Table of Contents.

Proprietary Statement: *This document contains proprietary information on Laboratory service unit, NCAH. It is intended solely for the information and use by the authorized staff only. The proprietary information may not be used, edited, reproduced, shared or disclosed to any other parties for any purpose in any form without expressed written permission of the undersigned authority.*

SOP No: NCAH/LSU/PARA 01
Title: SOP on Faecal Sample Collection
Version No: 1, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema.
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

The purpose of this SOP is to describe the procedure for collection of faecal samples from animals.

2. Objective

To identify and quantify the presence of parasitic eggs, larva and parasite in the fecal sample of different species of animal, to facilitate the diagnosis and surveillance purposes.

3. Principle

Fecal samples are collected in a clean and uncontaminated manner to obtain a fresh and representative specimen. Proper collection, handling, labeling, and transportation help preserve sample integrity and ensure reliable laboratory results.

4. Material Required

1) Rubber gloves
2) Swab
3) Face mask
4) Measuring cylinder
5) Container bottle
6) Distilled water
7) Marker pen
8) Faecal vials (Scintillation vials-8ml and 20ml plastic vials/ Plastic Sachet)
9) Laboratory submission forms
10) Spatula
11) Clean glass slides
12) Sterile forceps
13) Biohazard bag
14) Reagents, solution and buffer: 10% formalin; 70% methanol.

5. Procedure

Direct fecal sample

1) About 3grams of freshly voided faeces should be collected in the fecal vial (scintillation vials) and be sent to the laboratory within 24 hours.
2) If transport times are likely to be longer than 24 hours, the sample should be sent with proper cold chain maintained, or preserved in 10% formalin (general tests) and in 70% ethanol for PCR test, before sending to the laboratory to maintain sample integrity.
3) The faeces should be collected fresh either directly from the rectum or from the recently defecated patch.
4) The fecal vials should be filled with required volume.
5) The sampling procedure should be performed under proper PPE.

Swab Sample Collection

1) Swab sampling may be used only when direct fecal sample collection is not feasible. Fecal swabs are not generally recommended as swab samples are inadequate for parasite detection.
2) The swab should be adequately coated with fecal material to ensure a sufficient sample for laboratory testing and should be placed in sterile normal saline and examined in the laboratory as soon as possible.
3) Care should be taken when collecting swabs from small or delicate animals and birds to prevent injury; appropriately sized sterile swabs should be used.
4) Samples should be kept cool (approximately 4 °C) and processed promptly to maintain diagnostic accuracy.

Proprietary Statement: *This document contains proprietary information on Laboratory service unit, NCAH. It is intended solely for the information and use by the authorized staff only. The proprietary information may not be used, edited, reproduced, shared or disclosed to any other parties for any purpose in any form without expressed written permission of the undersigned authority.*

SOP No: NCAH/LSU/PARA 02
Title: SOP on Faecal Examination by Direct Method
Version No: 2, Total Pages:3
Issue Month/Effective Date: May 2026
Revision: Summary: New included
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema.
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

This method is used for the detection of gastrointestinal parasites in domestic and wild animals by direct microscopic examination of fecal samples.

2. Objective

To detect and identify eggs, larvae, cysts, and oocysts of parasites in animal feces, providing a rapid preliminary diagnosis of parasitic infections.

3. Principle

Fresh fecal material is suspended in saline or iodine solution to release parasite stages. Parasites are observed microscopically based on morphology, size, and internal structures.

4. Material required

1) Glass slides (preferably 25x 75mm)
2) Cover glass (preferable 22x40)
3) Tooth pick or applicator
4) Microscope
5) Lugol's Iodine solution
6) Saline or tape water

5. Test Procedure.

1) Place a small portion of feces on a clean glass slide.
2) Add a drop of saline solution and mix to form a uniform suspension.
3) Cover with a coverslip.
4) Examine under a microscope at 10× and 40× objectives.
5) For better visualization of cysts, prepare a separate portion with iodine solution and examine.
6) Identify parasite eggs, larvae, cysts, or oocysts based on morphology and internal features

6. Waste disposal

All tissue and fecal materials should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

7. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling. Fecal materials may contain infectious agents that can pose a potential zoonotic risk. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times

8. Trouble shooting

It can be difficult to observe or identify the eggs as they may be partly or completely covered by debris.

9. References

1) RVC/FAO guide to Veterinary Diagnostic Parasitology

SOP No: NCAH/LSU/PARA 03
Title: SOP on Faecal Examination by Direct Method – Mucosal Impression Smear
Version No: 3, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema.
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

Applicable for detecting intestinal parasites and their ova directly from the intestinal mucosa, especially in post-mortem examinations, to identify parasites that may not be present in feces**.**

2. Objective

To recover and identify intestinal parasites and their ova directly from the mucosal surface, ensuring accurate diagnosis of site-specific parasitic infections.

3. Principle

The mucosal lining is gently pressed onto a clean glass slide to transfer parasites or ova. The smear is examined microscopically using saline or iodine staining to visualize parasite morphology, size, and internal structures, allowing detection of parasites that are adherent to or embedded in the mucosa.

4. Materials Required

1) Clean glass slides and coverslips
2) Forceps and scissors
3) Saline solution (0.85%)
4) Lugol's iodine solution
5) Microscope
6) Gloves and PPE

5. Test Procedure

1) Open the intestine and gently rinse the mucosal surface with saline if needed.
2) Use forceps to select representative sections of the mucosa.
3) Press the mucosal surface gently onto a clean glass slide to make an impression smear.
4) Add a drop of saline or iodine solution to the smear.
5) Cover with a coverslip.
6) Examine under microscope at 10× and 40× objectives for parasite eggs, larvae, or protozoan cysts.
7) Identify parasites based on morphology.

6, Waste disposal

All tissue and fecal materials should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

7. Risk assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling. Fecal materials may contain infectious agents that can pose a potential zoonotic risk. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

8. Trouble shooting

It is mostly not applicable in live animals to obtain the mucosal smear from the different parts of the intestine.

9. Reference

1) Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food (MAFF/ ADAS) London, Her Majesty's Stationary Office (1971) Reference Book 418.
2) Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

Proprietary Statement: *This document contains proprietary information on Laboratory service unit, NCAH. It is intended solely for the information and use by the authorized staff only. The proprietary information may not be used, edited, reproduced, shared or disclosed to any other parties for any purpose in any form without expressed written permission of the undersigned authority.*

SOP No: NCAH/LSU/PARA 04
Title: SOP on Floatation technique for Taeniid eggs
Version No: 4, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format.
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema.
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

The flotation method is a qualitative diagnostic technique used to detect the presence of Taenia eggs in fecal samples. It works by separating the eggs from the fecal material and concentrating them using a flotation fluid with an appropriate specific gravity. This method allows for the eggs to rise to the surface, making them easier to identify under a microscope. Proper technique and careful sample handling are essential to ensure accurate results.

