Animal Health

Level - IV

Based On March 2022, Version 4 Occupational Standard



Module Title:-Applying General Laboratory Techniques and Procedures

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Introduction to the module

This module covers the knowledge, skills and attitude required to collect preserve and transport the right sample to the laboratory, process laboratory specimen for test and performing basic laboratory tests. The work functions within this module require the demonstration of relevant theoretical knowledge and skill to conduct preparation and processing of lab specimen, to conduct basic staining tests, microscopic examination and analysis and reporting of results.

LG #12	LO #1- Carry out Sample Collection, Preservation, and Shipment

Instruction sheet

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

- Relevant laboratory rules and guidelines
- Determination of specimen type and collection of sample
- Preparing materials and PPE
- Identification and preparation of sampling site
- Sample labeling, preserving and handling specimens
- Risks in sample collection and handling

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:

- Determine nature of specimen required to be collected
- Identify supplies, materials and PPE needed for specimen collection, preservation and transport
- Identify and prepare work area, animal and sampling site
- Collect specimens from animals
- Label and record sample for clear identification.
- Handle and preserve specimens.
- Perform samples packaging and transportation
- Recognize and report risks in sample collection and handling

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
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet 1

1.1 Relevant laboratory rules and guidelines

Laboratory technique has a range of associated risks from pathogens; equipment, chemicals and reagents. All duties should reflect awareness and precautions against such risks. Handling of samples, equipment, chemicals and reagents requires a guideline to ensure safe work practices.

Safe work practices are used in handling and processing laboratory samples.

Procedures to reduce the exposure to these hazards may include:

- Incident reporting,
- Cleaning,
- Removal of wastes and spillage,
- Containment or elimination of risk,
- The use of personal protective clothing and equipment,
- Seeking advice from supervisors.
- Use of safety cabinet (biohazard cabinet),
- Stringency in following safety and precaution rules in the laboratory

1.1.1. General Rules of laboratory

- Follow all written and verbal instructions carefully. If you do not understand ask the instructor before proceeding.
- Do not touch any equipment, chemicals, or other materials in the laboratory until you are instructed to do so.
- Perform only those experiments authorized by the instructor.
- Read all procedures thoroughly prior to attending that laboratory session.
- Work areas should be kept clean and tidy at all times. Bring only your laboratory instructions, worksheets, and/or reports to the work area but not other materials.

- Push your chair under the desk when not in use.
- Always work in a well-ventilated area. Use the fume hood when working with volatile substances or poisonous vapors.
- Dispose of all chemical waste properly.
- Labels and equipment instructions must be read carefully before use.
- Set up and use the prescribed apparatus as directed in the laboratory instructions or by your instructor.
- Return all equipment to the proper storage area.
- Handle all living organisms used in a laboratory activity in a humane manner.
- If you have a medical condition (e.g., allergies, pregnancy, etc.), check with your physician prior to working in lab.
- Walk; do not run, in the lab.
- Ensure that access to emergency equipment (eyewashes, safety showers, first aid kit and fire extinguishers) is not blocked.
- Use the flow bench only for inoculations and sterile work but not for general laboratory procedures.
- Conduct procedures involving the release of volatile toxic or flammable materials in a chemical fume hood.
- Perform procedures that liberate infectious bioaerosols in a biological safety cabinet
- All animal or human blood, body fluids and cultures should be handled as being potentially pathogenic.

1.1.2. General Safety Guidelines

Your prime concern the moment you start work in the laboratory should be to protect yourself and your fellow co-workers from exposure to harmful toxic substances and hazardous chemicals. Remember you are as much responsible for your own safety as safety of others around you. A majority of laboratory accidents are caused by overconfidence and non-adherence to safety guidelines.

Laboratory accidents often lead to cuts and bruises, severe burns, poisoning, corrosive damage from hazardous chemicals, diseases and long-term genetic disorders due to exposure to harmful microbes and radioactive radiations. Such situations are avoidable through adherence to safety guidelines. While it is not possible to cover the entire subject on the laboratory safety in a short article such as this one an attempt is made to provide some useful guidelines when carrying out different laboratory operations and processes. The followings are general laboratory guidelines

- Familiarize yourself with safety symbols and signs.
- Follow laboratory discipline such as non-consumption of eatables or drinks, non-smoking, distracting others and following prescribed dress code.
- Familiarize with MSDS (material safety data sheet) on chemicals you are likely to use
- Do not use mobile phase phones while working in the laboratory
- Wear protective clothing, safety glasses, gloves and facemasks when performing hazardous operations
- Avoid working alone in the lab
- Always keep your laboratory bench clean and keep reagents and chemicals at the designated places after use
- Immediately wash acid or alkali spills on your body with water before seeking medical attention
- Check validity of articles in the first aid kit from time to time and replace them accordingly
- Familiarize with the use of fire extinguishers, first aid kits and other safety gear
- Do not use cracked or partially broken glassware
- Ensure proper earthing of electrical instruments and do not overload electrical points
- Storage and handling of hazardous and flammable chemicals
- Corrosive chemicals, acids, alkalis, pesticides, etc should be stored separately in special storage cabinets made of all plastic, polypropylene or painted steel to prevent rusting or delamination of wood core materials.

- Cabinets should bear appropriate safety symbols
- Transfer large solvent bottles and carboys using special trays. Never hold such containers by the neck
- Always wear gloves when handling such containers
- Use liquid dispensing pumps fitted to mouth of bottles when transferring contents to other containers
- Use of hot ovens, muffle furnaces and digestions chambers
- Use fume hoods for sample preparation when digesting samples involving strong acids to prevent exposure to corrosive fumes
- Ensure that the exhaust system of fume hood is switched on during digestions
- Avoid naked flames and use heating mantles and and hotplates for digestions
- Add glass or ceramic beads to prevent bumping during boiling
- Handle muffle furnaces and items inside carefully using heat-proof mittens
- Do not place volumetric flasks with lids intact when drying in hot air ovens

1.2. Determination of specimen type and collection of sample

1.2.1. Humane method of sample collection from farm and pet animals

General Principles

The use of proper restraint and handling techniques reduces stress to animals and also to the researcher. Animals can inflict serious injuries to humans and to themselves as a result of improper handling.

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

Sample collection

General Guidelines in Sample Collection

Sample can be collected:

- Either from live clinical animal or recently dead animals as soon as possible after death /immediately (autopsy).
- From the affected site(s) and as early as possible.
- From clinical cases and in contact animals.
- From the edge of the lesion and include some macroscopically normal tissues.
- As aseptically as possible.
- Before the administration of any form of treatment.
- From individuals or groups depending on the nature of disease or purpose of samples collected
- Sterilized sample collection materials shall be used if intended for laboratory diagnosis purpose.
-

The following points should be considered during sample collection

- The body location from where sample to be taken (rectum, vein).
- Type of sample to be collected (blood, faeces).
- The amount of sample to be taken.
- The speed at which the collected sample shall be taken to laboratory to avoid deterioration and misreading of results.
- The specimens should be collected aseptically to avoid overgrowth of contaminating bacteria.
- The samples should be submitted individually.
- Fluid samples should be submitted in sterile container.
- Label should be completed with all necessary information.
- The specimens should be preserved with appropriate preservatives or special transporting media to keep the viability of microorganism.

I. Sample collection from live animals

a) Blood collection

- For hematology or for culture and/or direct examination for bacteria, viruses, or protozoa: Whole blood can be collected in tube containing anticoagulants, such as ethylene diamine tetra-acetic acid (EDTA) or heparin.
- Blood collected with anticoagulants mainly used for diagnosis of specific parasitic stages found in the circulatory system (Haemoparasites).
- Includes blood protozoa such like Trypanosomes spp, Babesia spp.,Dirofilaria spp etc
- Blood plasma is also used for some procedures.
- A blood sample is taken, as cleanly as possible, by vein puncture.
- Hemostasis should be achieved using gauze and direct pressure for several minutes following arterial puncture.

b. Faces

- Where possible, fecal samples should be collected from the rectum of the animal.
- If rectal samples cannot be obtained, then fecal samples can be collected from the pasture.
- In this particular case samples should be selected from the most recently dropped fecal part and several samples should be collected(only for parasitological examination)
- Collect enough quantities of fecal sample (about 10 gram).
- In large animals sample taken by hand but in small animals can be induced to defecate by inserting a moistened finger (spatula or thermometer) in to the rectum and gently massaging with a rotary motion until the external sphincter relaxes.
- Poultry from clean floor or cage, swabs from the rectum (or cloaca), with care to swab the mucosal surface.
- Faeces for parasitological should fill the container and be sent to arrive at the laboratory within 24 hours.

c. Skin scraps

- In diseases producing vesicular lesions, collect 2 g of affected epithelial tissue aseptically and place it in 5 ml phosphate buffered glycerine or Tris-buffered tryptose broth virus transport medium at pH 7.6.
- Vesicular fluid should be sampled where un ruptured vesicles are present and vesicular fluid should be aspirated with a syringe and placed in a separate sterile tube.
- Plucked hair or wool samples are useful for surface-feeding mites, lice and fungal infections.
- Take deep skin scrapings (until blood ooze out) from moist peripheral active lesions for burrowing mites (Sarcoptic mange) and hair shavings (not scraping) from hairy area for Chorioptic or Psoroptic mange.
- Clip hair and moisten the skin with liquid paraffin so that scrapings are adhered to the scalpel and not be blown away.
- In birds: feather tips can be taken for detection of viral antigen where Marek's disease is suspected**.**

d. Genital tract and semen

- Vaginal or preputial washing in buffered physical saline, or by the use of suitable swabs.
- Vaginal mucus or washings for cultural tests must be received within 24 hours of collection.
- The cervix or urethra may be sampled by swabbing.
- Mucus for agglutination test.
- Semen samples: using an artificial vagina or by extrusion of the penis and artificial stimulation.
- Avoid contamination of sperm with antiseptic washing solutions.
- Specific transport media and conditions often required**.**

e. Milk

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

f. Urine

- Urine samples should be collected in a clean, dry container that is free of any disinfecting or cleaning chemicals.
- Samples may be collected by free catch of voided sample, manual bladder expression, catheterization, or cystocentesis.

h) 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

II. Sample collection at post-mortem

- Samples of tissue from a variety of organs can be taken at post-mortem.
- Use biological safety cabinet; if this is not possible, an efficient face mask and eye protection to protect zoonotic diseases.
- If rabies or transmissible spongiform encephalopathies (TSEs) are suspected, detach the animal's head.
- Each piece of tissue should be placed in a fully labeled separate plastic bag or sterile screw-capped jar.

- Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents.
- Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation.
- The tissues may be sent to the laboratory dry or in bacterial or virus transport medium, depending on the type of specimen and the examinations required; swabs should be sent in transport medium.
- For histopathology, blocks of tissue not more than 0.5 cm thick and 1–2 cm long are cut and placed in neutral buffered 4–10% formalin, which should be at least ten times the volume of the tissue sample.
- For certain suspected diseases, larger portions of brain are required; the brain is sectioned using a sagittal cut, half is submitted fresh, on ice, and the other half is submitted in 10% buffered formalin.
- Store and pack formalin-fixed tissues separately from fresh tissues, blood and smears.
- Care should be taken to insure that formalin-fixed tissues are not frozen.

1.3. Preparing Materials and PPE

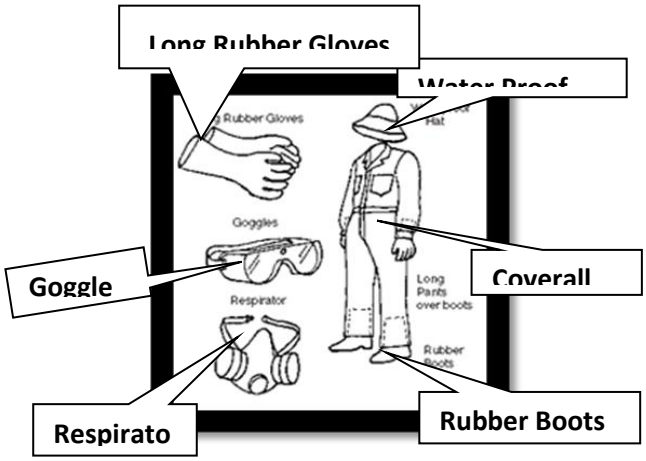
1.3.1. Personal protective equipment (PPE)

It acts as a barrier to minimize the risk of exposure to aerosols, splashes and accidental inoculation. The clothing and Equipment selected is dependent on the nature of the work performed (in lab.) Before leaving the laboratory, protective clothing should be removed, and hands should be washed. Whenever appropriate, use personal protective equipment, such as:

- **Coveralls**
- **Gloves**, surgical(latex, vinyl, or nitriyl), long heavy duty rubber, leather, lid etc.., specific to the materials being handled,

- **Face shields** when applying disinfectants, and goggles when handling concentrate powders or solutions
- None slip waterproof and rubber boot.
- **Aprons**

✓ Wear a waterproof apron when you pour and mix concentrated chemicals to protect yourself from splashes



Aprone

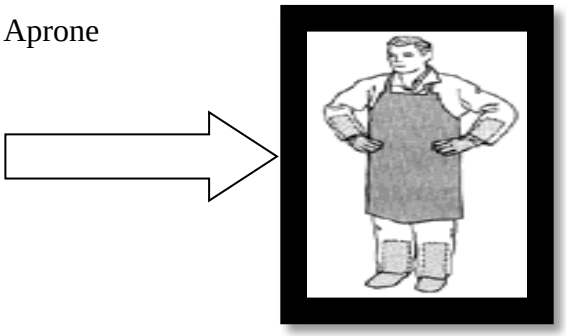


Fig.1.1. PPE

- **Respirators** -Protect your lungs by wearing a respirator to avoid inhalation of dust, vapors, or spray mist; or if there is a danger poison symbol on the label; or if you are performing cleaning and disinfection



Fig.1.2. Respirator

Types of Personal protective equipment

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

i. 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 airsupplied 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

ii. Skin protection

Any form of PPE that acts as a barrier between the skin and the agent of exposure can be considered as skin protection.

