

APPENDIX C
VETERINARY PARASITOLOGY DIAGNOSTIC TECHNIQUES

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1. Direct Fecal.

Use:

Utilized to diagnose intestinal Protozoan trophs.

Procedure:

- Students will be provided with fresh fecal specimens.
- Place a drop of saline on a microscope slide
- Stick an applicator stick into the fecal sample & withdrawal.
- Mix the **very small** amount of feces with the saline on the slide.
- Apply a cover slip.
- Step-up focus on the slide from 4X to 10X then 40X.
- Scan the slide in a stair-step path with 40X. (Scan for protozoa such as *Giardia*).
- Magnification with 100X may be required to verify protozoan identification.
- After examination for motile forms add a drop of Lugol's Iodine Solution to the edge of the cover slip. This will gradually stain organisms and reveal structural details.

2. Passive Fecal Flotation

Use:

Utilized to diagnose intestinal Helminth ova and Protozoan cysts in feces.

Procedure:

- Add a gram of feces (approx. size of a pea) to a (brown) flotation vial
- Fill the flotation vial $\frac{1}{2}$ full with flotation solution.
- Use a spit tongue depressor to thoroughly homogenize feces & solution.
- Add flotation solution until a meniscus forms slightly above the rim of the vial.
- Place a cover slip on top of the meniscus and allow it to stand for 10 -15 minutes.
- After 10-15 minutes, carefully **lift** the cover slip from the flotation vial and immediately place it on a microscope slide.
- Focus on the slide with 4X, then examine the slide with 10X.
(Scan for larvae & ova)
- Note: This lab utilizes Sodium nitrate with a specific gravity of 1:200, which usually causes the collapse of protozoan cysts. Further, trematode eggs are usually too heavy to be floated with this particular solution. Other flotation solutions such as Zinc sulfate, Sodium chloride or Sugar may be utilized to recover protozoan cysts or trematode eggs.
- Note: To remove excess fecal matter, the fecal-flotation mixture may be strained prior to pouring into the flotation vial.

3a. Fecal Centrifugation (with swing-head centrifuge)

Use:

Utilized to diagnose intestinal Helminth ova and Protozoan cysts in feces.

Procedure:

1. Label centrifuge tube near top of tube
2. measure 10-12 mls of float solution in tube and pour into clean beaker
3. Add a 1-2 grams of feces to the beaker
4. Mix thoroughly.
5. Pour mixture through a strainer into a 2nd clean beaker.
6. Next pour the fluid into a 15 ml centrifuge tube.
7. Fill tube with flotation solution until a slight meniscus forms
8. Carefully place coverslip on the tube
9. Carefully place tube in centrifuge and balance centrifuge.
10. Centrifuge at 1,200 - 1,300 rpm (approx. 280 X g) for 5 minutes.
11. Allow centrifuge to stop on its own, or coverslips will be displaced.
12. Remove tube, place in rack, and allow to stand for 10 minutes.
13. Carefully **lift** the cover slip from the tube and immediately place it on a microscope slide.
14. Focus on the slide with 4X, then examine the slide with 10X. (Scan for larvae & ova)

3b. Fecal Centrifugation (with fixed-head centrifuge)

Use:

Utilized to diagnose intestinal Helminth ova and Protozoan cysts in feces.

Procedure:

16. Label centrifuge tube near top of tube
17. measure 10-12 mls of float solution in tube and pour into clean beaker
18. Add a 1-2 grams of feces to the beaker
19. Mix thoroughly. Pour mixture through a strainer into a 2nd clean beaker.
20. Next pour the fluid into a 15 ml centrifuge tube.
21. Fill tube to within 1-2 centimeters of top (this will depend on the angle of the centrifuge head)
22. Carefully place tube in centrifuge and balance centrifuge.
23. Centrifuge at 1,200 - 1,300 rpm (approx. 280 X g) for 5 minutes.
24. Allow centrifuge to stop on its own.
25. Remove tube, place in rack
26. Fill tube with flotation solution until a slight meniscus forms
27. Carefully place coverslip on the tube
28. Allow to stand for 10 minutes.
29. Carefully **lift** the cover slip from the tube and immediately place it on a microscope slide.
30. Focus on the slide with 4X, then examine the slide with 10X. (Scan for larvae & ova)

4. McMaster's Quantitation Technique

Use:

Utilized to quantify intestinal Helminth ova expelled in feces.