2. Objective

To outline the procedure for detecting Taenia eggs in feces using flotation techniques. This includes sample collection, preparation, and the use of specific reagents to isolate and identify the eggs microscopically, ensuring accurate diagnosis of taeniasis.

3. Principles

The flotation technique is a qualitative diagnostic method employed for the identification of Taenia eggs in fecal specimens. It operates on the principle of segregating eggs from the fecal matter and concentrating them through the use of a flotation solution with an optimal specific gravity.

4. Material Required

1) Beakers or plastic containers
2) Tea strainer or cheesecloth
3) Mesh sieves with sizes: 100 µm, 41 µm, and 20 µm
4) Measuring cylinder
5) Stirring device (e.g., fork or tongue depressor)
6) 45 ml Centrifuge tube
7) Tube rack
8) Microscope slides and cover slips
9) Microcentrifuge tubes
10) Balance or teaspoon for measurement
11) Sugar solution (for flotation)

5. Procedure

1) About 3 g of faeces are taken in a 50 ml tube and added to the 45ml of (1:1) concentrated sugar solution.
2) After thoroughly shaking, the mixture is centrifuged at 1500 to 2000 RPM for 10 minutes.
3) The supernatant containing taeniid eggs (diameter about 32 µm) is sieved with mesh sizes 100 µm and then 41 µm, and then sieved with a mesh of 20µm, where eggs are retained.

4) Both the 100 µm and then 41 µm sieves are washed with water, and then the eggs are sucked by a Pasteur pipette from the 20 µm sieve.
5) Collected material is examined microscopically for taeniid eggs. If eggs were detected, the material is centrifuged at 15000 RPM for 2 minutes and the pellet will be stored at -80C for DNA extraction.
6) All Taeniid eggs will appear as circular structures featuring a radially striated eggshell when observed under the microscope.
7) The result should be interpreted as "Positive" or "Negative".

5. Trouble Shooting.

The eggs of Taenia spp and Echinococcus spp cannot be differentiated by microscopic examination; PCR methods are required. Direct microscopic examination of fecal samples or fecal flotation may reveal the eggs of Spirometra mansonoides, which are sometimes mistaken for trematode eggs, although they are larger and possess an operculum that is often difficult to see.

6. Waste Disposal

Fecal specimens must be preserved at -70°C to -80°C for a minimum of three days to inactivate the eggs, and subsequent to examination, hydatid cysts and all associated materials should be immersed in 2% sodium hypochlorite for one hour, thoroughly washed, and either disposed of safely or sterilized for reuse.

7. Risk Assessment.

There is a chance of infecting humans with taeniid eggs, so careful safety procedures and precautions should be followed when handling the samples.

8. References

1) Mathis, A., Deplezes, P., Eckert, J. (1996). An improved test system for PCR based specific detetction of Echinococcous multilocularis eggs. J. Helminthol.70. 219-222.
2) Bowman DD. Diagnostic parasitology. In: Bowman DD, editor. Georgi's parasitology for veterinarians. 9th ed. St-Louis: Elsevier; 2009. p. 295–371.

SOP No: NCAH/LSU/PARA 05
Title: SOP on Sedimentation technique & Modified Sedimentation Technique for the detection of Fasciola eggs
Version No: 5, Total Pages:4
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format. Revised waste disposal and risk assessment.
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema.
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

The sedimentation technique is a qualitative method used to detect trematode eggs, such as Paramphistomum, in feces by concentrating them in sediment due to their large and heavy nature.

2. Objective

To describe the procedure for detecting the trematodes eggs through sedimentation techniques.

3. Principles:

In a feces-water suspension, parasite eggs with relatively high specific gravity such as trematode eggs and heavy fecal particles tend to sink rapidly to the bottom, accumulating in the sediment. Through repeated sedimentation and decantation of the supernatant, the lighter fecal particles are gradually eliminated, allowing the parasite stages to be concentrated and detected within the final sediment.

4. Materials required

1) Beakers or plastic containers
2) A tea strainer or cheese cloth
3) Measuring cylinder
4) Stirring device (fork, tongue blade)
5) Test tubes
6) Test tube rack
7) Micro slide, cover slips
8) Balance or teaspoon
9) Microscope
10) Reagent, Solution, and Buffer -Methylene blue.

5. Procedure

Normal Sedimentation Method

1) Weigh or measure approximately 3-10g of faeces into Container 1.
2) Pour 45 ml of tap water into Container 1.
3) Mix (stir) thoroughly with a stirring device (fork, tongue blade)
4) Filter the faecal suspension through a tea strainer or double-layer of cheesecloth into Container 2.
5) Pour the filtered material into a test tube.
6) Allow to sediment for 5 minutes.
7) Remove (pipette, decant) the supernatant very carefully.
8) Re-suspend the sediment in 5 ml of water.

9) Allow to sediment for 5 minutes.
10) Discard (pipette, decant) the supernatant very carefully.
11) Stain the sediment by adding one drop of methylene blue.
12) Transfer the sediment to a micro slide Cover with a cover slip.

OR

B.Modified Sedimentation Technique for the detection of *Fasciola* eggs

1) Mix 20g faeces with around 50-100 ml water to make a homogeneous suspension
2) Pour suspension through a fine-meshed sieve (mesh size 100-300 μm) into a 500ml beaker
3) Wash the sieving residue with a strong water jet and fill the beaker up to 350 ml.
4) Let the suspension sediment for only 5 min, decant the supernatant, fill the beaker with water up to 250 ml and repeat the procedure once (a) or twice (b).
    a) Once for the investigation by microscope, in this case after the second wash step give the sediment in a 15 ml tube and again sediment it for 10 min, then carefully decant the supernatant. This sediment can be investigated on a slide.
    b) Twice for the investigation by Binocular: in this case add a few drops of methylene blue (1%solution) to the sediment, transfer the sediment to a Petri dish with counting grid and examine (one -3 Petri dishes have to be used depending on the purity of the sample
5) All Sediment has to be investigated in both a) or b) in negative cases or in cases with few eggs (under 20 eggs). In cases with many eggs, not all the sample has to be investigated. Calculate EPG by dividing the total number of eggs counted by 20 or depending on the amount of the sample (in grams) used.

5. Trouble shooting

In case of a time delay between processing the sample and reading the count, egg number may decline dramatically. Also, eggs may change their appearance, becoming crenated and “ghost-like”. It is therefore advisable to prepare only a few.