- Gloves-Because of much work is done with the hands, gloves are an essential item in providing skin protection include rubber gloves, cut-resistant gloves, chainsaw gloves and heat-resistant gloves.
- Lab coats 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.

iii. Eye protection

- 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 face-piece 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.

iv. 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.

B. 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.
- 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 face piece 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-inglove 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..

C. **Storage for PPE**

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. All PPE must be stored in a clean and sanitary condition ready for use. Accommodation may be simple. 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. Where PPE may

become contaminated during use, storage should be separate from any storage provided for ordinary clothing.

1.3.2. Materials, Equipments and Reagents for Sample Collection

- **Sample containers**

A sample collection container is a vessel, such as a cup or vial, used to hold the biological sample being collected for lab testing. A container may be used to hold a blood, oral fluid, urine, hair or other samples. Depending on the type of container, it could also be called a specimen bottle or specimen container. Depending on the type of sample being collected, a container may be an open cup, a vial, other type of bottle, or even a sponge. A container should be a sterile single-use vessel that is individually wrapped and sealed to prevent tampering

✓ **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 auto-sampler 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.

✓ **Test tubes and vacutainer 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

- ✓ **Capillary tube**

  Capillary tube is used to take blood from pierced ear vein or from blood holding vacutainer tube

- ✓ **Universal bottle/plastic container**

Used for collection of faeces, ectoparasite, adult parasite samples etc.

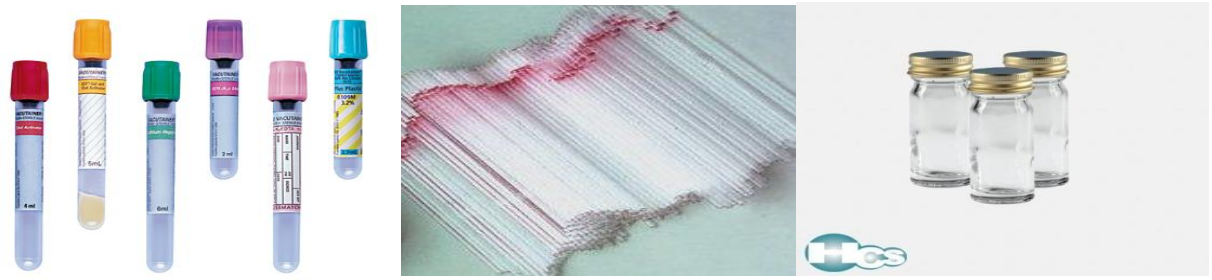


Fig 1.3 Sample containers

- ✓ **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

✓ **Cryovials**

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

- 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.

✓ **Microscope slides**

They 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).

✓ **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)

- 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 (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



Fig 1.4. Refrigerator

- **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.

- **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

- **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.

- **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.

1.4. Identification and Preparation of Sampling Site

1.4.1. Proper Animal Handling & Restraint

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.

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.

Proper Restraint Techniques are used to:

- 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:

- Remove 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!)

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.

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:

a. Muzzles

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



Fig. 1.5 Muzzle

b. Snake tongs or graspers

- Can be used to capture and restrain snakes and other animals, such as cats

c. 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'

d. Halter

- 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
- Used for equines



Fig. 1.6- Halter

e. Head collars

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

f. 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.

g. Hobbles – Hobbling

- To prevent kicking of equines and bovines
- Pass rope over neck (ring), ends through front legs, through hock hobbles (inside to outside) on back legs and through neck ring

h. Twitches

- Used for equines
- 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.



Fig. 1.7 Nose twitch

i. Cast by ropes – Casting

- Used for equines and cattle
- 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.

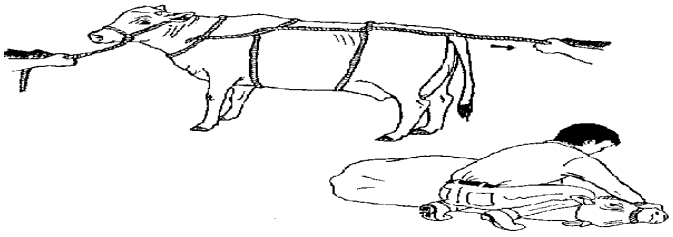


Fig. 1.8 Picture of casting

j. Snares

- Used for swine
- Cable or rope loop
- Place loop over snout behind canine teeth.

k. Hand restraining

- Used for goats and sheep
  - ✓ Halter
  - ✓ Collar
  - ✓ Flank hold
  - ✓ Side line

1.4.2. Preparation of Sampling Sites

a. Blood Sample Collection

- **Sites of Blood Sample Collection**

Blood sample is collected by puncture of vein from live animals or from left ventricle or other organs of dead animals. In laboratory, if blood sample is to make smear it is advisable to take a small amount of blood from the ear vein of horses, cattle, sheep, goats, pigs, rabbits, dogs and cats. In chicken it is better to take from wing vein (brachial vein).

In most large mammals: jugular vein, caudal vein, brachial veins and mammary veins are used.

In Pigs: caudal/ vena cava/ear veins.

Table 1: Common sites for blood collection

Species	**Site of collection and permitted conditions**
Dog, Cat	Cephalic, saphenous, femoral and jugular veins
Ruminants	Jugular and ear vein
Equine	Jugular and ear vein
Swine	Jugular vein, anterior vena cava, ear veins
Chicken	Brachial wing vein, right jugular vein, cardiac (anesthetized only)

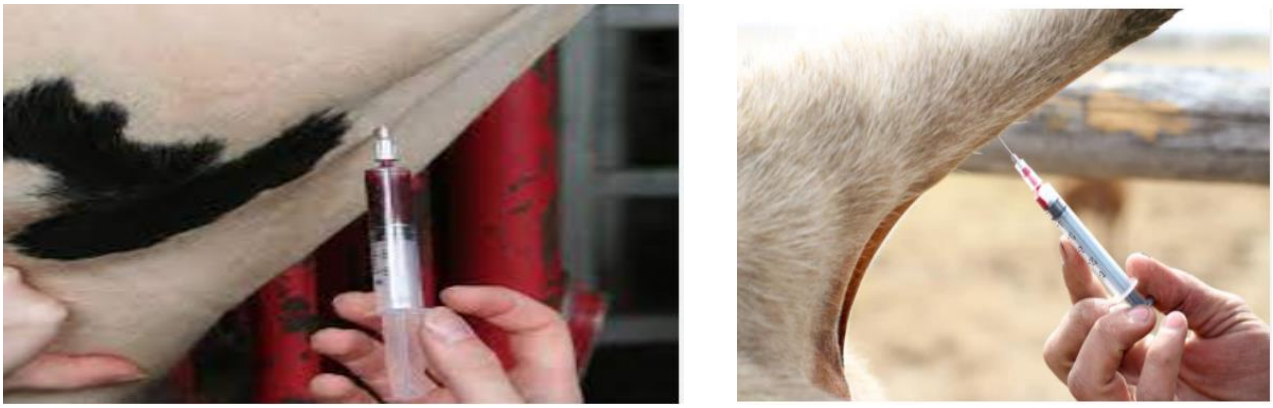


Fig. 1.9. Common blood collection sites from ruminants

b. Faces

Fecal samples should be collected from the rectum of the animal. If rectal samples cannot be obtained, then it can be collected from the pasture from the most recently dropped fecal part.

Collect enough quantities of fecal sample (about 10 gram). In large animals sample taken by hand but in small animals can be induced to defecate by inserting a moistened finger (spatula or thermometer) in to the rectum and gently massaging with a rotary motion until the external sphincter relaxes.In Poultry from clean floor or cage, swabs from the rectum (or cloaca), with care to swab the mucosal surface. Faeces for parasitological should fill the container and be sent to arrive at the laboratory within 24 hours Feces should be collected in airtight containers to prevent desiccation. If the processing of a fecal specimen must be delayed, it may be**:**

- Refrigerated (but not frozen) for several days (not recommended for samples with live larvae that you intend to examine using the Baermann technique)
- Fixed, e.g., 10% formalin)**. Never** use formalin in samples for faecal culture.

c. **Skin scrap**

- In diseases producing **vesicular lesions**, collect 2 g of *affected epithelial tissue* aseptically and place it in 5 ml phosphate buffered glycerine or Tris-buffered tryptose broth virus transport medium at pH 7.6.
- *Vesicular fluid* should be sampled where **un ruptured vesicles** are present and vesicular fluid should be aspirated with a syringe and placed in a separate sterile tube.
- *Plucked hair or wool samples* are useful for **surface-feeding mites, lice** and **fungal infections.**
- Take *deep skin scrapings* (until blood ooze out) from moist peripheral active lesions for **burrowing mites** (**Sarcoptic mange**) and *hair shavings* (not scraping) from hairy area for **Chorioptic** or **Psoroptic mange**.
- Clip hair and moisten the skin with liquid paraffin so that scrapings are adhered to the scalpel and not be blown away.
- In birds: *feather tips* can be taken for detection of viral antigen where **Marek's disease** is suspected**.**

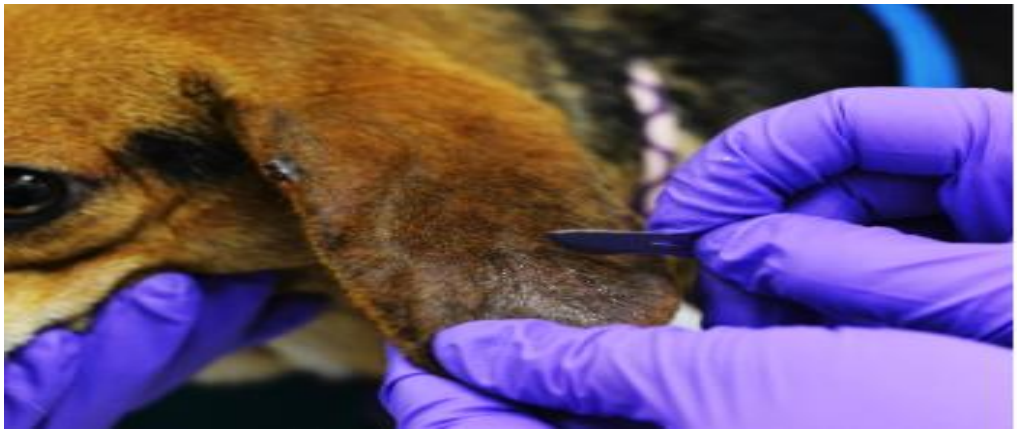


Fig. 1.10 Skin scraping

d. Genital Tract and Semen

Vaginal or preputial washing in buffered physical saline, or by the use of suitable swabs. Vaginal mucus or washings for cultural tests must be received within 24 hours of collection. The cervix or urethra may be sampled by swabbing. Mucus from vagina is used for agglutination test. Semen samples can be taken using an artificial vagina or by extrusion of the penis and artificial stimulation. Avoid contamination of sperm with antiseptic washing solutions. Specific transport media and conditions often required**.**

Preputial Wash Sample

The finding of this type of samples is important to diagnose infectious diseases which affect the male reproductive system.

e. Milk

Milk samples should be taken after cleaning and drying the tip of the teat. The initial stream of milk should be discarded and a tube filled with the next stream(s), a sample of bulk tank milk can be used for some tests. Milk for serological tests should not have been frozen, heated or subjected to violent shaking. If there is a delay in submitting to the laboratory, preservatives can be added to milk samples that will be collected for serological testing. If necessary, milk for bacterial examination can be frozen.



Fig. Milk sample collection

f. **Urine**

Urine samples should be collected in a clean, dry container that is free of any disinfecting or cleaning chemicals. Samples may be collected by free catch of voided sample, manual bladder expression, catheterization, or cystocentesis(puncture of urinary bladder).

g. Sample Collection at Post-Mortem

Samples of tissue from a variety of organs can be taken at post-mortem. Each piece of tissue should be placed in a fully labeled separate plastic bag or sterile screw-capped jar. For histopathology, blocks of tissue not more than 0.5 cm thick and 1–2 cm long are cut and placed in neutral buffered 4–10% formalin, which should be at least ten times the volume of the tissue sample. Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents. Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation. The tissues may be sent to the laboratory dry or in bacterial or virus transport medium, depending on the type of specimen and the examinations required; swabs should be sent in transport

medium. Use biological safety cabinet; if this is not possible, an efficient face mask and eye protection to protect zoonotic diseases. For certain suspected diseases, larger portions of brain are required; the brain is sectioned using a sagittal cut, half is submitted fresh, on ice, and the other half is submitted in 10% buffered formalin. If rabies or transmissible spongiform encephalopathy (TSEs) are suspected, detach the animal's head.

