1. Collection of Malarial Parasites

1. Preparation and Materials

Personal Protective Equipment (PPE): Gloves, lab coat, mask, and safety goggles to prevent exposure to bloodborne pathogens.

Sterile lancets or syringes: For drawing blood samples.

Alcohol swabs: For disinfecting the site of blood collection.

Capillary tubes or EDTA tubes: For collecting and storing blood samples (capillary tubes for finger-prick, EDTA tubes for venous blood).

Glass slides and coverslips: For preparing thick and thin blood smears.

Microscope: For later examination of blood smears.

Giemsa stain: For staining blood smears to visualize malarial parasites.

Sample labels: For recording information on sample containers and slides.

Sterile gauze or cotton: For post-collection care of the collection site.

Field logbook: To document patient/subject information, sample number, date, and collection details.

Cooler: To keep samples cool during transportation if needed.

2. Blood Sample Collection

A. Capillary Blood Collection (Finger/Heel Prick)

1. Select the site: Clean the fingertip (for adults) or heel (for infants) using an alcohol swab.

2. Prepare materials: Open sterile lancets and have sterile gauze or cotton ready.

3. Make the puncture: Use the lancet to prick the fingertip or heel, ensuring a clean and deep enough puncture for sufficient blood flow.

4. Collect blood:

For capillary tubes: Collect a small amount of blood using a capillary tube.

For blood smears: Collect a drop of blood directly onto a clean glass slide for preparing both thick and thin smears (described below).

5. Post-collection care: Apply pressure to the puncture site with sterile gauze or cotton until bleeding stops.

6. Label the sample: Immediately label the capillary tube and slides with the subject's ID, date, and other relevant information.

B. Venous Blood Collection

1. Site preparation: Clean the selected vein site (usually the forearm) with an alcohol swab.

2. Blood drawing: Use a sterile syringe or vacuum tube system to draw 2-5 mL of blood into an EDTA (anticoagulant) tube.

3. Post-collection care: Apply sterile gauze or cotton to the site, and properly dispose of sharps (needle and syringe).

4. Label the sample: Label the EDTA tube with patient information, sample number, and date of collection.

5. Preservation: For immediate analysis, blood can be used fresh. If the sample is to be transported, it should be kept in a cooler or refrigerated at 4°C.

3. Blood Smear Preparation

A. Thick Smear Preparation:

1. Place a drop of blood: Using a capillary tube or drop from a finger prick, place a small drop of blood (approx. 2-3 mm in diameter) onto the center of a clean glass slide.

2. Spread the blood: Use the edge of another slide or applicator to gently spread the drop into a thick layer approximately the size of a dime.

3. Label the slide: Clearly label the slide with patient ID and date of collection.

4. Air dry: Allow the thick smear to air dry completely. Do not fix with methanol, as this will be done after staining.

B. Thin Smear Preparation:

1. Place a drop of blood: Place a small drop of blood (smaller than for a thick smear) near one end of the slide.

2**. Spread the smear:** Use the edge of another slide to push the drop of blood across the slide in a smooth, even motion, creating a thin film.

3. Fix with methanol: After the smear has air-dried, dip the slide in methanol for about 10 seconds to fix the cells

4. Label the slide: Ensure the slide is labeled with the correct information (subject ID, date).

5. Air dry: Allow the methanol-fixed slide to air dry completely.

4. Staining and Examination

A. Giemsa Staining:

1. Prepare Giemsa solution: Dilute Giemsa stain (1:10 or 1:20 dilution in buffer or distilled water, depending on manufacturer's instructions).

2. Stain the thick and thin smears:

Place the slides in a staining rack and cover them with the Giemsa solution.

Leave the slides to stain for 20-30 minutes for proper differentiation of parasites.

3. Rinse: After staining, gently rinse the slides with buffer or distilled water and allow them to air dry completely.

B. Microscopy:

1. Examine under microscope: Use a microscope (with oil immersion for high magnification) to examine the stained slides.

2. Identify malarial parasites: Look for the characteristic stages of Plasmodium (rings, trophozoites, schizonts, gametocytes) in red blood cells (thin smear) or in the background (thick smear).

