing faulty or unsafe equipment laboratory equipments (5 points)
3. Once a piece of equipment is fully retired and it has been determined that it has no further use, it should be disposed of in an appropriate manner (2 points)

   a. true b. false

   Note: **Satisfactory rating – ≥ 5 points Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 4- Checking calibration status of equipment

4.1 Calibration Status of Equipment

Calibration is defined as adjustment or standardization of the accuracy of a measuring instrument, usually by comparison with a certified reference or standard. It can also defined as the comparison of two measurement devices or systems (one of known uncertainty (your standard) and one of unknown uncertainty (your test equipment. Instrument calibration and maintenance are an integral part of any operation in manufacturing/ laboratory and are vital for data quality assurance.

Follow the manufacturer's directions carefully when performing the initial calibration of the instrument. It is a good idea to calibrate the instrument with each test run, when first putting it into service. Determine how often the instrument will need to be recalibrated, based on its stability and on manufacturer's recommendation. It may be advantageous to use calibrators provided by or purchased from the manufacturer.

Prior to testing patient specimens, it is important to evaluate the performance of new equipment to ensure it is working correctly with respect to accuracy and precision. In addition, test methods using kits or laboratory instruments need to be evaluated for the ability to detect disease (sensitivity, specificity, positive and negative predictive value), and to determine normal and reportable ranges.

All measurement and testing equipment have a significant effect on the validity and accuracy of test results. All equipment that requires calibration shall be labelled, coded, or otherwise identified to indicate the status of calibration, including the last date of calibration and date of expiration or due date of next calibration. Equipment sent out of the laboratory must be recalibrated or checked and shown to be satisfactory before placing into service. Calibration and verification takes place at specified intervals through the use of traceable standards or appropriate reference materials or reference

cultures. Laboratory equipment that need calibration includes the following but not limited to:

- Balances
- Ovens
- Refrigerators
- Freezers
- Incubators
- Water baths
- Temperature measuring devices(thermometer)

4.2 Function checks of laboratory equipments

In order to verify that equipment is working according to the manufacturer's specifications, it is necessary to monitor instrument parameters by performing periodic function checks. This should be done before using the instrument initially, then with the frequency recommended by the manufacturer. These function checks should also be done following any instrument repairs. Some examples of function checks are daily monitoring of temperatures and checking the accuracy of wavelength calibration.

Calibration label: A label affixed to a measurement device, precision tool and/or test equipment that shows its calibration status. The label typically indicates the measurement device, identification, who performed the last calibration and when.

Calibration Interval

Working standards must be calibrated annually unless substantiation is provided over several calibration periods that reflect repeated accuracy. Calibration intervals are best established based on the performance on individual working standards including accessories with consideration given to their application and calibration history. The calibration intervals for working standards will also vary with the type of equipment, environment and use. Two similar working standards can be operated under very

different conditions. One may be outside, exposed to the sun, damp air and dirt and used two shifts per day, while the other may be in a fixed location within a workshop under a controlled environment, only one shift uses it per day and maybe used only occasionally. Considerations for acceptance of calibration intervals include but are not limited to the following:

- Manufacturer's recommendations for the type of equipment
- Calibration facility or laboratory past calibration history, as applicable
- Approved Maintenance Organisation intended use, appropriate training, environmental conditions, etc., and
- Adequate procedures developed by the approved maintenance organisation

Report of Calibration: A document describing a calibration, including the results, what was done, by whom, under what conditions and using what equipment and procedures. It could also include any attachments, such as, data sheet(s), graph(s), etc.

Self check 4- Written Test

Name…………………………………………… ID……………………… Date…….

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

Short answer questions

1. Write the definition of calibration (3 points)
2. What is the purpose of calibration of laboratory equipments? (2 points)
3. Explain calibration intervals (3 points)

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

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 5- Recording and comparing sample description with specification, and any discrepancies

5.1 Laboratory Sample Description

The quality of the work a laboratory produces is only as good as the quality of the samples it uses for testing. The laboratory must be proactive in ensuring that the samples it receives meet all of the requirements needed to produce accurate test results. Inaccuracies can also affect laboratory efficiency, leading to repeat testing with resultant waste of personnel time, supplies, and reagents.

5.2 Verification of quality

Once a sample enters the laboratory, there are a number of steps needed prior to testing. The sample description should be compared with specification present for the procedure requested before examination. These pre-examination steps include:

- Verifying the sample is properly labelled, adequate in quantity, in good condition, and appropriate for the test requested. The test request must be complete and include all necessary information
- Recording sample information into a register or log
- Enforcing procedures for handling sub-optimum samples, including sample rejection, when necessary.

5.3. Rejection of samples

The laboratory should establish rejection criteria (specification) and follow them closely. It is sometimes difficult to reject a sample, but remember that a poor sample will not allow for accurate results. It is the responsibility of the laboratory to enforce its policies on sample rejection so that patient care is not compromised. The following are examples of samples that should be rejected.

- unlabeled sample

- broken or leaking tube/container
- insufficient sample information
- sample collected in wrong tube/container; for example, using the wrong preservative or non-sterile container
- insufficient quantity for the test requested
- prolonged transport time, or other poor handling during transport
- When rejecting a sample, it is important to promptly inform authorized person that the sample is unsuitable for testing

Generally, presence of specimens discrepancies should compared with specification for each type of sample depending on procedures followed and objective of the test.

Self check 5- Written Exam

Name………………………………………… ID………………………… Date…….

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

Short answer questions

1. Explain cause of laboratory sample discrepancies (3 points)
2. Write pre-examination steps for verification of specimens quality to avoid discrepancies (4 points)
3. Presence of specimens discrepancies should compared with specification for each type of sample depending on procedures followed and objective of the test (1 point)

   a. false b. true

4. Discuss criteria used to evaluate and reject sample with discrepancies (2 points)

***Note:* Satisfactory rating – ≥ 5 points Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 6- Identifying and preparing samples accordance with appropriate standard methods

6.1 Sample collection and Submission

Specimens must be collected using appropriate biosafety and containment measures in order to prevent contamination of the environment, animal handlers, and individuals doing the sampling as well as to prevent cross-contamination of the specimens themselves. The procedures used to identify infectious agents vary widely. Before a detailed laboratory investigation is undertaken, a complete case history including a tentative diagnosis, should be submitted to the laboratory with the specimens. This will help laboratory staff to decide on the range of possible agents and so select the most appropriate tests and procedures that should identify the pathogens.

Samples are submitted to the laboratory in their raw form, therefore certain manipulations have to take place in order to prepare the sample for analysis. This involves the sample preparation and extraction. The procedures for sample preparation and extraction are vital in obtaining reliable results therefore proper procedures have to be followed in order to ensure that the sample obtained is homogeneous and representative of the original sample.

The reliability of the diagnostic testing is critically dependent on the specimen(s) being appropriate, of high quality, and representative of the disease process being investigated. 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.

Specimens must be collected according to a sound knowledge of the epidemiology and pathogenesis of the disease under investigation, or the disease syndrome to be diagnosed. While it is critical to collect specimens as aseptically as possible, equal care

must be taken to avoid contamination with detergents and antiseptic treatments used to clean the collection site on the animal, as these agents may interfere with the laboratory test procedures. Specific considerations regarding different specimen types are as outlined below.

Blood: Whole blood samples may be collected for haematology, clinical chemistry, toxicology, direct examination for bacteria or parasites, PCR testing, immunological testing, or for culture for bacteria or viruses. Dependent on testing needs, whole blood, blood cells, and/or plasma samples can be obtained from whole blood collected into appropriate anticoagulants. In selecting the anticoagulant to be used the collector must be aware of the laboratory tests, including PCR-based diagnostics, clinical chemistry, and toxicology, which may be negatively affected by the presence of specific anticoagulants or preservatives. To be effective anticoagulants require that the collected blood be thoroughly mixed with the chosen anticoagulant during or immediately following its sampling from the animal.

To obtain serum, whole blood is collected without anticoagulants and the clot is allowed to contract at ambient temperature protected from extremes of heat and cold for periods that may range from a few hours to overnight. Clear serum can be decanted or collected by pipette following physical removal of the clot, ideally following gentle centrifugation to separate cell components from the serum. In the absence of a centrifuge, separation of the clot can be facilitated by tilting the freshly collected blood tube at an approximate 45 degree angle until the clot has retracted, “ringing” the clot with a sterile rod or pipette to separate the clot from the tube surface, and then removing the clot with forceps. The results of serological testing can be compromised by the quality of the sample. Bacterial contamination and red blood cell debris in serum samples can produce false positive reactions in agglutination-type assays.