1.5. Sample labeling, preserving and handling specimens

1.5.1. Labeling specimens

- Ideally, sample labels will be produced and applied at the time the sample is drawn.
- This eliminates the possibility of applying the wrong label to the wrong sample.
- All specimens must be clearly labeled with a black indelible/permanent marker.
- Clearly mark specimens that are suspected of being hazardous to human health, i.e., rabies, mycoses, anthrax, brucellosis, leptospirosis, etc.
- Proper identification eliminates the possibility of a specimen becoming separated from its submission form or being switched with another specimen.
- Correct labeling and identification including:
  - ✓ Owner's name and address
  - ✓ Correct species, age, sex, breed, identification
  - ✓ Clinical signs of the patient
  - ✓ Number of animals at risk and details of any mortality and postmortem findings
  - ✓ The type of sample submitted
  - ✓ Tentative diagnosis wherever possible
  - ✓ Clinician's name, address and telephone number

1.5.2. Preservation of samples for prolonged storage

i. Preservation of sera

- Storage conditions should minimize loss of immunological and other biochemical properties of the sera.
- There are three methods:
  - ✓ deep freezing

- ✓ dry storage on paper disks at ambient temperature
- ✓ lyophilisation (freeze-drying).

- Deep freezing:
  - ✓ For long-term storage of sera a core temperature below –60°C should be maintained.
  - ✓ The lower the temperature the better, but too expensive to maintain.
  - ✓ Liquid phase N_2 at –196°C, vapour phase N_2 at –100°C and an ultra-low deep freezer will maintain –90°C.
- Paper disk storage*:*
  - ✓ Simple and inexpensive method, but it allows only a small quantity of serum to be stored and the eluted serum is only suitable for a limited number of tests.
  - ✓ The disks should be kept in a cool, dry atmosphere.
  - ✓ They can probably provide satisfactory results for up to about 5 years.
- Lyophilisation:
  - ✓ The best method for long-term storage of sera.
  - ✓ If freeze-drying conditions are optimized the loss of serum characteristics are minimized.
  - ✓ Requires expensive equipment and is a time-consuming process.
  - ✓ Lyophilised vials should be stored at 4°C.

ii. Preservation of parasitological samples

- Large insect specimens: mount them correctly in an entomological storage box.
- Add a little paradichlorbenzene or other preservative in each storage box to repel predators.
- Dried large insects are pinned through the thorax using fine entomological pine.
- Small insects such as biting midges (Simulium, Culicoides) are attached to the top of a card “point” using tragacanth gum $C_{741.}$

- Small and soft-bodied parasitic insects (small flies, insect larvae, lice): keep in 70% alcohol with 10% formalin or formal saline if they are soft bodied but 70% alcohol with 5% glycerine for hard bodied specimens.
- Ticks: keep in equal parts of glycerol and 70% alcohol
- Faecal samples can be cooled at 4^0 C and ice packed for shipment and should reach with in less than one or two days. This is to avoid the eggs developing and hatching.
- Don't keep fecal samples in a deep freezer.
- Faecal Samples can also be stored indefinitely in 3% formalin (1 part feces and 3 parts of 3 % formalin). This is to preserve parasite eggs.
- Such samples are not useful for larval culture as formalin prevents embryonation and hatching of eggs. N.B: Formalin-fixed faeces cannot be used for fecal cultures.
- When samples are received in the laboratory they should immediately be stored in the refrigerator (4°C) until they are processed.
- Samples can be kept in the refrigerator for up to *3* weeks without significant changes in the egg counts and the morphology of eggs.
- If samples are more than two hours old, samples should be stored at 4^oc until examine

1.5.3. Transportation (shipping) of specimen

- The specimens should be forwarded to the laboratory by the fastest method available.
- If they can reach the laboratory within 48 hours, samples should be sent refrigerated.
- If dry ice is used, the additional packaging requirements must be met.
- Whole blood can be collected in tubes containing anticoagulant such as EDTA and send to laboratory at $4\ ^O$C or in a chilled container.
- An alternative method for collecting and transporting blood that is to be used for serology is to place a drop of blood on to filter paper, the blood is dried at room temperature and the sample can then be shipped unrefrigerated.
- Fecal samples should be dispatched as soon as possible to a laboratory in suitable containers such as:
  - ✓ Screw cap bottles
  - ✓ Plastic containers with lids

- ✓ Disposable plastic sleeves/laboratory or rectal gloves used for collecting the samples
- ✓ Plastic bags

- However, screwed containers, plastic or glass are best if the sample is to be transported to another place.
- Faeces for parasitology should fill the container and be sent to arrive at the laboratory within 24 hours.
- If transport times are likely to be longer than 24 hours, the sample should be sent on ice or refrigerated to prevent the hatching of parasite eggs.
- For most blood parasites, blood smear on standard microscope slides can be made from fresh blood or blood with anticoagulant and sent to laboratory.
- Faeces are best stored and transported at 4°C.
- For routine shipment to the laboratory, samples can be cooled for 4^{o}c and then packed with ice or other coolant (blue ice) for shipment via any of the 24 to 48 hour transport services.
- All parasitological specimens should be dispatched in 5-10% formalin or 70% alcohol.
- If coolants are not available, samples can be stored indefinitely in 10% formalin (one part feces in 9 parts 10% formalin), but limitations must be noted.
- Sent to laboratory by placing in glycerin in a tightly sealed vial for shipment.
- Arthropod parasites can be most effectively stored in 70% ethyl alcohol or glycerin for shipment or long term preservation.
- Swabs should be transported in appropriate transport medium.

1.6. Risks in Sample Collection and Handling

1.6.1. Hazard identification and risk control

Hazard identification

A hazard is anything that has the potential to harm the health or Safety of a person or an animal. Hazards during sampling may include:

i. Physical hazards

It can be electrical equipment, open flames, and machinery operation and maintenance, solar radiation and electricity, breakages, and animal bites, kicks or scratches, confined spaces, tripping hazards, damaged or worn out equipment, manual handling items, poor surfaces, glassware and fire can all be hazardous if improperly used.

ii. Chemical hazards

Can be flammable, toxic (to skin contact or respiratory exposure), caustic (strong acids or bases that can cause skin and mucous membrane burn or destroy), corrosive, carcinogen (a substance with the potential to produce cancer) or mutagenic (a substance with the potential to make a stable change in a gene/pass to the offspring). All chemicals must be labelled with **hazard information** on the containers. Spillages; drift and volatility of chemicals, splash or scalding

iii. Biological hazards

Include contaminated bacteria, viruses, funguses, protozoa or parasites. In microbiology lab, making any bacteriological culture is recommended in the microbiological safety cabinet and avoids contact from biological culture.

Hazard Identification

Definition: is identifying and evaluating unsafe work conditions so that accidents, injuries, and job related illnesses are minimized, if not completely eliminated. It is the first step in the hazard identification and risk control process

- **Risk Assessment and Risk Control**

Persons working in clinical diagnostic laboratories are exposed to many risks. The goal of a safety program is to reduce 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. Is an interactive process consisting of steps, which and when undertake in sequence. It is a logical and systemic method of establishing the context, identifying, and analyzing, treating, monitoring and communicating risks to minimize the losses.

i. Risk Assessment

Assessing workplace risks means considering how likely problems will occur, and how serious the consequences might be. The identified risks should be ranked from highest priority to lowest priority and risks that are potentially most serious and/or most likely to cause health and safety problems should then be tackled first.

Five basic steps in the risk management process are as follows:

- Identifying the hazard
- Assess and prioritize the risks
- Decide on measures to control the risk
- Implement the appropriate control measures
- Monitor and review the risk.

ii. Risk Control

Controlling workplace risks means doing what can be done to prevent problems and protect people's health and safety. Once a hazard has been found it is then assessed as to its potential to cause damage and then a solution to the problem is sought. The most desirable way of managing risks of work injury is to eliminate the risk entirely, if this is possible. If not it should

be minimized in whatever ways are most likely to be practicable and effective. Remember-find it, check it out, get it fixed. In many cases it will be necessary to use more than one control method.

These are actions that are taken in response to a risk factor that has the potential to cause accident or harm in the workplace. The control measure can either designed to reduce the risk or eliminate them completely.

Self-Check – 1

Name…… ID…… Date…….

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

Part i. Choose the best answer and write your answer on the space provided (1 pt each)

_____1. One is a point should be considered during sample collection.

A) Appropriate restraining technique.

B) Appropriate selection of sample holding materials.

C) Body location from which sample to be taken from the animal.

D) All.

_____2. Blood sample collected for diagnosis of;

A) Blood parasite B) Protozoal parasites C) Ecto-parasite D) A and B

_____3. All are information's required during sample labeling except,

A) Address of owner, animal species. age, sex and breed

B) The name of laboratory technician who process the sample

C) Date of collection and type of specimen

D) Collectors name and laboratory test to be don

_____4. Where is the site of blood collection for poultry?

A. Jagular vein B. Wing vein C. Coccygeal artery D. facial artery

_____5. One of the following is not the material used for fecal sample collection.

A. Capillary tube B. vacutainer tube C. Glass slide D. All

Part ii. **Match correctly the following (2pts each)**

A	B
_____1. Blood sample	A) Heparin
_____2. Formalin	B) Collected for diagnosis of mastitis
_____3. Jugular vein	C) Preservative
_____4. Anticoagulant	D) Collection for diagnosis of haemoparasite
_____5. Milk sample	E) 70% alcohol
_____6. Preservative for tick sample	F) Site for blood collection
	G) Site for faeces collection

Part iii. Write short and precise answer.

1. Where milk sample could be collected?(1pt)

2. What is the difference between superficial and deep skin scraping?(1pt)

3. Faecal sample could be collected either from _____ or_____(1pt)
4. List 2 types of preservative for collected faecal sample.(2pt)

5. Write materials and procedures for faecal sample collection?(4pts)

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

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

Operation Sheet- 1

1.1.Procedures for Blood Collection from jugular vein

A. Materials

- PPE, Cotton, Syringes, Sample tube, Scalpel blades, Needle holder, Disinfectants , Ice box with ice packs, Hypodermic needles, puncturing needles or sterilized lancet

B. Procedures

I. Use PPE
II. Restrain the animal.
III. Locate the vein (bind if necessary).
IV. Shave the area.
V. Wash it with water and soap.
VI. Disinfect with 70 % Alcohol.
VII. Leave the skin to dry for some minutes.
VIII. Puncture with needle and syringe.
IX. Extract 5ml of blood, withdraw the needle and pass the blood to a tube containing any anticoagulant agent.
X. Mix inverting turning upside down the tube (5-7 times), till homogenizing the blood.
XI. Make duplicate smear if necessary.
XII. Identify and send the blood sample, use refrigerator if more than 2 hours elapse to take the sample to laboratory.

1.2.Procedures for Collecting Fecal Sample

A. Materials

- Include**:** Gloves, Lubricant, Labeling paper, Universal bottle, Restraining device, Preservatives (if necessary)

B. Procedures

I. Use PPE
II. Restrain the animals.
III. Wear arm length protective gloves (cut Sharpe nails and remove rings)
IV. Lubricate the gloved hand with lubricant.
V. Make the hand in conical shape
VI. Insert the hand directly to the rectum.
VII. Then, finally collect the fecal sample and place to the universal bottle (use preservative if necessary).

1.3. Procedures for Collecting Skin Scrap Sample

A. **Materials**

- Include: Petridish or test tube, Scalpel blade with handle, Disinfectants (Alcohol or Savlon), Cotton paraffin oil

B. **Procedures**

I. Clean the lesion with 70% alcohol.
II. Immerse the blade in a drop of paraffin oil.
III. Scrap the lesion with a scalpel blade till blood oozes. The scraping will adhere to the blade. Put the scraping material into a test tube/petridish.
IV. Finally identify the sample and send to the laboratory

1.4. Procedures for Collecting Preputial Wash Sample

A. Materials

Include: Plastic pipette connected to a syringe, sterile saline solution, Sterilized tube, Water and soap, Blade, PPE

B. Procedures

I. Use PPE
II. Shave, clean with water and soap all external parts, verifying that the external orifice of the preputial is kept dry.
III. Introduce a plastic pipette connected with syringe containing sterile saline solution to the preputial cavity.
IV. Tie the external orifice of the preputial with a rubber band then injecting the fluid into the cavity and massage it for some minute.
V. Aspire the wash at the interior of the syringe.
VI. Pass the sample to sterilized tube or seal the syringe with a rubber tap.
VII. Identify and submit to laboratory, take it refrigerated if the sample if takes more than 2 hours to reach the Lab.

1.5.Procedures for Collecting Milk Sample

A. Materials

Include: Golves antiseptics, towel, iodine tincture, alcohol, milk, vial, paper and pen, Water and soap, Blade, PPE

B. Preocedures

I. Wear PPE
II. Restrain the animal and Wear gloves.
III. Wash the udder with water and antiseptic solutions.
IV. Dry with clean towel.
V. Disinfect the teat with 70% alcohol or iodine tincture
VI. Discard the first 3-4 streams of milk.
VII. Open the collection vial and hold at a 45° angle to keep debris from accidentally falling into it.
VIII. Collect the milk sample from each teat in separate tubes about 5-10ml.
IX. Seal or stopper properly the tube immediately after collection.

X. Label the sample vials using a waterproof marker that will not come off during transport to the laboratory. Designate each quarter sampled as RF (right front), RR (right rear), LF (left front), or LR (left rear).

XI. Transport with ice.

Lab Test 1:

Name…….ID……Date……

Time started: _____ Time finished: _____

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

Task-1**:** Perform blood collection procedures

Task-2: Perform fecal sample collection procedures

Task-3: perform milk sample collection steps

Task-4: perform preputial wash sample collection steps

Task-5: perform skin scraping sample collection steps

LG #13	LO#2- Conduct General Laboratory Procedures

Instruction sheet

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

- Laboratory safety precautions, equipment and hazardous chemical
- preparing working solutions, reagents and solvent
- Laboratory sample handling and preparation
- Basic laboratory tests
  - ✓ Smear preparations
  - ✓ Hematological examination
  - ✓ Parasitological examinations
  - ✓ Bacteriological examinations
- Recording, calculation, interpretation and reporting of the test result

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

- Maintain personal hygiene and cleanliness standards
- Follow appropriate safety precautions for use of laboratory equipment and hazardous chemical materials
- Select and prepare working solutions, reagents and solvent of specified purity
- Handle the specimens in a manner that minimize the spread of pathogens to humans
- Prepare samples for examination
- Conduct basic laboratory tests for identification of parasitic and microbial infections
- Perform observation/Examination and decision of the test result.