3. Quantification: In the thick smear, count the number of parasites per field of view and compare it with the number of white blood cells to estimate parasite density.

5. Storage and Transportation

Blood samples: If blood samples need to be transported to a laboratory, store them in a cooler or refrigerator at 4°C and ensure they are delivered within 24-48 hours for analysis.

Slides: Ensure the slides are fully dried and stored in slide boxes to avoid damage during transportation.

6. Cleaning and Decontamination

Properly dispose of used lancets, needles, and other sharps in biohazard containers.

Wash hands and remove PPE after completing the collection process.

2. Collection and Identification of Eimeria Species

1. Objective

To collect, isolate, and identify Eimeria species from fecal or intestinal samples of infected animals.

2. Materials Required

Gloves and protective clothing

Fecal collection bags or containers

Microscope (preferably a compound microscope)

Centrifuge

Salt or sugar flotation solution (saturated sodium chloride or zinc sulfate)

Sterile water

Glass slides and cover slips

Lugol's iodine solution (optional)

Oocyst sporulation chamber (or petri dishes with moist filter paper)

3. Sample Collection

Fecal Samples: Collect fresh fecal samples from infected animals (chickens, cattle, sheep, etc.) into sterile containers. Avoid contamination with soil or water.

Intestinal Samples: If post-mortem examination is necessary, collect intestinal scrapings or the entire intestinal segment, especially from areas showing signs of coccidiosis (inflammation, thickening, lesions).

4. Processing of Samples

For Fecal Samples:

Mix 2-3 grams of feces in sterile water and filter to remove large particles.

Centrifuge the fecal suspension at 1500 rpm for 5 minutes.

Decant the supernatant and resuspend the pellet in flotation solution (saturated NaCl).

Centrifuge again at 1500 rpm for 5 minutes to concentrate the oocysts.

Transfer the top layer (containing oocysts) to a clean tube.

For Intestinal Samples:

Homogenize the intestinal scrapings in sterile water.

Proceed with the same flotation technique as for fecal samples to concentrate the oocysts.

5. Oocyst Identification

Prepare wet mounts of the concentrated oocysts on glass slides.

Observe the oocysts under a compound microscope at 40x to 100x magnification.

Morphological identification involves measuring the size and shape of oocysts. Some distinguishing characteristics include:

Shape: Ovoid, ellipsoidal, or spherical.

Size: Measure using a micrometer eyepiece (important to differentiate species).

Wall thickness and color.

Presence or absence of polar caps, micropyles, or other distinctive features.

6. Oocyst Sporulation

Place the concentrated oocysts in a sporulation chamber or petri dish with moist filter paper.

Keep at room temperature or around 28–30°C for 24-48 hours until sporulation occurs (oxygen and humidity are key factors).

Identify species based on the morphology of sporulated oocysts (e.g., number of sporocysts and sporozoites).

8. Reporting Results

Record the morphometric data, including size, shape, and distinguishing features of the oocysts.

Include photomicrographs where applicable.

3. for Collection of Coccidian Oocysts by Centrifugation Method

1. Objective

To collect and concentrate coccidian oocysts (such as Eimeria species) from fecal or intestinal samples using the centrifugation technique for subsequent microscopic examination or molecular identification.

2. Materials Required

Fresh fecal samples or intestinal scrapings

Clean containers for sample collection

Centrifuge tubes (15 ml or 50 ml)

Centrifuge

Flotation solution (saturated sodium chloride, zinc sulfate, or sucrose solution)

Sterile water or saline

Cheesecloth or fine mesh sieve

Microscope slides and cover slips

Compound microscope

Micropipette or transfer pipette

Gloves, lab coat, and protective eyewear

Labeling materials

3. Preparation of Flotation Solution

Saturated Sodium Chloride (NaCl) Solution: Dissolve 360 g of NaCl in 1 liter of distilled water. Stir until the salt is completely dissolved.

4. Sample Collection

Fecal Samples:

Collect fresh fecal material from animals using sterile containers. Preferably use samples within 24 hours of collection to ensure viability of oocysts.