7. Waste disposal

All biological waste generated during the procedure should be collected in designated biohazard waste containers and handled in accordance with the SOP on biological waste management.

8. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling. Fecal materials may contain infectious agents that can pose a potential zoonotic risk. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

9. References

1) Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016).
2) Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

SOP No: NCAH/LSU/PARA 06
Title: SOP on Floatation Method
Version No: 6, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format. Revised waste disposal and risk assessment
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU,NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema.
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

This procedure helps to find and identify eggs, cysts, and oocysts of parasites in fecal samples. It is used to diagnose parasitic infections, plan treatment, The method uses different microscopes, staining, and sample prep techniques to ensure accurate results.

2. Objective

To identify eggs of nematodes and cestodes, as well as coccidian oocysts, in fecal samples. The goal is to make it clear how to do the test properly so that parasites can be accurately detected and diagnosed.

3. Principles:

Feces are mixed with a flotation solution that has a higher specific gravity than water. This causes the heavier fecal particles to sink to the bottom or stay suspended in the liquid. Meanwhile, the lighter parasite eggs, cysts, and oocysts rise to the surface and gather there. This separation makes it easier to collect and examine the parasite stages under a microscope. Using this method helps to quickly and effectively detect parasitic infections in fecal samples.

4. Materials required

1) Beakers or plastic containers
2) A tea strainer (preferably nylon) or double layer cheese cloth
3) Measuring cylinder or other container graded by volume
4) Fork, tongue blades or other type of stirring rod
5) Test tube
6) Test tube rack or a stand
7) Micro slides, cover slips
8) Microscope
9) Reagent, solution and buffer: Floatation fluid, Saturated NaCl sG= 1.2g, Saturated Sugar solution sg=1.3g, ZnCl2=sG=1.3g.

5. Procedure

Floatation Method

1) Put approximately 3 g of faeces (weigh or measure with a pre calibrated tea spoon) into the tube.
2) Pour 50 ml flotation fluid into Container 1.
3) Mix (stir) faeces and flotation fluid thoroughly with a stirring device (tongue blade, fork).
4) Pour the resulting faecal suspension through a tea strainer or a double layer of cheese cloth into Container 2.
5) Pour the faecal suspension into a test tube from Container 2.

6) Place the test tube in a test tube rack or stand.
7) Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a cover slip on top of the test tube. Let the test tube stand for 20 minutes.
8) Carefully lift off the cover slip from the tube, together with the drop of fluid adhering to it, and immediately place the cover slip on a microscope slide.

6. Trouble shooting

In general, flotation techniques work effectively for detecting nematode and cestode eggs, as well as protozoan cysts. However, they may not be reliable for certain trematode eggs, which tend to sink rather than float. For detecting trematode eggs, sedimentation techniques are recommended as they are more effective in recovering these eggs from fecal samples.

7. Waste disposal

All tissue and fecal materials should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

8. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling. Fecal materials may contain infectious agents that can pose a potential zoonotic risk. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

9. References

1) Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016).
2) Parasitology in Veterinary Medicine. Wageningen Academic Publishers

Proprietary Statement: *This document contains proprietary information on Laboratory service unit, NCAH. It is intended solely for the information and use by the authorized staff only. The proprietary information may not be used, edited, reproduced, shared or disclosed to any other parties for any purpose in any form without expressed written permission of the undersigned authority.*

SOP No: NCAH/LSU/PARA 07
Title: SOP on Collection of Intestinal Content for Impression Smear
Version No: 7, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Added scope, objective, principle and reviewed waste disposal and risk assessment.
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. **Scope:**

Applicable for the collection of intestinal content from animals to detect parasitic stages (eggs, larvae, protozoan cysts) or other pathogens. This method is often used post-mortem for diagnostic parasitology and provides a rapid way to assess intestinal infections that may not be detectable in feces alone.

2. **Objective**

To recover and identify parasites or their ova present in the intestinal content by making impression smears, enabling accurate diagnosis of intestinal parasitic infestations.

3. **Principle**

Intestinal content is pressed gently onto a clean glass slide to transfer parasites or their ova. The smear is then examined microscopically, often with saline or iodine staining, to visualize parasite morphology, internal structures, and density. This technique allows detection of parasites that may be adherent to the intestinal wall or present in low numbers in feces.

4. **Materials Required**

1) Clean glass slides and coverslips
2) Sterile forceps and scissors
3) Saline solution (0.85%)
4) Lugol's iodine solution (for cysts)
5) Beakers or Petri dishes (for intestinal content collection)
6) Microscope
7) Gloves and personal protective equipment (PPE)
8) Biohazard disposal container

5. **Procedure**

1) Wear sterile gloves and protective equipment.Label the glass slide with patient/sample identification.
2) Expose the intestinal lumen under sterile conditions (e.g., during necropsy or surgical examination).
3) Collect samples using a sterile cotton swab or applicator stick, collect a small amount of intestinal content or mucosal material.
4) Gently press the collected material onto a clean glass slide to create a thin impression smear.
5) Proceed immediately for wet mount examination or
Label the slide properly and send it to the laboratory for microscopic examination. If delay occurs, keep slides in a clean slide box at room temperature.

6. Trouble shooting

For a valid analysis, the feces should be submitted to the laboratory within 24 hours of being passed by the animals, and preferably within 12 hours. If this is not possible, the sample should be kept in a cool area (but not frozen) out of direct sunlight or use 10% Formalin as preservative.

6. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling. Fecal materials may contain infectious agents that can pose a potential zoonotic risk. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

7. References

1) Clinical Veterinary Advisor, 2nd Ed. 2011
2) RVC/FAO guide to Veterinary Diagnostic Parasitology

8. Appendix

Preparation of preservative: (10% formalin)
Dilution Formula
C1 X V1 = V2
Or rearranged
V1 = C2 X V2/C1
•C1 =Concentration of stock (concentrated) solution required
•V1 = Volume of stock solution required
•C2 = Required concentration
•V2 = Total final volume
In words:
Required concentration X Total volume ÷ Actual concentrated solution

Example: Preparing 10% formalin from 40% formaldehyde (1000 ml)

V1 = 10 X 1000/ 40
V1= 25 ml

So:
•250 ml 0f 40% formaldehyde
•750 ml distilled water
= Final volume = 1000 ml of 10 % formalin

SOP No: NCAH/LSU/PARA 08
Title: SOP on Quantitative Faecal Examination – Stoll's Dilution Method
Version No: 8, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised the procedure
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, LSU, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

Applicable for the quantitative determination of parasite eggs or oocysts in animal feces, providing an accurate measure of infection intensity.