Serological assays can be negatively impacted by haemolysis in the serum sample. Microbial contamination and haemolysis are significant concerns especially when obtaining blood and serum samples from post-mortem animals. Frequent causes of

haemolysed serum and plasma include exposure to excessive temperatures or time delays prior to separating sera from the red blood cells, blood collection using a needle of too small gauge, or failure to remove the needle when transferring the blood sample from the collection syringe.

Blood and sera are typically shipped and stored cool (or frozen in the case of sera) in non-breakable vials, tubes, or bottles; however for some laboratory tests that require viable peripheral blood mononuclear cells, the blood must be packaged, transported and stored so as to prevent exposure to temperature extremes.

Faeces: Faeces can be collected freshly voided or preferably directly from the rectum/cloaca for tests such as culture for microorganisms, parasite examination, or faecal occult blood determination; or can be collected for culture and molecular-based diagnostics from the rectum/cloaca using cotton or gauze-tipped swabs, dependent on the volume of sample required by the specific test methodology.

Samples collected on swabs should be kept moist by placing them in the transport media recommended for use with the specific test to be performed, which may range from sterile saline to culture media containing antimicrobials or stabilisers. Faecal specimens should be kept chilled (e.g. refrigerated at 4°C or on ice) and tested as soon after collection as possible to minimise the negative impacts on test results caused by death of the targeted microorganism, bacterial overgrowth or hatching of parasite eggs. Double-packaging of faecal samples in screw cap or sealable containers that are subsequently contained within sealed plastic bags will help prevent cross-contamination of samples and associated packaging materials.

Epithelium: The specimens should be collected aseptically and preserved as specified for the intended test(s). Deep skin-scrapings obtained using the edge of a scalpel blade are useful for burrowing mites. Feather tips have been validated for use in the detection of viral antigen for Marek's disease, and used as a sample for molecular detection of additional avian diseases. Epithelial tissues, particularly those associated with vesicular

lesions and collected into viral transport media, can be critical in the laboratory diagnosis of specific viral infections such as foot and mouth disease.

Occular sample: The surface of the eye can be sampled by swabbing or ocular scraping, ensuring that cells rather than mucopurulent discharge or lacrimal fluids are collected for testing. Such swabs should be kept moist in saline or transport media specifically recommended for use with the testing to be performed.

Nasal discharge, saliva and vesicular fluids: Secretions can be collected directly into a vial or tube, or can be collected using swabs. Vesicular fluids provide a highly concentrated source of pathogen for diagnostic testing, and can be collected from unruptured vesicles using a sterile needle and syringe, and immediately transferred to a securely sealed vial or tube. Specifically developed sampling tools, such as probang cups, can be used for collecting cellular material and mucus from the pharynx of livestock.

Milk: The method of preservation of milk prior to testing varies with the requirements of the test; in some cases it will be critical to avoid freezing or addition of chemical preservatives.

6.2. Specimens Processing

Specimens are processed according to the study design and methods most appropriate for preserving the analytes of interest. For a particular specimen type and analysis, several processing methods may be appropriate.

- Blood sample processing – separation into fractions (e.g. plasma, serum, buffy coat and red blood cells. The processing method used for blood specimens depends on the laboratory analyses to be performed.
- Tissue- processing after surgery and autopsy: specimens resected specifically for research may be either processed in the operating room or

pathology suite, shortly after the time of collection, or may be transported to the repository for processing.

- Urine: processing of urine before storage is fairly straight forward. If the analytes are stable to freeze cycles then larger aliquites can be stored.

Self check 6- Written Exam

Name…………………………………………… ID………………………… Date…….

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

Choose the correct answers from the given alternatives (2 points)

1. Samples submitted to the laboratory in their raw form needs certain manipulations in order to prepare the sample for __________

   a. report b. discarding c. analysis d. store

2. Whole blood sample required for serological test must be collected into:

   a. test tube having EDTA b. universal bottle c. test tube without EDTA

3. Samples collected on swabs should be kept moist by placing them in the transport media recommended for use with the specific test to be performed

   a. false b. true

Part 2: Short answer questions

1. Define sample processing (2 points)
2. Mention whole blood sample processing steps for serological test (2 points)

   Note: **Satisfactory rating – ≥ 5 points Unsatisfactory - below 5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

LG #56	LO 3- Conduct basic laboratory procedures

Instruction sheet

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

- Conducting basic laboratory tests for identification of microbial infections
- Conducting basic laboratory tests for identification of internal and external parasitic infections
- Recording laboratory data
- Performing calculations on data
- Recording and giving laboratory test result feedback
- Reporting out of specification or atypical results
- Shutting down of equipments after completion of work
- Cleaning work places and equipment after accomplishment of tasks

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:

- Conduct basic laboratory tests for identification of microbial infections
- Conduct basic laboratory tests for identification of internal and external parasitic infections
- Record laboratory data
- Perform calculations on data
- Record and give laboratory test result feedback
- Report out of specification or atypical results appropriate personnel
- Shut down of equipments after completion of work
- Clean work places and equipment after accomplishment of tasks

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- Conducting basic laboratory tests for identification of microbial infections

1.1 Common Staining Technique

Staining is technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biological tissues for viewing, often with the aid of different microscopes.

I. Differential Staining

a. Gram's staining

Gram staining (or Gram's method) is an emprical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell wall. The Gram stain is almost always the first step in the identification of a bacterial organism.

Gram staining Principles

Gram staining is used to determine gram status to classify bacteria broadly. It is based on the composition of their cell wall. Gram staining uses crystal violet to stain cell walls, iodine as a mordant, and a fuchsin or safranin counterstain to mark all bacteria. Gram status is important in medicine; the presence or absence of a cell wall will change the bacterium's susceptibility to some antibiotics. Gram-positive bacteria stain dark blue or violet. Their cell wall is typically rich with peptidoglycan and lacks the secondary membrane and lipopolysaccharide layer found in Gram-negative bacteria

Components: Gram staining consists of four components:

- Primary stain (Crystal violet, methyl violet or Gentian violet)
- Mordant (Gram's Iodine)
- Decolourizer (ethyl alcohol, acetone or 1:1 ethanol-acetone mixture)

- Counterstain (Dilute carbol fuchsin, safranin or neutral red)

Procedures of Gram staining

1. Primary staining of heat fixed smear of specimen or bacterial culture is made with a pararosaline dye, e.g. crystal violate, gentian violet or methyl violet solution for one minute.
2. Pour off crystal violet and add dilute solution of iodine- keep for one minute.
3. Wash with water.
4. Decolourisation with an organic solvent (alcohol or acetone) – 10 to 30 seconds.
5. Wash with water.
6. Counterstain with a dye of contrasting colour (dilute carbol fuchsin, safranin or nutral red) 20-30 seconds.

b. Acid-fast Staining

The Ziehl–Neelsen stain, also known as the acid-fast stain, widely used differential staining procedure. In this type some bacteria resist decolourization by both acid and alcohol and hence they are referred as acidfast organisms. This staining technique divides bacteria into two groups namely acid-fast and non acid-fast. This procedure is extensively used in the diagnosis of tuberculosis and leprosy. Mycobacterium tuberculosis is the most important of this group, as it is responsible for the disease called tuberculosis (TB) along with some others of this genus.

Principle

Mycobacterial cell walls contain a waxy substance composed of mycolic acids. These are β-hydroxy carboxylic acids with chain lengths of up to 90 carbon atoms. The property of acid fastness is related to the carbon chain length of the mycolic acid found in any particular species

Ziehl- Neelsen Procedure

1. Make a smear. Air Dry. Heat Fix.
2. Flood smear with Carbol Fuchsin stain
   - ✓ Carbol Fuchsin is a lipid soluble, phenolic compound, which is able to penetrate the cell wall
3. Cover flooded smear with filter paper
4. Steam for 10 minutes. Add more Carbol Fuchsin stain as needed
5. Cool slide
6. Rinse with distilled water
7. Flood slide with acid alcohol (leave 15 seconds). The acid alcohol contains 3% HCl and 95% ethanol, or you can declorase with 20% H2SO4 8.
8. Tilt slide 45 degrees over the sink and add acid alcohol drop wise (drop by drop) until the red color stops streaming from the smear
9. Rinse with distilled water 10. Add Loeffler's Methylene Blue stain (counter stain). This stain adds blue color to non-acid fast cells. Leave Loeffler's Blue stain on smear for 1 minute
10. Rinse slide. Blot dry.
11. Use oil immersion objective to view.