- Carry out recording, calculation, interpretation and reporting of the test result.

Learning Instructions:

7. Read the specific objectives of this Learning Guide.
8. Follow the instructions described below.
9. Read the information written in the information Sheets
10. Accomplish the Self-checks
11. Perform Operation Sheets
12. Do the “LAP test”

Information Sheet 2

2.1.Maintaining Personal Hygiene and Cleanliness Standards

Sanitation, personal hygiene and cleanliness

Sanitation is the act or process of making sanitary or the promotion of hygiene and prevention of disease by maintenance of sanitary condition as (by removal of sewage and trash) often used attributively a sanitation equipment's and sanitation worker.

Hygiene refers to behaviors that can improve cleanliness and lead to good health or is a series of practices performed to preserve health or conditions and practices that help to maintain health and prevent the spread of diseases. Personal hygiene refers to maintaining the body's cleanliness. Objectives of sanitation include:

- Reducing the number of people practicing open waste disposed container (e. g. Do not open defecation area, tools that contain pus, blood, necrotic organ etc.).
- Increasing the number of people with access to basic sanitation facilities.
- Improving the affordability and availability of sanitation products and services.
- Increasing the amount of specimens waste effectively captured and treated on-site, or collected, transported, and treated offsite.
- Improving the ability of educational and health systems to manage sanitation and hygiene facilities in the laboratory institutions.
- Increasing the number of people with safely managed sanitation services.
- Increasing washing laboratory equipment with detergent (soap, Ajaksi, Omo, Barkina) at critical times.
- Increasing the rate of safe management of household drinking water and nutrition.

Cleanliness: is both the abstract state of being clean and free from germs, dirt, trash, or waste, and the habit of achieving and maintaining that state. Many people equate hygiene with 'cleanliness,' but hygiene is a broad term. It includes such personal habit choices as how frequently to take a shower or bath, wash hands, trim fingernails, and wash clothes. It also includes attention to keeping surfaces in the home and workplace, including bathroom facilities, clean and pathogen-free (Alp *et al.*, 2006). Personal hygiene in the laboratory is directed mainly toward the prevention of occupational acquired disease or physical injury (Brown *et al.*, 2008). The following guidelines are standard operating procedures for personal hygiene and cleanliness:-

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

2.2. Laboratory Safety Precautions, Equipments and Hazardous Chemicals

2.2.1. Laboratory Safety Precautions

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

- First, pay attention to what you are doing and what you are working with.
- Always wear safety equipment when working with dangerous chemicals.
- Recognize any accidents immediately.
- Keep food and drinks out of the laboratory work area.
- Always read labels of chemicals carefully.
- Never do unauthorized experiments.
- Never work alone in laboratory. Keep your lab space clean and organized.
- Do not leave an on-going experiment unattended.
- Always inform your instructor if you break a thermometer.
- Do not clean mercury yourself!!
- Never taste anything.
- Never pipette by mouth; use a bulb.
- Never use open flames in laboratory unless instructed by lab rule.
- Check your glassware for cracks and chips each time you use it as cracks cause the glassware to fail during use and cause serious injury to you.
- Do not use corridors for storage or work areas.
- Do not store heavy items above table height.
- Areas containing lasers, biohazards, radioisotopes, and carcinogens should be posted accordingly.

- However, do not post areas unnecessarily and be sure that the labels are removed when the hazards are no longer present.

- Be careful when lifting heavy objects.
- Clean your lab bench and equipment, and lock the door before you leave the laboratory.
- Never mix two unknown chemicals.
- Wipe spill of chemicals immediately.
- If contact with chemical, rinse it off immediately with lots of water.
- Never taste, smell or touch anything chemical.
- Coats, backpacks, etc., should not be left on the lab benches and stools.
- Always wash your hands before leaving lab.
- Learn where the safety and first aid equipment is located (fire extinguishers, fire blankets, and eye-wash stations)
- Always pour acids into water.
- Never leave burners unattended.
- Label all materials clearly.
- Never pipette anything by mouth.
- Clean up your work area before leaving.
- Do not use flammable liquids near open flames (Trant, 2020; Van Noorden, 2013).
- Treat every chemical as if it were hazardous.
- Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
- Never return chemicals to reagent bottles.
- Try for the correct amount and share any excess.

- Comply with fire regulations concerning storage quantities, types of approved containers and cabinets, proper labeling, etc.
- If uncertain about regulations, contact the building coordinator.
- Use volatile and flammable compounds only in a fume hood.

- Procedures that produce aerosols should be performed in a hood to prevent inhalation of hazardous material.
- Never allow a solvent to come in contact with your skin.
- Always use gloves.
- Never "smell" a solvent!!
- Read the label on the solvent bottle to identify its contents.
- Dispose of waste and broken glassware in proper containers.
- Clean up spills immediately.
- Do not store food in laboratories.
- Do not use any equipment unless you are trained and approved as a user by your supervisor.
- Never eat, drink, or smoke while working in the laboratory.
- Shorts and sandals should not be worn in the lab at any time.
- Properly dispose wastes and used material in appropriate containers.

2.2.2. Laboratory Glassware and Measuring Equipment

- **Uses and Maintenance of Equipment and Glassware**

Good equipment maintenance is essential for safe and efficient operations. Laboratory equipment should be regularly inspected, maintained, and serviced on schedules that are based on the manufacturer's recommendations, as well as the likelihood and hazards of equipment failure. Maintenance plans should ensure that any lockout procedures cannot be violated. Carefully handle and store glassware to avoid damage. Discard or repair chipped or cracked items. Handle vacuum-jacketed glassware with extreme

care to prevent implosions. Evacuated equipment such as Dewar flasks or vacuum desiccators should be taped, shielded, or coated. Only glassware designed for vacuum work should be used for that purpose.

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

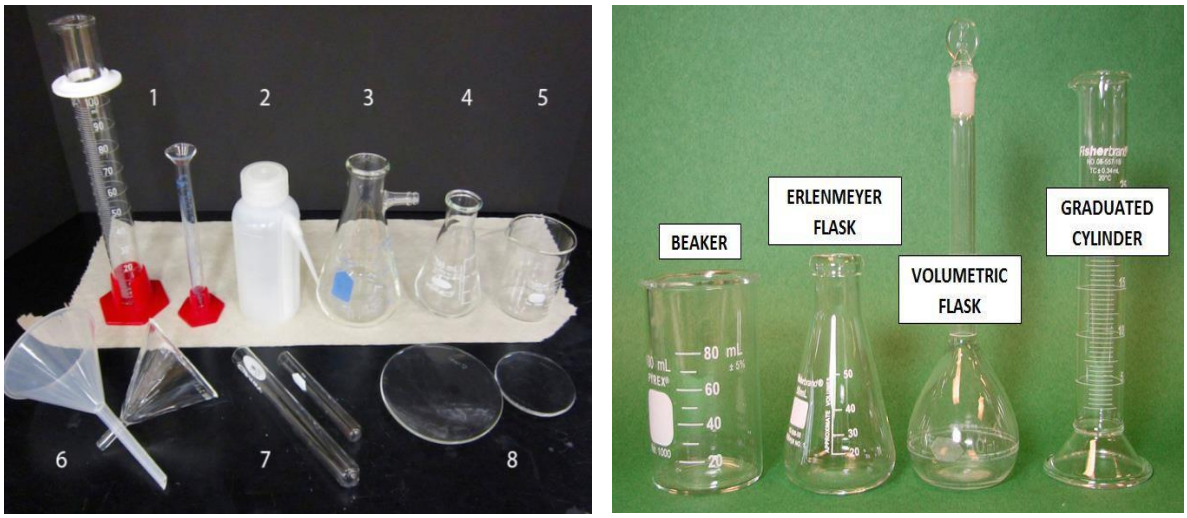


Fig. 2.1: Glass ware types used in laboratory

- **Major Glass ware used in Laboratory**

Cylinders, Wash bottle, Side arm, Flask, Beaker, Funnels, Test tubes, Watch glasses, Universal bottle, Slide and cover slip, Microscope are the major used glass ware. When used glass ware Always wash new glasses before first use, Hand wash glasses individually, do not soak with other glasses to reduce the chance of mechanical shock and use a suitable detergent and warm water with a non-abrasive sponge or cloth.

A. Microscope

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

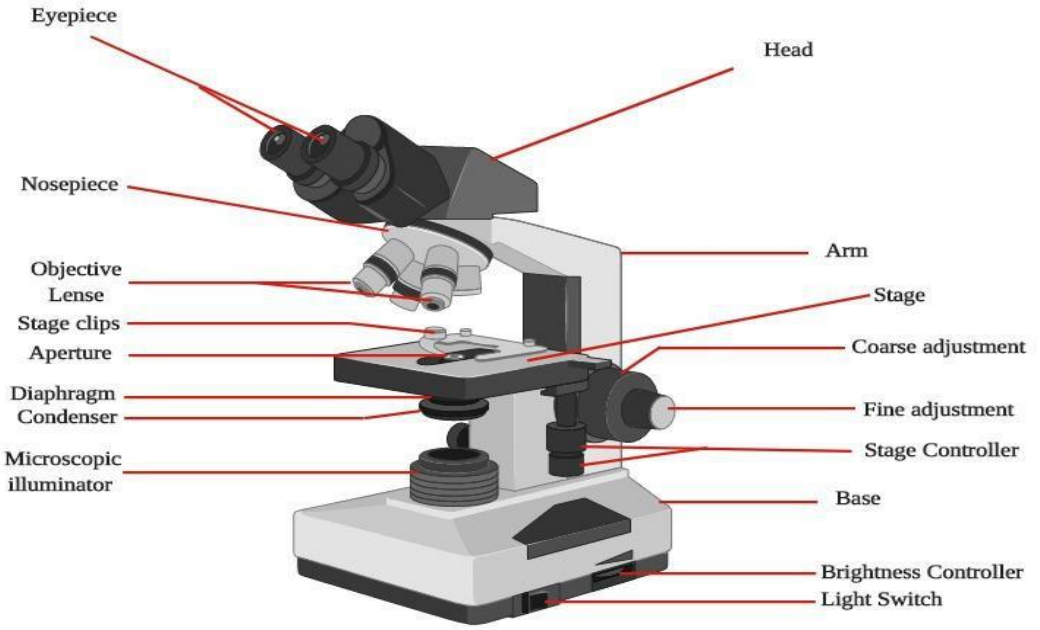


Figure: Components of Microscope | Image Credit : Microbiologynote.com

Fig. 2.2: Microscope body parts

B. Bakers

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

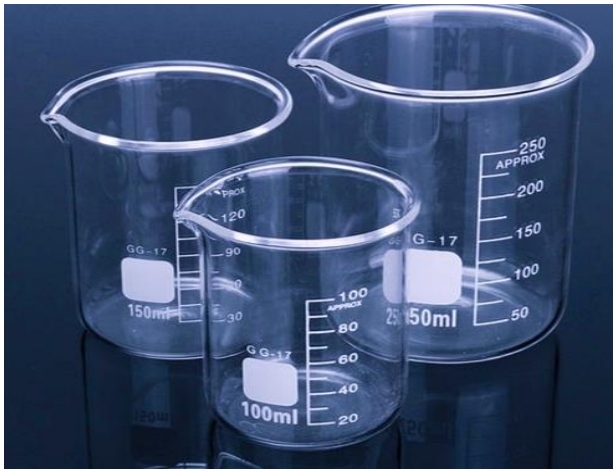


Fig. 2.3. Beakers

C. Flasks

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



Fig. 2.4. Flasks

D. Test Tubes

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



Fig. 2.5. Test Tubes

E. Pipettes

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

F. Micropipettes

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

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

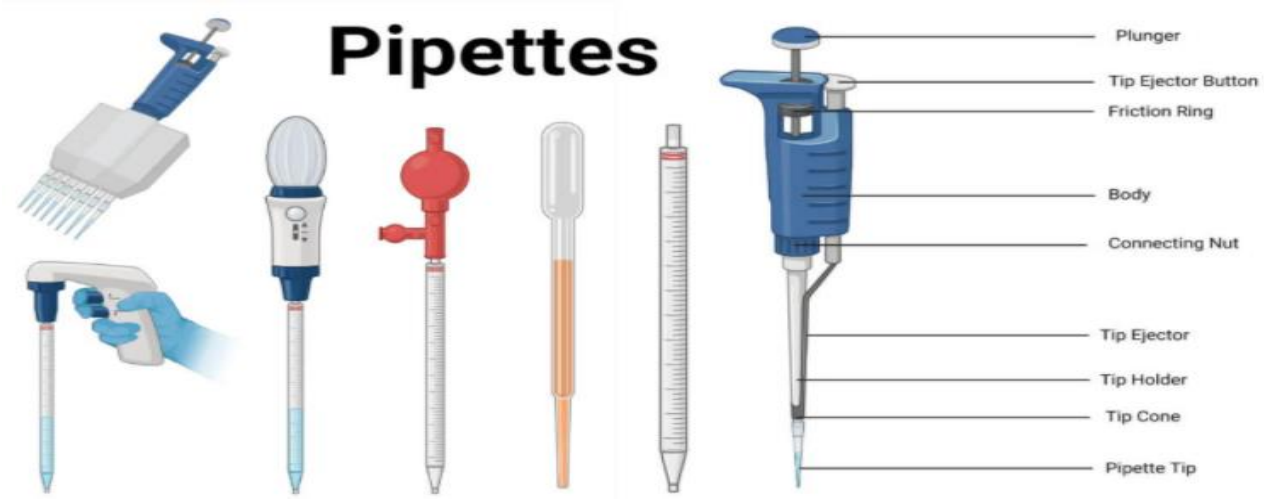


Fig. 2.6. Different Pipettes

G. Funnels

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

i. Ceramics

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

ii. Crucible

A crucible is made of ceramic and holds small amounts of chemicals during heating at high temperatures. Depending on the specific type, the crucible can withstand temperatures above 1,000 °C

and is used in conjunction with a Bunsen burner or furnace. Common uses include heating a hydrated solid to remove water or combusting a compound to determine organic content.



