Animal Health Care Service

Level-III

Based on March, 2018, Version 3 Occupational standards (OS)

Module Title: - Carrying out Sample Collection, Preservation and Shipment

LG Code: AGR AHC3M22 0121 LO (1-4) LG (85-88)

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Table of Contents

LG #85	LO #1- Follow OHS practices

Instruction sheet

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

- Following Safe work.
- Maintaining personal hygiene and cleanliness standards.
- Recognizing and reporting risks in sample collection.
- Using, maintaining and storing PPE clothing and equipment.
- Collecting specimens from farm
- Handling the specimens

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:

- Follow Safe work.
- Maintain personal hygiene and cleanliness standards.
- Recognize and report risks in sample collection.
- Use, maintain and store PPE clothing and equipment.
- Collect specimens from farm
- Handle the specimens

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 1 and 2 on page 30 and 31 respectively’’
7. Perform “the Learning activity performance test” which is placed following “Operation sheets “on 33 page ,
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- Following Safe work. Practice

Introduction

Safety is the state of being "safe", the condition of being protected from harm or other non-desirable outcomes. Safety can also refer to the control of recognized hazards in order to achieve an acceptable level of risk.

Safety is the condition of a “steady state” of an organization or place doing what it is supposed to do. For any organization, place, or function, large or small, safety is a normative concept. It complies with situation-specific definitions of what is expected and acceptable.

Security is the process or means, physical or human, of delaying, preventing, and otherwise protecting against external or internal, defects, dangers, loss, criminals, and other individuals or actions that threaten, hinder or destroy an organization’s “steady state,” and deprive it of its intended purpose for being.

1.1. Work Health And Safe (WHS)) Policy And Procedure

These usually include important information regarding:

- personal protective clothing and equipment
- standard and safety precautions
- handling hazardous/dangerous materials and goods, including completing safety data sheets (SDSs)
- emergency procedures
- standard housekeeping
- hazard identification and control systems
- manual handling
- staff development and training programs
- waste management
- WHS personnel.

Personal protective equipment procedures

PPE is clothing and equipment designed to protect workers from direct exposure to blood, body fluids, potentially infectious materials and other harmful agents in the work environment. Workers must use PPE correctly to protect their own health and safety. Your supervisor must be notified immediately if PPE requires repair or replacement. Using PPE to eliminate or reduce risks to health and safety is a last resort. PPE should only be used when particular risks cannot be eliminated or reduced.

Standard precautions

Standard precautions are meant to reduce the risk of transmission of blood borne and other pathogens from both recognized and unrecognized sources. They are the basic level of infection control precautions which are to be used, as a minimum, in the care of all patients.

- Hand hygiene is a major component of standard precautions and one of the most effective methods to prevent transmission of pathogens associated with health care.
- In addition to hand hygiene, the use of personal protective equipment should be guided by risk assessment and the extent of contact anticipated with blood and body fluids, or pathogens
- Respiratory hygiene and cough etiquette

Emergency procedure

An emergency procedure is a plan of actions to be conducted in a certain order or manner, in response to a specific class of reasonably foreseeable emergency, a situation that poses an immediate risk to health, life, property, or the environment. Where a range of emergencies are reasonably foreseeable, an emergency plan may be drawn up to manage each threat. Most emergencies require urgent intervention to prevent a worsening of the situation, although in some situations, mitigation may not be possible and agencies may only be able to offer palliative care for the aftermath. The emergency plan should allow for these possibilities

Self-check 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. _____is the state of being "safe", the condition of being protected from harm or other non-desirable outcomes.(1pt)
2. Mention important information regarding work health and safe (whs)) policy and procedure(7pts)

   _____
   _____
   _____
3. _____ is a plan of actions to be conducted in a certain order or manner, in response to a specific class of reasonably foreseeable emergency, a situation that poses an immediate risk to health, life, property, or the environment.(1pt)
4. Explain PPE(1pt)

   _____
   _____
   _____

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

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

Information Sheet 2- maintaining Personal hygiene and cleanliness standards.

Sanitation addresses the cleaning—and disinfection when necessary—of people, equipment, animals and material entering a farm. Routine farm operations such as feeding, milking, animal handling, medical treatments, contact with vehicles and equipment, interactions with service providers and outside visitors, are all possible contact points for the transfer of diseases and pests. Entry and exit routes from buildings and a property have the potential to bring and take away disease-causing organisms.

Some form of cleaning and disinfection should be done before people and their clothing, equipment, supplies, and larger items such as vehicles and heavy equipment cross from dirty or low risk (the farm perimeter) to clean or higher risk areas (animal housing, animal transport vehicles, feed, water and other items that come into close contact with livestock).

Proper Personal Hygiene:

- Wash hands before and after animal handling.
- Do not eat or drink in the animal housing areas.
- Wear coveralls, farm specific clothing or laboratory coats when handling animals.
- Avoid handling sick animals or animals with lesions unless gloved.
- Wear a mask if you are allergic to animal hair or dander or if feed or bedding dust is present
- If you are sick, DO NOT enters the agricultural animal facilities. You are more susceptible to other infective agents and you may transfer pathogens to the animals!
- Routinely wear gloves when cleaning animal area.
- Note progression of any illness. Report illnesses to your supervisor.
- Inform physician of your animal related activities.

Self-check 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Sanitation addresses the _____and _____ when necessary—of people, equipment, animals and material entering a farm.
2. Describe personal hygiene

   _____
   _____
   _____
   _____

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

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

Information Sheet 3- Recognizing and Reporting Risks in sample collection and handling

3.1 Risk

Persons working in clinical diagnostic laboratories are exposed to many risks. Whether the patients are humans or animals and whether laboratorians work in microbiology or elsewhere in the laboratory, the human and animal diagnostic laboratory is a challenging environment. The more those laboratorians become aware of and adhere to recommended, science-based safety precautions, the lower the risk. The goal of a safety program is to lower the risk to as close as possible to zero, although zero risk is as yet unattainable as long as patient specimens and live organisms are manipulated. Protection of laboratorians, coworkers, patients, families, and the environment is the greatest safety concern.

3.2 Laboratory Exposures

Laboratory exposures occur more often than is generally suspected. Other laboratory incidents such as minor scrapes or cuts, insignificant spills, or unrecognized aerosols occur even more frequently and might not cause an exposure those results in an LAI (laboratory animal infection). In this report, "laboratory exposures" refer to events that put employees at risk for an LAI and events that result in actual acquisition of LAIs. Except for reporting requirements imposed by CDC's Select Agent Program, which deals with handling of specific, potentially hazardous biological agents and toxins, no national surveillance system is in place to which medical laboratory exposures and subsequent work-related infections are reported. Increased attention has been focused on laboratory biosafety and biosecurity since 2001 but has been largely limited to precautions required for agents of bioterrorism. Other laboratory exposures and LAIs continue to occur, almost always because of a breakdown of established safety protocols. Because of the lack of an official surveillance mechanism for reporting LAIs and because of the fear of punitive action by an oversight agency if injuries are

reported, the data needed to determine the extent and cause of LAIs are unavailable. In addition, there is a dearth of science-based insights on prevention of LAIs.

The Blue Ribbon Panel recognizes the need for a voluntary, no punitive surveillance and reporting system with the potential for anonymity to be implemented in the United States. Such a system would allow for reporting and evaluation of all LAIs and would potentially lead to training and interventions to facilitate a negligible incidence rate.

3.3 Routes of Laboratory Infection

The five most predominant routes of LAIs are

- parenteral inoculations with syringe needles or other contaminated sharps;
- spills and splashes onto skin and mucous membranes;
- ingestion or exposure through mouth pipetting or touching mouth or eyes with fingers or contaminated objects;
- animal bites and scratches (research laboratories or activities); and
- Inhalation of infectious aerosols.

Self-check 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Explain laboratory exposure (2)

   _____
   _____
   _____

2. Write the most predominant routes of laboratory Infection

   _____
   _____
   _____

You can ask you teacher for the copy of the correct answers.(4)

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

Information Sheet 4- Using, maintaining and storing PPE clothing and equipment

4.1. **Introduction**

Personal protective equipment (PPE) is protective clothing, helmets, goggles, or other garments or equipment designed to protect the wearer's body from injury or infection. The hazards addressed by protective equipment include physical, electrical, heat, chemicals, biohazards, and airborne particulate matter. Protective equipment may be worn for job-related occupational safety and health purposes, as well as for sports and other recreational activities.

The purpose of personal protective equipment is to reduce employee exposure to hazards when engineering controls and administrative controls are not feasible or effective to reduce these risks to acceptable levels. PPE is needed when there are hazards present. PPE has the serious limitation that it does not eliminate the hazard at the source and may result in employees being exposed to the hazard if the equipment fails.

Any item of PPE imposes a barrier between the wearer/user and the working environment. This can create additional strains on the wearer, impair their ability to carry out their work and create significant levels of discomfort. Any of these can discourage wearers from using PPE correctly, therefore placing them at risk of injury, ill-health or, under extreme circumstances, death. Good ergonomic design can help to minimise these barriers and can therefore help to ensure safe and healthy working conditions through the correct use of PPE.

Practices of occupational safety and health can use hazard controls and interventions to mitigate workplace hazards, which pose a threat to the safety and quality of life of workers. The hierarchy of hazard controls provides a policy framework which ranks the types of hazard controls in terms of absolute risk reduction. At the top of the hierarchy are elimination and substitution, which remove the hazard entirely or replace the hazard with a safer alternative. If elimination or substitution measures cannot be applied, engineering controls and administrative controls – which seek to design safer

mechanisms and coach safer human behavior – are implemented. Personal protective equipment ranks last on the hierarchy of controls, as the workers are regularly exposed to the hazard, with a barrier of protection. The hierarchy of controls is important in acknowledging that, while personal protective equipment has tremendous utility, it is not the desired mechanism of control in terms of worker safety

4.2. Types of Personal protective equipment (PPE)

Personal protective equipment can be categorized by the area of the body protected, by the types of hazard, and by the type of garment or accessory.

- Respirators
- Skin protection
- Eye protection
- Hearing protection
- Protective clothing and ensembles

Respirators

A respirator is a device designed to protect the wearer from inhaling hazardous atmospheres, including fumes, vapours, gases and particulate matter such as dusts and airborne microorganisms. There are two main categories: the air-purifying respirator, in which respirable air is obtained by filtering a contaminated atmosphere, and the air-supplied respirator, in which an alternate supply of breathable air is delivered. Within each category, different techniques are employed to reduce or eliminate noxious airborne contaminants.

Figure1: White, disposable Standard N95 filtering face piece respirator

Figure2: A half-face elastomeric air-purifying respirator (reusable)

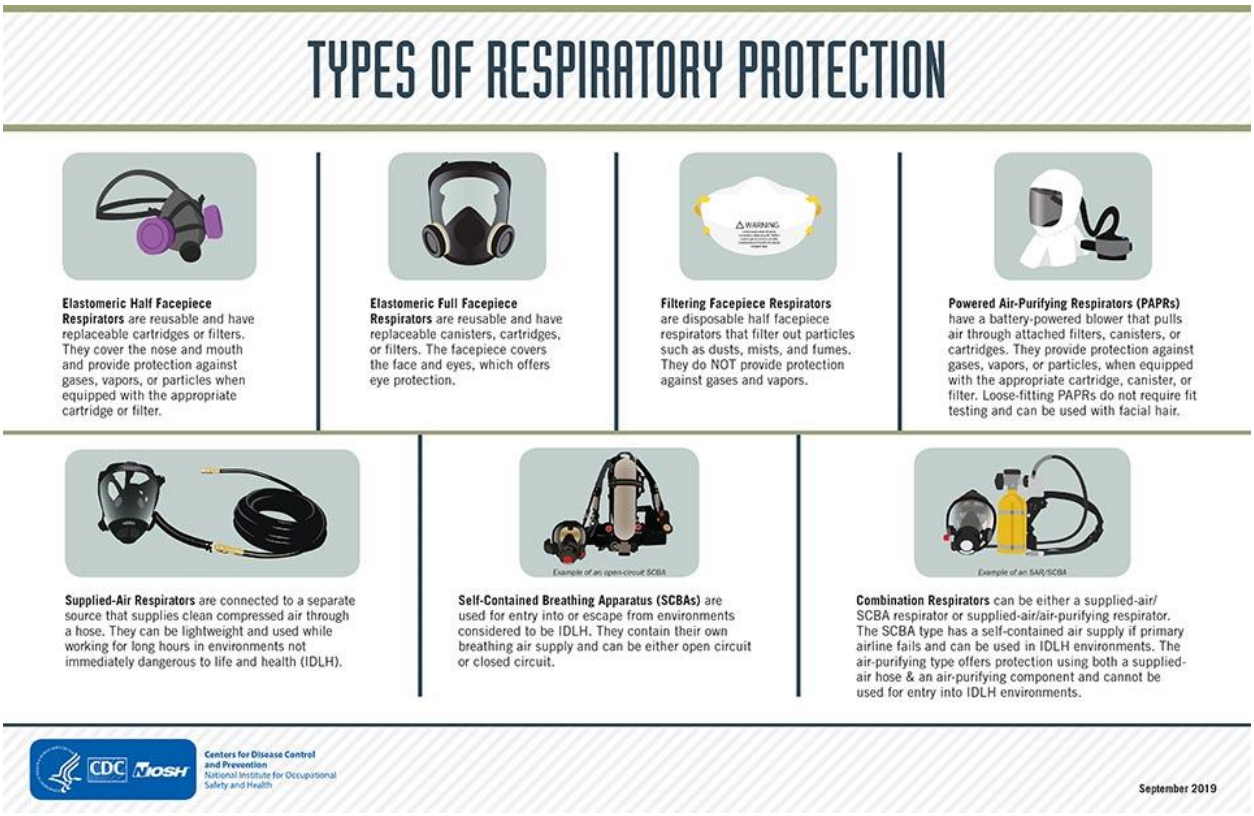

Figure3: types of respiratory protection

Skin protection

Any form of PPE that acts as a barrier between the skin and the agent of exposure can be considered skin protection. Because much work is done with the hands, gloves are an essential item in providing skin protection. Some examples of gloves commonly used as PPE include rubber gloves, cut-resistant gloves, chainsaw gloves and heat-resistant gloves.

Other than gloves, any other articles of clothing or protection worn for a purpose serve to protect the skin. Lab coats for example, are worn to protect against potential splashes of chemicals. Face shields serve to protect one's face from potential impact hazards, chemical splashes or possible infectious fluid.