2. Objective

To determine the number of parasite eggs or oocysts per gram of feces, facilitating assessment of parasitic burden for diagnostics, monitoring, and epidemiological studies.

3. Principle

Stoll's method is a quantitative fecal examination technique used to estimate the number of parasite eggs per gram (EPG) of feces.
It works on the principle of diluting a known quantity of feces and counting eggs in a measured aliquot, which is then multiplied by a factor to estimate total egg load.

4. Materials Required

1) Fresh fecal sample
2) Saline solution (0.85%)
3) Measuring cylinder or graduated tube
4) Beaker or conical flask
5) Stirring rod or spatula
6) Microscope
7) Counting chamber or slide with coverslip
8) Pipette or dropper.
9) Gloves and PPE

5. Test Procedure

1) Weigh 4 grams of feces accurately.
2) Add feces into a container and mix with 56 ml of diluent (water or saline). → This makes a 1:15 dilution**.**
3) Mix thoroughly to make a uniform suspension**.**
4) Filter through sieve/gauze to remove debris.
5) Stir the filtrate continuously to keep eggs evenly distributed.
6) Using a pipette, take 0.15 ml of the suspension.
7) Place it on a glass slide and cover with a cover slip.
8) Examine under a microscope (10× objective).
9) Count the number of eggs present.
10) Calculation: EPG=Number of eggs counted×100

6. **Waste disposal**

All tissue and fecal materials should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

7. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample handling. Fecal materials may contain infectious agents that can pose a potential zoonotic risk. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

7. Referrence

1) Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture,
2) Fisheries and Food, Her Majesty's Stationery Office London, UK

SOP No: NCAH/LSU/PARA 09
Title: SOP on Adult Worms and Cyst Sample (Identification)
Version No: 9, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

This procedure applies to the collection, preservation, and laboratory submission of adult parasites (worms) and parasitic cysts obtained from animals for identification in the laboratory.

2. Objective

To ensure proper collection and preservation of adult worms and cyst samples for accurate morphological identification of parasites.

3. Principle

Adult parasites or cysts recovered from animals or fecal material are collected and preserved appropriately to maintain their morphological characteristics, which are essential for accurate laboratory identification.

4. Materials required.

1) Petri dish
2) Rubber gloves
3) Marker pen
4) Spatula
5) Reagents, solution and buffer
6) 0.85 % NaCl solution
7) 70% alcohol containing 5% glycerine
8) 10 % formalin solution

5. Procedure

Nematodes

1) Collect worms into a tube/ petri dish containing 0.85 % NaCl solution.
2) Shake gently to remove debris.
3) Transfer worms into hot 70% alcohol. (It fixes the worms in stretched position for easier identification)
4) Store in 70% alcohol containing 5% glycerine or helminth preservative fluid.

Trematodes

1) Collect the worms in Petri-dish and shake vigorously for 1 min in 1% Sodium chloride.
2) Add an equal quantity of saturated mercuric chloride solution and allow it to stand for 10 minutes. (This kills the worm in an out stretched position)
3) Wash properly in running water.
4) Store in 70 % alcohol, 5% glycerine added or helminth preservative fluid.

Cestodes

1) Collect worms in a petri dish and relax the worm in a water bath at 40°C for about 15 to 30 min.
2) Drop in fixative. (Example-10 % formalin solution or helminth preservative fluid). This will preserve the worm in a stretched position.
3) Flukes, tapeworms and roundworms should be washed in water and preserved in 5% formalin. Always include the head of the tapeworm. When identification of a parasitic cyst is required, tissues should be submitted chilled in a plastic bag or jar.

6. Waste disposal

All tissue, specimen not requiring further preservation should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

7. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling.Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

8. Reference

1) CDC (2023). Stool Specimen Examination Guideline.
2) FAO (Parasitology Techniques Manual).
3) Garcia et al., Laboratory Diagnosis of Gastrointestinal Parasites

SOP No: NCAH/LSU/PARA 10
Title: SOP *on Skin Scrapping*
Version No: 10, Total Pages:4
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format
Supersedes Version No: 2028.1
Prepared by:Parasitology Section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. **Scope:**

This procedure applies to the collection of skin scraping samples from animals for laboratory examination of ectoparasites, mites, and other dermatological pathogens.

2. **Objective:**

To obtain adequate skin scraping samples for the detection and identification of ectoparasites and other skin-related pathogens in the laboratory.

3. **Principle:**

Skin scrapings are collected from affected areas of the skin, particularly at the edge of lesions, to obtain epidermal debris, mites, eggs, or other pathogens. The skin scrapings are mainly collected from those animals suffering from skin problems like scabies, alopecia and dermatitis in order to identify the different types of mites, nematodes, and fungus that cause the skin problem. Proper collection and handling of the sample help ensure accurate microscopic examination and diagnosis.

4. **Materials required**

1) Rubber gloves
2) Marker pen
3) Glycerol,
4) Scissor,
5) Scalpel blade,
6) Cotton,
7) Spirit
8) Aluminium foil paper
9) Glass slides and cover glass
10) Collecting containers (glass tubes, small plastic bags, paper etc)
11) Water bath, test tube, Centrifuge
12) Microscope

5. **Sampling Procedure**

1) The skin scrapings are collected from the affected parts of the skin.
2) The hair over the area should be clipped short and discarded. The site should be cleaned and disinfected.
3) The area selected should be the moist part, "active" – reddened, weeping fluid, covered with new scab material.

4) Hold the blade at an acute angle and scratch until the blood oozes out freely. For suspected demodicosis, scrape deep enough to draw blood.
5) In pustular form of demodecosis, mites can be demonstrated by examination of the cheesy contents of the incised pustule.
6) Specimens are to be sent in 10 % KOH. If ecto-parasites are seen, fixed them in alcohol and send to the laboratory.

6. Test Procedure

Direct examination (recommended if Demodex is suspected)

1) Place a suitable quantity of the scraped materials on a clean glass slide.
2) Mix the scraping with oil or water.
3) Dip on a cover glass and press gently.
4) Examine carefully with a microscope using low power.

Digestion method (Alkali maceration technique)

This method is usually recommended where large amounts of samples are available. The individual or pooled scrapings are placed in a boiling tube and covered with 10% caustic potash and kept in water bath for digestion of tissues.

1) Place the specimens in a test tube and add 5 ml of 10% potassium hydroxide solution.
2) Heat gently to boiling point for about 5 minutes or put in water bath till a homogenous suspension is obtained.
3) Examine a drop of the sediment under a low magnification for mites or fungi.

Permanent mounts are made by first mixing the deposit with water, spinning down and decanting the supernatant, then mixing with the deposit 0.5 ml of melted glycerine jelly. The mixture is aspirated on to slides and covered with cover slips. The jelly sets rapidly, and slides may be examined almost immediately using a low magnification. Any mites or suspicious mites are marked and the cover sealed with varnish.