Fig. 2.7 Funnels

H. Mortar and Pestle

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



Fig. 2.8 Pestle and Mortar

I. Microscope Slides/Glass Slides and Cover Slip

Glass slide (Microscope slide) is a thin flat piece of glass, typically 75 by 26 mm (3 x 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.Glass slides (Microscope slide) 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. Some models of glass slides have a frosted area at one end, for labeling with a pencil or pen.



Fig. 2.9. Glass slides and cover slip

- **Washing and Sterilization of Glass and Plastic Materials**

There are 6 major effective ways to ensure sterile lab equipment

i. Steam Sterilization

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

ii. Dry Heat Sterilization

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

iii. Other Heat Sterilization Methods

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

iv. Sterile Filtration

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

v. Chemical Sterilization

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

vi. Radiation Sterilization

Electromagnetic radiation such as UV, X-ray and gamma rays are extremely effective at penetrating organisms and killing them. UV sterilization is effective for sterilizing small sample areas such as laminar

hoods, whereas X-ray and gamma rays can be used to sterilize large samples during manufacturing (Rubbo and Gardner, 1965; Sinha *et al.*, 2020).

2.2.3. Cleaning of Splashes of Chemicals

A chemical spill is considered to be minor only if:

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

The recommended steps for dealing with a minor spill include:

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

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

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

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

2.2. Preparing Working Solutions, Reagents And Solvent

2.2.1. Identifying Relevant Standard Methods for Solution Preparation

- **Terminologies related with Solution Preparation**

  - ✓ **Solution** is a homogeneous mixture of two or more substances. OR a mixture of substance dissolved in another so the properties are the same throughout. Solution: composed of a solute and the solvent.
  - ✓ **Solute** is the dissolved substance, OR the substance found in small amount
  - ✓ **Solvent** is a substance in which solutes dissolves to make the mixture or the substance that is present in the greatest amount.
  - ✓ **Water** is the Universal Solvent but there are many things it cannot dissolve. For example water and oil do not mix. We say oil is immiscible in water. Water is a good solvent due to its polarity.
  - ✓ **Mixtures:** combinations of different substances where each substance retains its chemical properties.
  - ✓ **Concentration-** amount of a substance dissolved in a given amount of solvent

- ✓ **Compound-** composed of two or more substances (elements) but in a ratio that cannot vary.
    - E.g. Water; there are 8 grams of oxygen for each gram of hydrogen. It won't be water if that ratio changes.

- **Ways of Preparing Solutions**

A. Dissolution

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

Factors affecting dissolution

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

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

B. Dilution of the solution

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

- A working solution from the stock

- Measurable concentration of a sample (for reporting the actual concentrations of body fluid constitutes)
- If the specimen at hand is less than a procedure calls for
- If the concentration of substances (analyte) is too high to be accurately measured.
- Whenever a solution is diluted, it is volume is increased and its concentration decreased, but the total amount of solute remains unchanged
- They are of two types of dilution. This is simple dilution and serial dilution

Materials used to prepare solution

Balance	Labeling Materials	Glass Rod
Flask	Reagent Bottles	Glass bead
Measuring cylinder	Burette Stand	Spatula
Funnel	Beaker	Scoop
Incubator	Water Bath	Pipettes
Decicators	Mortar	Pestles
Salt	Sugar	Formaldehyde
Water	Giemsa powder	KOH stock

- **Dilution preparation**

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

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

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

Types of dilution

1. Simple dilution
2. Serial dilution

1. Simple dilution:

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

Total volume of solution

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

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

$$D = Vs/Tv$$

Where: D= dilution

Vs= volume of solute (sample)

Tv= Final volum

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

C1xV1=C2V2

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

Using Proportion

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

V =C/A+B

Where: C – total volume of final reagent
A – Total parts of solution A
B – Total parts of solution B
V – Volume of each part

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

- Formula: C/A+B V = 70mL required 2 parts of A + 5 part of B = 70 mL
- 7 part = 10 volume of one part
- A = 2 x 10 = 20 mL
- B = 5 x 10 = 50 mL

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

Using CV1=C2V2

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

Example1. What volume is needed to make 500ml of 0.1M solution of tris-buffer from a solution of 2 M tris-buffer?

Example: 2: To make 45 ml of 30% Solution from 70% solution

C2 =30% V1 = C2V2

V2 = 45ml, C1 = 70%

V1= 30 X 45 = 19.3 ml 70 %

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

Diluting body fluids/standards

Example: 1.To make 8ml of a 1 in 20 dilution of blood.

C1xV1=C2V2 =>20xV1=1x8V1 (blood volume) =0.4

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

2. To make 4ml of a 1 in 2 dilution of serum in physiological saline.

C1xV1=C2V2 => 2xV1=1x4 =>V1 (serum volume) =0.4

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

Calculating the dilution of a body fluid

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

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

Sample: total 50:100, 1 in 2 dilutions

Calculate the dilution of urine using 0.5 ml of urine and 8.5 ml of diluting fluid (Physiological line)

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

Serial dilutions

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

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

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

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

Dilution fold total volume

- Volume transferred = is equal to the constant volume transferred to each successive tubes in the serial dilution system.
- Total volume = is equal to the volume being transferred plus the volume of diluents already in the tube.

E.g.1. what is the dilution fold of the following serial dilution system consisting of five tubes?

The following amount of diluents have been added to the tubes; 0.5 mL to tube 1 & 0.5 mL to tube 2 to 5. Next, 0.5 mL of patient serum is added to tube 1 and 0.5 mL is serialy transferred through tube 5. Finally, 0.5 mL is discarded from tube 5.

- 1/Y = 0.5/1.0
- Y x 0.5 = 1
- Y = 1/0.5 = 2

E g. 2. it is often desirable to determine the dilution of a given tube (Y) in a serial dilutions ystem. This dilution can be calculated by Solution of tube 1 = dilution of Y x [1/dilutionfold]

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

Y = ½ x (½) (Y-1)

= ½ x (½)2

= ½ x ½ x ½=1/8; The dilution of serum in the tube 3 is 1/8.

E.g. In a typical microbiology exercise the students perform a three steps

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

A. **Prepare reagent solution**

- **Reagent preparation**

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

Weigh balance

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

- **Media Preparation for bacterial growth**

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

Types of media

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

Types of Culture Media

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

Ingredients commonly used in media

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

I. Nutrient Agar: Composition, Preparation and Uses

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre of distilled water.
2. Heat this mixture while stirring to fully dissolve all components.
3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.
6. Replace the lid of each Petri dish and store the plates in a refrigerator.

II. MacConkey AgarComposition, Principle, Uses, Preparation and Colony Morphology

Principle of MacConkey Agar:

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium Preparation.

Preparation of MacConkey Agar

1.Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water.
2.Heat to boiling to dissolve the medium completely.
3.Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4.Cool to 45-50°C.
5.Mix well before pouring into sterile Petri plates.
6.Pour MacConkey gar into each plate and leave plates on the sterile surface until the agar has solidified.
7.Replace the lid of each Petri dish and store the plates in a refrigerator.

III. Inoculation of the Sample to the Prepared Media

Principle of the laboratory technique employed: The streak plate method is the most common ways of separating bacterial cell on the agar surface to obtain well isolated colony. Small amount of sample is transferred onto the surface of solid agar medium by inoculating loop and streaked gently.

Material and equipment: solid media, milk, Bunsen burner, inoculating loop, incubator

Procedure:

1. Collect the sample
2. Sterilize the inoculating loop by flame of Bunsen burner and cooled
3. Pick a small amount of sample by the loop and place in one corner of Petri dish containing media
4. Sterilize the loop and a loop streak full sample gently across the medium
5. Sterilize the loop by flaming and cooled by stabbing at the corner the medium
6. Pass the loop once across one end of the primary streak and streake onto the free region
7. Sterilize the loop and cooled again and streak starting from the end of the secondary streak
8. Label and incubate the inoculums in inverted position at $37C^0$ for 24 hours

Result (s): The well separated colony will be observed in the area of the third quadrant.

Interpretation of laboratory finding: The colonies will be well recognized and it is possible to perform other test for bacterial identification if properly done.

B. **Preparing Labels and Log Reagent Solution in Details**

- **Laboratory Reagent Labels**

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

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

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

Purpose of labeling: Workplace reagent labeling primarily serves two purposes

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

Employers must ensure that:

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

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

MARKED CAUTION:

- do not use unknown substance
- Stored in isolation until the contents can be identified
- If contents cannot be identified, the contents should be suitably disposed of

(With advice from relevant authorities).

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

- the substance changes (including new ingredients)
- new information becomes available that affects the information provided on the label (often instigated through a change of MSDS)

Example of format lab and log recorded are:

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

2.3. Laboratory Sample Handling and Preparation

2.3.1. Specimen Handling in Safe Manner

Specimen: means the group of elements (sampling units) drawn from a population, on which tests are performed or parameters measured to provide surveillance information.

A **sample** is an unbiased number of observations taken from animal. So the sample, in other words, is a portion, part, or fraction of the whole group, and acts as a subset of the population. Samples are used in a variety of settings where research is conducted. When handling specimens, universal safety precautions should always be employed. Appropriate protective clothing and personal protective items should be worn. No tissue, even fixed material, should ever be handled with bare hands. It must be kept in mind that noxious agents are present in the laboratory.

2.3.2. General Consideration of Specimen Handling

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

General consideration for taking and handling sample are:-

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

- These samples should be directly labelled as final samples. Samples are packed and sealed according to the packing requirements.
- Care should be taken not to erase or conceal the information on the original packing.
- Sampling method of animal

The sampling method depends on:

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

2.4. Basic Laboratory Tests

2.4.1. Preparing samples smear for subsequent staining

Smear means to spread specimen (Blood, pus, fecal etc.) over a surface slide to identify an agent or normal physiological detection. The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure. A smear can be prepared from a solid or broth medium. In clinical settings, light microscopes are the most commonly used microscopes. There are two basic types of smear preparation used to view specimens with a light microscope.

A. Wet smear

The simplest technique, in which the specimen is placed on the slide in a drop of liquid. Some specimens, such as a drop of blood, are already in a liquid form and can be deposited on the slide using a dropper. Solid specimens, such as a skin scraping, can be placed on the slide before adding a drop of liquid to prepare the wet mount. Sometimes the liquid used is simply water, but often stains are added to enhance contrast. Once the liquid has been added

to the slide, a coverslip is placed on top and the specimen is ready for examination under the microscope

B. Fixed Smear

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

2.4.2. Hematological Examination

2.4.2.1. Packed Cell Volume (PCV) Determination

The packed cell volume (PCV) is the measure of the ratio of the volume occupied by the red cells to the volume of whole blood in a sample of capillary, venous, or arterial blood. The ratio is measured after appropriate centrifugation and is expressed as a decimal fraction. It is measured by hematocrit reader.