Skin hazards, which lead to occupational skin disease, can be classified into four groups. *Chemical agents* can come into contact with the skin through direct contact with contaminated surfaces, deposition of aerosols, immersion or splashes. *Physical agents* such as extreme temperatures and ultraviolet or solar radiation can be damaging to the

skin over prolonged exposure. *Mechanical trauma* occurs in the form of friction, pressure, abrasions, lacerations and contusions. *Biological agents* such as parasites, microorganisms, plants and animals can have varied effects when exposed to the skin.

Figure 4: A worker wearing a respirator, lab coat, and gloves while weighing carbon nanotubes

Eye protection

While the required eye protection varies by occupation, the safety provided can be generalized. Safety glasses provide protection from external debris, and should provide side protection via a wrap-around design or side shields.

- Goggles provide better protection than safety glasses, and are effective in preventing eye injury from chemical splashes, impact, dusty environments and welding. Goggles with high air flow should be used to prevent fogging.
- Face shields provide additional protection and are worn over the standard eyewear; they also provide protection from impact, chemical, and blood-borne hazards.
- Full-facepiece respirators are considered the best form of eye protection when respiratory protection is needed as well, but may be less effective against potential impact hazards to the eye.

Hearing protection

A hearing protection device, also known as a HPD, is an ear protection device worn in or over the ears while exposed to hazardous noise to help prevent noise-induced

hearing loss. HPDs reduce (not eliminate) the level of the noise entering the ear. HPDs can also protect against other effects of noise exposure such as tinnitus and hyperacusis. There are many different types of HPDs available for use, including earmuffs, earplugs, electronic hearing protection devices, and semi-insert devices.

Figure 5: Earmuff hearing protection device

Figure 6: Different styles of earplugs are pictured. Left, pre-molded earplugs. Center, formable earplugs. Right, roll-down foam earplugs.

Protective clothing and ensembles

This form of PPE is all-encompassing and refers to the various suits and uniforms worn to protect the user from harm. Lab coats worn by scientists and ballistic vests worn by law enforcement officials, who are worn on a regular basis, would fall into this category. Entire sets of PPE, worn together in a combined suit, are also in this category.

- PPE gowns are used by medical personnel like doctors and nurses.
- Chainsaw protection (especially a helmet with face guard, hearing protection, kevlar chaps, anti-vibration gloves, and chainsaw safety boots).
- Bee-keepers wear various levels of protection depending on the temperament of their bees and the reaction of the bees to nectar availability. At minimum most bee keepers wear a brimmed hat and a veil made of fine mesh netting. The next level of protection involves leather gloves with long gauntlets and some way of keeping bees from crawling up one's trouser legs. In extreme cases, specially fabricated shirts and trousers can serve as barriers to the bees' stingers.
- Diving equipment, for underwater diving, constitutes equipment such as a diving helmet or diving mask, an underwater breathing apparatus, and a diving suit.
- Firefighters wear PPE designed to provide protection against fires and various fumes and gases. PPE worn by firefighters include bunker gear, self-contained breathing apparatus, a helmet, safety boots, and a PASS device.

4.3. Using Personal Protective Equipment (PPE)

Putting On (Don) PPE Gear

More than one donning method may be acceptable. Training and practice using your healthcare facility's procedure is critical. Below is one example of donning.

- Identify and gather the proper PPE to don.
- Ensure choice of gown size is correct (based on training).
- Perform hand hygiene using hand sanitizer.
- Put on isolation gown. Tie all of the ties on the gown. Assistance may be needed by other healthcare personnel.
- Put on NIOSH-approved N95 filtering facepiece respirator or higher (use a facemask if a respirator is not available).
- Put on face shield or goggles.
- Put on gloves. Gloves should cover the cuff (wrist) of gown.

- Healthcare personnel may now enter patient room.

Taking Off (Doff) PPE Gear

More than one doffing method may be acceptable. Training and practice using your healthcare facility's procedure is critical. Below is one example of doffing.

- Remove gloves. Ensure glove removal does not cause additional contamination of hands. Gloves can be removed using more than one technique (e.g., glove-in-glove or bird beak).
- Remove gown. Untie all ties (or unsnap all buttons). Some gown ties can be broken rather than untied. Do so in gentle manner, avoiding a forceful movement. Reach up to the shoulders and carefully pull gown down and away from the body. Rolling the gown down is an acceptable approach. Dispose in trash receptacle.
- Healthcare personnel may now exit patient room.
- Perform hand hygiene.
- Remove face shield or goggles. Carefully remove face shield or goggles by grabbing the strap and pulling upwards and away from head. Do not touch the front of face shield or goggles.
- Remove and discard respirator (or facemask if used instead of respirator). Do not touch the front of the respirator or facemask.*
- Perform hand hygiene after removing the respirator/facemask and before putting it on again if your workplace is practicing reuse.

Maintaining PPE

An effective system of maintenance of PPE is essential to make sure the equipment continues to provide the degree of protection for which it is designed. Therefore, the manufacturer's maintenance schedule (including recommended replacement periods and shelf lives) must always be followed. Inspect PPE before each use. With most PPE, it only takes a few minutes to inspect the equipment for any breaks, tears and visible signs of stress or damage. Maintenance may include: cleaning, examination, replacement, repair and testing. You may be able carry out simple maintenance (e.g. cleaning), but more intricate repairs must only be carried out by competent personnel.

Immediately remove any damaged equipment from service until a competent person or a manufacturer's representative can certify the equipment for use. If not authorized by a manufacturer to repair PPE, do not attempt to fix it. The costs associated with the maintenance of PPE are the responsibility of the employer.

Storage for PPE

Where PPE is provided, adequate storage facilities for PPE must be made available for when it is not in use, unless the employee may take PPE away from the workplace, e.g., footwear or clothing. All PPE must be stored in a clean and sanitary condition ready for use. Accommodation may be simple, e.g., pegs for waterproof clothing or safety helmets, and it need not be fixed, e.g., a case for safety glasses, a container in a vehicle, or zip-lock bags on a designated shelf. Storage should be adequate to protect the PPE from contamination, loss, damage, water or sunlight. Proper storage often requires a dry and clean place that is not subject to temperature extremes. A hard hat hanging in the back window of a truck, for example, may suffer sun and heat damage that prematurely ages the shell, reducing worker protection. Where PPE may become contaminated during use, storage should be separate from any storage provided for ordinary clothing.

Self-check 4	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. What is the importance of PPE in animal sample collection? (1)

   _____
   _____

2. Write the major category of PPE(5)

   _____
   _____
   _____

3. Give examples for each PPE (5)

   _____
   _____
   _____
   _____

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

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

Information Sheet 5- Collecting specimens from farm and pets

Taking Samples from live animals

Blood

This is the most common sample collected from a live animal. The jugular vein is the preferred location for small ruminants and horses. The wing vein is the site for birds. The tail vein is the easiest site for sample collection in cattle.

For small ruminants and horses, apply digital pressure at the lower end of the jugular to fill it. Insert the needle, bevel up, and draw back on the syringe. When finished withdraw the needle and hold a finger over the site for a few seconds.

In adult cattle, the tail vein is the easiest site for blood sampling. Elevate the tail, palpate for the junction of the vertebrae, and insert the needle here.

For poultry, blood can be taken from the wing vein in small birds or from the jugular in larger birds. The wing vein can be found on the underside of the wing. Pluck some of the feathers for better visibility. Hold off the end of the vessel to fill it. Insert the needle, parallel to the skin; be sure to have the bevel up. Go through the skin first, then go into the vein. In larger birds, the jugular is easy to use. Again, it might be helpful to pluck some feathers to more easily visualize the vessel. Hold it off below to help fill it, and hen insert needle with the bevel up.

Swabs

Swabs are often used to collect exudates from lesions, for example taking a swab from an abscess. Additionally, swabs are used to collect tracheal and oropharyngeal fluid samples from birds to test for avian influenza.

Feces

Preferably, fecal samples should be taken directly from the rectum or just after defecation. This is particularly important for the diagnosis of lungworms and protozoans such as *Giardia* and trichomonads.

Urine

In cattle, massaging the area under the escutcheon should result in a flow of urine within one minute. In sheep, occluding the nares for a short period sometimes

precipitates urination. In all other cases, catheterization is necessary unless the animal can be confined and monitored constantly to catch the next instance of urination.

Skin scrapings

Larger external parasites can simply be picked off and placed into a container. Ticks and fleas should be submitted for identification or stored in 70% alcohol. For smaller parasites, such as skin mites, scrape with a razor blade to be sure you go deep enough to get the parasite.

Scrape the skin with a razor blade and put the collected material onto a slide with some mineral oil. Then you can put the slide under a microscope

to see the mites.

Impression smears

Take the tissue and touch gently to a glass slide. Allow to air dry. If the tissue is very bloody, you might want to blot a few times on a paper towel prior to making the smear.

Self-check 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Write the location of veins from which the blood sample is collected for cattle, birds, horses, sheep and dog.(5)

   _____
   _____

2. What are the samples collected from live animals?(5)

   _____
   _____
   _____

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

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

Information Sheet 6 - Handling the specimens

Handling Laboratory Specimens

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

Collecting specimens

1. Gather personal protective equipment –depending on symptoms and history of the patient: • Gloves- when handling any body fluids or risk of contaminating hands • Masks/respirators- if respiratory symptoms or initiating a cough from the patient with specimen collection, aerosolized excretions, risk of splash or spray • Goggles- if risk of splash or spray to eyes
2. Care should be taken when collecting and handling specimens to avoid contamination of the outside of the container.
3. Secure lids tightly to prevent leakage.
4. Place the specimen(s) into a plastic, zip-lock type bag. Requisition should be outside the pouch that the specimen is shipped in.
5. Hand hygiene must be performed following any direct contact with blood or body fluids, after the handling or transporting of laboratory specimens and after glove removal.

If airborne spread disease is suspected specimens should be collected in a negative pressure room, if available (e.g. TB). If there is no negative pressure room then a room with good air circulation or outdoors may be the best alternative. The collector of sputum for TB testing should wear an N95 respirator or separate themselves from

the area where the person is providing the sputum specimen. Even if the patient has a controlled or non-productive cough, the irritation of having a nasopharyngeal swab done could bring on a deeper, productive cough, increasing the risk of contamination of the person taking the swab. Respiratory protection should be worn. Make sure you are aware of correct collection method, container (with or without stabilizing solution), storage and transportation so that the specimen will provide the most accurate results in which to base diagnosis and treatment decisions.

Handling specimens

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

Self-check 6	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Write the importance of proper specimen handling.(1)

   _____
   _____

2. Mention how the specimens handled (4)

   _____
   _____
   _____

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

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

Operation sheet 1– Using a Classic Needle and Syringe from the tail-head for blood collection

The following are the steps to be followed while using a classic needle and syringe from the tail-head for blood collection:

- Order your blood test kit and have it shipped to you.
- Confine and restrain the animal.
- Raise the tail and locate the coccygeal blood vessels underneath the tail.
- Clean the area to be sampled.
- Insert the needle directly into the vein.
- Draw slowly back on the plunger of the syringe.
- Remove the needle the same way you inserted it.
- Take a rubber-capped test tube (called a "vacutainer tube") and insert the needle into the top of the cap
- Label the tube.
- Put the tube back in its place
- Usually the box that contained the kit has a built-in tray to place the vacutainer tubes in. Place the tubes in that box with the bubble wrap surrounding each tube to protect them.

Operation sheet 2– milk sample collection

The following are the steps to be followed to collect milk sample for mastitis test or somatic cell count:

- Label tubes prior to sampling (date, farm, cow, quarter).
- Brush loose dirt, bedding, and hair from the udder and teats.
- Thoroughly wash and dry grossly dirty teats and udders before proceeding with sample collection. Udders should be washed as a last resort.

- Discard several streams of milk from the teat (strict foremilk) and observe milk and mammary quarters for signs of clinical mastitis.
- Record all observations of clinical signs.
- Dip all quarters in an effective premilking teat disinfectant and allow at least 30 seconds contact time.
- Dry teats thoroughly with an individual towel.
- Beginning with teats on the far side of the udder, scrub teat ends vigorously (10 to 15 seconds) with cotton balls or gauze pledgets moist (not dripping wet) with 70% alcohol.
- Teat ends should be scrubbed until no more dirt appears on the swab or is visible on the teat end.
- Begin sample collection from the closest teat and move to teats on the far side of the udder.
- To collect a composite sample (milk from all four quarters in the same tube), begin sample collection with the nearest teats and progress to the teats on the far side of the udder. One to2 ml of milk should be collected from each quarter of the udder.
- When samples are taken at the end of milking or between milkings, teats should be dipped in an effective germicidal teat disinfectant following sample collection
- Store samples immediately on ice or in some form of refrigeration. Samples to be cultured at a later date (more than 48 hours) should be frozen immediately.

Lap Test	Performance test

Name_____ ID_____ Date_____

Time started: _____ Time finished: _____

Instructions: Given necessary tools and materials you are required to perform the task within **1** hour. It is expected from each student to do.

During your work: You can ask all the necessary tools and equipment

Task 1: collect blood sample from tail head vein

Task 2: collect milk from cow

LG #86	LO #2- Prepare supplies, materials, tools and equipment

Instruction sheet

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

- identifying and preparing supplies and materials needed for specimen collection, preservation and transport
- sterilizing and cleaning materials to be used
- preparing tools and equipment needed to restrain animals for sample collection

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

- identify and prepare supplies and materials needed for specimen collection, preservation and transport
- sterilize and clean materials to be used
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Information Sheet 1- Identifying and Preparing Supplies and Materials

1.1 Sample container

A collection container is a vessel, such as a cup or vial, used to hold the biological sample being collected for lab testing. A collection container may be used to hold a blood, oral fluid, urine, hair or other samples. Depending on the type of container, a collection container could also be called a specimen bottle or specimen container.

Depending on the type of sample being collected, a collection container may be an open cup, a vial, other type of bottle, or even a sponge. When used for workplace drug testing, a collection container should be a sterile single-use vessel that is individually wrapped and sealed to prevent tampering.

1.2 Laboratory refrigerators

Laboratory refrigerators (or lab fridges) are refrigerators designed with special features and options optimized for use in the laboratory, as opposed to simpler models intended for purely domestic, catering or other use. Together with laboratory freezers, laboratory refrigerators are essential items of equipment in most laboratories.