5. Sample Processing

Fecal Samples:

1. Weigh approximately 2-5 grams of feces into a container.

2. Add 10 ml of sterile water or saline to the fecal sample.

3. Mix thoroughly using a spatula or stirring rod to form a uniform suspension.

4. Filter the suspension through a cheesecloth or fine mesh sieve to remove large debris and undigested material.

Intestinal Scrapings:

1. Homogenize the intestinal scrapings in 10 ml of sterile water or saline.

2. Filter the homogenate through cheesecloth or a sieve.

6. Centrifugation Protocol

1. First Centrifugation (Debris Removal):

Transfer the filtered suspension into a 15 or 50 ml centrifuge tube.

Centrifuge at 1500-2000 rpm for 5 minutes.

Carefully decant the supernatant, leaving the pellet of fecal debris at the bottom of the tube.

2. Flotation Centrifugation:

Resuspend the pellet in a small amount of sterile water (5 ml).

Add an equal volume (or more) of flotation solution (saturated NaCl)

Centrifuge at 1500-2000 rpm for 5 minutes. The oocysts will float to the top due to the high density of the flotation solution.

3. Oocyst Collection:

Using a micropipette or transfer pipette, carefully collect the top layer of the liquid (meniscus) where the oocysts are concentrated.

Transfer this liquid to a clean centrifuge tube.

4. Washing Step (Optional, to remove flotation solution):

Add sterile water to the tube containing the collected oocysts.

Centrifuge again at 1500-2000 rpm for 5 minutes to pellet the oocysts.

Decant the supernatant and resuspend the pellet in a small amount of sterile water. Repeat if necessary.

7. Microscopic Examination

1. Prepare a wet mount by placing a drop of the oocyst suspension on a microscope slide.

2. Place a cover slip over the drop and examine under a compound microscope at 40x to 100x magnification.

3. Observe and identify the oocysts based on their morphology (shape, size, and wall thickness). Record any distinguishing features.

8. Storage of Oocysts

If the oocysts need to be stored for further analysis, keep them in sterile water at 4°C.

For sporulation (necessary for species identification), place the oocyst suspension in a petri dish with moist filter paper and incubate at room temperature or 28-30°C for 24-48 hours.

Wear appropriate personal protective equipment (PPE) when handling animal samples and chemicals.

Dispose of fecal material, slides, and other waste in biohazard containers.

Use standard parasitology guides for coccidian oocyst identification.

4. Parasitic Diseases

1. Objective

The objective of this SOP is to provide standardized procedures for the diagnosis, treatment, and prevention of parasitic diseases in a clinical setting.

2. Scope

This SOP applies to healthcare personnel involved in the diagnosis and treatment of parasitic diseases, including laboratory technicians, nurses, and physicians.

4. Materials and Equipment

Diagnostic kits (e.g., blood smears for malaria, stool analysis kits for intestinal parasites)

Microscopes

PPE (gloves, masks, gowns)

Anti-parasitic medications (e.g., antimalarials, anthelmintics)

Educational materials for patients

5. Procedure

5.1 Diagnosis

1. Patient History and Examination:

Assess patient history, including recent travel, exposure to endemic areas, and symptoms like fever, gastrointestinal issues, or anemia.

Perform physical examination to check for signs of parasitic infections (e.g., enlarged spleen, skin lesions).

2. Laboratory Diagnosis:

Malaria: Perform a thick and thin blood smear for microscopic detection of Plasmodium species.

5.2 Treatment

1. Antimalarials:

For severe malaria, use intravenous artesunate.

2. Anthelmintics:

Administer albendazole or mebendazole for soil-transmitted helminths.

Praziquantel for schistosomiasis or liver fluke infections.

3. Other Parasite-Specific Medications:

Treat specific parasitic infections according to national or international treatment protocols.

5.4 Infection Prevention and Control

1. Patient Education:

Educate patients on preventive measures such as proper hygiene, use of insecticide-treated bed nets (for malaria), and safe food and water practices.

2. Community Health:

Conduct mass drug administration (MDA) in endemic areas for controlling helminth infections.

Promote vector control measures (e.g., insecticide spraying) in malaria-endemic regions.