II. Simple Stain

a. Giemsa staining

The Giemsa stain is used to stain spirochaetes such as *Borrelia anserine*, to to demonstrate the capsule of *Bacillus anthracis*, to stain rickettsial organisms such as *Haemobartonella felis* and *Cawudria ruminantum,* and to demonstrate the morphology of *Dermatophilus congolensis* more clearly than the Gram method.

Giemsa staining preparations

- Giemsa powder 1.0 g

- Glycerol 60.0 ml
- Absolute methanol 66.0 ml

The Giemsa powder is dissolved in the glycerol at 55-60 ^{0}C about two hours. Methanol is added and mixed thoroughly. One part of the solution diluted with 9 part buffer solution before use.

Procedure of Giemsa staining

- Make smear and fix in methanol for 2 minutes
- Stain in 10% Giemsa in buffered ph 7.2 solution for 30 minutes in a coplein jar or upside down on a staining plate.
- Wash off stain with a stream of buffer. (phosphate buffer PH 7.2)
- Flood with buffer and leave until differentiation is complete (1-2 minute)
- Dry by standing up right on the bench

b. Methylene blue stain

Methylene blue stain is used to demonstrate reaction of *B. anthracis*. The blue bacilli are surrounded by irregular purple (pink) capsular material.

Preparation of methylene blue stain

- Methylene Blue 0.2g
- Absolute alcohol or rectified spirit 10.0ml
- Distilled water 90.0ml

Dissolve the dye in alcohol and then add water. Filter through a filter paper.

Procedures for methylene blue staining

1. Put a large drop of polychrome methylene blue on the smear to cover it completely.

2. Leave for 30–60 seconds and air dry.
3. Wash off (into hypochlorite solution, 10,000 ppm). A wash bottle is better than a tap.
4. Examine under oil immersion. The capsule is seen clearly as pink amorphous material surrounding the blue-black bacilli.

1.2 Blood smear preparation

Examination of blood films is important in the investigation and management of anemia, Heamoparasite infections, and other conditions which produce changes in the appearance of blood cells and differential white cell count.

Principle of smear preparation

A small drop of blood is placed near the frosted end of a clean glass slide. A second slide is used as a spreader. The blood is streaked in a thin fi lm over the slide. The slide is allowed to air-dry and is then stained. EDTA anticoagulated blood is preferred. Blood smears can also be made from finger stick blood directly onto a slide. Smear Preparation can be:

- Wet smear
- Thin smear
- Thick smear

Procedures for each preparation

a. Wet blood smear/film preparation

1. A dropof blood is placed atthe centre of a clean slide
2. Cover with a clean, dry cover slip
3. Examine the film under the microscope (40×objective)

The method does not require staining. It is rapid and simple to perform.

Extracellular blood parasites such as Trypanosomes and Microfilaria of filarial nematodes can be diagnosed. Note that you can see only the movement of Trypanosomes species.

b. Thin blood smears preparation

It can be made by spreading a drop of blood evenly across a clean grease free slide using a smooth edged spreader.

- Make a drop of blood on one end of glass slide
- Place the end of second glass slide/spreader slide/ against the surface of the first slide, holding at an angle of 30-45 degrees
- Draw the spreader slide gently into the drop of blood and when the blood has alo ng 2/3 of width of the spreader slide by capillary action, push the spreader slide forward with a steady even motion
- Dry by waving rapidly in the air
- Fix with methyl alcohol for 2 min.
- Stain with Giemsa diluted 1:10 in neutral phosphate buffer for 30 min. in a coplein jar or upside down on a staining plate.
- Wash with water
- Allow it to dry by standing upright on the rack.
- Examine under the microscope(x100)

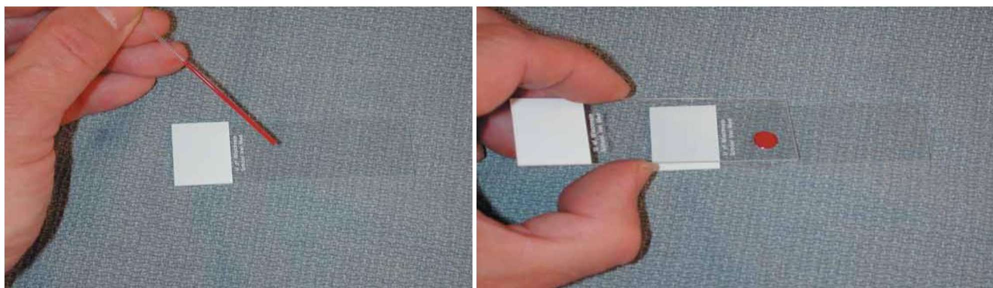

Figure 2: Blood Smear preparation

Thick bloods smear preparation

- A large drop of blood is put at the centre of a clean dry slide
- The drop is spread with an applicator stick, needle or corner of another slide to cover an area of ½ an inch square

- The smear is thoroughly dried in a horizontal position so that the blood could not ooze to one edge to the film and protected from dust, insects and direct sunlight.
- Dehemoglobinize by gently running distilled water on the smear or by immersing the smear in distilled water for 5 to 10 minutes
- Fix with methyl alcohol for 2 minutes
- Stain with Giemsa diluted in buffered distilled water 1:10 for 30 minutes
- Wash with buffered distilled water till it assumes abluish purple color
- Examine under the microscope (X40 and X100)

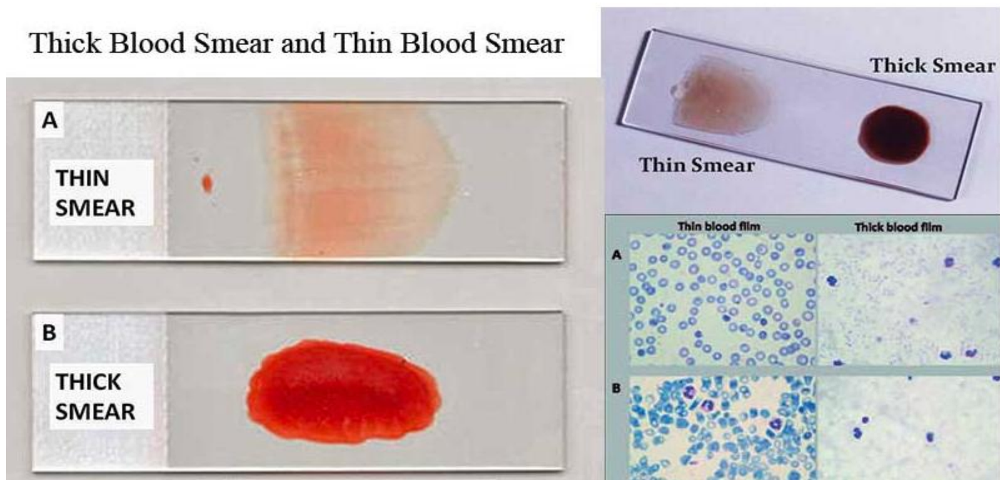

Figure 3: Thin and thick blood smear

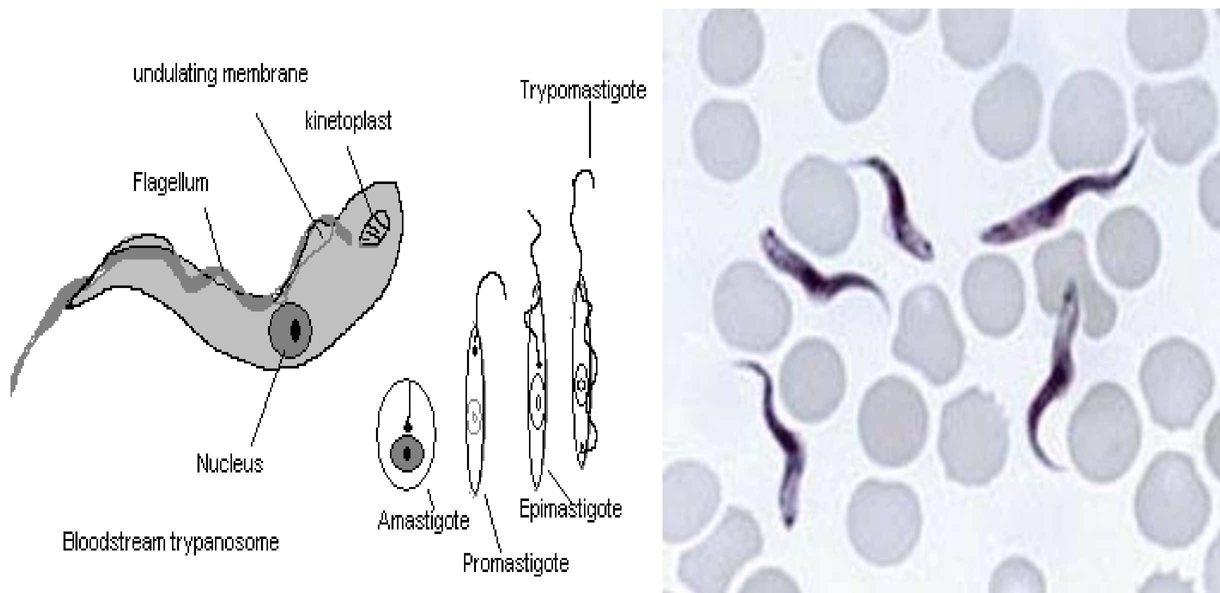

Figure 4: Morphology of Trypanosomes in positive sample

California Mastitis Test

The California mastitis test (CMT) is a simple cow-side indicator of the somatic cell count of milk. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel.