The primary function of a laboratory refrigerator is to maintain a defined, internal storage temperature (selectable from anything between 1° to 15°C) for the secure storage and protection of temperature-sensitive products, samples, specimens, chemicals, drugs, solutions and other substances.

Laboratory refrigerators are manufactured in a range of capacities and dimensions, and are usually supplied complete with one or more shelves, compartments and/or drawers, which may often be height-adjustable or reconfigurable to suit the needs of the user. Bench top, under-counter or upright floor-standing models are also available, depending on overall storage requirements and installation space, plus a range of refrigerator and freezer accessories.

1.3 Cold chain

A cold chain is a temperature-controlled supply chain. An unbroken cold chain is an uninterrupted series of refrigerated production, storage and distribution activities, along with associated equipment and logistics, which maintain quality via a desired low-temperature range. It is used to preserve and to extend and ensure the shelf life of products, such as fresh agricultural produce, seafood, frozen food, photographic film, chemicals, and pharmaceutical products. Such products, during transport and when in transient storage, are sometimes called cool cargo. Unlike other goods or merchandise, cold chain goods are perishable and always en route towards end use or destination, even when held temporarily in cold stores and hence commonly referred to as "cargo" during its entire logistics cycle. Adequate cold storage, in particular, can be crucial to prevent quantitative and qualitative food losses.

Figure1: Cold chain being maintained using ice box while transporting polio vaccine

1.4 Mineral oils

Mineral oils are usually seen as a mixture of liquid hydrocarbons. It is derived from crude oil by distillation and refining.

Application

Mineral oil has been used:

- to suspend the gels for capillary contraction assay
- in polymerase chain reaction (PCR) reactions mixture to form a vapor barrier in order to prevent the evaporation of the reaction at elevated temperatures
- as a continuous phase to prevent droplet coalescence in emulsification system

Mineral oil is suitable for use as an overlay to control evaporation and cross contamination of samples in a variety of molecular biology applications.

1.5 Formaldehyde

Formaldehyde is a colorless poisonous gas synthesized by the oxidation of methanol and used as an antiseptic, disinfectant, histologic fixative, and general-purpose chemical reagent for laboratory applications. Formaldehyde is readily soluble in water and is commonly distributed as a 37% solution in water; formalin, a 10% solution of formaldehyde in water, is used as a disinfectant and to preserve biological specimens. Environmentally, formaldehyde may be found in the atmosphere, smoke from fires, automobile exhaust and cigarette smoke. Small amounts are produced during normal metabolic processes in most organisms, including humans.

1.6 Formalin

Formalin: 30-40% aqueous solution of formaldehyde is called formaline. It is used as preservative in biological laboratory to preserve different dead animal.

Formaline is used as an antiseptic, disinfectant and preservative. It is a 37 percent aqueous solution of the pungent gas formaldehyde and has the chemical formula HCHO.

1.7 vial

A vial (also known as a phial or flacon) is a small glass or plastic vessel or bottle, often used to store medication as liquids, powders or capsules. They can also be used as scientific sample vessels; for instance, in autosampler devices in analytical chromatography. Vial-like glass containers date back to classical antiquity; modern vials are often made of plastics such as polypropylene. There are different types of vials such as a single dose vial and multi-dose vials often used for medications. The single dose

vial is only used once whereas a multi-dose vial can be used more than once. The CDC sets specific guidelines on multi-dose vials.


Figures 2: vials

1.8 Test tubes

A test tube, also known as a culture tube or sample tube, is a common piece of laboratory glassware consisting of a finger-like length of glass or clear plastic tubing, open at the top and closed at the bottom. They are usually placed in special-purpose racks.

A Vacutainer blood collection tube is a sterile glass or plastic test tube with a colored rubber stopper creating a vacuum seal inside of the tube, facilitating the drawing of a predetermined volume of liquid. Vacutainer tubes may contain additives designed to stabilize and preserve the specimen prior to analytical testing. Tubes are available with a safety-engineered stopper, with a variety of labeling options and draw volumes. The color of the top indicates the additives in the vial.

Figure 3: vacutainer tube

Glass slide

A microscope slide is a thin flat piece of glass, typically 75 by 26 mm (3 by 1 inches) and about 1 mm thick, used to hold objects for examination under a microscope. Typically the object is mounted (secured) on the slide, and then both are inserted together in the microscope for viewing. This arrangement allows several slide-mounted objects to be quickly inserted and removed from the microscope, labeled, transported, and stored in appropriate slide cases or folders etc.

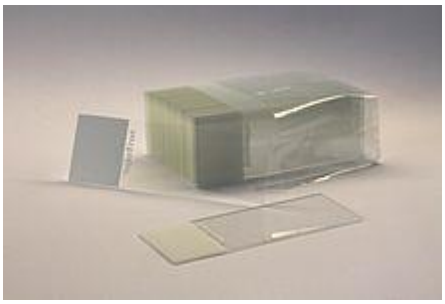


Figure 4: A set of standard 75 by 25 mm microscope slides. The white area can be written on to label the slide.

Figure 5: A microscope slide (top) and a cover slip (bottom)

Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips, slide clamps or a cross-table which is used to achieve precise, remote movement of the slide upon the microscope's stage (such as in an automated/computer operated system, or where touching the slide with fingers is inappropriate either due to the risk of contamination or lack of precision).

Cryovials

Cryovials are designed for storage of cells, specimens and solutions. Two different cap types, External & Internal are avilable.

Storable in ultra low freezers.

Use only in vapor-phased liquid nitrogen

Distinctive external & Internal cap design.

Self-standing bottom

External cap: External thread of the body fits perfectly into internal thread of the cap in helical form

Internal cap: Internal thread of the body fits perfectly into the external thread of the cap in helical form

Figure :both internal and external cryovials

Other supply and equipments

This may include:-

Syringe, Needle, lancets, staining jar, Scalpel with blade, Styropor, Covered box, slide box, rack, wire basket, Cotton, Cork, Rubber stopper, universal bottle, swabs, water proof marker, labler, Sterile forceps, scissors, and scalpels. Bottles for collection of faeces, blood, and other samples that do not require transport medium, Notebook and equipment for labeling specimens, Cool box (Thermos flask)

Self-check 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. List supply and materials used for laboratory sample collection, transportation and preservation.(5pt)

   _____
   _____
   _____

2. Write the importance and types of Cryovials(5pt)

   _____
   _____
   _____

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

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

Information Sheet 2- sterilizing and cleaning Materials to be used are according to standard operating procedure

Cleaning -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

General Principles of Cleaning

In general the following applies for all areas that provide care to service users:

- Wash hands before and after all procedures and after removing gloves.
- Cleaning where possible, should take place in a dedicated area away from patient care. Use a designated sink (not a hand wash basin)
- Equipment should be dismantled where necessary in line with the manufacturers' instructions before cleaning.
- A clean, disposable cloth should be used and discarded immediately after use.
- Use neutral detergent and warm water (maximum 42-43°C) for general cleaning, rinse thoroughly to remove detergent residue.
- Dry thoroughly after cleaning using disposable towels or paper roll (where appropriate). Items should NOT be left on surfaces to air dry!
- If item is visibly soiled with blood or body fluids, clean first and then disinfect with a chlorine releasing agent (see section below on blood spillages).
- Wear protective clothing as appropriate
- Decontaminate any cleaning equipment after use e.g. bowl/bucket/sink.
- A written cleaning schedule should be devised specifying the persons responsible for cleaning, the frequency of cleaning, and the expected outcomes. These schedules should be publicly displayed and followed.
- Keep mops and buckets clean, dry and store inverted.
- Mop heads should be removable for laundering daily or disposable/single use.

- Ensure colour coding, in line with the National Cleaning guidelines, (see Appendix 1) is used for equipment used to clean, toilets, kitchens, general areas and isolation rooms.
- Store all non-disposable cleaning equipment clean and dry between uses.

Disinfection is a process of removing or killing most, but not all viable organisms. The aim of disinfection is to reduce the number of micro-organisms to a level at which they are not harmful. Spores are not destroyed.

Disinfection is used as part of the decontamination process for moderate risk items. It include thermal and chemical processes. Moist heat may be used for items such as crockery, linen and bedpans e.g. automated processes in a machine.

Disinfectants are chemical agents designed to inactivate or destroy microorganisms on inert surfaces. Specific chemical disinfectants can be used to decontaminate heat sensitive equipment and the environment. Disinfectants are not cleaning agents as they are generally inactivated by organic material, therefore all items must be cleaned thoroughly prior to disinfection.

General Principles of Disinfection

- Do not use disinfection as a substitute for sterilisation.
- Only use chemical disinfectants if absolutely necessary.
- Choose an appropriate disinfectant, compatible with the surface being disinfected and approved by the Infection Prevention and Control Team.
- Read the relevant COSHH assessment sheet before using any chemical disinfectant.
- Wear protective clothing (and respirators if required).
- Ensure adequate ventilation.
- Check the expiry date of the disinfectant.
- Ensure that the correct dilution is used (check manufacturer's instructions).
- Never dilute a disinfectant by guesswork.
- Never use two disinfectants together, do not add anything to a disinfectant (including detergent) as this may result in a dangerous chemical reaction.

- Clean equipment / surfaces thoroughly before disinfection.
- Ensure sufficient contact time between disinfectant and equipment being decontaminated (as per product manufacturer's instructions).
- Rinse thoroughly after disinfection (if alcohol is used to disinfect then rinsing is not required). Discard disinfectant solution after use.
- Do not 'top up' solutions of disinfectant.
- Ensure that containers used for disinfection are stored clean, dry and inverted between uses.

Methods of Disinfection

Chemical Disinfectants

- ✓ Alcohol
- ✓ Chlorine and chlorine compounds
- ✓ Formaldehyde
- ✓ Glutaraldehyde
- ✓ Hydrogen peroxide
- ✓ Iodophors
- ✓ Ortho-phthalaldehyde (OPA)
- ✓ Peracetic acid
- ✓ Peracetic acid and hydrogen peroxide
- ✓ Phenolics
- ✓ Quaternary ammonium compounds

Miscellaneous Inactivating Agents

- ✓ Other germicides
- ✓ Metals as microbicides
- ✓ Ultraviolet radiation
- ✓ Pasteurization
- ✓ Flushing- and washer-disinfectors

Sterilization This is a process of removing or killing all viable organisms including spores. Dead microorganisms and toxins (pyrogens) may remain. Prions will not be effectively destroyed by this process.

All instruments that penetrate skin or mucous membranes or are used in sterile body cavities must be sterilised prior to use. Sterilisation of reusable items of equipment must

be carried out in the local acute Hospital's Sterile Services Department (HSSD), local processing of instruments must not be undertaken within the Trust and as far as possible staff must use single use sterile disposable instruments. Single Use Items Single use items may be divided into two groups:

- Single-use: Single-use items should be used once only and discarded
- Single patient use: Single patient use items may be reused for the same service user after appropriate decontamination as per the manufacturer's instructions. ALWAYS check the information on the packaging.

Common Laboratory Sterilization Methods

1 Wet Heat (Autoclaving)

The laboratory sterilization method of choice in most labs is autoclaving: using pressurized steam to heat the material to be sterilized. This is a very effective method that kills all microbes, spores, and viruses, although, for some specific bugs, especially high temperatures or incubation times are required.

Autoclaving kills microbes by hydrolysis and coagulation of cellular proteins, which is efficiently achieved by intense heat in the presence of water.

The intense heat comes from the steam. Pressurized steam has a high latent heat; at 100oC it holds 7 times more heat than water at the same temperature. This heat is liberated on contact with the cooler surface of the material to be sterilized, allowing rapid delivery of heat and good penetration of dense materials. At these temperatures, water does a great job of hydrolyzing proteins… so those bugs don't stand a chance.

Autoclave:is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure.

- Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period of time.
- The autoclave is also called a steam sterilizer that is commonly used in healthcare facilities and industries for various purposes.

- The autoclave is considered a more effective method of sterilization as it is based on moist heat sterilization.

The simplest form of the autoclave is the pressure cooker types or laboratory bench autoclaves. The following is the detailed description of different components/ parts of an autoclave:

Figure: Autoclave Parts or Components. Image Source: pharmawiki.

Pressure Chamber

- The pressure chamber is the main component of a steam autoclave consisting of an inner chamber and an outer jacket.
- The inner chamber is made up of stainless steel or gunmetal, which is present inside the out chamber made up of an iron case.
- The autoclaves used in healthcare laboratories have an outer jacket that is filled with steam to reduce the time taken to reach the sterilization temperature.
- The inner chamber is the case where the materials to be sterilized are put.
- The size of the pressure chamber ranges from 100 L to 3000 L.

Lid/ Door

- The next important component of an autoclave is the lid or door of the autoclave.
- The purpose of the lid is to seal off the outside the atmosphere and create a sterilized condition on ht inside of the autoclave.
- The lid is made airtight via the screw clamps and asbestos washer.
- The lid consists of various other components like:

Pressure gauge

- A pressure gauge is present on the lid of the autoclave to indicate the pressure created in the autoclave during sterilization.
- The pressure gauge is essential as it assures the safety of the autoclave and the working condition of the operation.

Pressure releasing unit/ Whistle

- A whistle is present on the lid of the autoclave is the same as that of the pressure cooker.
- The whistle controls the pressure inside the chamber by releasing a certain amount of vapor by lifting itself.

Safety valve

- A safety valve is present on the lid of autoclave, which is crucial in cases where the autoclave fails to perform its action or the pressure inside increases uncontrollably.
- The valve has a thin layer of rubber that bursts itself to release the pressure and to avoid the danger of explosion.

Steam generator/ Electrical heater

- An electrical steam generator or boiler is present underneath the chamber that uses an electric heating system to heat the water and generate steam in the inner and the outer chamber.
- The level of water present in the inner chamber is vital as if the water is not sufficient; there are chances of the burning of the heating system.
- Similarly, if the water is more than necessary, it might interfere with the trays and other components present inside the chamber.

Vacuum generator (if applicable)

- In some types of autoclaves, a separate vacuum generator is present which pulls out the air from the inside of the chamber to create a vacuum inside the chamber.
- The presence of some air pockets inside the chamber might support the growth of different microorganisms. This is why the vacuum chamber is an important component of an autoclave.

Waste water cooler

- Many autoclaves are provided with a system to cool the effluent before it enters the draining pipes.
- This system prevents any damage to the drainage pipe due to the boiling water being sent out of the autoclave.