3. Environmental Measures:

Implement sanitation improvements and water treatment programs to reduce transmission of waterborne parasites.

6. Quality Control

Ensure that laboratory tests and medications used meet quality standards.

Regularly calibrate diagnostic equipment like microscopes and rapid tests.

Monitor treatment outcomes and resistance patterns in parasitic infections.

7. Records and Reporting

Maintain records of diagnostic results, treatments administered, and patient outcomes.

5.DETERMINATION OF ADULTERATION IN MILK (STARCH & UREA).

Aim:

To detect the starch, glucose and urea adulterations in milk.

Back ground Information:

Milk sold loose in the local market is sometimes adulterated with starch, urea, ammonium sulfate or glucose to increase the thickness of the milk and to sweeten it. It is adulterated with water. Starch and urea are used to increase the specific gravity of milk so that lactometer fails to detect the adulteration of milk with water. Following test can be performed to detect the presence of starch and urea in milk.

A) Detection of starch as adulterant:

Principle: Since starch reacts with iodine to form a blue coloured complex, Lugol's iodine can be used as detecting agent.

Requirement: Lugol's iodine, 5% starch solution, Pure sealed packet of milk of two different sources/Brands

Procedure:

1) Take two test tubes;
2) In test tube 1 take 2 to 5ml of sample 1 and in test tube 2 take sample 2.
3) To each test tube add few drops of Lugol's iodine and shake the test tube. If needed add few more drops of Lugol's iodine.

Result: lf the milk turns blue; it indicates that the milk is adulterated with starch. If the milk remains white it is unadulterated.

B) Detection of Urea as an adulterant.

Principle : Since Urea is highly soluble in water and cools the water, when it is added to milk it not only maintains the temperature of the milk cold during transport by acting as a refrigerant and preservative but also helps in maintaining the specific gravity of milk when adulterated with- water. To test whether the milk is adulterated with urea, following tests can be performed.

Requirement: Conc. HCl, 1% $NaNO_2$. Acetate buffer pH 4.6, 2% NaOH, 2% sodium hypochlorite, 5% phenol, Pure milk sample (sealed packet) (Sample No .1), Milk sample adulterated with urea (1 to 2% urea solution 75:25) (Sample No.2)

Procedure: Deproteinize the milk by adding 10ml of acetate buffer pH 4.6 to 20ml of milk. Filter the sample to get the filtrate.

a) Test for Ammonia release: Take two test tubes. In both tubes add 2 to 5ml filtrate of milk To each add 1 ml of 2% NaOH and boil the contents, smell the vapour.

Result: If the vapours arising from the test tube smells of ammonia, it indicates that the sample is adulterated with urea.

b) Test for Nitrogen release: Take two test tubes and add 2 to 5 ml of filtrate of milk in one test tube and in other test tube take 2 to 5ml filtrate of

sample no.2.To each add 1 ml of conc. HCl and 1ml of 1% $NaNO_2$ shake the contents. Observe the reaction.

Result: If the bubbles of nitrogen arise from the sample, the sample is adulterated with urea.

c) **Colour test for urea:** Take two test tubes and add 2 to 5 ml of filtrate of pure milk of two sample. To each add 1 ml of 2% NaOH + 0.5 ml of 2 % sodium hypochlorite solution + 0.5 ml of 5% phenol solution. Shake the contents and heat. Observe the colour. The colour is stable for 12 hrs and can detect urea as low as 0.1 %. **Result:** Blue or bluish green colour develops, which indicates the milk is adulterated with urea.

d) **Test for ammonium sulfate:** The test is very similar to colour test for urea except that there is no need to obtain protein free filtrate from milk. As in the case of urea, a bluish colour forms on heating for 20 seconds.

Result: The bluish colour turns deep blue subsequently and indicates the presence of ammonium sulfate.

6.Study of Vermiculture Techniques- vermi composting.

Principle

This process is mainly required to add nutrients to the soil. Compost is a natural fertilizer that allows an easy flow of water to the growing plants. The earthworms are mainly used in this process as they eat the organic matter and produce castings through their digestive systems.