7. Waste disposal

All tissue and fecal materials should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

8. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection to personnel during sample collection and handling.Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

9. References

1) Peter deplazes, johannes eckert, alexander mathis, georg von samson-himmelstjerna and horst zahner (2016). Parasitology in veterinary medicine. Wageningen academic publishers.
2) Oie terrestrial manual 2016, chapter 2.9.7

10. Appendix

Preparation of preservative **(**10% Potassium hydroxide)

To prepare 10% potassium hydroxide solution, dissolve 10 g of potassium hydroxide pellets in 100 mL of distilled water.

Proprietary Statement: *This document contains proprietary information on Laboratory service unit, NCAH. It is intended solely for the information and use by the authorized staff only. The proprietary information may not be used, edited, reproduced, shared or disclosed to any other parties for any purpose in any form without expressed written permission of the undersigned authority.*

SOP No: NCAH/LSU/PARA 11
Title: *SOP on Blood Sampling*
Version No: 11, Total Pages:3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. **Scope**
   This procedure applies to the collection, preservation, and processing of blood samples from various animals for detection of hemaprotozoa, for diagnostic purposes.
2. **Objective:**
   To ensure proper and standardized methods are followed for safe, accurate, and reliable collection of blood samples to maintain sample integrity for laboratory analysis.

3. **Principle:**
   Blood samples are collected using appropriate techniques and equipment for each animal species. Appropriate sample, proper handling, preservation, and processing are essential to prevent sample contamination or degradation, to ensure proper testing using proper test procedures to enable relevant diagnostic results.

4. **Materials required**
   1) Vacutainer with EDTA (Purple cap)
   2) Vacutainer with Heparin (Green cap)
   3) Vaccutainer needle
   4) Vaccutainer needle adaptor
   5) Scissors
   6) Razor blade
   7) Alcohol swab
   8) Sterile gloves

5. **Sampling Procedure and transportation**

1) Site for collection of blood samples : Jugular Vein - The most commonly used site in the horse, cattle, sheep, goat, and large wild mammal; Cephalic Vein - The most commonly used site for collection of blood in the dog and cat (dorsal aspect of the forelimb at the level of the elbow).
2) Remove the hair from the sampling site with the help of a shaving blade or scissors.
3) Clean the shaved area with spirited gauze or alcohol.
4) Collect the blood sample using a vacutainer needle or syringes with appropriate needle gauge.
5) Blood samples should be maintained in a proper cool chain, not frozen, while being held prior to dispatch and during transport to the laboratory.

6. **Waste disposal**

All the materials used should be disposed of in accordance with the Standard Operating Procedures (SOP) for biological waste management.

7. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection or injury to personnel during sample collection and handling. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

SOP No: NCAH/LSU/PARA 12
Title*: SOP on Test Procedure for the detection of Haemoprotozoa*
Version No: 12, Total Pages: 4
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format. Added Leishman Staining procedure
Supersedes Version No: 2018.1
Prepared by: Parasitology section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

Describes standard methods for preparation, fixation, staining, and microscopic examination of blood smears for the detection of haemoprotozoan parasites such as Babesiosis, Theileriosis, and Trypanosomiasis in domestic animals.

2. Objective

To detect and identify haemoprotozoa in blood samples in order to support the diagnosis of parasitic diseases affecting livestock and guide appropriate treatment and control measures.

3. Principle

Romanowsky-type stains such as Giemsa stain and Leishman stain contain acidic and basic dyes that differentially stain cellular components, with parasite nuclei and cytoplasm appearing blue to purple and red blood cells staining pink, thereby allowing clear visualization of intracellular and extracellular haemoprotozoa under microscopy.

4. Materials required

1) Clean grease-free glass slides
2) Lancet/needle and syringe
3) EDTA blood collection tubes
4) Methanol (absolute)
5) Giemsa stain / Field's stain / Wright's stain
6) Buffered water (pH 7.2)
7) Microscope with oil immersion lens (100x)
8) Immersion oil
9) Slide rack and staining tray

5. Procedure

1) Place the glass slides on an even surface.
2) Mix blood thoroughly (if not a fresh sample). Place a small drop of blood on one end of one glass slide. Hold the top and bottom edges of the slide with the thumb of your non-dominant hand
3) Using your dominant hand, place the edge of the other slide at an approximately 35-45º angle on the first glass slide, in front of the blood drop. Using gentle pressure, gently pull the second slide back into the blood drop and allow the blood to spread to the edge of the slide.
4) To spread the blood, rapidly but gently push the top slide forward through the remainder of the slide. It is important to keep gentle, equal pressure throughout the whole process,

and do not lift the top slide before it reaches the edge of the bottom slide. A feathered edge should be present

5) After preparation, the smear should be labeled and dried (air dryer or waving method)

6. Staining Methods

Giemsa Staining (Preferred Method)

1) Air-dry the blood film, protecting it from flies and other insects if it is not to be stained immediately.
2) Fix in absolute methanol for 5 minutes and air-dry.
3) Dilute stock Giemsa stain 1:10 with Phosphate Buffer Solution (PBS) and flood the film (or place slide in a staining jar). Prepare a fresh stain at least every 2 days.
4) Stain for 30 minutes.
5) Wash gently with tap water.
6) Air-dry
7) Examine under oil immersion (100x objective).
8) Parasite cytoplasm stains blue and nuclei stain magenta.

Leishman Staining

1) Prepare a thin blood smear on a clean slide and allow it to air-dry.
2) Flood the smear with undiluted Leishman stain and leave for 1–2 minutes for fixation.
3) Add an equal volume of Phosphate Buffer solution (pH 6.8) and mix gently.
4) Allow the stain to act for 8–10 minutes.
5) Gently wash the slide with buffered water or clean tap water.
6) Drain excess water and air-dry the slide in a vertical position.
7) Examine the stained smear under oil immersion (100× objective).
8) Parasite cytoplasm stains blue, while nuclei stain purple to reddish.

7. Trouble shooting

1) Use clean, grease-free slides
2) Maintain stain pH at 7.2
3) Include known positive slide (if available)
4) Ensure proper staining time.

8. Waste Disposal

All used materials such as blood-contaminated slides, lancets/needles, cotton, and gloves should be treated as biohazardous waste and disposed of in designated containers; sharps must be discarded in puncture-proof sharps containers, while other contaminated waste should be properly disinfected and handled as per the SOP on waste management.

9. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection or exposure to personnel during sample handling. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

Reference

1) MSD Manual – Collecting and Handling Specimens for Microscopic Parasitic Diagnosis (includes blood smears and use of Wright/Giemsa stain)https://www.msdmanuals.com/professional/infectious-diseases/laboratory-diagnosis-of-infectious-disease/microscopy#blood-smears-and-stains.

SOP No: NCAH/LSU/PARA 13
Title*: SOP on Blood Sample for Heartworm Detection (Dirofilaria immitis)*
Version No: 13, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary: Revised to standard SoP format.
Supersedes Version No: 2018.1
Prepared by: Parasitology Section, Laboratory Services Unit,NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution: NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

This procedure applies to the collection of blood samples from dogs for the laboratory detection of Dirofilaria immitis (heartworm) infection using microscope and serology test.

2. Objective

To obtain high-quality blood samples for the accurate detection and diagnosis of heartworm infection in dogs.

3. Principle

Microfilariae or antigens of Dirofilaria immitis circulate in the peripheral blood. Proper collection of blood, usually from the cephalic, jugular, or saphenous vein, allows detection of microfilariae by microscopic examination or antigen detection by serological assays, enabling early and accurate diagnosis of heartworm infection.

4. Material required

1) Fresh anticoagulated blood (EDTA blood)
2) 2% formalin solution
3) Centrifuge
4) Test tubes
5) Pipettes
6) Microscope slides and coverslips
7) Stain (e.g., methylene blue or Giemsa)
8) Microscope
9) Gloves and PPE

5. **Procedure**

Direct blood Film

1) Place 1 drop of fresh blood or heparinized blood on a micro slide and cover with coverslip.
2) Examine under low power (10X or 40X) for live microfilariae.

3) The result should be interpreted as “Positive” or “Negative’.

Modified Knott's test

1) 1 mL of anticoagulated blood (EDTA or heparin) is added to 9 mL of 2% formalin in a 15mL test tube.
2) The blood is immediately mixed with the formalin by inverting the test tube up and down 5–6 times.

3) The sample is centrifuged (1500 rpm for 5 minutes).
4) The supernatant is poured off. Be careful not to disturb the sediment
5) 1-2 drops of 0.1% methylene blue (1:1000) are added, and the sediment is resuspended into the dye by stirring with an applicator stick.
6) The solution is pipetted onto a slide, coverslipped, and examined using the 10X or 40X objective of a compound microscope.
7) Microfilaria will be fixed in an extended position with nuclei stained blue.
8) The result should be interpreted as "Positive" or "Negative'.

6. **Hematocrit method**

1) Fill a micro hematocrit tube with blood and centrifuge for 1 minute.
2) Wait a few minutes and then examine the buffy coat with a dissecting microscope. Look for live microfilariae.
3) Break the tube at the buffy coat and dab the buffy coat on a micro slide. Add coverslip and examine for microfilariae on low power on the microscope.
4) The result should be interpreted as "Positive" or "Negative'.

7. Waste disposal

All used materials such as blood-contaminated slides, lancets/needles, cotton, and gloves should be treated as biohazardous waste and disposed of in designated containers; sharps must be discarded in puncture-proof sharps containers, while other contaminated waste should be properly disinfected and handled as per the SOP on waste management.

8. Risk Assessment

Appropriate precautions should be taken to minimize the risk of infection or exposure to personnel during sample handling. Standard biosafety measures, including the use of personal protective equipment (PPE) and proper hygiene practices, should be strictly followed at all times.

9. Reference

1) Western College of Veterinary Medicine (procedure overview) Modified Knott's Test for Microfilariae in Blood

SOP No: NCAH/LAB/PARA 14
Title: SOP on collection and Identification of ticks
Version No: 14, Total Pages: 4
Issue Month/Effective Date:May 2026
Revision: Summary: Added the 2023 version.
Supersedes Version No: 2023.1
Prepared by: Parasitology Section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution:NCAH/RLDC/RVH&ECs/SVL/DVHs

1. Scope

This procedure is used for identification of adult ticks by studying the morphology of body parts using stereo-zoom microscope and to understand various types of ticks present in animal population and environment.

2. Objective

The objective of the document is to guide in collection and identification of ticks from various animal species and environment by using stereo-zoom microscope.

3. Principles

The main principle in the identification of adult ticks is learning morphological features such as the shape and color of their body, legs, mouthparts and the presence or absence of certain structures like festoons or spurs by using stereo-zoom microscope.

4. Material Requird

1) Rubber gloves
2) Needle-nose forceps and fine paint brush for transferring ticks
3) Soft cloth
4) pole or stick
5) clean leak proof container
6) Collection tubes
7) 70% ethanol
8) Cool box
9) Ice Pack
10) Paper towels
11) Marker Pen
12) Stereo-zoom microscope or stereo microscope
13) Laboratory submission forms

4. Procedure

Sampling method from animals

1) Put on disposable gloves to protect yourself from tick-borne diseases.
2) Carefully examine the affected area to locate the tick. Ticks are commonly found in warm, moist areas, such as the neck, armpits, and behind the ears.
3) A small hairbrush dipped in ethanol and applies on the skin, use fine-tipped forceps, grasp the tick as close to the skin's surface as possible.
4) Do not squeeze or crush the tick's body
5) Gently pull upward with steady and even pressure

6) Avoid twisting or jerking the tick, as this may cause the mouthparts to break off
7) Place the tick in a clean leak proof container with 70% ethanol

Sampling method from the environment

1) Put on disposable gloves to protect yourself from tick-borne diseases
2) Choose a suitable location: ticks are commonly found in grassy or wooded areas, so choose a location that fits this description.
3) Attach the tick drag cloth or flag to a pole or stick, making sure it is secure and can be easily dragged along the ground.
4) Drag the cloth slowly and steadily over the vegetation and ground, making sure to cover a sufficient area. This will dislodge any ticks that may be present.
5) After dragging the cloth for a certain distance or time, carefully inspect it for ticks. Ticks will often attach themselves to the cloth.
6) If you find ticks on the cloth, carefully use forceps as close to the cloth as possible.
7) Slowly pull the ticks straight off the cloth, making sure to avoid twisting or crushing them. Be careful not to leave any parts of the ticks' mouthparts embedded in the cloth.
8) Once the ticks are removed, carefully place them in a clean, dry vial or container. Make sure to seal the container tightly to prevent the ticks from escaping
9) Clearly label the vial with the date, location, and any other relevant information.
10) Continue dragging the cloth and inspecting it for ticks, repeating steps 7-10 as necessary.
11) Store the tick samples in a cool, dry place until they can be transported to a laboratory for analysis.
12) Place the tick in a clean leak proof container with 70% ethanol after reaching the laboratory.