In general, an autoclave is run at a temperature of 121° C for at least 30 minutes by using saturated steam under at least 15 psi (Pounds per square inch) of pressure.

Dry Heat (Flaming, Baking)

Dry heating has one crucial difference from autoclaving. You've guessed it – there's no water, so protein hydrolysis can't take place.Instead, dry heat tends to kill microbes by oxidation of cellular components. This requires more energy than protein hydrolysis so higher temperatures are required for efficient sterilization by dry heat. For example, sterilization can normally be achieved in 15 minutes by autoclaving at 121oC, whereas dry heating would generally need a temperature of 160oC to sterilize in a similar amount of time.

1. Filtration

Filtration is a great way to quickly sterilize solutions without heating. Filters, of course, work by passing the solution through a filter with a pore diameter that is too small for microbes to pass through. Filters can be sintered glass funnels made from heat-fused glass particles or, more commonly these days, membrane filters made from cellulose esters. For the removal of bacteria, filters with an average pore diameter of 0.2um are

normally used. But remember, viruses and phages can pass through these filters so filtration isn't a good laboratory sterilization method if these are a concern.

2. Solvents

Ethanol is commonly used as a disinfectant, but isopropanol is a better solvent for fat and is probably a better option. Both solvents work by denaturing proteins through a process that requires water, so they must be diluted to 60–90% in water to be effective.

Again, it's important to remember that although ethanol and IPA are good at killing microbial cells, they have no effect on spores.

3. Radiation

UV, x-rays, and gamma rays are all types of electromagnetic radiation that have profoundly damaging effects on DNA, so make excellent tools for sterilization. The main difference between them, in terms of their effectiveness, is their penetration. UV has limited penetration in air so sterilization occurs in only a fairly small area around the lamp. However, it is relatively safe and is quite useful for sterilizing small areas, like laminar flow hoods. (It's really important to remember to sterilize your equipment too.) X-rays and gamma rays are far more penetrating, which makes them more dangerous but very effective for large-scale cold sterilization of plastic items (e.g. syringes) during manufacturing.

4. Gas Sterilization

Ethylene oxide can be used to sterilize equipment that is sensitive to heat or moisture and is often used to sterilize medical equipment such as catheters and stents. Ethylene oxide essentially prevents cell metabolism and replication by alkylation. Because ethylene oxide is easily absorbed, equipment must be aerated after sterilization to remove any residue. Ethylene oxide is also highly toxic and can present a number of health risks. As it's generally used for healthcare products, you're not very likely to be using it in the lab.

Self-check 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. _____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. (1pt)
2. Mention the general principles of cleaning.(5pt)

   _____
   _____
   _____
3. _____ is a process of removing or killing most, but not all viable organisms.(1pt)
4. _____ are chemical agents designed to inactivate or destroy microorganisms on inert surfaces.(1pt)
5. _____ is a process of removing or killing all viable organisms including spores.
6. _____: is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure.(1pt)
7. Mention some laboratory material sterilization methods.(5pts)

   _____
   _____
   _____
   _____
   _____

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

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

Information Sheet 3- Preparing Tools and equipment needed to restrain animals for sample collection

Proper Animal Handling & Restraint

Effective restraint is an important part of veterinary medicine that we simultaneously take for granted but also need to be successful. Restraint techniques are passed on in an almost cultural fashion with sometimes little thought given to their logic and effectiveness. Historically, animals were restrained by physically overpowering them. This often resulted in injury to the animal or involved personnel, and/or the restraint attempt failed which prevented successful completion of the intended procedure.

General Principles

- A number of factors are involved in triggering aggression and/or escape responses in animals. The most common include fear, pain, punishment (which induces fear and anxiety) and excessive physical contact. Most animals show fear/defensive aggression because they find some aspect of the processes threatening. This may be the environment, the personnel, the equipment, the procedure, the restraint technique used or any combination of these.
- Animals are particularly likely to react to handling of certain body regions as well. These include the head/neck, the legs and feet, the groin/perineum, the abdomen, and any area that is painful. These areas are natural targets in serious attacks because they are areas where it is relatively easy to deliver an incapacitating or fatal injury. Restraint techniques should be chosen with these factors in mind. In particular, avoid directly restraining the animal's legs whenever possible as this universally induces even more struggling and aggression. Protection of the legs is a biologically hard-wired behavior.
- It also is important to remember that what matters is whether the animal finds an interaction threatening — not whether the veterinary professional does. Often, in the process of trying to be friendly to an animal, we portray body signals that actually mean the opposite. This is particularly true around horses and dogs. The

way the average person greets a dog is a perfect example. Most people approach dogs from the front, lean over, and extend their hand to allow the dog to sniff it or to try to pet the dog. There are several elements in this approach that directly threaten the dog: the direct, frontal approach; making eye contact; leaning over; and reaching out over the dog's head. These are intensified if they are done in a quick, tense or agitated manner.

- Good restraint is all about empathy, finesse, and technique – it has little to do with strength. If any procedure requires more than two people to actually hold the animal, there is something wrong with that technique for that procedure on that animal for that day. The more people that are involved, the more threatened the animal will feel and the more easily someone will be injured.

Physical control or restraint of the animal

It may be necessary to control or restrain the animal using physical methods; this may need to be carried out in conjunction with chemical restraint methods.

Physical control or restraint may be essential in some situations, for example:

- To prevent worsening of an incident, especially if human life or safety is involved
- To enable an entrapped animal to be released
- To remove the animal from a place of danger to a place of safety
- To prevent injury to emergency responders
- When administering first aid to the animal

Before attempting to physically gain control or restraint of an animal, the activity should be risk assessed, including the following considerations:

- The species, size and behaviour of the animal
- The impact on the animal in terms of potential injury or distress
- The environment
- The resources and equipment available

The species, size and behaviour of the animal Physically controlling or restraining an animal may include using equipment such as:

- Muzzles

- ✓ May be purpose-made or improvised, and often used for dogs
- ✓ Assessment of the animal will determine the type of muzzle required

- Slip leads
  - ✓ Can be used to control a non-aggressive dog
  - ✓ Can be used as a pair, one from each side of the animal, (known as double leading) to provide additional control
- Rigid leads or graspers
  - ✓ Should be used if the behaviour of a dog is unknown
  - ✓ Can be used for some wild animals, such as foxes and badgers
  - ✓ Can be used as a pair to provide additional control
- Snake tongs or graspers
  - ✓ Can be used to capture and restrain snakes and other animals, such as cats
- Nets
  - ✓ Can be used for many species of smaller animals, but need to be suitable for the size and strength of the animal
  - ✓ May be of a traditional hoop, triangle or square type
  - ✓ Other types, such as throw nets or 'walk toward' nets can be used
- Extension poles
  - ✓ Lightweight, interlocking, aluminium poles that can provide additional reach when controlling or restraining an anima
  - ✓ Capture or restraining equipment, such as graspers or nets, can be attached to the extension poles
- Towels, cloths or blankets
  - ✓ May be purpose-made or improvised, and can assist with the capture and control of a range of smaller animals and deer
  - ✓ Covering the head or eyes may calm some species
- Halters

- ✓ May be purpose-made or improvised, and are mainly used to control the head of larger domesticated animals, or if the animal is being chemically restrained
- ✓ Can be used for smaller livestock such as goats, sheep and young bovines

- Head collars
  - ✓ Purpose-made devices, in a range of sizes, primarily for the head control of equines

Restraint Methodology

- **Appropriate restraint is all about empathy, finesse, and technique – it has little to do with strength.** If any procedure requires more than two people to actually hold the animal, there is something wrong with the employed technique for that procedure on that animal for that day. The more people that are involved, the more threatened the animal will feel and the more easily someone will be injured.
- Restraint also does not necessarily mean immobilization. Animals have **5 basic reactions to stress or threat.** These include fight, flight, freeze, faint, and fidget (or fooling around). The latter is an often overlooked sign of stress. Staff members frequently assume animals that are obnoxious and hyper in the room are just plain stupid or untrained, but this is a simplistic outlook considering the situation the animal is in. Keep open minded that the animal's hyperactivity might actually be a reaction to stress. Punishing these animals usually raises their stress level and exacerbates the behavior.
- Restraint dictates that we move into the animal's personal space without the animal's permission. This puts personnel in the "critical zone" where animals are often more likely to attach rather than to try to run away, especially if escape is blocked. Making wise choices as to *how* you invade the animal's space can reduce this reaction in most animals.
- **Always use the least amount of restraint necessary for the procedure and invade the animal's "intimate space" as little as possible.** Restrain the animal

for the behavior that it is currently showing – not for the behavior that you think it may show in the near future.

- **Restraint requires patience!** Some animals need time to adapt to the process; trying to rush the animal will only increase its anxiety.

Patient Restraint Considerations

- Restraint is the process of; holding back, checking, or suppressing an action and/or keeping something under control using safety and some means of physical, chemical, or psychological action.
- Restraint is a necessary tool used by veterinary staff to allow an animal to be controlled for various procedures
  - ✓ Safety of the patient and staff are paramount!
- Sedatives/Tranquilizers – are sometimes necessary to keep a patient calm or pain free during certain stressful procedures or circumstances.

Animal Safety

- The patient's safety is of the highest concern when selecting and implementing restraint techniques.
- Patients who are not well socialized or accustomed to human contact, will become easily stressed in a new environment
- Young animals must always be handled with care, as they can be very fragile
- Older patients should also be handled with care, as they may be arthritic and have increased pain
- The safety of the patient and staff must be considered every time restraint is necessary
- Never allow non-veterinary staff or an owner to restrain any animal
  - ✓ This can have potential legal repercussions

Why Proper Restraint Techniques

- Prevents injury
- Necessary for examinations and treatments
- More comfortable for animal and handler
- A First Aid Kit should be available for all bites and scratches

- All Animal bites must be reported to the NY Dept. of Health

Planning the restraint procedure

- When preparing to restrain a patient, always make sure the area has enough room, is clean, dry, and well lit
- A plan should be discussed:
  - ✓ Move any costly equipment
  - ✓ Nonslip area
  - ✓ Temperature should be considered
  - ✓ What should be done if animal happens to get away from restrainer
  - ✓ Backup plan (Plan B!)

Rabbit Restraint & Handling

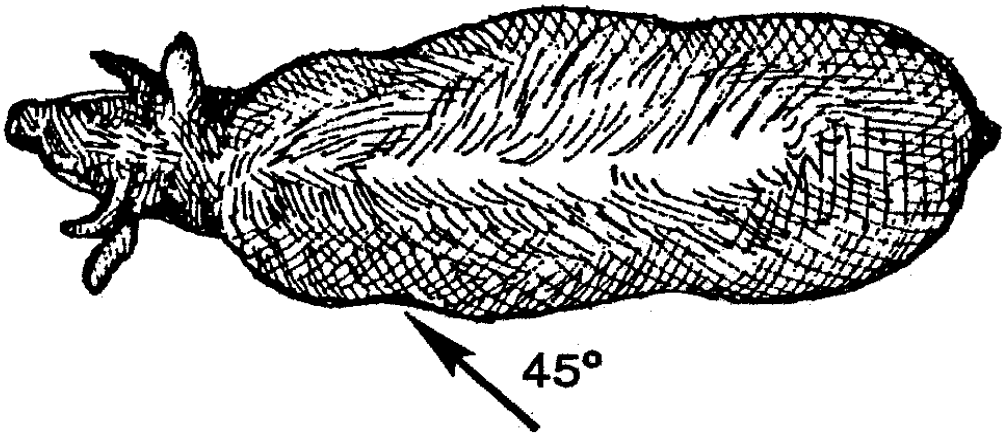
- Grab scruff of the neck with one hand and lifting up while placing the other hand under the rump for support or wrap patient securely in a towel.
- Holding
  - ✓ Use the same technique but the hand under the rump is moved to support the abdomen.
- Rabbits seldom bite but many cause injury with their hind legs or may be injured if placed on a smooth surface
- Rabbit's foot pads are covered with fur which causes a lack of traction

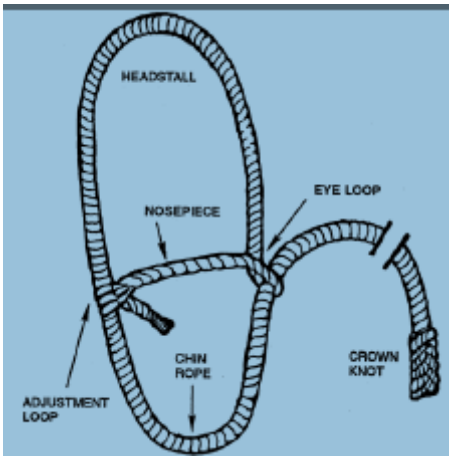
Can lead to dislocation of their hip or spinal fracture, when they try to move or hop

Dairy Cattle Restraint

- Excessive restraint frightens.
- Halters – Haltering
  - ✓ Loosen chin rope.
  - ✓ Go over nose and under chin with left hand.
  - ✓ Tighten chin rope by pull of lead rope with left hand.
  - ✓ Place headstall with right hand over poll and behind ears.
- Stanchions
- Milking parlor

- Treatment pen



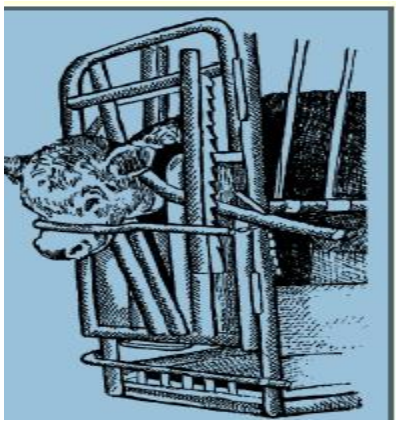


Beef Cattle

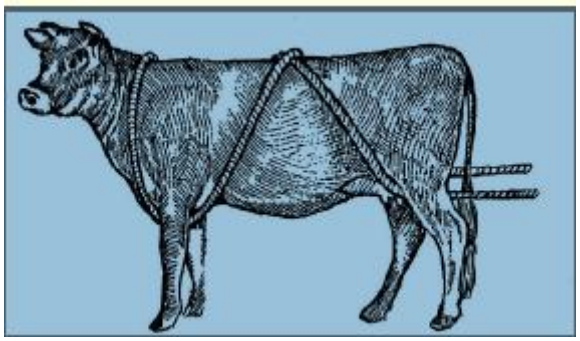
- Squeeze chutes
  - ✓ Close head gate.
  - ✓ Close tail gate.
  - ✓ Close sides.
  - ✓ Apply nose bar or nose tong to work head.