Procedure:

- Fill McMaster's Graduated Vial (clear vial with 2 lines) to the bottom line with flotation solution (= 26 mls).
- Add feces, about 4 gm, until the fluid level rises to the top line.
- Pour this mixture into a clean beaker and mix thoroughly.
- Pour mixture through a strainer into a 2nd clean beaker.
- Withdraw a small amount of the well-mixed, strained suspension with a pipette and load this into the McMaster's counting chamber.
- Wait 1 minute for eggs to rise to the top of the chamber.
- Focus on the lines of the McMaster's chamber with 4X, then examine the chamber with 10X. (Scan for ova).
- Examine the entire ruled area, counting all the eggs within the ruled areas
- Add the total egg from each side of the chamber.
- Multiply the sum of the 2 chambers by 25 to determine the eggs per gram (epg).
- Note: The McMaster's Quantitation Techniques is mainly for the quantitation of Strongyle-type ova, thus only strongyle-type eggs should be counted. However, a general idea (i.e. none, few, many...) of the number of other nematode ova, cestode ova & coccidian oocysts should be noted.
- Note: The McMaster's Chambers can **NOT** be examined with the 40X or 100X objectives.

5. Sedimentation.

Use:

Utilized to diagnose intestinal Helminth ova (mainly Trematode eggs) or helminth larvae expelled in feces.

Procedure:

- 8) Mix about one gm of feces with 15 - 20 ml of saline solution.
- 9) Strain fecal solution into a plastic beaker.
- 10) Pour the strained solution into a 15 ml centrifuge tube and allow to stand 20 minutes.
- 11) After 20 minutes, use a pipette to aspirate and discard all but the bottom 0.5 to 1ml of solution from the centrifuge tube
- 12) Mix this bottom aliquot.
- 13) Place a drop of the sediment on a slide, cover with a cover slip, and examine with 4X & 10X.
- 14) Scan for operculated trematode ova and nematode larvae

6. Baermann technique.

Use:

Utilized to collect nematode larvae from soil, fecal or tissue samples.

Funnel Method:

1. Sample Preparation

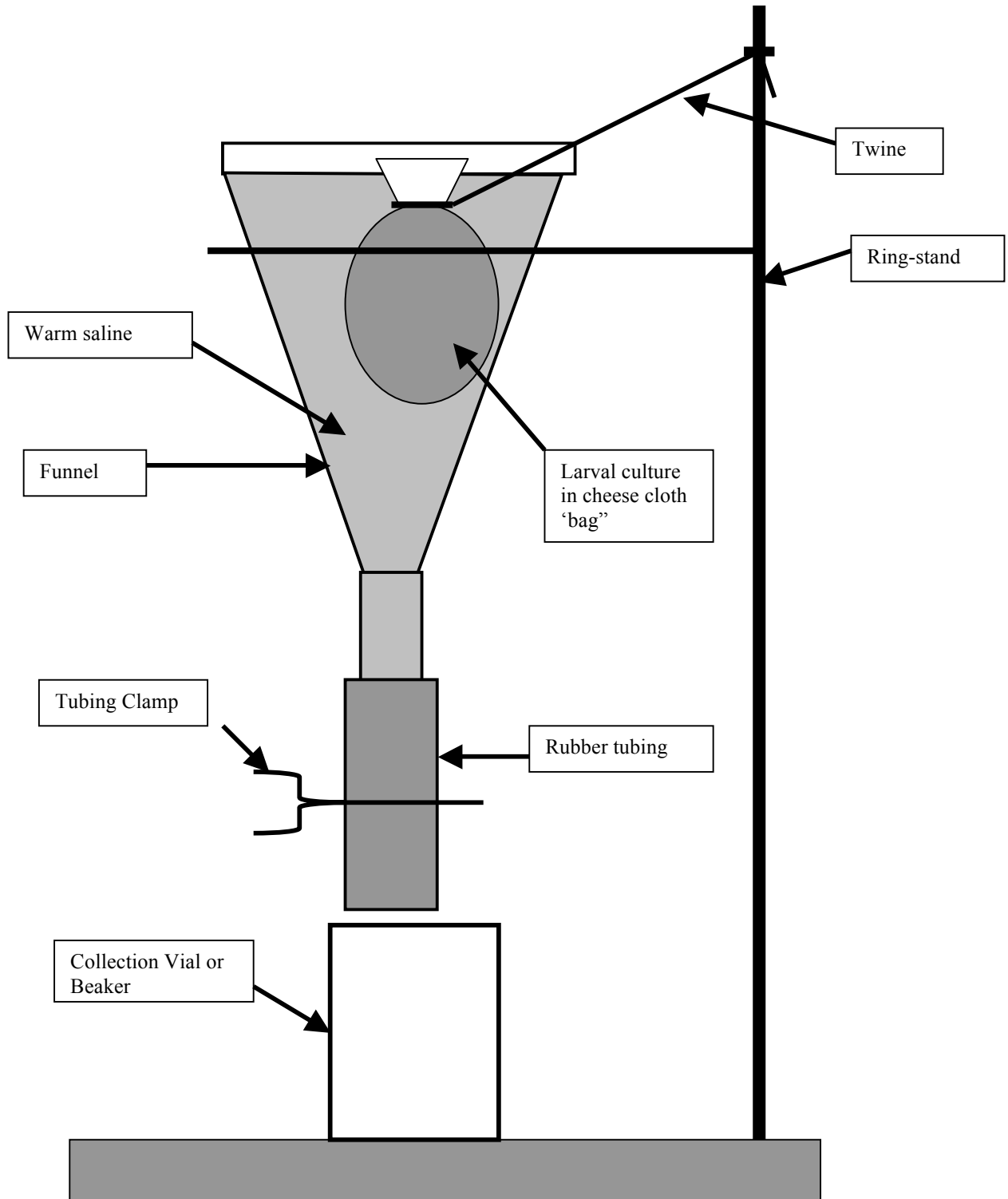
- Fecal samples
 1. Feces is mixed with vermiculite and/or charcoal and allowed to culture for approximately 10 days. (Duration of culture depends on the length of time needed for the larvae to develop & hatch from ova passed in the feces.)
 - Tissue samples
 1. Tissue should be well macerated before being placed in the baermann apparatus. (See Figure #1)
- #### 2. Larval Collection
- Fecal culture or macerated tissue is enclosed in a “bag” of cheesecloth or other porous cloth.
 - The “bag” of feces is then placed in a funnel that contains warm (35-40°C) saline.
 1. The warm saline cause the motile larvae to “wiggle” through the sample & cheese cloth & fall free into the saline.
 - After 15 to 30 minutes the larvae that have settled into the stem of the funnel are drawn from the bottom of the funnel through the rubber tubing.
 - Larvae are then examined by Dissecting Microscope and/or placed on a slide for examination & identification with a Compound Microscope.

Cup Method:

1. Sample Preparation

- Fecal Samples
 1. Feces is mixed with vermiculite and allowed to culture for approx. 10 days.
- #### 2. Larval Collection
- Plastic cups containing about 2 gm of feces were mixed with vermiculite 10 days prior to lab.
 - Fill the cup containing the cultured feces with warm water.
 - Place a petri dish on top of the cup and invert while holding the petri dish in place.
 - Leave the inverted cup in a warm and lighted location for 30 minutes.
 - Examine the rim for the cup using the dissecting microscope.
 - Remove larvae with a pipette and place some of them on a microscope slide for examination with the compound microscope.

Figure #1: Baermann Apparatus



7. Blood Drop Test

Use: Utilized to diagnose parasitic helminthes that live within the blood.

Procedure:

- 8) Place a small drop of whole blood on a microscope slide.
- 9) Cover with a cover slip
- 10) Examine with 4X & 10X.
- 11) Note: This is mainly used as a quick diagnosis for Heartworm infection. The microfilariae that occur in the blood stream are seen as small motile worms among the blood cells.