5. Tick identification procedure

1) Place a drop of a small amount of water on the center of the petri dish.
2) Use forceps to carefully pick up the tick specimen and place it in a Petri dish. (Ensure they are free from any contaminants)
3) Using forceps and carefully transfer the tick specimen onto the drop water on the petri dish.
4) Ensure the tick is positioned in a way that allows for clear observation of its features
5) Place petri dish under stereo zoom microscope and adjust the focus and magnification settings to obtain a clear view of the tick specimen.
6) Observe different parts of the tick, such as the mouthparts, legs, body segments, and any other features of interest (refer annexure).

6. Quality control

1) Use Key guidelines as reference for identification of ticks
2) Preserve the samples in 70% alcohol at room temperature for further analysis and future reference
3) Ticks received in damaged conditions cannot be analyzed for identification.
4) The specific identification of ticks can be challenging, particularly if dealing with larval or nymph forms

7. Waste Disposal

Autoclave all the ticks and contaminated items before disposal.

8. References

1) Salomon J, Hamer SA, Swei A. A Beginner's Guide to Collecting Questing Hard Ticks (Acari: Ixodidae): A Standardized Tick Dragging Protocol. J Insect Sci. 2020 Nov 1;20(6):11. doi: 10.1093/jisesa/ieaa073. PMID: 33135760; PMCID: PMC7604844. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7604844/
2) Centers for disease control and preventions, CDC. Tick removal and testing. https://www.cdc.gov/
3) National centre for animal health, laboratory Services unit, Standard Operating Procedure Parasitology, Version 2018.01
4) Practical veterinary physiology pdf
5) Pem Rinzin, Rai Doj Bir, Namgay Ugyen, Chhoden Deki, Tsheten Karma ,Tenzin Sangay year 2018, Pictorial Keys to identification of adult ixodid ticks of cattle in five agro agro-ecological zones of West-central Bhutan, Regional Livestock Development Centre, DoL, email: rldcwangduee@yahoo.com

SOP No: NCAH/LAB/PARA 15
Title: SOP on Shipment of samples
Version No: 15, Total Pages: 3
Issue Month/Effective Date: May 2026
Revision: Summary:New added
Supersedes Version No:
Prepared by: Parasitology Section, Laboratory Services Unit, NCAH.
Reviewed by: Dr. Kinzang Chedup, Mr. Norbula, Mr. Lungten Dorji, Mrs. Tshewang Dema
Approved by: Head, LSU
Application/Distribution:NCAH/RLDC/RVH&ECs/SVL/DVHs

1. **Scope**

This procedure covers the packaging and shipment of fecal, blood, skin scraping, and other relevant animal samples to the diagnostic laboratory for the detection and identification of parasites, including gastrointestinal helminths, haemoprotozoa, ectoparasites, and other relevant agents.

2. **Objective**

To ensure safe, standardized packgaing, and timely transport of samples while maintaining their integrity and viability, enabling accurate diagnosis of parasitic infections and supporting effective disease surveillance and control measures.

Packaging Procedure and transportation

Step 1: Prepare the Sample (Primary Container)

1) Collect the sample (feces, soil, and blood) in a clean, leak-proof container
2) Fill only 2/3 of the container (do not overfill)
3) Close the lid tightly
4) Seal with parafilm or adhesive tape
5) Label clearly:
    a) Animal/Patient ID
    b) Sample type
    c) Date & time
6) Wrap the container with absorbent material (cotton/tissue)

Step 2: Place in Secondary Packaging

1) Take a zip-lock or biohazard specimen bag
2) Put the wrapped primary container inside the bag
3) Add extra absorbent material inside the bag
4) If required, place an ice pack (for samples needing cooling)
5) Seal the bag tightly
6) Ensure the biohazard label is visible on the bag

Step 3: Prepare the Outer Container

1) Take a cool box (thermocol/Styrofoam)
2) Place the sealed secondary package inside
3) Add cushioning material (foam, paper, bubble wrap) to prevent movement
4) Close the cool box lid securely

Figure 1: Triple packaging of samples.

Label the outer box clearly with:

Documentation

Place request/record form in a separate pouch (not inside with sample). Should include sender details, receiver (lab) details, sender details and type of sample

Transport

Transport the sample as soon as possible. Maintain temperature (cool but do not freeze fecal samples) and avoid shaking or rough handling