- ✓ Drop bottom side plank to work feet.
- ✓ Drop side bars to work neck, body and legs.
- ✓ Tilt calf chute (calf table) to work calves.

- Lane chute
  - ✓ Crowd multiple cattle.
  - ✓ Chock single animal with pole in front and rear.



- Cast rope – Casting (Burley Method)
  - ✓ Halter tie head
  - ✓ Pass rope over withers, ends through forelegs, cross over back and through hind legs
  - ✓ Pull both ends of rope from rear to fall cow

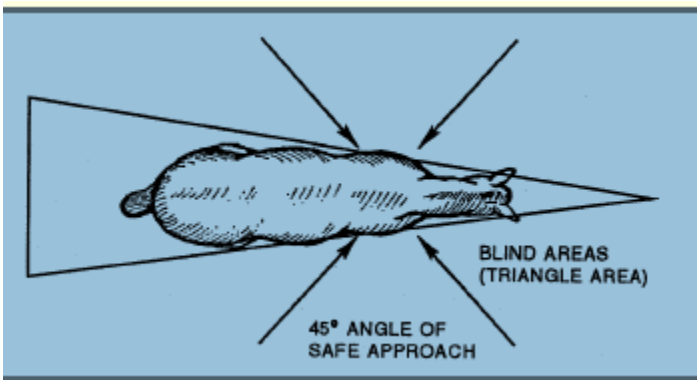


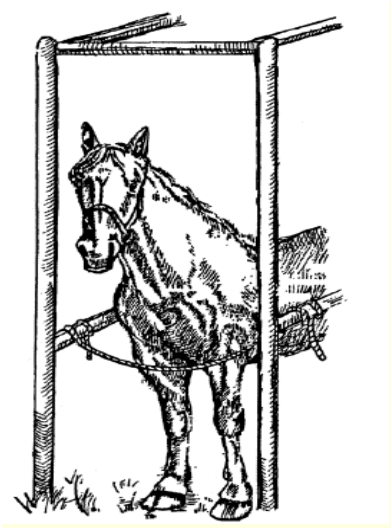
- Flanking (Calves)
  - ✓ Reach over calf.

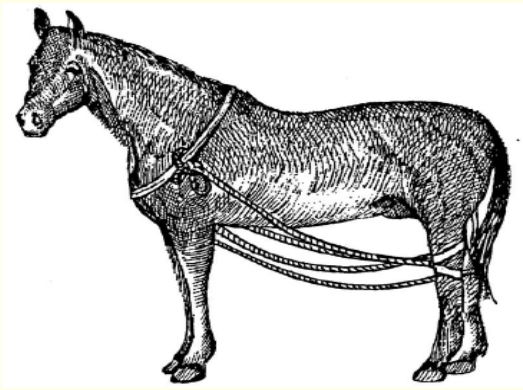
- ✓ Reach down flank and grasp nearest hind leg with one hand.
- ✓ Reach between forelegs and grasp nearest foreleg with other hand.
- ✓ Lift and slide calf to ground.
- ✓ Kneel on neck and thigh.
- ✓ Bottom foreleg from ground.

Horses

- Excessive restraint frightens.
- Halters – Haltering
- Stocks
  - ✓ Place on halter and lead in stock.
  - ✓ Tie rope to stock in front and rear of horse:-.
  - ✓ Double clove hitch ties
  - ✓ Kick gate
- Hobbles – Hobbling
  - ✓ To prevent kicking
  - ✓ Pass rope over neck (ring), ends through front legs, through hock hobbles (inside to outside) on back legs and through neck ring.

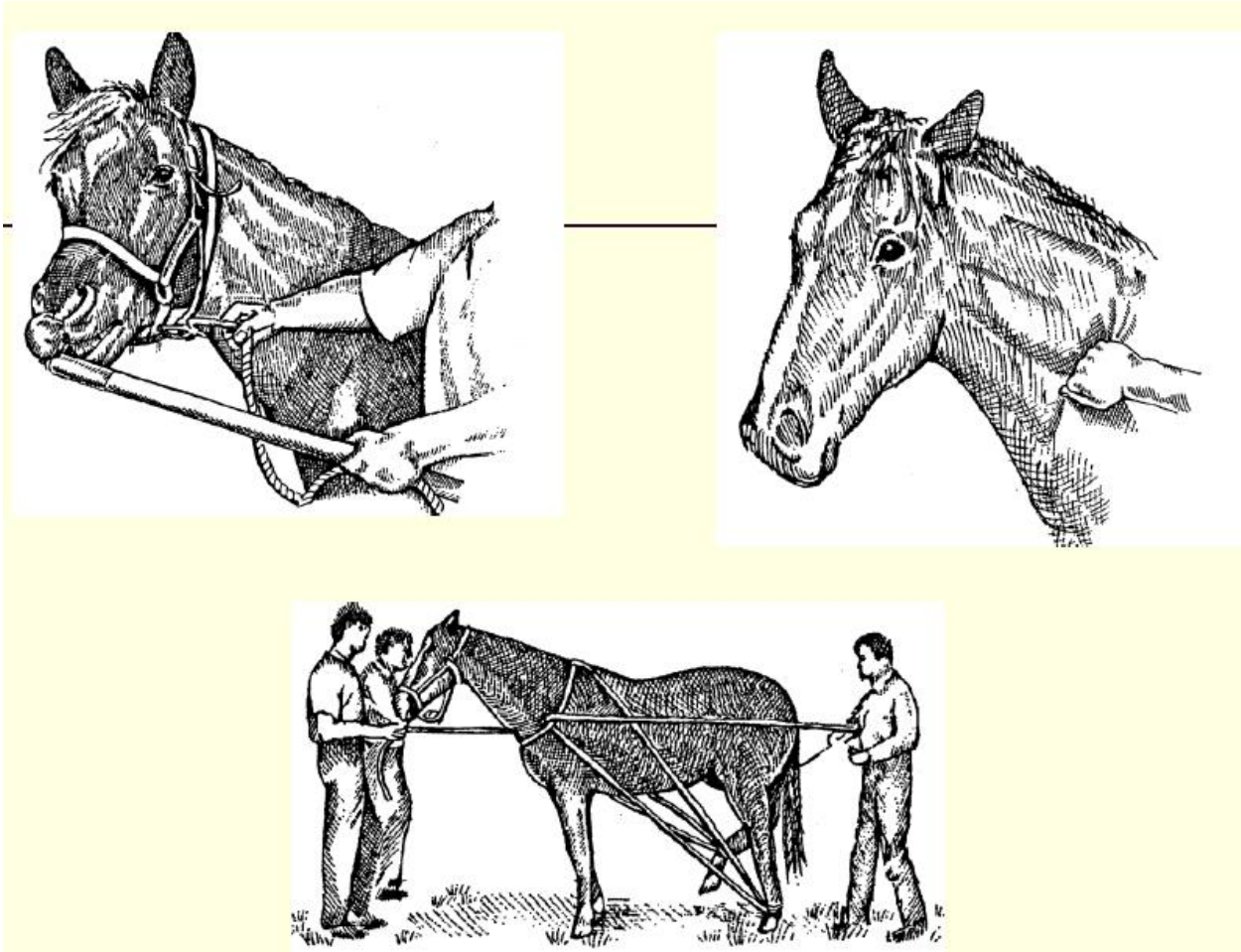




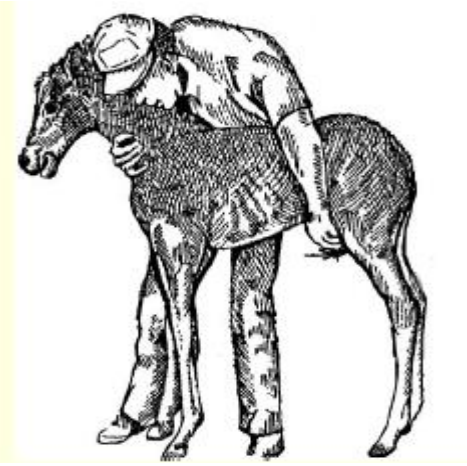


- Twitches
  - ✓ Place nose twitch (rope or chain loop on upper lip).
  - ✓ Place nose clamp on upper lip.
  - ✓ Place skin twitch by hand grasp and roll of skin on neck or shoulder.
- Cast ropes – Casting
  - ✓ Place on halter
  - ✓ Pass rope ring over neck, ends from withers through pastern hobbles (inside to outside) on back legs and through neck ring.
  - ✓ Pull opposite side rope end around rump to assist fall.
  - ✓ Pull fall side rope end from front to fall horse to off side.
  - ✓ Pull halter lead rope to near side and kneel on neck.

- ✓ Tie pasterns with rope ends with double clove slip hitch.



- Hand cast – Casting foal
  - ✓ Place one hand under neck.
  - ✓ Reach over and pull tail between hindlegs with other hand to slump foal to ground.
  - ✓ Kneel on neck and maintain tail hold.



Swine

- Nose snare

✓ Cable or rope loop

✓ Place loop over snout behind canine teeth.

- Rope-board cast

✓ Rope tie foreleg and hindleg together.

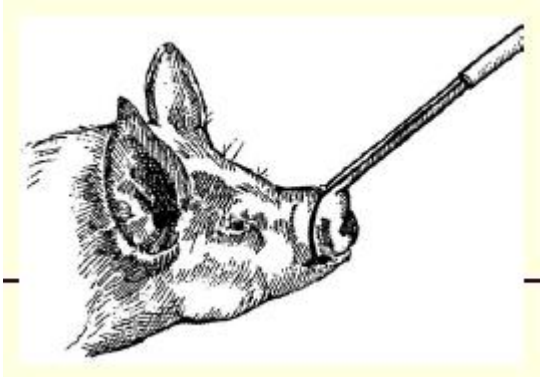
✓ Cast with vertical board.

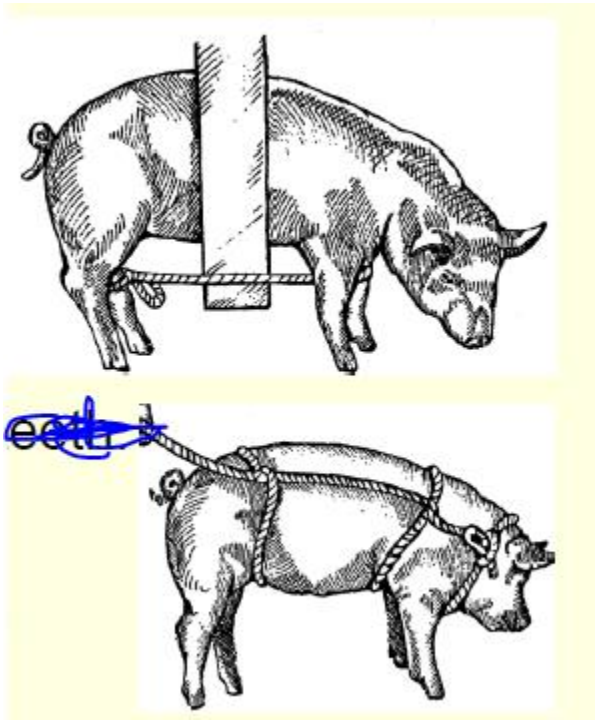
✓ Apply pressure with board against body to ground.

- Rope cast

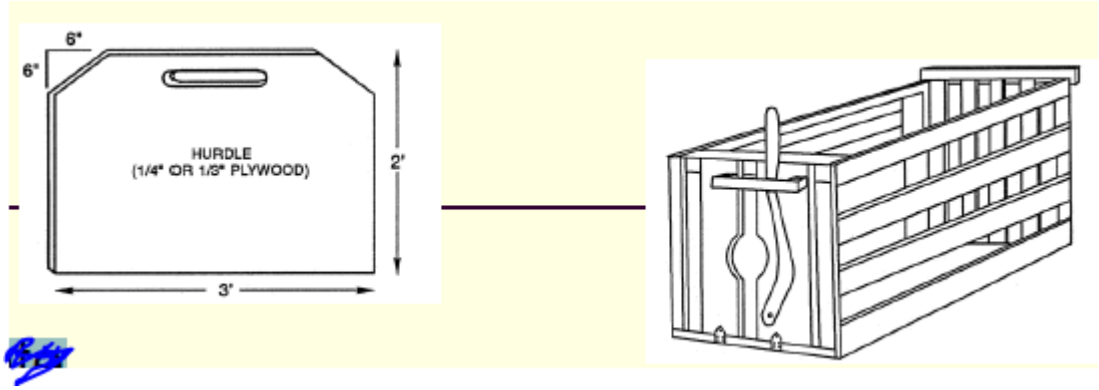
✓ Place rope ring on neck and run rope end along body with half hitches behind forelegs and front of hindlegs.

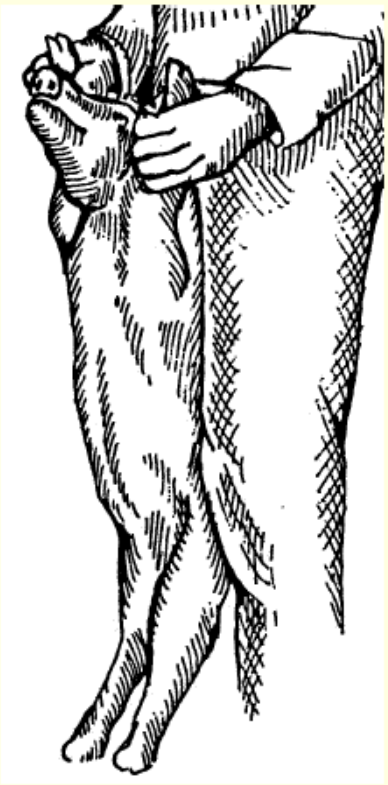
✓ Pull rope end from rear to fall hog.