Procedure of California Mastitis Test

- Collection of milk samples from each quarter of udders in the CMT paddle. The paddle has four shallow cups marked in different to identify the individual quarter from which milk sample was obtain.
- Take about 2 ml or 1 teaspoon of milk from each quarter
- Add an equal amount of CMT solution to each cup in the paddle
- Rotate the CMT Paddle in a circular motion to mix the milk contents do not mix more than 10 seconds.
- The result is observed after about 20 seconds. The result is more gel formation.

Figure 5: California mastitis test

1.3 Microbiological Media Preparation

Growth medium or culture medium is a gel or liquid designed to support the growth of microorganisms or cells. There are different types of media for growing different types of organisms or cells. Most bacteriological culture Medias are commercially available as pre-made or as bases, which can be easily prepared in the laboratory. These commercial products are entirely acceptable and should be made and stored according to the manufacturer's recommendations.

1.3.1 Types of bacteriological culture media

Culture media are divided in to various types. According to the physical state media may be:

- ✓ Liquid media
- ✓ Solid media
- ✓ Semisolid

Liquid Media: Often called broths, milks, or infusions, these media have a liquid or fluid consistency that allows them to be easily poured or pipetted from one container to another. A liquid medium is usually prepared in tubes, flasks, or bottles and consists of various solutes dissolved in distilled water. Once inoculated, microbial growth can occur

throughout this liquid, transforming a transparent medium into a cloudy (turbid) suspension.

Solid Media: These media, usually prepared in tubes or Petri plates, provide a firm surface for microbes to grow on or within. Unlike a liquid medium, bacteria dispersed on a solid medium can grow as a continuous layer or as separate colonies. A solid medium is typically prepared by adding a solidifying agent to a liquid medium.

Semisolid Media: A medium having more of a “jellylike” consistency is considered a semisolid medium. Unlike a liquid medium that flows freely, this medium cannot be poured. However, to give it less body or firmness than a solid medium, the solidifying agent used in its preparation is added in smaller amounts. One of the useful features of a semisolid medium is it can be used for determining motility

I. Plate Media Preparation

a. Prepare media in stainless steel beakers or clean glassware according to manufacturer instructions. Media must be boiled for one minute to completely suspend agar.
b. Cover beaker with foil or pour into clean bottles being sure to leave lids loose. Sterilize according to manufacturer’s instructions when given, or at 121° C for 15 minutes
c. Cool media to 50°C in water bath.
d. Alternatively, media can be autoclaved and cooled to room temperature and refrigerated for later use. Store bottles labeled with media type, date, and initials.
e. Before pouring media, disinfect hood or counter thoroughly and place sterile petri dishes on the disinfected surface. Mix any added ingredients into the media at this temperature.
f. Label the bottom of each plate with medium type and date prepared.

g. Remove bottle cap and pour plates or dispense with a sterile Cornwall pipette, lifting each petri dish lid as you go. Pour approximately 15 to 20 mL per 100 X 15mm petridish. Replace lids as soon as the plate is poured.
h. Invert plates when the media has cooled completely (~ 30 to 60 minutes) to prevent excessive moisture and subsequent condensation on the plate lid.
i. Allow plates to sit overnight at room temperature. Store plates upside down in the refrigerator in a tightly sealed plastic bag or plate storage tin.
j. Follow manufacturer's recommendation for storage period of prepared media. Each batch should be labeled with the date of preparation

Figure 6: Culture Media Prepared on Plate

II. Tube Media Preparation

a. Prepare media in stainless steel beakers or clean glassware according to manufacturer's instructions. If the medium contains agar, boil for one minute to completely suspend the agar.
b. Arrange test tubes in racks. Disposable, autoclavable, screw cap tubes can be used for all tube media.
c. Use an automatic pipetter or Pipette-aid to dispense the medium. Dispense approximately 5 to 10 mL media in 16 X 125 mm or 20 X 125 mm tubes. Close caps loosely.
d. Immediately after use, rinse the automatic pipetter in hot tap water followed by distilled water to remove all media and prevent clogging of the instrument.

e. Loosely place screw caps on tubes. Do not tighten caps. It is necessary to allow pressure to release from tubes while heating in the autoclave.
f. Follow manufacturer's recommendation for autoclave time and temperature
g. If making slants, put tubes in slant racks after autoclaving. Adjust the slant angle to achieve the desired slant angle and butt length. Then tighten caps.
h. Cool completely to room temperature in the slanted position.
i. Label the tubes or the tube rack with type of medium and date made.
j. Store at 2 to 8°C, following manufacturer's recommendation for period of long-term storage

Figure 7: Microbiological media prepared in tube

Self check 1- Written Exam

Name………………………………………… ID……………………… Date……

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

Test 1: Short answer questions

1. __________ is staining technique used to identify bacteria into Gram positive and Gram negative characteristics (2 points)
2. Write the principles of Gram staining (4 points)
3. The staining techniques used for mycobacterium bovis is known as___ (2 points)

Test 2: Choose the best answers from the given alternatives (2 points each)

1. A type of laboratory techniques employed for screening of mastitis case is:

   a. gram staining b. blood smear c. California mastitis test d. acid-fast stain
2. __________ is a gel or liquid designed to support the growth of microorganisms or cells.

   a. milk b. culture medium c. growth medium d. B and C
3. One of the following laboratory tools is used for preparation of bacteriological growth medium

   a. petridish b. refrigerators c. incubator d. pistol and mortar
4. Wet blood smear/films is carried out to check________

   a. morphology of Heamoparasite b. motility of trypanosome species

   c. staining characteristics of *bacillus anthrax* d. babesiosis

Note: **Satisfactory rating – ≥ 8 points Unsatisfactory - below 8 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 2- Conducting basic laboratory tests for internal and external parasitic infections

2.1. Laboratory techniques for identification of internal parasitic infections

After fecal sample is collected from domestic animals, it would present to laboratory for examination. The methods for examination internal parasitic infections are direct smear, flotation, and gross examinations.

a. Direct fecal smear

This technique is effective only where the concentration of parasite stages is high. It is frequently difficult to identify them since they are partially covered by debris and Quantitative results cannot be obtained.

Note: This method is used when adequate laboratory facilities are lacking.

Procedure:

- Place a small drop of water/saline solution on a clean slide.
- A small amount of faecal sample is emulsified with a loop or glass rod and mixed with the saline/water
- Cover the smear with cover slip
- Examine the slide under microscope with low powers(X4 and X10) and then the high dry power(X40) to confirm.

b. Flotation technique

It is a method of separating the eggs from faecal debris by floating them on a variety of solutions. When faeces are emulsified in liquids of high specific gravity and either centrifuged or allowed to stand, the worm, eggs and many protozoan oocysts float to the top while the heavy coarse then be removed and examined. Nematode and Cestode eggs float in a liquid with a specific gravity of between 1.10 and 1.20.

Procedure

1. Take 3 grams of well mixed faecal sample into a beaker or plastic container 1. If the faeces are pelleted grind it using mortar & pestle.
2. Add approximately 40-50 ml of flotation fluid and stir/mix thoroughly until all the faecal material is broken down.
3. Pour the faecal suspension through a tea strainer or sieve /a double layer of gauze into container 2 to remove large faecal debris. The strained fluid is caught and the strainer is rinsed with water and the debris left on the strainer is discarded.
4. Place the tube in a test tube rack and gently topped off with the suspension leaving a convex meniscus at the top of the tube.
5. Carefully place a coverslip on top of the test tube, ensuring that no bubble is trapped under it (or take the smear on the slide with a glass rod from the supernatant).
6. Leave the test tube to stand for 10-20 minutes.
7. Carefully lift the coverslip off the test tube together with the drop of fluid adhering to it.
8. Place the coverslip on a clean slide.
9. Examine using a compound microscope at x 10 magnification.