Purpose: This is a benefit test for diagnosis of certain blood disorders for example. Increased PCV values indicate polycythemia, Diabetes mellitus, etc while Decreased P.C.V values indicate anemia, Leukemia and Hypothyroidism, etc

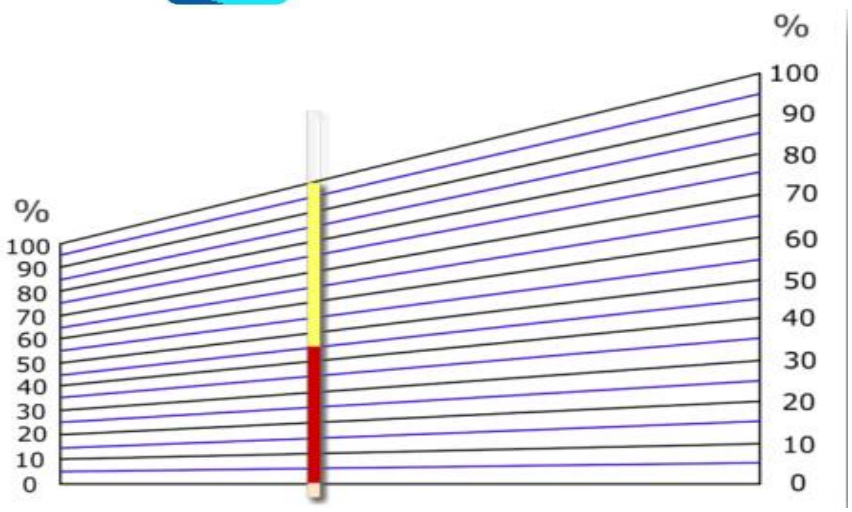


Fig. 2.10. Micro hematocrit reader

Normal ranges of PCV in different animals

	Bovines	Equines	Ovine	Caprine	Swine	Guinea pig	Canine	Feline	Rabbit	Camel
PCV (%)	24-46	40-75	24-45	24-45	32-50	37-48	43-80	25-77	30-50	35-75

2.4.2.2. Blood Smear Preparation and Examination

There are some methods of blood smear preparation and examination:

a) Wet blood smear preparation & examination

This technique is used to determine the movement of the parasite in the blood. We can detect the movement of the parasite, but the morphology of the parasite cannot see, it is difficult identify the species.

b) Thin and thick smears

If you are using venous blood, blood smears should be prepared as soon as possible after collection (delay can result in changes in parasite morphology and staining characteristics).

i. Thin smears

This smear allows the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms or morphological characteristics. This method is the most effective method, but when the animal has a low parasitaemia the infections cannot be detected. Thus it is commonly used when an animal is highly infected by blood parasites. It identifies the blood parasites up to it's species level.

ii. Thick smears

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

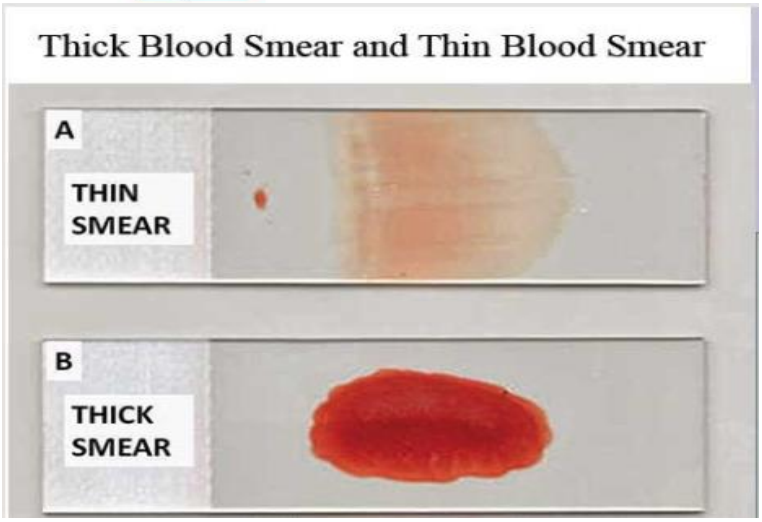


Fig. 2.11. Thin and thick smears

2.4.3. Parasitological Examinations (Coproscopy)

The fecal sample should be taken directly from the rectum of the animal or if not possible fresh and not contaminated sample can be taken from the ground and the amount should be around 10gm/sample. If more than two hours has elapsed after the sample is collected, helminthes eggs and larvae can be preserved at 4^0c for 15 months or at 15^0c for 72 hours. It is also possible preserve by mixing the fecal sample with 10% formalin.

The fecal examination can be of two types:

A. Macroscopic examination

Fecal sample is taken and put on Petri dish, and can be examined by naked eye. The visual identification of the sample consists on the form, consistency, color and presence of blood or mucus. It is also possible to see different worms, immature stages of trematodes, tape worm segments and nematodes. Abnormal constituents in faeces like blood, mucus and epithelial tissues reveal different intestinal disease conditions.

B. Microscopic examination

It includes qualitative (direct smear and concentration methods) and Quantitative (obtaining accurate information regarding the severity of infection).

I. Qualitative Examination

a) Direct Smear Examination

Features: The sensitivity of this test is very low. For this reason it is not recommended for routine fecal examination. This test is used to identify heavier infections only. Result interpretation is expressed as positive for the presence of eggs and Negative for absence of eggs. It would be necessary to revise at least 3 fields for accurate results and to conclude that the result is positive. For a negative diagnosis, it is required to revise the whole preparation.

Principle: Direct fecal smear is a qualitative method used to examine eggs of parasites in the fecal sample within a short period of time.

b) Simple flotation method

The simple **flotation** method is a **qualitative** test for the detection of **nematode** (except Diphillobothrium) and **cestode eggs** and **coccidia oocysts** in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a flotation fluid. The most commonly used are NaCl, Sodium Nitrate, and Magnesium Sulphate or Sugar solution. Most parasitic stages float efficiently at a specific gravity of 1.2 to 1.3.

Principle: This method is based on the determination of worm eggs, mixing the faeces with a solution with a specific gravity higher than that of the eggs than can be present in the faeces; the eggs float congregated at the surface of the suspension.

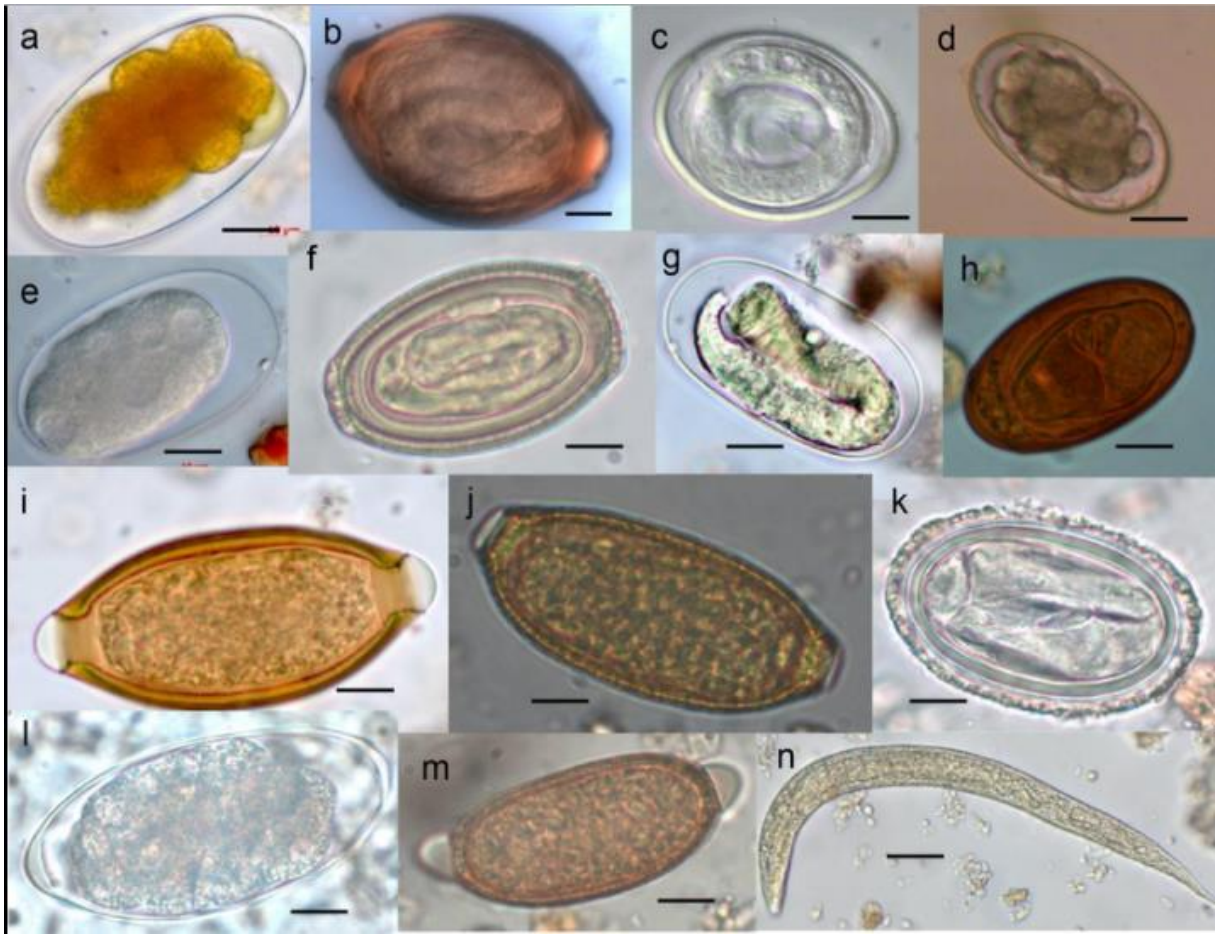


Fig. 2.12. Images of Nematode parasitic eggs

c) Sedimentation technique

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

Table 2. Magnification Levels for Examining Prepared Samples

Magnification	Parasites
10 x 10	Nematode and cestode eggs
10 x 40	Coccidia oocysts
10 x 4	Trematode eggs

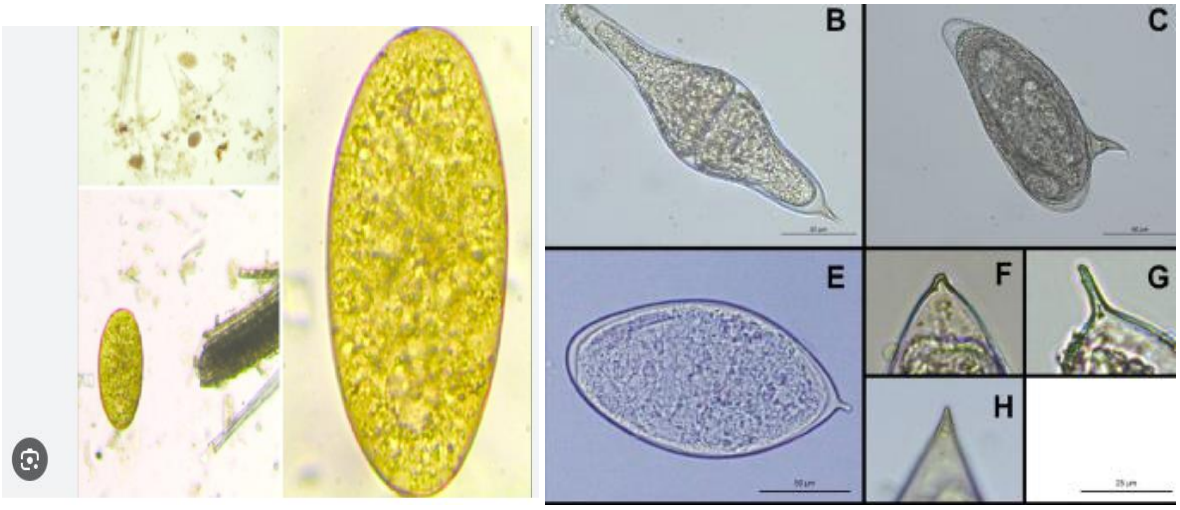


Fig. 2.13. Eggs of trematode parasites

d) Baermann Technique (Fecal culture)

This method is used for isolation and identification of Lungworm larvae and infective larvae harvested from fecal cultures

Principle: The Baermann technique is used to isolate lungworm larvae from fecal samples and infective larvae from fecal cultures**.** It is based on the active migration of larvae from faces suspended in water and their subsequent collection and identification.

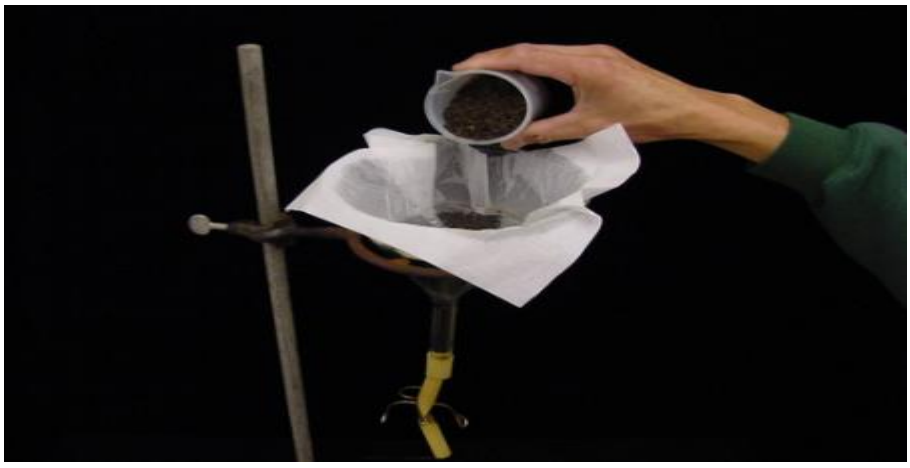


Fig. 2.14. Baerman apparatus

I. Quantitative examination

It is used to know the severity of infections in animals; it counts the eggs in the field throughout the slide. Those techniques are determining the eggs per gram (EPG) of faeces.

Mc Master Technique

Principle: The McMaster counting technique is a quantitative technique to determine the number of eggs present per gram of faeces (e.p.g.). A flotation fluid is used to separate eggs from faecal material in a counting chamber (McMaster egg counting chamber) with two compartments. Eggs present in the counting chamber is counted and multiplied by either 100 (if only one part of the chamber is counted) or 50 (if two of the chambers are counted). The eggs observed out of the chamber cannot be included in the number of eggs per gram.