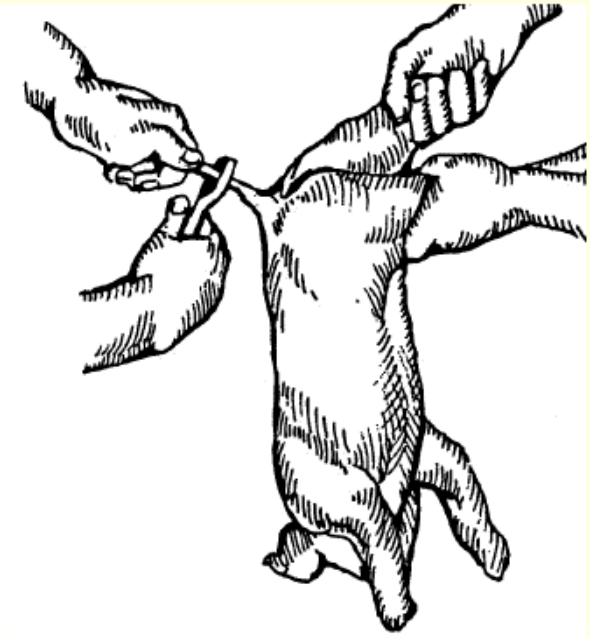




- Hurdle board
- Head gate crate
- Vertical hold legs with hands (head up, belly out)
  - ✓ Additional restraint between knees
- Vertical hold legs with hands (head down, belly out)
  - ✓ Additional restraint between knees
- Horizontal hold on side with knee on shoulder and with hands hold on top legs
- Horizontal hold on back in V-board trough with hind legs pulled forward



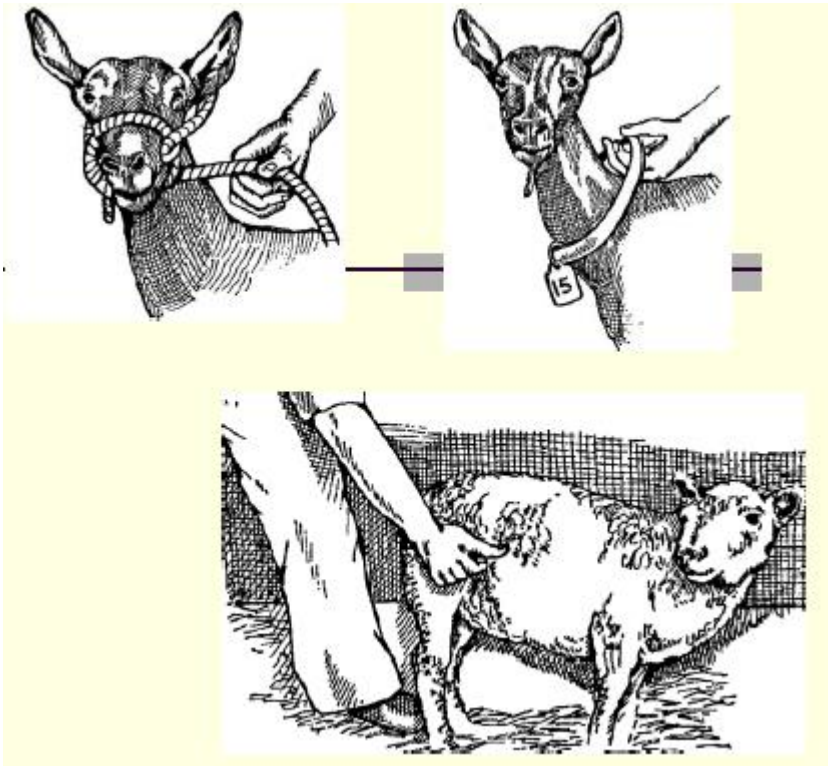


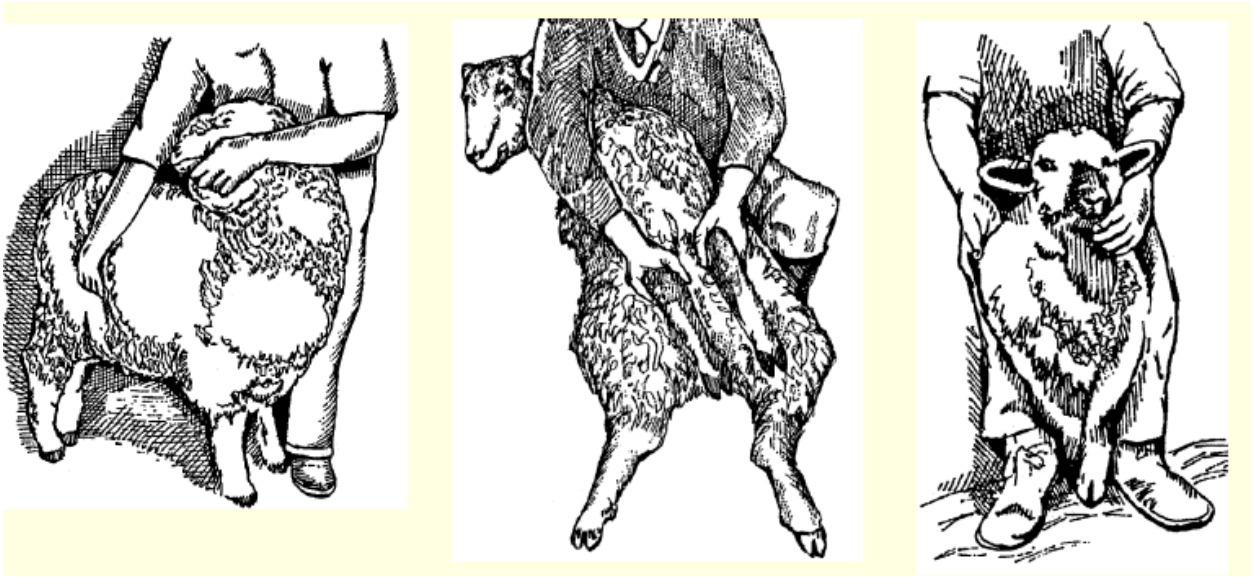


Sheep and Goats

- Halter
- Collar
- Flank hold
- Jaw restraint
- Rumping
  - ✓ Pull jaw and push flank to roll back on rump.
  - ✓ Grasp front legs.
- Saddle restraint

- ✓ Straddle and grasp body with knees.





- Vertical hold with hands (head up, belly out)
  - ✓ Additional restraint between knees
- Horizontal hold on side with knee on shoulder and with hands hold on top legs

Self-check 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. List some equipments by which animals physically restrained.

   _____
   _____
   _____
   _____

2. Animals have 5 basic reactions to stress or threat and mention them.

   _____,_____,_____,_____, and _____

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

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

Operation sheet – Procedures for autoclaving

The following are the steps to be followed while running an autoclave:

- check for any items left from the previous cycle
- Put a sufficient amount of water inside the chamber.
- Place the materials to be sterilized inside the chamber.
- Close the lid, and tight the screws to ensure an airtight condition, and switched on the electric heater.
- Adjust the safety valves to maintain the required pressure in the chamber.
- Allow the air-water mixture to escape through the discharge tube to let all the air inside to be displaced.
- Close the drainage pipe and allow the steam inside to reach the desired levels (15 lbs in most cases).
- Remove the whistle blows to excess pressure from the chamber.
- Run the autoclave for a holding period, which is 15 minutes in most cases.
- Switch off now, the electric heater, and allow the autoclave to cool until the pressure gauge indicates the pressure inside has lowered down to that of the atmospheric pressure.
- open the discharge pipe then to allow the entry of air from the outside into the autoclave.
- Open the lid and take the sterilized materials out of the chamber.

Lap Test	**Autoclaving**

Name_____ ID_____ Date_____

Time started: _____ Time finished: _____

Instructions: Given necessary tools and materials you are required to perform the task within **1** hour. It is expected from each student to do.

During your work: You can ask all the necessary tools and equipment

Task: sterilize Tools and materials for sample collection

LG #87	LO #3- Prepare for sampling

Instruction sheet

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

- Wearing required protective clothing
- Preparing Work area and animal
- Selecting the appropriate restraining technique
- labeling and recording sampling containers/equipment
- sterilizing the sampling site and equipment

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:

- Wear required protective clothing
- Prepare Work area and animal
- Select the appropriate restraining technique
- label and record sampling containers/equipment
- sterilize the sampling site and equipment

Learning Instructions:

19. Read the specific objectives of this Learning Guide.

20. Follow the instructions described below.

21. 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.

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

23. 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).

24. If you earned a satisfactory evaluation proceed to to the next learning guide

Information Sheet 1- Wearing required protective clothing

1.1 Functions Protective clothes

- Protect personnel who enter animal facilities and/or perform animal procedures from exposure to animal dander, hair, secretions, and excretions that may cause allergic respiratory and skin responses, or that may cause illness.
- Prevent exposure (to animal dander, hair, secretions, and excretions) of persons by contact with persons who have been in animal facilities or have conducted animal procedures.
- Minimize the risk of possible ocular, oral, or dermal exposure to chemical disinfectants.
- Minimize the transmission of disease agents among animals.
- To protect personnel work with macaques or macaque tissues from the risk of Herpes B virus infection and other naturally occurring infectious diseases of macaques.
- This procedure applies to anyone entering an animal or procedure room occupied by one or more animals.

1.2 Protective Equipment

Scrub suit: This is clothing, typically referred to as “scrubs,” worn in place of street clothing when handling potentially contaminated, and/or mildly messy, materials to avoid contamination of street clothing. Scrubs are usually a lightweight, loose-fitting cotton shirt and pants. Typically, scrubs are provided and laundered by the institution.

Cloth lab coat: Cotton fabric coat that covers the torso and arms, usually worn over street clothing. Laboratory coats minimize contamination of clothing and skin beneath the gown by particulates and mists. Should be removed once contaminated.

Disposable lab coat: This is a lightweight fabric that covers the torso and arms, usually worn over scrubs or street clothing. Laboratory coats minimize contamination of clothing and skin beneath the gown by particulates and mists. Should be removed once contaminated.

Face mask: A variety of masks are used in the animal facilities. For the purpose of this SOP, a face mask refers to a surgical mask or cone mask.

Face mask and eye splash shield combination

This protects the user's mouth and eyes from potential splashes or particulates.

This protects the wearer's hair from particulate contamination and minimizes contamination of the environment by the wearer.

Disposable exam gloves: Gloves typically made of latex, nitrile, synthetic material, or vinyl, that protect the wearer's hands from contamination. Exam gloves are important in minimizing contamination from animal to animal or from area to area, provided they are removed and replaced appropriately.

Shoe covers: Lightweight fabric or plastic booties worn over shoes to prevent contamination of the shoes and to prevent contamination of the environment by material that may be on the shoes. Shoe covers are especially important in minimizing the spread of contamination from area to area, provided they are removed and replaced appropriately.

Waterproof shoe covers: These are booties worn over shoes that are resistant to water.

Face shield: This is eye protection that provides splash protection from the sides and top. The top portion of the shield is composed of soft foam that rests against the wearer's forehead, creating a protective barrier.

Safety glasses: Hard plastic eye protection worn to protect the wearer's eyes from splashes and particulates.

Tyvek® (or equivalent) jumpsuit: This is made of a lightweight and water-resistant fabric that covers legs, torso, and arms.

Sleeve covers: These are lightweight, water-resistant fabric covers for the lower arms.

Rubber boots: These are waterproof boots made from lightweight rubber.

N-95 respirator

Self-check 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Write the function of protective clothes

   _____
   _____
   _____
   _____

2. List down at least 5 protective clothes /equipments

   _____,_____,_____,_
   _____,_____,_____,_____
   _____,_____

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

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

Information Sheet 2- Preparing Work area and animal

2.1 Introduction

Laboratory tests contribute vital information about a patient's health. Correct diagnostic and therapeutic decisions rely, in part, on the accuracy of test results. Adequate patient preparation, specimen collection, and specimen handling are essential prerequisites for accurate test results. The accuracy of test results is dependent on the integrity of specimens.

2.2 Safety and Disposal Considerations in Specimen Collection

In all settings in which specimens are collected and prepared for testing, laboratory and health care personnel should follow current recommended sterile techniques, including precautions regarding the use of needles and other sterile equipment. Treat all biological material as material that is potentially hazardous as well as contaminated specimen collection supplies. For all those who are involved in specimen collection and preparation, the responsibility to adhere to current recommendations designed to maintain the safety of both patients and health care workers does not end when the patient is dismissed.

There are four steps involved in obtaining a good quality specimen for testing: (1) preparation of the patient, (2) collection of the specimen, (3) processing the specimen, and (4) storing and/or transporting the specimen. Since information related to any of these areas may change as clinical laboratory technology changes, please refer to the latest edition of the LabCorp *Directory of Services and Interpretive Guide* for current instructions.

2.3 Preparation

Prior to each collection, review the appropriate test description, including the specimen type indicated, the volume, the procedure, the collection materials, patient preparation, and storage and handling instructions.

Preparing the Patient: Provide the patient, in advance, with appropriate collection instructions and information on fasting, diet, and medication restrictions when indicated for the specific test.

Preparing the Specimen: Verify the patient's identification. Proper identification of specimens is extremely important. All primary specimen containers must be labeled with at least two identifiers at the time of collection. Submitted slides may be labeled with a single identifier, but two identifiers are preferred. Examples of acceptable identifiers include (but are not limited to): patient's name (patient's first and last name exactly as they appear on the test request form), date of birth, hospital number, test request form number, accession number, or unique random number. A location such as a hospital room number is not an appropriate patient identifier. If chain of custody documentation is necessary for the procedure, follow the appropriate protocol. All specimens should be labeled in the presence of the patient. Process and store the specimen(s) as required. Appropriate storage and handling are necessary to maintain the integrity of the specimen and, consequently, the test results.

2.4 Avoiding Common Problems

Careful attention to routine procedures can eliminate most of the potential problems related to specimen collection. Materials provided by the laboratory for specimen collection can maintain the quality of the specimen only when they are used in strict accordance with the instructions provided. To collect a sufficient quantity of each type of specimen indicated for the procedures to be performed, please consult the volume requirements published in this *Directory.*

General Specimen Collection. Some of the common considerations affecting all types of specimens:

- Please examine specimen collection and transportation supplies to be sure they do not include **expired containers**.
- Label a specimen correctly and provide all pertinent information required on the test request form

- Submit a quantity of specimen sufficient to perform the test and avoid a QNS (quantity not sufficient), as indicated in the test requirements.
- Use the container/tube indicated in the test requirements for appropriate specimen preservation.
- Follow patient instructions prior to specimen collection.
- Carefully tighten specimen container lids to avoid leakage and/or potential contamination of specimens.
- Maintain the specimen at the temperature indicated in the test requirements.