OR

1. If centrifuge is available, pour the suspension into a test tube
2. Use a centrifuge briefly (at 1500 rpm for 3 minutes) and discard the supernatant carefully to the remaining sediment
3. Almost fill the tube with saturated floatation fluid
4. Resuspend the sediment with a spatula

5. Place the tube in a test tube rack and to up with the floatation solution to just fill the tube (**Do not over-fill**).

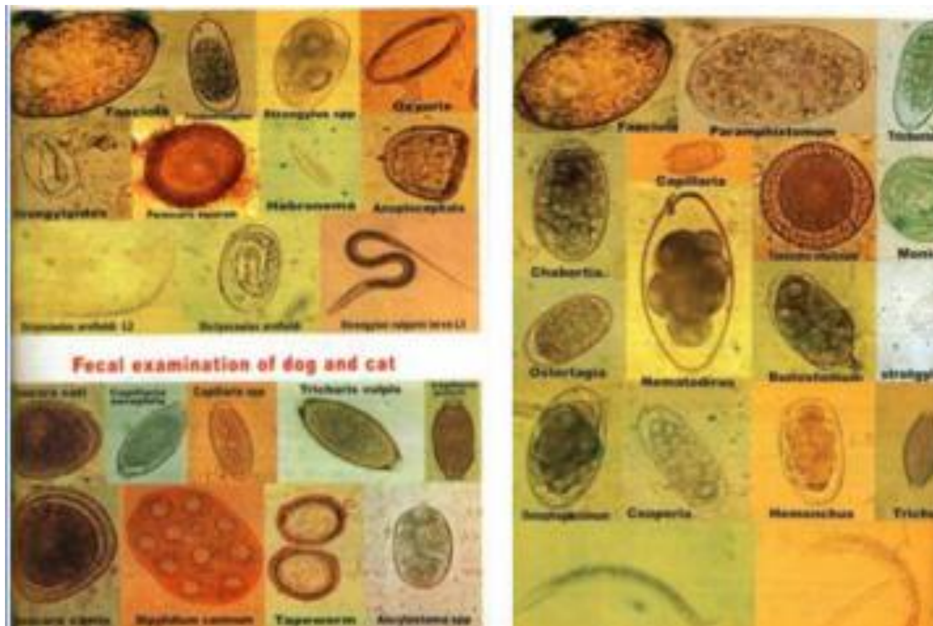

Figure 8: Larvae and eggs of endoparasites under floatation technique

c. Sedimentation technique

This method is mostly commonly used for Helminth eggs (mostly Trematode) which do not float well in common floatation fluid.

Procedure

1. Weigh or measure about 3 grams of faeces into a mortar and pistol
2. Pour 40-50ml of tap water into mortar
3. Mix thoroughly with a pestle/stirring rod
4. Sieve the mixture through a tea strainer or double-layer of gauze into a beaker
5. Pour the filtered material into a test tube.
6. Centrifuge the filtrate for 3 minutes at 1500 rpm (let to sediment for 5-10 minutes)
7. Remove the supernatant (pipette, decant) very carefully (by resuspending the sediment in 5 ml water repeat the procedure (6 & 7) until the suspension become clear)

8. Add a drop of 1% methylene blue to the sediment and mix (the dyes stain the faecal particles a deep blue or green leaving the trematode eggs unstained)
9. Take a drop of the sediment on the slide
10. Cover the smear with a cover slip and examine it under low power microscopic magnification.
    - Fasciola eggs appear yellowish and Paramphistomum eggs appear grayish with dark granules in the egg against the pale blue background.

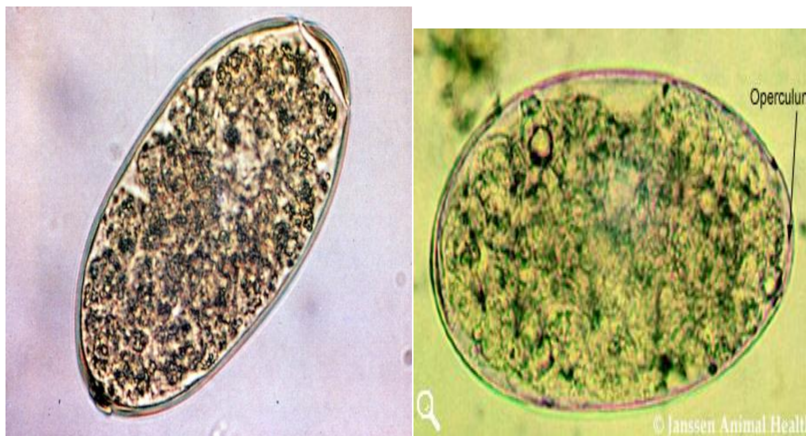

Figure 9: appearance of Fasciosala egg.

d. McMaster egg counting technique

McMaster is the quantitive methods for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. Advantage of this technique is quick as the eggs are floated free of debris before counting.

Equipment required:

- Beakers or plastic containers
- Balance
- Tea strainer or cheesecloth
- Measuring cylinder
- Stirring device (fork)
- Pasteur pipettes and rubber teats

- Flotation fluid
- McMaster counting chamber
- Microscope

Procedure of McMaster techniques

- Weigh out 2 gm of feces and transfer in to container 1
- Add 60 ml of saturated salt solution into container 1
- Mix feces with saturated salt solution by stirring device
- Filter fecal from container 1 into container 2 or cylinder by gauze or sieve
- Take a sub- sample with a Pasteur pipette from container 2
- Fill both side of the McMaster counting chamber with the sub sample
- Allow the counting chamber to stand for 3-5 minutes
- Examine the sub sample of filtrate under a microscope at 10x 10 magnification
- Count all eggs and coccidian oocytes within the engraved area of both chambers
- Focus first on the etched lines of the grid, then go down a tiny bit, the egg will be fl oating just below the top of the chamber.
- The calculation of egg from chambers is: Multiply the total number of eggs in the 2 chambers by 100= eggs per gram (EPG) or multiply the total by 50. This gives the EPG (egg per gram of feces) of faeces. (Example: 50 eggs seen in chamber 1 and 100 eggs seen in chamber 2 = (50+ 100) x 50 = 25,000 epg.)
- Formula for calculation of Egg per Gram of Feaces (EPG)

$$\text{EPG}= \frac{\textbf{Egg counted x Total volume/Volume counted}}{\textbf{Grams of Feces used}}$$

Figure 10: Mcmaster egg counting chamber

e. Baermann apparatus technique

The Baermann technique is used to isolate lungworm larvae from faecal samples and infective larvae from faecal cultures. It is based on the active migration of larvae from faeces suspended in water and their subsequent collection and identification. Therefore, the following events take place when the sieve is placed in the water. The larvae will be moving around in a random fashion and within any given time interval. Some of them will migrate through the tissue and fall into the water. Because they can't swim they sink to the bottom and over time a number accumulate there. The more active the larvae are (i.e. the warmer the water) the greater the number of larvae that accumulate at the bottom in a given time interval. The longer you wait, the more larvae will fall to the bottom of the dish, but with time, the fecal sample breaks down and begins to pass through the tissue leading to an accumulation of sediment along with the larvae.

Equipment required:

- Funnel
- Funnel stand
- Rubber or plastic tubing
- Rubber bands
- Clamp or spring clip

- Cheese cloth
- Simple thin stick
- Strainer
- Microscope
- Test tube
- Pasteur pipette
- Small petridish

The procedure of baermann apparatus technique

- First of all, construct the funnel with its standing; fi t a short piece of tubing which is closed at one end with a clamp or string clip to the stem of a funnel
- Support the funnel by a stand
- Weigh the fecal culture/ feaces about 5- 10 gm and place on a piece of double layer cheesecloth Form the cheesecloth around the faeces as a pouch
- Close the pouch with a rubber band
- Fix a supporting stick under the rubber band
- Place the pouch containing faecal culture material or feaces in the funnel and trim the surplus cheesecloth off
- Fill the funnel with lukewarm water and covering the faecal material
- Leave the apparatus in place for 24 hours, during time larvae actively move out of faeces and ultimately collect by gravitation in the stem of the funnel.
- Draw 10-15 ml of fl uid from the stem of the funnel into a test tube or petri dish.