The nutrients profile of vermicompost's are:

- 1.6 per cent of Nitrogen.
- 0.7 per cent of Phosphorus.
- 0.8 per cent of Potassium.
- 0.5 per cent of Calcium.
- 0.2 per cent of Magnesium.
- 175 ppm of Iron.
- 96.5 ppm of Manganese.
- 24.5 ppm of Zinc.

Materials Required

- Water.

- Cow dung.
- Thatch Roof.
- Soil or Sand.
- Gunny bags.
- Earthworms.
- Weed biomass
- A large bin (plastic or cemented tank).
- Dry straw and leaves collected from paddy fields.
- Biodegradable wastes collected from fields and kitchen.

Procedure

1. To prepare compost, either a plastic or a concrete tank can be used. The size of the tank depends upon the availability of raw materials.
2. Collect the biomass and place it under the sun for about 8-12 days. Now chop it to the required size using the cutter.
3. Prepare a cow dung slurry and sprinkle it on the heap for quick decomposition.
4. Add a layer (2 – 3 inch) of soil or sand at the bottom of the tank.

5. Now prepare fine bedding by adding partially decomposed cow dung, dried leaves and other biodegradable wastes collected from fields and kitchen. Distribute them evenly on the sand layer.
6. Continue adding both the chopped bio-waste and partially decomposed cow dung layer-wise into the tank up to a depth of 0.5-1.0 ft.
7. After adding all the bio-wastes, release the earthworm species over the mixture and cover the compost mixture with dry straw or gunny bags.
8. Sprinkle water on a regular basis to maintain the moisture content of the compost.
9. Cover the tank with a thatch roof to prevent the entry of ants, lizards, mouse, snakes, etc. and protect the compost from rainwater and direct sunshine.
10. Have a frequent check to avoid the compost from overheating. Maintain proper moisture and temperature

7. SERICULTURE TECHNIQUES

MULBERRY CULTIVATION -

Propagation of mulberry

- Mulberry is mostly propagated through cuttings.

- Cuttings may be planted straight away in the main field itself or nursery may be raised and the sprouted and rooted saplings may be planted in the main field.
- The latter method is advisable because of its easy establishment in the main field.

Selection of planting material

- Generally, the mulberry plants are raised from semi-hardwood cuttings.
- Cuttings are selected from well established garden of 8-12 months old.
- Only full grown thick main stems, free from insect and disease damages having a diameter of 10-12mm are chosen for preparation of cuttings.
- The cuttings should be of 15-20 cm with 3-4 active buds and should have 45o slanting cut at the bottom end.
- Care should be taken to make a sharp clean cut at both the ends of cuttings without splitting the bark.
- Manually/power operated mulberry cutter (stem cutting machine) is available for quick cutting of propagation material.

Nursery

Nursery bed preparation

- Select 800 sq.m. area of red loamy soil near water source for raising saplings for planting one hectare of main field.
- Apply 1600 kg of Farm Yard Manure (FYM) @ 20 t/ha and mix well with the soil.
- Raise nursery beds of 4m x 1.5m size.
- The length may be of convenient size depending upon the slope, irrigation source, etc.
- Provide a drainage channel and avoid shady area.

Pre-treatment of cuttings

- Mix one kilogram of *Azospirillum* culture in 40 liters of water.
- Keep the bottom end of the cuttings for 30 minutes in it before planting. *Azospirillum* is applied for inducement of early rooting.

Nursery planting

- Apply VAM @ 100 g/m2 of nursery area.
- Irrigate the nursery bed. Plant the cuttings in the nursery at 15 cm x 7 cm spacing at an angle of 45o.
- Ensure exposure of one active bud in each cutting.

Nursery management

- Irrigate the nursery once in three days.
- Dust one kg of any one of the following chemicals around the nursery bed to avoid termite attack.

1.malathion 5D

2. quinalphos 1.5D

To avoid root rot and collar rot, drench the soil with carbendazim 50 WP (2 g/l) or apply *Trichoderma viride* 0.5 g/m2 using rose can.

- After weeding, apply 100 g of urea/m2 between 55 and 60 days after planting at the time of weeding.

Age of sapling

- The saplings are ready for transplanting in the main field after 90-120 days of planting.