Self-check 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Write the common considerations affecting all types of specimens.

_____
_____
_____
_____
_____
_____
_____

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

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

Information Sheet 3-Selecting the appropriate restraining technique

3.1 Types of Restraints

- Physical Restraints
- Chemical Restraints
- Psychological Restraints
- Electro-Immobilization

3.2 Purpose of Restraining

- Done for examination, collection of samples, drug administration, therapy and manipulation.
- The method should provide least restraint to minimize stress, pain, suffering and fear for the animal.
- Restraining is also done to ensure Human Safety.
- It ensures that you can safely examine your animal without causing injury to animal or to you.

3.3 Precautions during Restraining

- Animals should be approached in a calm manner, avoiding sudden movements, such as waving of hands and arms.
- Stay alert at all times and observe the animal's response.
- Restrain animals properly.
- Wear personal protective equipments.
- Have an exit strategy.

Restraining of Cattles Techniques;

Various Methods are used for restraining of cattles depending upon the purpose. Some of them are described below;

- Haltering
- Casting
- Hobbles
- Crush
- Nose holder
- Chemical Restraints
- Drugs
- Kick Bars

Restraining of sheep

- Sheep squeezing into a narrow chute Restraining of sheep 3
- Holding a Sheep
- Parting Wool
- Setting Up Restraining of sheep
- Hoof trimming from,
- Oral examination from, setup position
- Shepherd's crook
- Gramble

Restraining Of Equidae Family -Horse (Equus caballus)

There are three main techniques of restraining;

- Physical
- Verbal
- Chemical

Physical Restraints

- Halter and Lead Rope
- Chain over Nose and Lip
- Twitching with hand
- Twitching with Device
- Lifting Limb and Stocks

Restraining of Donkey

Donkeys are also restrained like horses by using many techniques, some of them are mentioned below;

- Knots
- Halter
- Head Collar
- Chin Holder
- Blind Folding

Holding & Restraining Technique of Poultry

#correct chook handling techniques

- To handle a chook you need to be calm & sensible.
- You need to talk softly to the chook & keep talking gently to it while you catch it.To catch it you need to try carefully and quickly grab hold of it by the legs and body.
- Once you have the bird hold it by putting your middle finger in between its legs and squeeze the legs so the chook doesn't go flying away.

- Stroke your cook gently with your other hand. To put the chook down simply make sure its feet are touching the ground and reverse the catching process.
- If your chook goes crazy do not panic just firmly hold onto your chook and talk quietly to reassure it.If you feel scared while holding your chook don't worry your confidence will grow and the chook will quiet down over time.

Restraining Of Camel

Techniques;

There are two main types of restraining;

- Physical Restraining
- Chemical Restraining

Physical Restraining is further divided into sub-techniques, which are following;

- Halter
- Body harness
- Nose Plugs
- Stocks
- Hobbles

Chemical Restraining utilizes the same drugs as used in cattle, buffalos, and horses with special considerations for dosages.

For Examples: Xylazine, Butorphanol, Detomidine, Meditomedine, etc.

Self-check 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Write the types of restraints

   _____
   _____
   _____

2. What are the purposes of restraints?

   _____
   _____
   _____
   _____

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

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

Information Sheet 4- Labeling and recording Sampling containers/equipment

Identifying information can be provided by writing directly onto the vials in indelible ink. If labels are used, they should be secured to insure retention during freezing. To protect patients from adverse errors made due to improperly labeled specimens, the laboratory policy demands that proper labeling criteria are always met. Every specimen brought to the laboratory must have a label on the container in which it is held. It is not acceptable to label only the lid, transport bag, or other container used to transport the specimen. Enclose specimens in a secure container and label the container with a waterproof pen. Place this container in a waterproof bag with tissue, towels or other blotting material to absorb any leakage. Put all specimen containers in an insulated box packed with ice or frozen refrigerant packs and deliver them to the laboratory as soon as possible. If sending specimens by post or courier ensure that they are delivered during business hours on a weekday the label must contain the following legible information:

- Patient (species, breed, sex)
- Owner name
- Patient medical record number, with check digit
- Patient location
- Collection date and time
- Specimen type and/or source
- Test required (note any special handling required)
- Ordering veterinarian

Self-check 4	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1 write the information labeled on the sample collection.(6pts)

_____

_____

_____

_____

_____

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

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

Information Sheet 5- sterilizing the sampling site and equipment

Blood spillage may occur because a laboratory sample breaks in the phlebotomy area or during transportation, or because there is excessive bleeding during the procedure. In this situation, clean up the spillage and record the incident, using the following procedure.

1. Wear a pair of non-sterile gloves.
2. Use tongs or a pan and brush to sweep up as much of the broken glass (or container) as possible. Do not pick up pieces with your hands.
3. Discard the broken glass in a sharps container. If this is not possible due to the size of the broken glass, wrap the glass or container in several layers of paper and discard it carefully in a separate container. Do not place it in the regular waste container.
4. Use disposable paper towels to absorb as much of the body fluids as possible.
5. Wipe the area with water and detergent until it is visibly clean.
6. Saturate the area again with sodium hypochlorite 0.5% (10 000 ppm available chlorine). This is a 1:10 dilution of 5.25% sodium hypochlorite bleach, which should be prepared daily.
7. Rinse off the tongs, brush and pan, under running water and place to dry.
8. Remove gloves and discard them.
9. Wash hands carefully with soap and water, and dry thoroughly with single-use towels.
10. Record the incident in the incident book if a specimen was lost, or persons were exposed to blood and body fluids.

Self-check 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1 Mention procedures to clean up the blood spillage and record the incident. (6)

_____
_____
_____
_____
_____
_____

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

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

LG #88	LO #4- Collect, preserve, store and transport specimen

Instruction sheet

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

- collecting appropriate specimen and volume
- Ensuring that any sampling procedure have conformed with the requirements of the sampling plan
- Placing collected specimen in appropriate container
- maintaining required temperature of collected sample
- using appropriate physical and chemical methods to preserve and store samples
- transporting specimen to the diagnostic laboratory

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:

- collect appropriate specimen and volume
- Ensure that any sampling procedure have conformed with the requirements of the sampling plan
- Place collected specimen in appropriate container
- maintain required temperature of collected sample
- use appropriate physical and chemical methods to preserve and store samples
- transport specimen to the diagnostic laboratory

Learning Instructions:

25. Read the specific objectives of this Learning Guide.
26. Follow the instructions described below.
27. 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.
28. Accomplish the “Self-checks” which are placed following all information sheets.
29. 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).
30. If you earned a satisfactory evaluation proceed to to the next learning guide

Information Sheet 1- collecting appropriate specimen and volume

1.1 Equipment required for collection of samples

- Sterile forceps, scissors, and scalpels.
- Sterile swabs
- Vials for containing transport medium for collection of samples for isolation or identification
- Bottles for collection of faeces, blood, and other samples that do not require transport medium
- Bottles containing formalin saline for tissues to be examined histologically.
- Blood collection equipment- without additive for serum, and with anticoagulant for isolation
- Notebook and equipment for labeling specimens
- Swabs and transport medium for bacteriological investigation
- Cool box (Thermos flask)
- Heavy duty plastic bags for postmortem material.

1.2 Collection of Samples

Tissues (in general): 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 appropriate to the nature of the sample required must be available, and also labels, and report forms. Special media may be required for transport of samples from the field. The operator should wear protective clothing: overalls, rubber gloves and rubber boots. If rabies is suspected, it is usual to detach the animal's head, and the operator should wear a face mask and goggles, gloves and a plastic apron.

Tissues may be collected for culture or for histopathology and occasionally for use as antigen in serological tests. The person removing the tissues should be experienced in

post-mortem technique and have knowledge of pathology sufficient to select the right organs and the most promising lesions for sampling. The skin of the dead animal may be removed with ordinary instruments, but the body cavities should be opened with sterile instruments, and a fresh set of sterile instruments should be used to collect the pieces of the various organs required. Each piece of tissue should be placed in a separate sterile screw-capped jar or plastic bag, fully labeled with the date, tissue and animal identification. Care must be taken not to contaminate one tissue with another. Instruments can be heated on a burner with portable packs of liquid gas or by using local fuel to light a fire. Disinfectants must not be used on or near tissues to be sampled for bacterial culture or virus isolation.

The fresh samples should be forwarded to the laboratory by the fastest direct route. If they can reach the laboratory within 24 hours they should be forwarded in a wide-mouthed vacuum flask with wet ice. An alternative is to use polystyrene containers and chemical refrigeration bricks. Only if the samples are likely to take more than 24 hours to reach the laboratory, it is necessary to freeze the samples and send them in this state. The tissues may be sent to the laboratory dry or in bacterial or virus transport medium depending on the examinations required. For histopathology, blocks of tissues not more than 0.5 cm thick and 1-2 cm2 are cut and placed in neutral buffered 10% formalin, which should be at least 4 times the volume of the tissue sample. Samples for histology should not be frozen. For some procedures, e.g. rabies, larger portions of brain are required, some fresh and some in fixative, and for Scrapie and BSE whole brains may be required.

Blood:

Blood samples may be taken for hematology or for culture and/or direct examination for bacteria, viruses, or protozoa, in which case the blood is added to anti-coagulants such as heparin. They may also be taken for serology, in which case a clotted sample is required. A blood sample is taken, as cleanly as possible, by venepuncture. In most large mammals, the jugular vein or a caudal vein is selected, but brachial veins and mammary veins are also used. In birds, a wing vein (brachial vein) is usually selected.

Blood may be taken by syringe and needle or by needle and vacuum tube (not easy in delicate veins but convenient in strong veins). Ideally the skin at the site of venepuncture should first be shaved (plucked) and swabbed with 70% ethyl alcohol and allowed to dry.

Whole blood samples can have antibiotics added to reduce bacterial growth, taking care that the antibiotics are chosen so as to avoid interference with the growth of the pathogens concerned. For samples with anti-coagulant and/or antibiotics, thorough mixing is necessary as soon as the sample has been taken. It may be also necessary to make a smear of fresh blood on a microscope slide. For serum samples, the blood should be left to stand at ambient temperature (but protected from excessive heat) until the clot begins to contract. The clot can then be ringed round with a rod and the bottles then placed in a refrigerator at 4º C. Later, the serum can be decanted or removed after centrifugation. Chemical preservatives, such as boric acid or merthiolate, should be avoided in sera to be used in virus neutralization tests. An alternative method is to transport a drop of dried blood on a filter paper disk that contains enough material for sensitive antibody assay systems.

Disease	Preservative for blood
Blue tongue	OCG, Sod. Citrate, heparin
African horse sickness	OCG
Rinderpest	Heparin
Equine rhinopneumonitis	Heparin
Equine infectious anaemia	EDTA
Swine, fever	EDTA
Marek's disease	Heparin
Malignant catarrhal fever	EDTA (1 mg/ml of blood)

Faeces:

Freshly voided faeces should be selected, and sent with or without a transport medium. An alternative and sometimes preferable method is to take swabs from the rectum (or cloaca), taking care to swab the mucosal surface. Swabs may also be transported either

dry or in transport medium. Faeces for Parasitology should fill the container to reduce air and prevent hatching of parasite eggs.

Skin

In diseases producing vesicular rashes or where lesions are exclusively in the skin, samples are taken from the lesions themselves. Scrapings of the lesion may be taken, and additionally the vesicular fluid should be sampled where unruptured vesicles are present.

Genital tract:

Samples may be taken by vaginal or prepucial washing, or by the use of suitable swabs. Sometimes the cervix or urethra is also sampled by swabbing.

Eye:

A gentle swab of the surface of the conjunctiva is taken and is broken off into transport medium. Scrapings may also be taken onto a microscope slide. Metal-handled swabs are useful to ensure sufficient cells are removed for microscopic examination.

Nasal discharge (saliva, tears)

Samples may be taken by soaking cotton swabs that are wetted with transport medium and sent to the laboratory at 4º C.

Milk:

Samples of milk should be taken after cleansing the tip of the teat. The initial stream of milk is discarded and a tube filled with the next stream(s). In severe mastitis, there may be little fluid present.

Environmental sample

Samples may be taken to monitor hygiene or as part of a disease inquiry, for example, from litter, ventilation ducts, feed troughs, drains, soil, hatcheries and slaughter houses.

Serum:

Serum samples are the most commonly collected specimens from live animals for conducting various serological tests. Generally serums samples early in the course of disease (acute, within 1-4 days) and during convalescence (convalescent, around 21 days) are collected. Such samples are called paired serum samples and are used to

demonstrate the rising antibody titre. In comparing the antibody titres of acute and convalescent phase sera, a minimum four fold rise is considered significant.

Collection of appropriate volume specimen

Blood sample volume

On average, the total circulating blood volume is equal to 5.5 -8.0 % of the animal's body weight. Non-terminal blood collection without additional monitoring (see below) should be limited to 10% of the total circulating blood volume on a single collection or every 2 week period for serial collections.

Example (Using mean blood volume table below): a 4 kg rabbit is calculated to have a total blood volume of 224 ml (56 ml/kg x 4.0 kg). Thus, 22.4 ml (10% of 224 ml) may be collected without giving replacement fluids once every two weeks.

Estimated Total Blood Volume and Safe Bleeding Volume of Selected Species:

Species	Blood volume (ml/kg)		One Bleeding - max - 10% of blood volume (ml/kg)
	Mean	Range	
Cat	55	55	5.5
Cattle	55	55	5.5
Chicken	60	60	6.0
Dog	86	86 (79-90)	8.6
Ferret	75	75	7.5
Frog	95	95	9.5
Gerbil	67	67	6.7
Goat	66	--	6.6
Guinea Pig	75	75 (67-92)	7.5
Hamster	78	78	7.8
Minipig	65	65 (61-68)	6.5
Monkey (Cynomolgous Macaque)	65	65 (55-75)	6.5
Monkey	54	54 (44-67)	5.4

Species	Blood volume (ml/kg)		One Bleeding - max - 10% of blood volume (ml/kg)
	Mean	Range	
(Rhesus Macaque)			
Mouse	79	79 (63-80)	7.9
Pig	65	65 (61-68)	6.5
Rabbit	56	56 (44-70)	5.6
Rat	64	64 (58-70)	6.4
Sheep	66	66 (60-74)	6.6

Source: Adapted from Formulary for Laboratory Animals, Hawk, Leary, and Morris 2005

Milk volume sample: Collect milk until the sample tube is ⅓ to ½ full, holding the tube at an angle to prevent loose dirt or hair from falling into it.