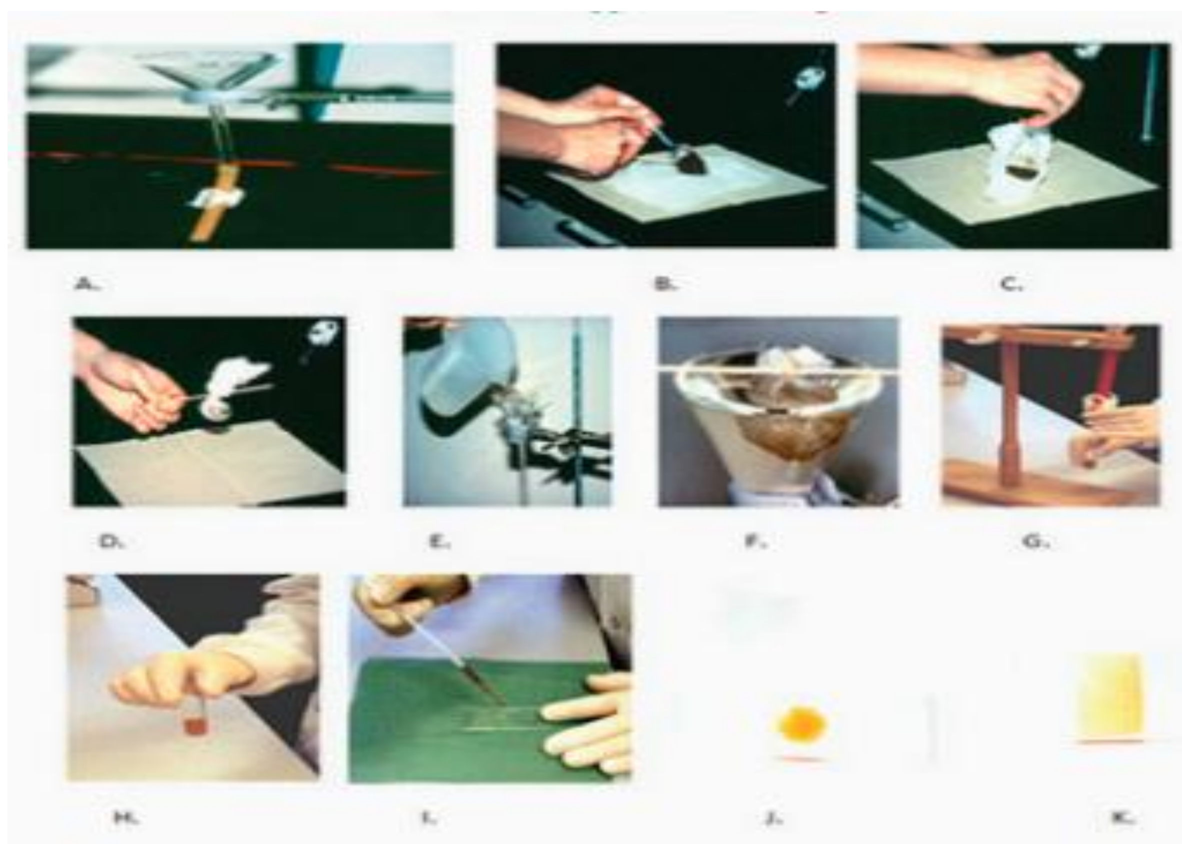

Figure 11: Procedures of Bearman apparatus technique

2.2 Laboratory technique for examination of External Parasitic infections

Skin scraping

Skin scrapings are part of the basic database for all skin diseases. There are two types of skin scrapings, superficial and deep. Superficial scrapings do not cause capillary bleeding and provide information from the surface of the epidermis. Deep skin scrapings collect material from within the hair follicle; capillary bleeding indicates that the sampling was deep enough. Skin scrapings are used primarily to determine the presence or absence of mites. Skin scrapings help diagnose fungal infections and scabies. For fungal infection, scale is taken from the border of the lesion and placed onto a microscope slide. Then a drop of 10 to 20% potassium hydroxide is added. Hyphae,

budding yeast, or both confirm the diagnosis of tinea or candidiasis. For scabies scrapings are taken from suspected burrows and placed directly under a cover slip with mineral oil; findings of mites, feces, or eggs confirm the diagnosis.

Superficial skin scraping superficial scraping used to identify surface mites and multiple scrapings should be taken to increase the likelihood of ectoparasites (Cheyletiella, Sarcoptes, Psoroptes, Otodectes, and Demodex mites, fleas and lice) detection.

The procedure of superficial scraping

- Remove the hair coat in the area by gentle clipping and after clipping the hairs they are has to be sterilized with antiseptics (chlothrixidine, soap).
- A few drops of liquid paraffin can be applied and spread over the skin scraping site , then scraping with a blunt scalpel blade
- Holding the blade perpendicular to the skin or slightly deviated away from the direction of scraping, the blade is gently passed over large areas of the skin in a sweeping pattern.
- Don't be apply 10% KOH directly to skin.
- Take the scrabed hair and skin content into petridish
- Emulsion of scant material(superficial epidemis) is spread over a microsope slide or petridish
- Add 2 -3 drops of 10% KOH into scrabed sample and mix it
- It is warmed for about 3 minutes
- Covered with a cover slide and the slide is examine under microscope at 10x low power, to see adult, larvae, nymph and egg of parasite

Deep skin scraping

Deep skin scraping is important in the diagnosis of burrowing and deep follicular mites such as Sarcoptes scabiei and Demodex sp. The technique is repeated until capillary blood oozes out and multiple sites should be scraped to maximize detection of ectoparasites.

Procedure

- The hair should be clipped with scalpel blade. Holding the blade perpendicular to skin and blade is gently passed over a small area of lesional skin in a sweeping pattern. The skin should be pinched fi rmly between the thumb and forefinger to help extrude the parasite from deeper epidemal layer.
- Place the scalpel blade with material removed into petridish or other container
- The collected material is mounted onto glass slide in liquid paraffin or potassium hydroxide.
- Cover a slide should be applied into sample collected on slide and examine it under low power objective of microscope

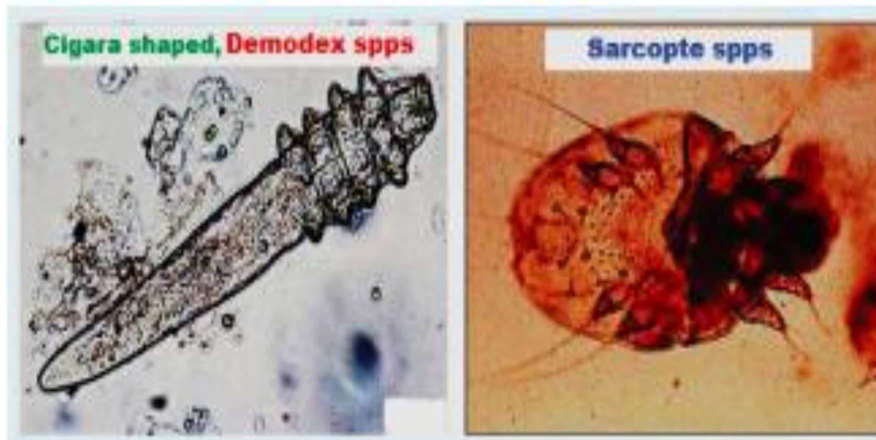

Figure 12: The structure of mites obtained from scrapping of skin

Self check 2- Written Exam

Name…………………………………………… ID………………………… Date…….

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

Test 1: Short answer questions

1. _______technique of fecal examination is effective only where the concentration of parasite stages is high (2 points).
2. Write the quantitative and qualitative technique of fecal examination (1 point)

Test 2: Choose the correct answers from the given alternatives (2 points each)

1. A faecal examination technique used to identify trematod parasite egg is:
   a. floatation b. sedimentation c. bearman technique d. skin scrapping
2. Skin scrapping is conducted to identify ________ parasite infections
   a. ectoparasite b. endoparasite c. lung worm d. fasciolosis
3. The primary objectives of bearman technique is:
   a. to identify adult parasite in fecal sample
   b. to identify larvae in fecal sample
   c. to identify eggs of parasite in fecal sample
4. __________ is the quantitive methods of fecal examination used for determining the number of nematode eggs per gram of feces

a. Floation technique b. sedimentation technique c. Mcmaster egg counting

Note: **Satisfactory rating – ≥ 5.5 points Unsatisfactory - below 5.5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 3- Recording laboratory data

3.1 Introduction

A laboratory data is a designated data science system that is intended to uncover all that your data has to offer. As a space that facilitates data science and accelerates data experimentation, data labs uncover which questions businesses should ask, and then help to find the answer.