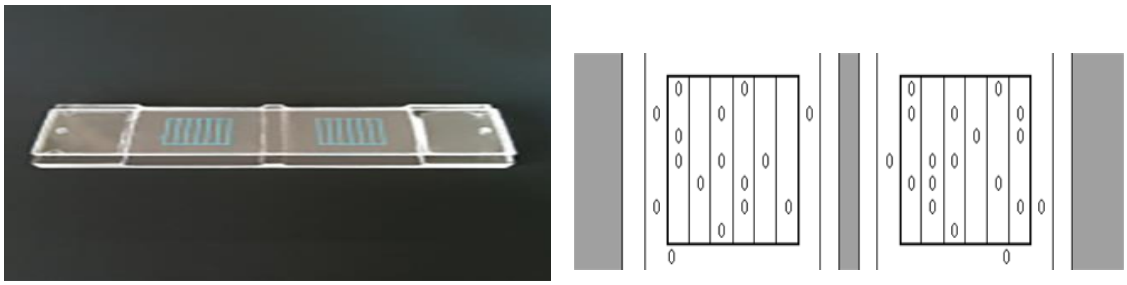


Fig.2.15. McMaster egg counting chamber

2.4.4. Bacteriological Examinations

A. Gram staining

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

Principle of Gram Staining

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

Reagents Used in Gram Staining

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

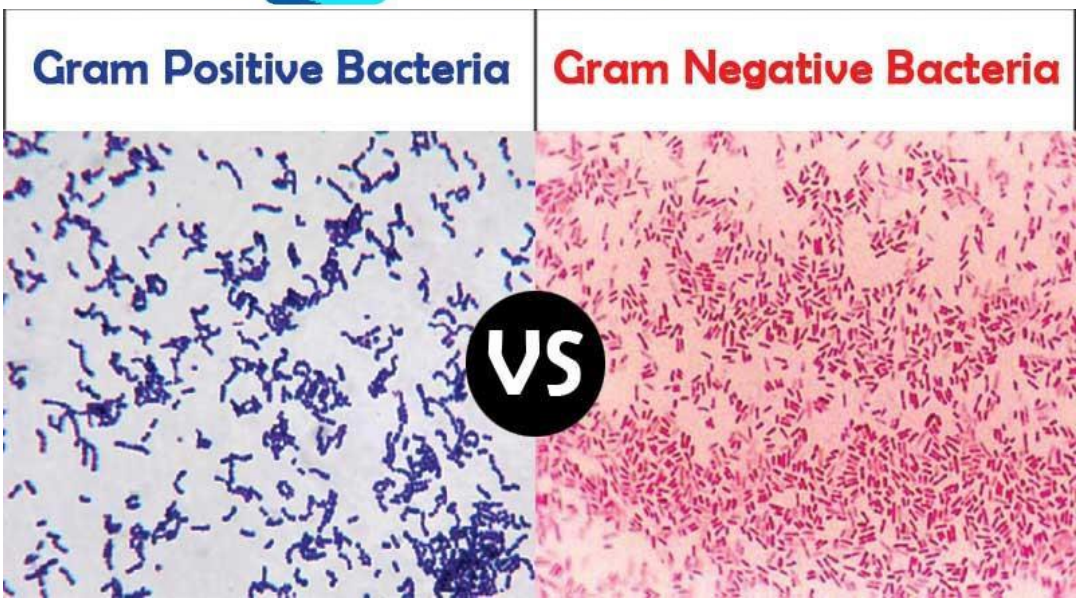


Fig. 2.16. Microscopic vision of gram positive and gram negative bacteria

B. Acid fast staining

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

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

Principle of Acid-Fast Staining

When the smear is stained with carbol fuchsin, it *solubilizes* the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is

decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appears blue while acid-fast cells retain the red color.

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

Table: 1. Summary of Acid-Fast Stain

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

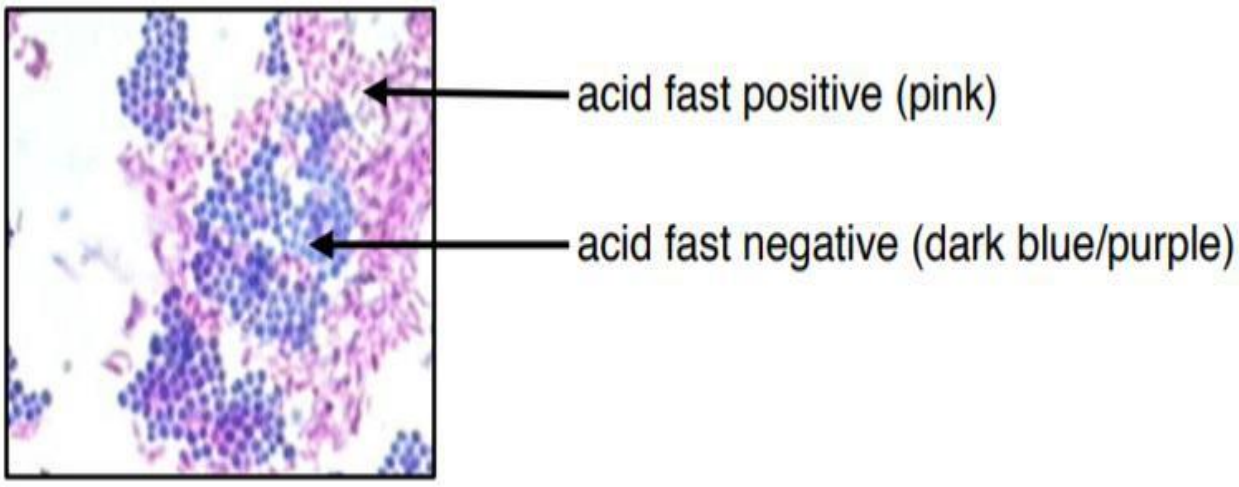


Fig 2.17 Colors of bacteria in acid fast staining

C. Giemsa Staining

Giemsa stain is a gold standard staining technique that is used for both thin and thick smears to examine blood for parasites, a routine check-up for other blood parasites and to morphologically differentiate the nuclear and cytoplasm of Erythrocytes, leucocytes and Platelets and parasites. It is composed of both the Acidic and Basic dyes, in relation to affinities of acidity and basicity for blood cells. Azure and methylene blue, a basic dye binds to the acid nucleus producing blue-purple color. Eosin is an acidic dye that is attracted to the cytoplasm and cytoplasmic granules which are alkaline-producing red coloration. The stain must be buffered with water to pH 6.8 or 7.2, to precipitate the dyes to bind simple materials.

Principle of Giemsa staining

Giemsa stain is a differential stain and contains a mixture of azure, methylene blue, and eosin dye. It is specific for the phosphate groups of DNA and attaches itself to where there are high amounts of adenine-thymine bonding.

Azure and eosin are acidic dye that variably stains the basic components of the cells like the cytoplasm, granules, etc. Methylene blue acts as the basic dye, which stains the acidic components, especially the nucleus of the cell. Methanol act as a fixative as well as a cellular stain. The fixative does not allow a further change in the cells and makes them adhere to the glass slide.

D. California Mastitis Test (CMT)

California Mastitis Test (CMT) is a quick, simple test that accurately predicts the somatic cell count of milk from individual quarters or on composite milk samples. The California Mastitis Test is a simple, inexpensive, rapid screening test for mastitis.

The test is based upon the amount of cellular nuclear protein present in the milk sample. Since inflammatory cells associated with mastitis are the predominant cell type present in milk the CMT reflects the SCC level quite accurately and is a reliable indicator of the severity of

infection. The test is appropriate for cow-side evaluation of udder health and the procedure can be taught quickly to producers and the milking crew.

Principle of CMT

CMT reagent (sodium lauryl sulphate) reacts and rupture leukocytes (WBCs) and thus deoxyribonucleic acid (DNA) is released from their nuclei which result in gel formation. Thickness of gel indicates the severity of inflammation. The CMT will only trigger a visible reaction with a concentration of 400,000 cells/ml or more. The degree of gelling indicates the presence and severity of mastitis. The change in color indicates the pH variation of the milk and therefore, the level of inflammation.

Interpretation

CMT score	Definition	Total cell count	Visible reaction	Interpretation
0	Negative (-)	0-200,000 (0-25% neutrophils)	Normal milk fluid	Healthy Quarter
T	Trace (+)	200,000-400,000 (30-40% neutrophils)	Slight precipitation	Subclinical Mastitis
1	Weak positive (++)	400,000- 1200000/ 1,500,000 (40-60% neutrophils)	Distinct precipitation (thickening) but no gel formation	Subclinical Mastitis
2	Distinct positive (+++)	1,500,000-500,000 (60-70% neutrophils)	Mixture thickened immediately and a gel formation	Serious Mastitis Infection
3	Strong positive (++++)	Over 5,000,000	Viscosity greatly increased strong gel that is cohesive with a convex surface.	Serious Mastitis Infection



Fig. 2.18. CMT paddle

2.5. Recording, Interpretation and Reporting of the Test Result

2.5.1. Recording and documentation

Laboratory needs to have evidence and documentation will form a major part of the evidence sought by external accreditation bodies. This can be very broad in nature and ranges from mission statements, quality manuals and detailed laboratory procedures, to minutes of meetings and work sheets, depending upon the particular accreditation system.

Hierarchy of documentation

Because of the wide range of documents involved in the preparation for accreditation it is useful to think in terms of a hierarchy of documentation as illustrated below

Forms and records: - Recording any information or data such as patient's results, quality control data or the result of an audit should be done in a systematic way on forms of an agreed format. Records made on forms 'provide evidence of fulfillment of intent'. If a procedure requires something to be recorded on a form, the form must be referred to in the procedure.

Document control

Document control requires a procedure for the control of all documents, including the quality manual, procedures and forms. It requires that all documents are uniquely identified and with documents produced on computer this can be by a unique file name. It is also required that date of issue, revision version, the total number of pages and authorizing signatories be included in the document.

2.5.2. Interpretation and Reporting of Laboratory Results

Clinical laboratory test results are a very important parameter in diagnosis, monitoring and screening. 70-80 % of decisions in diagnosis are based on laboratory results and more and more laboratory analyses are requested. Thus a lot of data are provided and it is therefore imperative for patient care (and safety) that the clinicians are familiar with the tests and with interpretation of the results.

The laboratory result must be interpreted on the background of a reference interval that is used to distinguish between "health" and "disease". The clinician must also evaluate the result from the knowledge of biological variation and be aware of the potential risk of false interpretation. Likewise, influence of random errors and systematic errors on the result is of importance as well as the diagnostic sensitivity and specificity.

Self-Check – 2

Name…… ID…… Date…….

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

Part I. Choose The Best Answer And Write Your Answer On The Space Provided (1 Pt Each)

_____1. The movement of blood parasite can be identified by:

A) Thick smear B) Wet film/smea C) Thin smear D) Giemsa stain

_____2. The best technique for identification of cestods and trematode parasite eggs is:

A. Floatation B) Sedimentation C) Direct fecal smear D) Barman

_____3. Which one is the **factor affecting dissolution?**

A) PH B) Size of particle C) Temperature of solvent D) All

_____4. One of the following is not used for gram staining?

B. Carbon fuschine B. Crystal violet C. Grams iodine D. Safranin

_____5. One of the following is not glass ware material used in the laboratory.

B. Slide B. Pestle and mortar C. Pippete D. Non

Part ii. **Match Correctly The Following (2pts Each)**

A	B
_____1. Thick blood smear	A) Parasite movement
_____2. Thick blood smear	B) Trematode
_____3. Wet film	C) Acid alchol
_____4. Sedimentation technique	D) Sub clinical mastitis test
_____5. Acid fast staining	E) Low infection
_____6. CMT test	F) Used for high infection

Part iii. Write short and precise answer.

1. Write at least 10 laboratory safety precautions?(6 pts)

2. What is the difference between sedimentation and Barman technique?(3pt)

3. _____is a process by which the concentration or activity of a given solution is decreased by the addition of solvent (3 pt)

4. What is the principle of acid fast staining.(3pt)

5. Mention at least two floatation fluids ?(4pts)

Note: **Satisfactory rating – 15 points** **Unsatisfactory - below 15 points**

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

Operation Sheet- 2

2.1. Procedures for wet blood smear

A. Materials

- **PPE, Fresh** blood samples, slides, cover slips and microscope.

B. Procedures

I. Use tools and materials used for wet blood smear: (

II. Take 3 drop of fresh blood on the slide.

III. Cover with cover slip.

IV. Examine immediately under high dry magnification power (40x objective).

2.2. Procedures for thin blood smear (Romanowsky stain)

A. Materials

- **PPE,** Blood sample, slides, methanol, Giemsa solution, cover slips, microscope, ether, ethanol

B. Procedures

I. Use PPE

II. Take a small drop of blood sample

III. Put it on the clear slide

IV. The slide should be hold firmly

V. Place the end of second glass slide /spreader slide / against the surface of the first slide, holding at an angle of 30-45 degrees

VI. Draw the spreader slide gently into the drop of blood and when the blood has along 2/3 of width of the spreader slide by capillary action, push the spreader

slide forward with a steady even motion. A quicker movement results a good smear

VII. Dry it in air

VIII. Fix by **methanol** not less than 2-3 minutes or ethanol-ether (1:1) not less than 10 minutes

IX. Allow to be dry the slide

X. Stain with Giemsa solution for 15 minutes

XI. Wash with tap water

XII. Allow to be dry the slide

XIII. Examine with oil immersion under high magnification power

Result interpretation: Positive=>The parasite, its morphology and also the spp of the parasite will be observed and identified, if it is present we can also observe the morphology of RBC and WBC.

2.3. Procedures for thick blood smear

A. Materials

- PPE, Blood sample, slides, methanol, giemsa solution, cover slips, microscope, plastic/wire loop

B. Procedures

I. Use PPE

II. Put a drop of blood at the center of the clean slide

III. Smear it using the sterilized wire or plastic loop

IV. Dry it in air

V. Fix by **methanol** not less than 5 minutes or ethanol-ether (1:1) not less than 10 minutes

VI. Allow to be dry the slide

VII. Stain with Giemsa solution for 30 minutes

VIII. Wash with tap water

IX. Allow to be dry the slide

X. Examine under oil immersion objective lens.

2.4. Procedures for PCV Determination

A. Materials

- PPE, Hematocrit tube, hematocrite centrifuge, hematocrit reader and sealer

B. Prodedures

I. Use PPE

II. The blood is filled in to a micro hematocrit tube ($3/4^{th}$) and seals it with sealer.

III. Centrifuge the filled hematocrit tube in a hematocrite centrifuge at 2000 rpm for 4-5 minutes.

IV. Read the value (the tube) with hematocrit reader and record the result.

Interpretation: (PCV) = erythrocyte mass; the animal is anemic when PCV falls dawn from the normal.