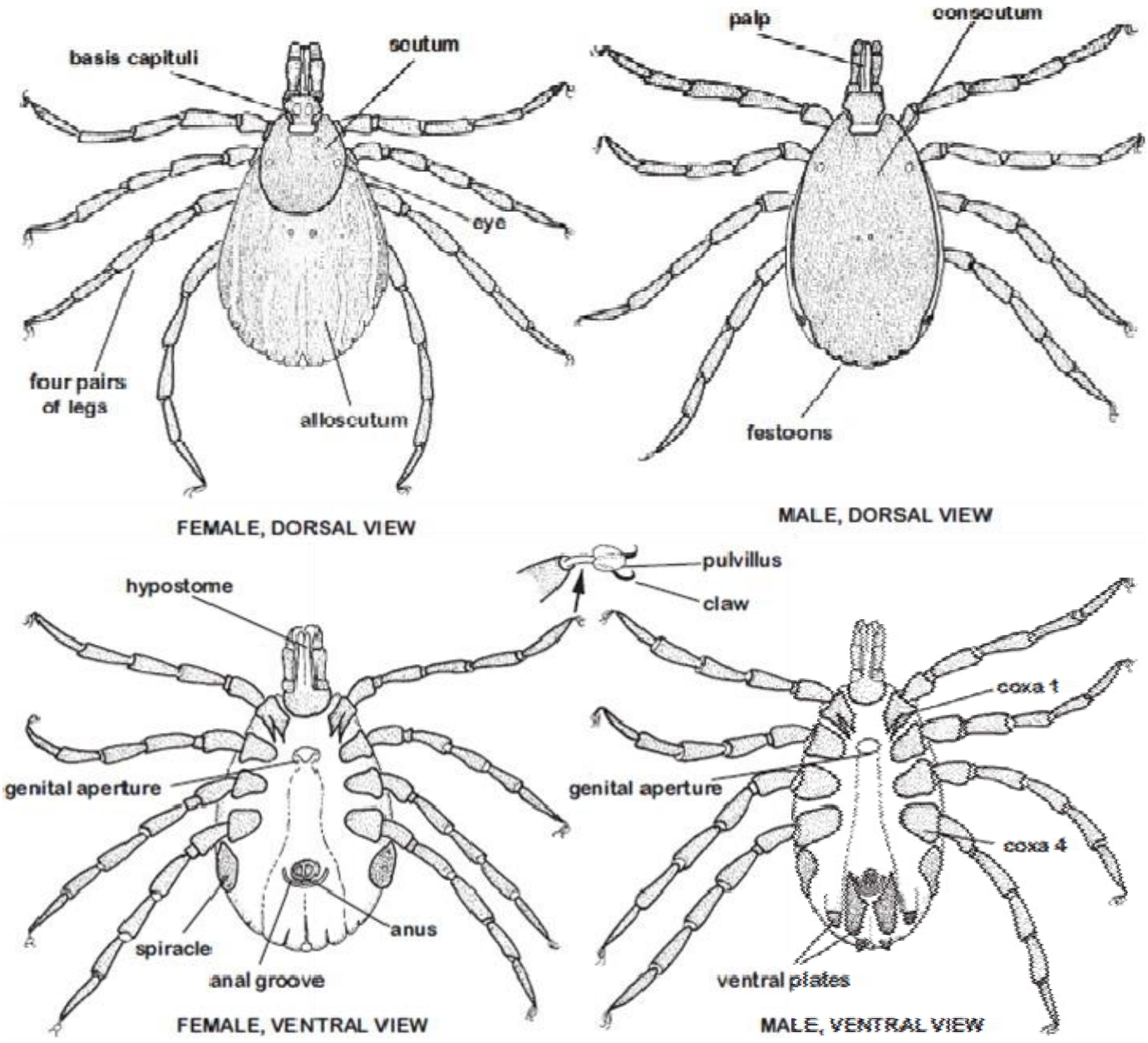
Annexure I

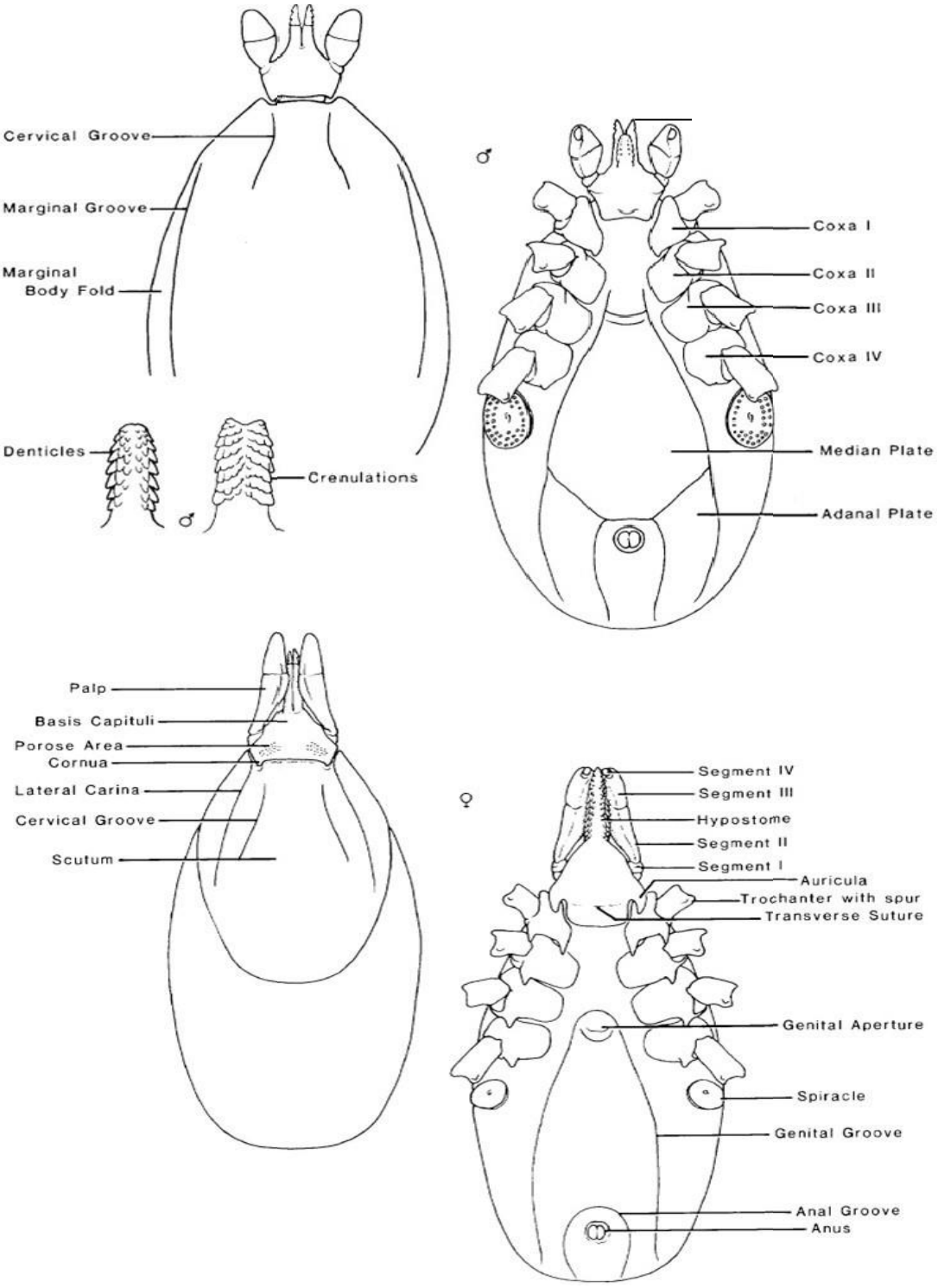
A. Identification keys.

Important Characteristics of Soft and Hard Ticks

Character	Argasidae (Soft Ticks)	Ixodidae (Hard ticks)
Scutum	Absent	Present • Males –covers entire dorsal surface • Females-covers part of dorsal surface
Mouthparts	Hidden when viewed from the dorsal surface	Visible when viewed from the dorsal surface
Feeding	Larvae feed slowly ,over several days ,nymphs and adults feed quickly, several Times	Larvae, nymphs and adults feed once, requiring several days to repletion
Life stages	Egg, larva, two or more nymphs, adult	Egg, larva, nymph, adult

General morphological features of adult Ixodidae ticks.




Cervical Groove
Marginal Groove
Marginal Body Fold
Denticles
Crenulations
♂
♂
Coxa I
Coxa II
Coxa III
Coxa IV
Median Plate
Adanal Plate
Palp
Basis Capituli
Porose Area
Cornua
Lateral Carina
Cervical Groove
Scutum
♀
Segment IV
Segment III
Hypostome
Segment II
Segment I
Auricula
Trochanter with spur
Transverse Suture
Genital Aperture
Spiracle
Genital Groove
Anal Groove
Anus

Differentiating features between soft ticks and hard ticks.