Large clinical databases are becoming increasingly available every day to researchers as more laboratories and practices adopt electronic record systems. These records may cover a range of clinical fields, including infectious and non-infectious diseases with all specific and non-specific tests. Analyzed information can be used in the process of patient care (i.e. diagnosis of diseases, treatment, screening and prevention). Analysis of laboratories data consequently yields information that is necessary for the evidence-based planning and decision making in the health care system.

Another issue is the accessibility that should be easy for every stakeholder, while new technologies are now more and more implemented in the clinical settings. Besides, accessibility of the data can be through the entire health sector.

Inventories are most often organised by assigning a unique identifying number or alphanumeric code to each sample (sample container) that is cross-referenced to a database or inventory log. Inventory records can be manual data logs, computerised spreadsheets, or specialised computer programs. However the records are managed, they must be kept current and the information entered must be traceable to its source. The identification of the individual making an entry or modification to the sample inventory should be recorded.

Self check 3- Written Exam

Name………………………………………… ID……………………… Date……

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

Short answer questions

1. Write the purpose of recording laboratory data (4 points)
2. Explain basic laboratory data need to be recorded (2 points)
3. ________ is a designated data science system that is intended to uncover all that your data has to offer (2 points)

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

Answer Sheet

Score = ___________
Rating: ____________

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

Information sheet 4- Performing calculations on data

4.1 Recording, storage and interpretation of data

Because of the volume and complexity of the information to be stored and the need to keep these data available for an undetermined period of time, careful consideration should be given to database design. The storage of raw (primary, non-interpreted) data is essential to allow the evaluation in response to various kinds of questions, including those arising in the future. Laboratory results should be collected in a suitable national database and recorded quantitatively. The information to be recorded should include, where possible, the following aspects:

- sampling programme
- sampling date
- animal species and production type
- type of sample
- purpose of sampling
- type of antimicrobial susceptibility testing method used
- geographical origin (geographical information system data were available) of herd, flock or animal
- animal factors such as age, condition, health status, identification, sex;
- exposure of animals to antimicrobial agents
- bacterial isolation rate

The reporting of laboratory data should include the following information:

- identity of laboratory
- isolation date
- reporting date
- bacterial species and where relevant, other typing characteristics, such as:
  - ✓ serotype or serovar
  - ✓ phage type

- ✓ antimicrobial susceptibility result or resistance phenotype
- ✓ genotype

Ideally, laboratory data should be collected at the individual isolate level

Self check 4: Written Exam

Name…………………………………………… ID………………………… Date…….

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

Short answer questions

1. Write some example of laboratory data that should be recorded and help for analysis (4 points)
2. Discuss some example of calculations performed in the laboratory (2 points)

***Note:* Satisfactory rating – ≥ 3 points Unsatisfactory - below 3 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 5- Recording and giving laboratory test result feedback

5.1 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.

5.2 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.

Agents and tissues maintained in an archive must be correctly identified and sufficient supporting data that characterizes the sample or agent must be recorded. For reference materials, further documentation that authenticates the agent or tissue is required. The unique identity of the tissue, fluid, or agent and the storage location are best maintained in an electronic or paper inventory record which also documents the

date the material was obtained, date and method of preservation, volume of material stored, source of the material including associated species, geographical location, and the clinical history of the donor animal and the disease situation of the flock or herd.

However the records are managed, they must be kept current and the information entered must be traceable to its source. The identification of the individual making an entry or modification to the sample inventory should be recorded.

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 5- Written Exam

Name…………………………………………… ID………………………… Date…….

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

Short answer questions

1. Explain the main purpose of clinical and client record keeping (4 points)
2. Write tips on writing a report on health care quality for clients (2 points)

Note: **Satisfactory rating – ≥ 3 points   Unsatisfactory - below 3 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information sheet 6- Reporting out of specification or atypical results

6.1 Handling of out of specifications results

The term out of specifications, are defined as those results of in process or finished product testing, which is falling out of specified limits, which are mentioned in compendia. The Out of Specification may arise due to deviations in product manufacturing process, errors in testing procedure, or due to malfunctioning of analytical equipment. When an Out of specification (OOS) has arrived, a root cause analysis has to be performed to investigate the cause for out of specification. The reasons for Out of specification can be classified as assignable and non-assignable. When the limits are not in specified limits, it is called out of specifications. There are two phases of out of specification investigation process. These are phase I and phase II investigation process.

Sample rejection and discrepancies between the sample and accompanying paperwork should be resolved by contacting the sender immediately to resend a duplicate sample or to clarify paperwork.

Phase I (laboratory investigation)

The purpose of the laboratory investigation is to identify the cause for out of specification (OOS) result. The reason for the out of specification (OOS) may be defect in measurement process or in manufacturing process. Irrespective of the rejection of batches, the OOS results must investigate for their trend. The investigation can be done to only those batches that are resulted in OOS, or also to other batches and even other products associated with OOS. The OOS investigation should be thorough, timely, unbiased, well documented and scientifically sound. There are two phases of out of specification result investigation.

Phase II investigation

When there is no possible outcome has obtained from the phase I investigation, the phase II investigation should be commenced in context to investigate the errors occurred in manufacturing processes, sampling procedures along with other additional laboratory testing.

The additional laboratory testing at phase II investigation should involve retesting and resampling.

a. Retesting

The main objective behind retesting of the same sample is to determine the analytical or dilution error. The sample for retesting should be taken from the same lot of the initial test. The person, who is going to retest the sample should be more or at least equally qualified and experienced as of the first analyst. If the retest results falls within limits, then the initial results should be replaced with later, but should be included in the report along with explanation regarding failure at the first time. If the retested results are also out of limits, then the batches should be re injected and the investigation should further expanded to other associate batches and products.

b. Resampling

While retesting refers to analysis of the original, homogenous sample materials. Resembling involves analyzing a specimen from any additional units collected as part of the original sampling or from a new sample collected from the same batch. When the results of resampling or within specified limits, then the initial results should be superseded. If the error is due to improper sampling, then the sampling procedures should be validated, and new sampling procedure should be proposed, if needed, and documented.

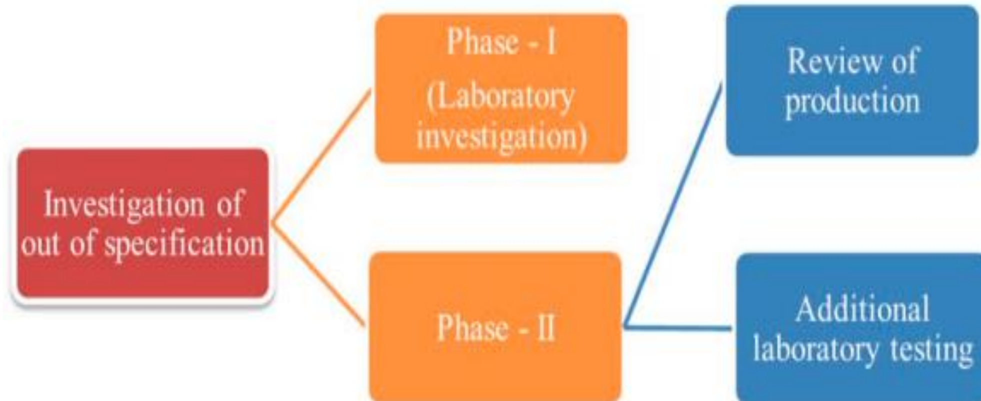

Diagram show Investigation process Out of specification (OOS) result

Self check 6- Written Exam

Name…………………………………………… ID……………………… Date…….

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

Short answer questions

1. __________are defined as those results of in process or finished product testing, which is falling out of specified limits (2 points)
2. Discuss the two phases of out of specification result investigation methods (3 points)
3. _________involves analyzing a specimen from any additional units collected as part of the original sampling or from a new sample collected from the same batch (2 points)

Note: **Satisfactory rating – ≥ 3.5 points Unsatisfactory - below 3.5 points**

Answer Sheet

Score = ___________

Rating: ____________

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

Information Sheet 7- Shutting down of equipments after completion of work

7.1 Shutting down of laboratory equipments after completion of work

At that time of completing laboratory activities, the following shutdown checklist, with any lab-specific additions, should be implemented.