2.5. Procedures for Giemsa staining

A. Materials

- **Include:** prepared smear, methyl alchol, giemsa stain, microscope, buffered distilled water

B. Procedures

I. Use PPE

II. Fix the dried smear in **methanol** (methyl alcohol) for 2-3 minutes.

III. Allow the smears to air-dry.

IV. Dilute 1 part of **stock Giemsa stain** with 9 parts buffered distilled water, pH 7.0.

V. Flood the smear with the diluted stain (prepared in step 2 above named **Gram reagents preparation**) for 30-45 minute.

VI. Wash with buffered distilled water.

VII. Blot, dry in air and examine under oil immersion.

2.6. Procedures for Gram Staining

A. Materials

1. **Include:** prepared smear, crystal violet (primary stain), microscope, acetone (decolorizer), safranin, Bunsen burner, Grams iodine (mordant) & plot paper.

B. Procedures

I. Cover air-dried, heat-fixed smear with **crystal violet** for 1 min.
II. Wash with clean water and drain
III. Cover the smear with **Grams iodine** (mordant) for 1 min.
IV. Wash with clean water and drain
V. Decolorized the smear for 15 seconds with 95% **ethanol, acetone** or acetone-alcohol.
VI. Wash immediately with clean water
VII. Counter the stain the smear with dilute **safranin** for 1 min
VIII. Wash slide in a gentile and indirect stream of tap water until no color appears
IX. Allow to dry and examining under oil immersion using a Bright field microscope

Result interpretation: Gram positive---appear in **blue/purple**, Gram negative-----appear in **red/pink** color.

2.7. Procedures for Acid fast staining

A. Materials

- **PPE,** prepared smear Carbol fuchsine, acid alchol, methyline blue, microscope, filter paper, Bunsen burner

B. Procedures

I. Use PPE
II. Prepare bacterial smear on clean and grease free slide, using sterile technique.
III. Allow smear to air dry and then heat fix.
IV. Cover the smear with **carbol fuchsin** stain.
V. Heat the stain until vapor just begins to rise (i.e. about 60 C).
VI. Wash off the stain with clean water or clean boiled rainwater.
VII. Cover the smear with 3% v/v **acid alcohol** for 5 minutes or until the smear is sufficiently decolorized.

VIII. Wash well with clean water.

IX. Cover the slide with solution of methylene blue for 30 seconds

X. Wash off the stain with clean water.

XI. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry (do not blot dry).

XII. Examine the smear microscopically, using the 100 X oil immersion objective.

2.8. Procedures for CMT

A. Materials

- **PPE,** Milk, CMT paddle, California reagent, pipette

B. Procedures

I. Use PPE

II. Take about 2-3 ml milk from each quarter in each paddle compartment

III. Add CMT solution to each cup in the paddle.

IV. CMT reagent is added to each compartment in volume equal to the milk quantity.

V. The milk reagent mixture is swirled in a circular motion with presence of gel or slime being recorded for each quarter.

VI. Rotate the CMT Paddle in a circular motion to thoroughly mix the contents. Do not mix more than 10 seconds.

VII. Read the test quickly. Visible reaction disintegrates after about 20 seconds. The reaction is scored visually. The more gel formation, the higher the score.

2.9. Procedures for Direct fecal smear

A. Materials

- **PPE,** Hand gloves, Petri dish or sample container, Mortar and pestle, Glass rod or wire or plastic loop, microscope, microscopic slides, cover slip, water, feces

B. Procedures

I. Use PPE

II. Put a Small amount of feces on microscopic slide.

III. Add a drop of water on the feces and emulsify thoroughly,

IV. Avoid the debris and spread the mixture of water and feces on the slide,

V. Place the cover slip on the smear and examine first under low power (10x) and then by high dry power (40x) microscope.

Results interpretation: Positive=> presence of eggs will be observed, Negative=> no eggs.

2.10. Procedures for Floatation technique

A. Materials

- **PPE,** Feces, digital balance, beaker, measuring cylinder, test tube, test tube rack, si eve (cheesecloth) microscope, slide, cover slip, pestle and **mortar**, floatation fluid ($MgSo_4$), plastic loop and glove.

B. Procedres

I. Use PPE

II. Put approximately 3 g of faeces into mortar and crush with pestle (if pleated)

III. Pour 42 ml flotation fluid into mortar with crushed feces.

IV. Mix (stir) faeces and flotation fluid thoroughly with a stirring device (plastic loop).

V. Pour the resulting faecal suspension through a tea strainer or a double-layer of cheesecloth into a beaker.

VI. Pour the faecal suspension into a test tube.

VII. Place the test tube in a test tube rack or stand.

VIII. Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a coverslip on top of the test tube.

IX. Let the test tube stand for 15 minutes.

X. Take the cover slip gently and examine under microscope at low power objectives.

Result interpretation: Positive=> presence of eggs will be observed, Negative=> no eggs. If egg is found further examination will be done.

2.11. Procedures for Sedimentation technique

A. Materials:

- **PPE,** Microscope, Centrifuge, Balance, Hand gloves, Petri dish or sample container, Slide, Sieve, Cover slip, Mortar and pestle, Beaker, Centrifuge tube, Graduated cylinder, plastic loop, Pasteur pipettes, Teaspoon & methylene blue)

B. Procedures

I. Use PPE
II. Take 3gm of faecal sample in to mortar and crush with pestle (if pleated)
III. Emulsify (mix) with 42ml of water
IV. Sieve through strainer (cheesecloth)
V. Pour the suspension in to a test tube up to 3/4th
VI. Centrifuge at 1500 rpm for 3 minutes
VII. Decant the supernatant
VIII. Repeat the above procedure (No 5, 6, & 8) until the supernatant is clear
IX. Stain the sediment by adding one drop of **methylene blue**.
X. Transfer a small drop sediment to a microslide
XI. Cover with a coverslip and examine under microscope at low power objectives.

Result interpretation: Positive=> presence of eggs will be observed, Negative=> no eggs

2.12. Procedures for Mc Master Technique

A. Materials

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

B. Procedures

I. Wear PPE
II. Weigh 4 g of faeces and place into mortar and crush with pestle.
III. Add 56 ml of flotation fluid.
IV. Mix (stir) the contents thoroughly with a stirring device (fork, tongue blade).

V. Filter the faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2. While stirring the filtrate in a beaker, take a sub-sample with a Pasteur pipette.
VI. Fill both sides of the McMaster counting chamber with the sub-sample.
VII. Allow the counting chamber to stand for 5 minutes (this is important)
VIII. Examine the sub-sample of the filtrate under a microscope at 10 x 10 magnification.
IX. Count all eggs and coccidia oocytes within the engraved area of both chambers.
X. The number of eggs per gram of faeces can be calculated as follows

- Count the number of eggs within the grid of each chamber, ignoring those outside the squares
- Multiply the total by 50 – this gives the eggs per gram of faeces (e.p.g.)

2.13. Procedures for Baermann technique

A. Mareials

- **PPE** Funnel (size according to need),Funnel stand, Rubber or plastic tubing, Rubber bands, Clamp or spring clip, Cheesecloth or screen, Simple thin stick (about 15 cm long), Strainer, Microscope, Test tube, Pasteur pipette and Small petri dish(es)

B. Procedures

I. Use PPE
II. Fit a short piece of tubing which is closed at one end with a clamp or spring clip, to the stem of a funnel of appropriate size.
III. Support the funnel by a stand.
IV. Weigh or measure about 5-10 g of faecal culture/faeces and place it on a piece of double-layer cheesecloth.
V. Form the cheesecloth around the faeces as a "pouch".
VI. Close the pouch with a rubber band.
VII. Fix a supporting stick under the rubber band - Step 1
VIII. Fix a supporting stick under the rubber band - Step 2
IX. Place the pouch containing faecal culture material or faeces in the funnel. Trim the surplus cheesecloth off.

X. Fill the funnel with lukewarm water, covering the faecal material.

XI. Leave the apparatus in place for 24 hours, during which time larvae actively move out of faeces and ultimately collect by gravitation in the stem of the funnel.

XII. Draw a few ml of fluid from the stem of the funnel into a small petri dish.

XIII. Examine under dissecting microscope for live lungworm larvae (L_1).

LAP TEST-2

Name…….ID……Date……

Time started: _____ Time finished: _____

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

Task-1: Perform thin and thick blood smear preparation procedures

Task-2: Perform Gram staining procedures

Task-3: perform Acid fast staining procedures

Task-4: perform sedimentation technique procedures

Task-5: perform nutrient agar preparation procedures

Task-6: perform CMT procedures

Task-7: Perform Baerma technique procedures

Task-8: Perform floatation technique procedures

Task-9: Perform mac master technique procedures

Task-10: Perform PCV determination procedures

Task-11: Perform Direct fecal smear procedures

LG #14	**LO#3- Accomplish Close up Tasks in the Laboratory**

Instruction sheet

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

- Segregate, treat and disposal wastes
- Cleaning and storage of laboratory materials

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:

- Segregate disposable and non-disposable materials
- Collect, treat and dispose disposable materials
- Collect, clean and store reusable laboratory materials
- Clean fixed laboratory materials used in the examination process and laboratory room

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
4) Accomplish the Self-checks
5) Perform Operation Sheets
6) Do the “LAP test””.

Information Sheet 3

3.1. Segregate, Treat and Disposal Wastes

3.1.1. Segregation of waste materials

Waste segregation means the aggregation of waste material into designated containers by you for collection and designation for Recycling, Recovery or Disposal. Waste segregation is the sorting and separation of waste types to facilitate recycling and correct onward disposal. When waste is sorted correctly, it can save your company money. Waste segregation should be based on:

- The type of waste.
- The most appropriate treatment and disposal.

Waste can be categorized as**:**

- Liquid waste (blood, solutions, etc)
- Solid waste (paper, plastics, bottles, wood, media, guaze etc)
- Glassware Waste (slides, cover slip, broken beakers etc)

3.1.2. Treatment for Reusable Laboratory Materials

Empty glass containers and bottles, aluminum cans, most plastic containers and bottles, and paper can be recycled. Empty chemical containers that contained hazardous materials must be triple rinsed and dried before submitted to recycling. Empty solvent bottles must be dried before submitted to recycling.

Sterilization of Reusable Materials

i. Steam Sterilization

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

ii. **Dry Heat Sterilization**

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

iii. **Other Heat Sterilization Methods**

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

iv. Sterile Filtration

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

v. Chemical Sterilization

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

vi. **Radiation Sterilization**

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

3.1.3. **Waste Disposal**

Waste disposal refers to the collection, processing, recycling, or deposition of waste materials generated by human society. Waste (also known as trash, rubbish, refuse, garbage, junk, litter and ort) is unwanted or useless material. Waste disposal refers to the collection, processing, recycling, or deposition of waste materials generated by human society.

Laboratory waste disposal typically involves following established protocols and adhering to local regulations to ensure the safe and responsible handling of various types of laboratory waste. The specific methods of disposal vary depending on the nature of the waste, but here are some common methods:

1. Segregation and recycling: Lab waste is often separated into different categories, such as glass, plastic, metal, and paper, for recycling purposes. This helps reduce the volume of waste that needs to be disposed of in other ways.
2. Chemical treatment: Some liquid laboratory waste, such as certain types of chemical solutions, may undergo chemical treatment to neutralize or deactivate hazardous components before disposal. This process typically involves adding specific reagents to the waste to render it less harmful.
3. Incineration: Incineration is a common method for the disposal of certain types of laboratory waste, including combustible materials, contaminated organic waste, and certain chemicals. Incineration involves controlled burning of waste at high temperatures to reduce it to ash and gas. Specialized incinerators designed for laboratory waste are used to ensure complete combustion and minimize the release of pollutants.
4. Autoclaving: Autoclaving is a process of sterilization that uses high-pressure steam to treat laboratory waste, particularly items contaminated with biological agents. This method is effective in inactivating microorganisms and rendering the waste safe for disposal. After autoclaving, the waste is often disposed of as regular solid waste or subjected to further treatment as necessary.

5. Solid waste disposal: Non-hazardous laboratory waste, such as plastic consumables, paper, and other non-contaminated materials, can be disposed of as regular solid waste. This waste is typically collected and sent to landfills, following local waste management guidelines.
6. Hazardous waste disposal: Hazardous laboratory waste, including certain chemicals, heavy metals, and biological materials, requires special handling and disposal procedures. This may involve contracting with licensed waste management companies that specialize in hazardous waste disposal. These companies will collect, transport, and dispose of the waste in compliance with applicable regulations.

It is important to note that waste disposal practices and regulations can vary between countries, regions, and institutions. Therefore, it is crucial to consult local guidelines and regulations to ensure proper and compliant laboratory waste disposal

Self-Check – 3

Name…… ID…… Date…….

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

WRITE SHORT AND PRECISE ANSWER.

1. What is waste disposal (2 pts)?

2. Write at least 5 methods of waste management/disposal?(5pts)

3. _____ the process of using worms for the degradation of organic matter into nutrient rich manure (3 pts)
4. How can we categorize waste materials(5pt)

5. Mention at least 5sterilization of laboratory materials?(5pts)

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

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

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