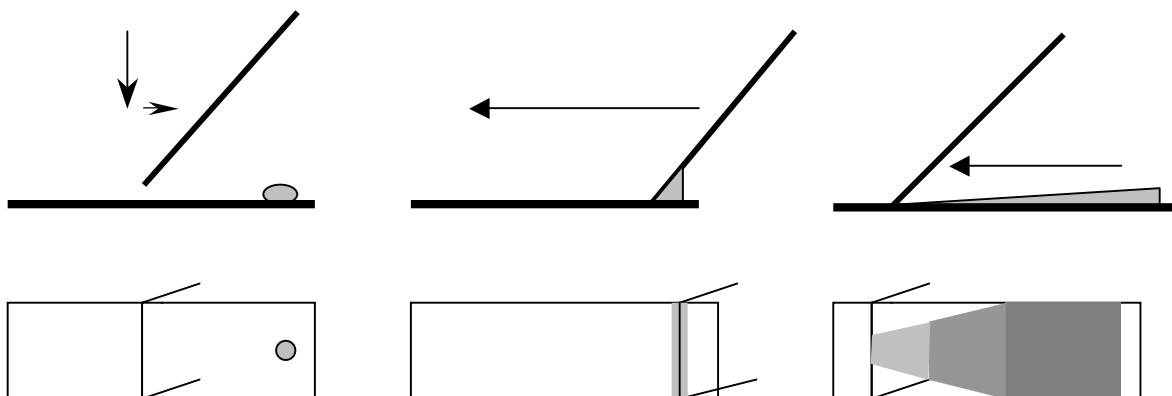
8. Thin Blood Smear

Use: Utilized to diagnose parasitic protozoa that live within the blood stream or within blood cells.

Procedure:

- Place a small drop of whole blood near the end of a clean slide.
- Touch the end of a second slide to the center of the first slide.
- The second slide should be held at a 20-40° angle to the first.
- Slide the second slide to the blood. Upon contact the blood will spread along the edge of the second slide.
- Push the blood with the second slide over the 1st slide with an even, smooth motion; dragging the blood behind.
- Allow blood smear to air-dry.
- Blood smear may be examined directly or stained prior to examination.

Figure #2: Technique for Thin Blood Smear



9. Skin scraping

Use: Utilized to diagnose sarcoptic mange mites that live within skin.

Procedure:

- Clean the surface of the integument area to be scraped.
- Dip a scalpel or razor in glycerin or mineral oil. (This promotes the adherence of skin, debris, & mites to the blade.)
- With the blade perpendicular to the skin surface, scrap the skin until blood appears. (Scrapings should get down to the dermis (thus blood supply), as this is where Sarcoptic mites live.)
- Place the scrapings in a petri dish or on a slide and examine with a Dissecting Microscope or Compound Microscope.

10. “Scotch-tape” Technique for diagnosis of pinworms

Use: Diagnosis of Horse (& human) pinworms.

Procedure:

- If necessary, clean the peri-anal region of the horse
- On the following day, gently press scotch-tape (clear type) on the peri-anal skin.
- Put 1-2 drops of saline on a slide, then stick the tape to the slide.
- Examine slide under microscope.
- The larvated eggs have a single operculum and are somewhat flat on one side.

11. Modified Knott’s Test

Use: Utilized to diagnose Heartworm microfilaria that live within the blood.

Procedure:

- Pipette 1.0 ml of blood into a 15 ml centrifuge tube containing 10 ml of lysing solution (2% formalin) and mix thoroughly.
- Centrifuging for 5 minutes.
- Pour off & discard the supernatant
- Add one drop of stain to the sediment and mix
- Place one drop of the sediment on a microscope slide, cover, and examine with 4X, 10X.

12. Filter Test

Use: Utilized to diagnose Heartworm microfilaria that live within the blood.

Procedure:

- Unscrew filter assembly, then place clear filter membrane on screen, then O-ring on top of membrane, and re-assemble filter assembly.
- Remove plunger from 15 ml syringe
- Pour 10 ml of lysing solution (2% formalin) into syringe.
- Drop-wise add 1 ml of whole blood into the same syringe, reinsert plunger and mix thoroughly.
- Push blood solution through the filter assembly provided.
- Use forceps to remove filter from filter assembly.
- Place filter on a slide, add 1 drop of stain to edge of cover slip
- Cover with a cover slip.
- Examine the filter on which microfilaria are trapped, with 4x or 10X objectives.

13. Oocyst culture.

Use: Utilized to sporulate and identify coccidian oocyst passed in the feces.

Procedure:

- Mix a small amount of feces with 1% potassium dichromate solution.
- In a petri dish spread the mixture very thin over the bottom of the dish. (Oxygen is required for sporulation, thus mixture must be thin.)
- Incubate at room temperature.
- Keep mixture moist by adding potassium dichromate as needed.
- At room temperature, sporulation is usually complete in 2 to 4 days, although some species require a week.
- After incubation period is complete, make a direct smear or fecal float of the culture.
- Examine specimens with 4x, 10X, and 40X objectives.