General

- Clean glassware and store appropriately, do not leave dirty equipment out
- Turn off the lights
- Turn off plumbed natural gas
- Cancel deliveries, if possible
- Lock all lab doors
- Return all animals to proper housing area

Biological

- Samples that can be stored at -80, -20 or 4 C should be frozen or stored as appropriate.
- For cultures that cannot be frozen down, ensure you have enough supplies to maintain cultures, and personnel to do the work.
- Dispose of all biological materials appropriately.
- Ensure the cryostorage units have enough liquid nitrogen.
- Disinfect and empty aspirator collection flasks by the biosafety cabinets and bench tops.
- Turn off UV light

Chemicals

- Be diligent in returning chemicals to their proper storage location immediately after use; don't leave cleanup for tomorrow.
- Move chemicals from laboratory benches and store in secondary containment with compatible chemicals.
- Label and securely cap every container.
- Move hazardous waste with completed waste tag to the proper waste storage area.
- Store compressed gas cylinders, not in use, with their valve caps tightly secured and double chained.

Equipment

- Electrical equipment
  - ✓ Review proper shut down procedures to prevent surges.
- Incubators
  - ✓ Consider the availability of CO_2, and plan to consolidate and shut down unneeded incubators to conserve supplies.
- Fridges/Freezers
  - ✓ Check that essential equipment is on red power supply for emergency power.
- Shut down microscopes, hot plates, sterilizers, water baths, and all other equipment that is not being used. Unplug from energy source, if possible.
- Autoclaves: close doors or shut down completely.
- Check water distillation units.
- Communicate with all delivery personnel and set a time for essential deliveries if needed.

Self check 7- Written Exam

Name…………………………………………… ID……………………….. Date…….

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

Short answer questions

1. Discuss checklist that should implemented to prevent biological hazards after completion of laboratory activities (6 points)
2. List laboratory equipments need shutting down after completion of work (2 points)

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

Answer Sheet

Score = ___________
Rating: ____________

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

Information sheet 8- Cleaning workplaces and equipment after accomplishment of tasks

8.1 Cleaning Workplaces and Equipments

Cleaning is a process that removes dirt, dust, large numbers of microorganisms and the organic matter using detergent and warm water or disposable detergent wipes, such as blood or faeces that protects them. Cleaning is a pre-requisite to disinfection or sterilization. Cleaning removes grease, soil and approximately 80% of micro-organisms. It is an important method of decontamination and may be safely used to decontaminate low risk items of equipment such as those coming onto contact with intact skin. In most clinical areas a daily clean with a detergent based fluid is adequate. The aim is to remove organic matter and dust and to reduce the bacterial load in the environment. The cleaning of floors and hand wash basins and emptying of waste paper bins are the basic tasks that it is reasonable to expect a cleaner to undertake without any specialised training. However, in order to work safely, the cleaner must be made aware of the need always to follow some basic precautions.

Lab personnel are responsible for cleaning the surfaces in their laboratories and offices. Cleanliness and tidiness is widely recognised as an essential and effective risk control measure within a laboratory environment. The checklist below provides essential and practical information aimed at helping staff and students ensure that their work area is maintained in a clean and tidy condition.

8.2 Laboratory housekeeping checklist

- keep your work area free from clutter and organise materials and equipment so as not to present a hazard
- plan new work carefully and use the risk assessment process to consider necessary safety precautions or control measures that may be required prior to commencing work

- tidy work area and clean-up work surfaces after each project or at the end of each day
- clean up equipment after use to ensure it is kept in good working order
- ensure that any chemicals, materials or equipment not in immediate use are properly stored
- ensure that all laboratory wastes (e.g. chemical, biological, radioactive, sharps or mixed) are properly segregated and disposed of at point of use in accordance with Laboratory Waste Management Procedures;
- avoid the accumulation of paper waste as it provides a ready source of fuel for fire
- clean up spills immediately and thoroughly using appropriate equipment, materials or spill kits
- chemicals shall be segregated according to their dangerous goods class and stored separately to minimise risk of interaction
- Upon completion of work, PPE used should be immediately disposed of as contaminated waste
- Lab. Coats or gown should be treated with disinfectant prior to laundering separately from other clothing.
- Laboratory gowns must be removed before washing hands when leaving the laboratory. Hands should be washed with a suitable disinfectant hand-wash before leaving the laboratory
- cultures of microorganisms must be clearly identified, dated and appropriately stored
- benches must be wiped with disinfectant before and after work. The production of aerosols should be minimised, particularly when working on an open bench
- microscopes and objectives should be cleaned after use. Immersion oil must be thoroughly removed

- wastes containing live micro-organisms should be autoclaved or treated with an appropriate disinfectant before disposal
- do not leave cultures on the table or in unmarked areas when the laboratory session is completed
- microbiological waste should be autoclaved before leaving the laboratory
- wash hands thoroughly with liquid soap and water
- dry hands thoroughly after washing

8.3 Safety Data Sheets (SDS)

A Safety Data Sheet (SDS) provides critical information required for the safe handling of chemicals used in the workplace, including chemical and physical properties, health hazard information, emergency procedures and safe storage, use, handling and disposal procedures. A Safety Data Sheet must be provided with all first purchases or deliveries of hazardous substances and dangerous goods. There is no need to include a SDS with every delivery, unless the information contained in the SDS has been revised.

Self check 8- Written Exam

Name…………………………………………… ID……………………….. Date…….

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

Short answer questions

1. Give the definition of material safety data sheet (2 points)
2. __________is a pre-requisite to disinfection or sterilization (2 points)
3. Discuss laboratory housekeeping checklist after accomplishment of tasks (2 points)

***Note:* Satisfactory rating – ≥ 3 points Unsatisfactory - below 3 points**

Answer Sheet

Score = ___________

Rating: ____________

Operation sheet 1- Conducting Gram staining

Procedures for Gram staining technique

1. Prepare smear of specimen or bacterial culture on clean microscopic slide
2. Primary staining of heat fixed crystal violate, gentian violet or methyl violet solution for one minute
3. Wash with tap water
4. Add dilute solution of iodine and keep for one minute.
5. Wash with water.
6. Decolourisation with an organic solvent (alcohol or acetone) – 10 to 30 seconds.
7. Wash with water.
8. Counterstain with a dye of contrasting colour (dilute carbol fuchsin, safranin or nutral red) 20-30 seconds.
9. Dry the slide on rack by putting upside down of the slide or gauze
10. Observing under 100X objective lenses by adding oil immersion

Operation sheet 2- Blood smear examination

Steps for thin blood smear preparation

1. Make a drop of blood on one end of glass slide
2. Place the end of second glass slide/spreader slide/ against the surface of the first slide, holding at an angle of 30-45 degrees
3. Draw the spreader slide gently into the drop of blood and when the blood has alo ng 2/3 of width of the spreader slide by capillary action, push the spreader slide forward with a steady even motion
4. Dry by waving rapidly in the air
5. Fix with methyl alcohol for 2 min.
6. Stain with Giemsa diluted 1:10 in neutral phosphate buffer for 30 min.
7. Wash with water
8. Allow it to dry by standing upright on the rack
9. Examine under the microscope(x100)

Operation sheet 3- Performing floatation technique

Procedures for sedimentation techniques

1. Take 3 grams of well mixed faecal sample into mortar & pestle. Grind if the faeces is pelleted.
2. Add approximately 40-50 ml of flotation fluid and stir/mix thoroughly until all the faecal material is broken down.
3. Pour the faecal suspension through a tea strainer or sieve /a double layer of gauze into test tube
4. Place the tube in a test tube rack and gently topped off with the suspension leaving a convex meniscus at the top of the tube.
5. Carefully place a coverslip on top of the test tube, ensuring that no bubble is trapped under
6. Leave the test tube to stand for 10-20 minutes.
7. Carefully lift the coverslip off the test tube together with the drop of fluid adhering to it.
8. Place the coverslip on a clean slide.
9. Examine using a compound microscope at x 10 magnification

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. The project is expected from each student to do it.

Note: Time allowed for task 1= 15 minutes, task 2= 40 minutes and task 3= 30 minutes

Materials required for Gram staining: Bunsen burner, forceps, microscope, microscopic slide, rack, oil emersion, crystal violet, iodine tincture, methyl alcohol and safronin (carbol fusion)

Materials required for blood smear preparations: microscopic slide, coverlip, giemsa stain, methyl alcohol, rack, uncoagulated blood, glove, water, gauze and microscope.

Materials required for Gram staining: Sensitive balance, pistol and mortar, test tube, test tube rack, cover slip, microscope, microscopic slide, sieve, gauze and glove

Task 1: Perform gram staining on milk sample

Task 2: Perform thin and thick blood smears from cattle to identify morphology of trypanosomes

Task 3: Perform floatation technique from fecal sample for examination of nematode parasite eggs.

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