Urine sample volume collects at least 10 ml of urine. We try and standardize the volume of urine used for urinalysis. This is impossible to do if samples ranging from 0.5 ml (way too little to do anything useful with) to 100 ml are collected. In addition, we need a minimum of 10 ml of urine for electrophoresis.

Self-check 1	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. List out equipments used for sample collection.

   _____
   _____
   _____

2. Write down the the types of samples collected from animal.

   _____
   _____
   _____

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

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

Information Sheet 2- Ensuring that any sampling procedure have conformed with the requirements of the sampling plan

Samples may be taken from animals, or their environment, for the purpose of establishing a disease diagnosis, for health surveillance, or for the monitoring of response to vaccines. In order to get timely and correct diagnosis of a suspected infectious disease, it is imperative on the part of a clinician to collect the most suitable material from live or dead animals. A great variety of different combinations of samples and species of animal may occur. The knowledge of pathogenesis of infectious disease is single most important factor in order to collect the most suitable specimen. In the face of an outbreak where animals in various stages of the clinical disease may be seen, it is better to collect specimen from fresh cases of the disease. In all cases, the samples need to be appropriate for the purpose required, and adequate in number and amount to provide a statistically valid result. Samples must be taken with care, to avoid undue stress or damage to the animal or danger to the operator. For example, a carcass suspected of being infected with Bacillus anthracis should not be opened, but a drop of blood should be obtained from a superficial vein. It is usually important to adopt aseptic techniques, and care must be taken to avoid cross-contamination between samples. Just prior to death and shortly thereafter, a number of intestinal bacteria may invade the host tissues. The significance of these organisms, some of which are potential pathogens is difficult to assess when tissues have been invaded. For best results, fresh tissues must be collected as soon as it is feasible. Live sick animals presented for necropsy, invariably provide the best source of samples/specimens. For microbiological investigations, strict sterile precautions must be observed meticulously while collecting and handling materials for isolation studies. It requires at least as much effort, and often more, to process a negative specimen as it does one from which microorganism is isolated. The chance of isolating a microbe depends critically on the knowledge, care, and attention of the veterinarian who collects the specimen. Specimens taken as a last resort when days or weeks or empirically chosen antibiotic therapy have failed are almost invariably a waste of effort.

Having obtained suitable material, it must be carefully packaged, labeled, and transmitted to the laboratory by the fastest practicable method. Relevant shipping regulations must be obeyed. If material is sent to a laboratory in another country, this laboratory must be consulted in advance to ensure that it is willing to receive the material. An import license may be required. All samples must be accompanied by a written note indicating the origin of the material, the relevant history, and the tests required.

Self-check 2	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Why samples taken from animal or from their environment?

   _____
   _____
   _____

2. Explain what the samples need to be during sample collection

   _____
   _____
   _____

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

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

Information Sheet 3- Placing collected specimen in appropriate container with cap or proper closure and labeling properly

Keeping the tissues cool

Bacteria are usually present in ALL collected samples. Unfortunately the nonspecific bacteria will replicate even faster than any bacteria that might be causing a disease. So it is important to keep the tissue cool because the bacteria grow at warm temperatures and the nonspecific bacteria will quickly outgrow any specific bacteria we might be trying to find. Also, the nonspecific bacteria will generate toxic products that might kill any viruses or fungus we are looking for in the sample.

Try to keep the various tissues separated from one another

As tissues get grouped together, bacteria from one can quickly overtake another. This is especially true if intestinal samples or fecal materials are included. Use plastic bags that can be sealed - “ziplock” or “whirlpak” bags are the best.

Samples should be kept moist –

If the sample dries out, any agents in there might dry out as well, making it difficult to isolate the infectious organism.

Self-check 3	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

3. Why tissue samples are kept in cool?

   _____
   _____
   _____

4. What is the importance keeping various samples separate from each other?

   _____
   _____
   _____

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

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

Information Sheet 4- maintaining required temperature of collected

Safe Storage Temperatures for biological samples

Good storage practices of biological materials are an essential component of any laboratory. Biological samples often degrade over time when stored at room temperature, but some samples may also lose integrity at low temperatures if subjected to multiple freeze-thaw cycles. The best storage temperature for a given biological sample or reagent often varies depending on the type of biological material, the solution it is suspended in, the sample's intended use, and how long the material will be stored. The most common storage temperatures are bench top/room temperature, refrigerated, freezer, ultra-lowfreezer, and cryogenic freezer storage.

Room Temperature Storage (15ºC to 27ºC)

Biological materials that have fixed with a preservative such as Bouin's, formalin or alcohol, such as paraffin embedded tissues or biological specimens, can typically be stored at room temperature in a climate controlled building. While room temperature storage is typically not ideal for samples from which molecular data is desired, it is sometimes possible to obtain DNA results from preserved or dried tissues that have been kept at ambient temperatures. However, the DNA in these tissues is often highly degraded, and only short read lengths are obtained. RNA degrades rapidly at room temperature and typically cannot be isolated from tissues that have not been kept in freezer storage.

Refrigerated Storage (2ºC to 5ºC)

While typically a poor option for long-term storage, refrigerated temperatures are optimal for short-term storage of frequently used biological reagents, such as enzymes and antibodies. These reagents will quickly lose integrity if repeatedly frozen and thawed during routine experimental use and typically will remain viable at refrigerated temperatures when used within manufacturer recommended timeframes. Biological

materials that will not be used in a short timeframe can be aliquoted and frozen until needed, reducing the number of freeze-thaw cycles they are subjected to.

Freezer Storage (-20ºC)

Many biological materials can be stored at standard freezer temperatures, preferably in appliances without frost free cycles (these cycles require brief periods of thaw to prevent frost accumulation and can degrade biological materials). -20ºC freezer storage is ideal for short-term storage of samples and reagents that are not stable at warmer temperatures. DNA and RNA can typically be obtained from tissues that have been suspended in appropriate solutions before freezing at -20ºC, though colder temperatures are recommended for long-term storage or for the storage of tissues or cells that are not suspended in a stabilizing solution.

Ultra-low Freezer Storage (-80ºC)

Ultra-low -80ºC freezers are a practical option for long-term storage of biological materials. Ultra-low temperatures prevent the degradation of nucleic acids, proteins, endocrine molecules, and many other biological molecules. These temperatures have been shown to maintain the viability of numerous biological assays and reagents through long-term storage. When samples are stored at ultra-low temperatures, it is important to consider freeze-thaw protocols. Cells typically preserve best when frozen slowly (about 1ºC a minute), but thawed quickly (such as in a water bath).

Cryogenic Freezer Storage (-150ºC to -190ºC)

Cryogenic freezer storage is often deemed the gold standard for long-term storage of biological samples. At these extremely low temperatures all biological activity is suspended and no degradation occurs. Cryogenic freezing is ideal for sensitive samples and specimens which cannot be suspended in a preservative find more information. Like ultra-low temperature freezing, it is important to consider freezing and thawing protocols when utilizing cryogenic freezer storage.

Self-check 4	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. Write the following Safe Storage Temperatures for biological samples

a. Room Temperature Storage _____

b. Refrigerated Storage_____

c. Freezer Storage _____

d. Ultra-low Freezer Storage _____

e. Cryogenic Freezer Storage_____

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

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

Information Sheet 5- using appropriate physical and chemical methods to preserve and store samples

Preservation of specimens

Various preservatives are used for different specimens, e.g. phosphate buffered glycerin for tissues; EDTA, sodium citrate, heparin or OCG mixture for whole blood and transport media (TPB) for swabs. The preserved specimens are most frequently transported on ice in a thermos flask or other suitable containers.

A. Phosphate buffered glycerin (PBG):

It is prepared by mixing equal parts of phosphate buffered saline and neutral glycerin (pH 7.0).

Phosphate buffered saline	(PBS)
Sodium chloride (Nacl)	8.00 g
Pot. chloride(Kcl)	0.20 g
Di-Sod. hydrogen phosphate (Na2 HPO4)	1.15 g
Pot. dihydrogen orthophosphate (KH2 PO4)	0.20 g
Glass Dist. water	1000 ml.

The final pH of PBG sol. is adjusted between 7.2 to 7.4, before autoclaving.

B. Oxalate-Carbolic acid-Glycerin (OCG) Mixture:

Potassium oxalate	5.0 g
Phenol (Carbolic acid)	5.0 g
Glycerin	500 ml
Dist. water	500 ml

The pH is adjusted to 7.2 before autoclaving.

C. Tryptose phosphate broth (TPB):

It is used as a transport medium for nasal, eye, rectal swabs etc.

Tryptose (Difco)	20.0 g
Dextrose	2.0 g
Sod. chloride	5.0 g
Di-sod. hydrogen phosphate	2.5 g
Dist. water	1000 ml.

The pH is adjusted to 7.2-7.4 before autoclaving. Antibiotics (Penicillin, Streptomycin, Mycostatin) are added before collection of swabs to check bacterial contamination.

Self-check 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

5. What are the preservatives used for different specimens?(5pts)

_____

_____

_____--

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

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

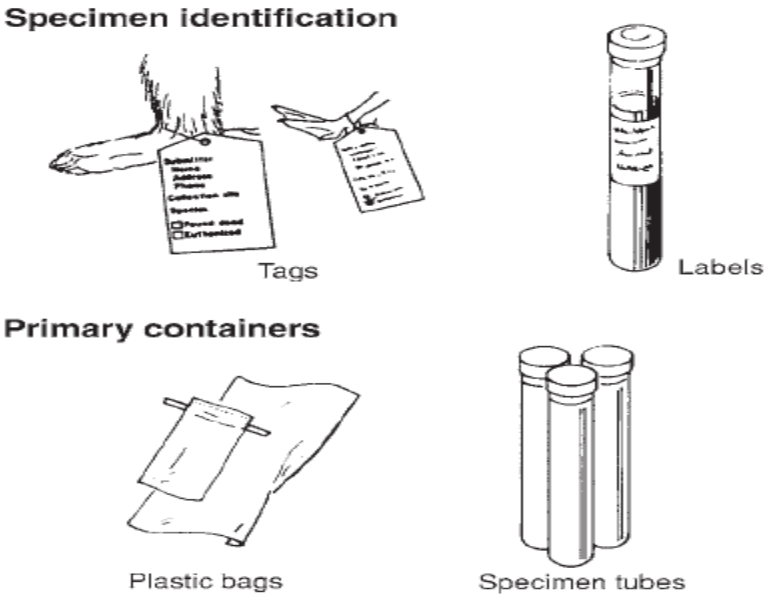
Information Sheet 6- transporting specimen to the diagnostic laboratory

Specimen Shipment

Procedures for shipping specimens can vary with different disease diagnostic laboratories and regulatory requirements but most have instructions available online. Following the laboratory's shipping instructions will facilitate the processing of specimens when they reach the laboratory and assure that the quality of specimens is not compromised. Time spent on field investigation, specimen collection, and obtaining an adequate history will be of little value if specimens become contaminated, decomposed, or otherwise spoiled during shipping to the diagnostic laboratory. Pay attention to whether the laboratory requires notification prior to shipment as this will ensure proper handling and facilitate processing once they arrive.

There are five important considerations for proper specimen shipment:

- prevent cross-contamination from specimen to specimen,
- prevent decomposition of the specimen,
- prevent leakage of fluids,
- preserve individual specimen identity, and
- properly label the package.



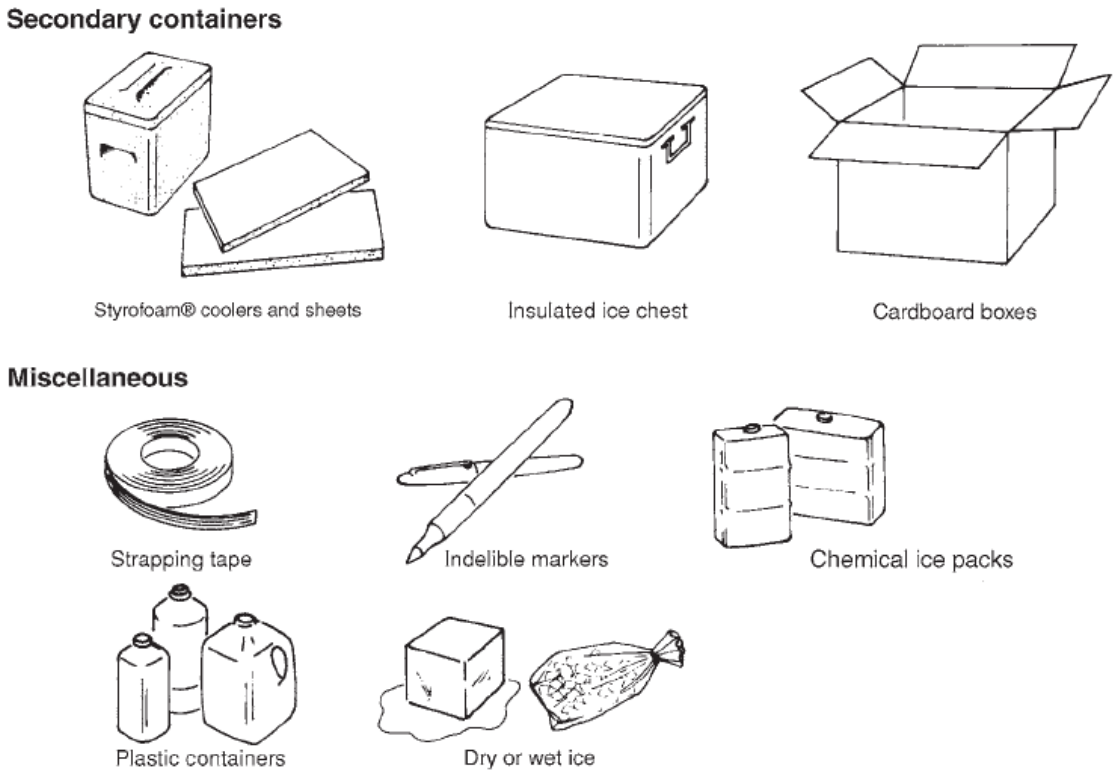


Figure: Basic specimen-shipment supplies

Self-check 5	Written test

Name…… ID…… Date…….

Directions: Answer all the questions listed below.

Short answer

1. list and describe the important consideration during laboratory sample shipment.

_____
_____
_____
_____
_____

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

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

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