Pruningmethods-

i)Bottom pruning -The plants are cut at ground level leaving 10-15 cm stump above the ground. This type of pruning is done once in a year. **ii)Middle pruning**-The branches are cut at 40-60 cm above the ground level. After bottom pruning, subsequent cuts are made at 45-50 cm height.

iii) Kolar or Strip system - In closely planted area, this type of pruning is done. The branches are cut at ground level every time. Thus, it receives five prunings every year. This type of severe pruning requires heavy fertilization and irrigation.

Harvesting -

The method of leaf harvest depends on the type of rearing practiced. It is preferable to harvest the leaves during morning hours. There are three methods of harvesting of mulberry leaves

Leaf picking -Individual leaves are harvested with or without petiole. Leaf picking starts 10 weeks after bottom pruning and subsequent pickings are done at an interval of 7 - 8 weeks.

Branch cutting-The entire branches are cut and fed to the worms. Before that, topping is done to ensure uniform maturity of the lower leaves.

Whole shoot harvest-the branches are cut at ground level by bottom pruning. Shoots are harvested at an interval of 10-12 weeks and thus 5 to 6 harvests are made in a year.

Time of harvest - It is preferable to harvest the leaves during morning hours.

Preservation of leaves- Use leaf preservation chamber or wet gunny bags to store the leaves or cover the bamboo basket with wet gunny bags to keep it cool and fresh.

8. ANALYSIS OF FAECAL SAMPLE FOR STUDY OF DIFFERENT TYPES OF EGGS IN HELMINTHES

1. Sample Collection

- **Materials Needed**: Clean, dry containers (preferably sterile), gloves, labels.
- **Procedure**:
    - Collect fresh fecal samples, ideally within 24 hours of analysis.
    - Use a clean spatula or scoop to avoid contamination.
    - Label each container with sample ID, date, and time of collection.

2. Sample Preparation

- **Materials Needed**: Fecal flotation solution (e.g., sodium nitrate, zinc sulfate), centrifuge, microscope slides.
- **Procedure**:
    - Mix a small amount of feces (about 1-2 grams) with the flotation solution in a container.
    - Stir well to break up clumps and dissolve debris.

- Pour the mixture into a centrifuge tube and centrifuge for 5-10 minutes at low speed (e.g., 1500-2000 rpm).
- Carefully decant the supernatant, leaving sediment behind.

3. Flotation and Recovery of Eggs

- **Materials Needed**: Flotation solution, microscope slides, cover slips.
- **Procedure**:
  - Add more flotation solution to the sediment and mix gently.
  - Place a cover slip on top of the solution in a dish and allow it to sit for 10-20 minutes to allow eggs to float to the surface.
  - Remove the cover slip carefully and place it on a microscope slide.

4. Microscopic Examination

- **Materials Needed**: Microscope, slides, cover slips.
- **Procedure**:
  - Examine the slide under the microscope starting at low power (10x) and then switch to high power (40x or 100x) for detailed observation.
  - Identify and count the different types of helminth eggs present in the sample.

- Document findings, noting the type and number of eggs per gram of feces (EPG).

5. Identification of Eggs

- **Materials Needed**: Identification key or reference guide for helminth eggs.
- **Procedure**:
  - Compare observed eggs against the identification guide.
  - Record the characteristics (size, shape, color, operculum presence, etc.) to confirm species identification.

6. Data Recording and Reporting

- **Materials Needed**: Data sheets, computer for data entry.
- **Procedure**:
  - Record all findings systematically in a laboratory notebook or database.
  - Include sample ID, date, type of eggs identified, and quantification.
  - Generate a report summarizing the results for further analysis.

7. Quality Control

- **Procedure**:
    - Regularly calibrate and maintain microscopes and centrifuges.
    - Include positive and negative controls in each batch of samples to ensure accuracy.
    - Conduct periodic training for personnel on identification techniques.

8. Disposal of Samples

- **Materials Needed**: Biohazard waste containers.
- **Procedure**:
    - Dispose of all biological waste according to local regulations and institutional guidelines.
    - Ensure that all materials used in the analysis are decontaminated or disposed of appropriately.