

LABORATORY

1

INTRODUCTION TO THE PARASITOLOGY LABORATORY

Introduction

This lab is an introduction to some techniques used by veterinarians to detect eggs, cysts, and larvae of parasites in the feces of animals. The proper use of the microscope is vital to these techniques .

Objective:

The purpose of this first laboratory is to introduce you to some of the techniques that a veterinarian uses to detect the eggs, cysts, and larvae of parasites in the feces of animals. The examination of blood for parasites is also described in this handout, although you will not be doing this procedure today. Since most of the diagnostic stages of parasites are microscopic, the proper use of your microscope is very important.

Checklist of Objectives:

- Be able to run a passive fecal flotation.
- Be able to run a centrifugational fecal flotation.
- Be able to make a “wet mount” slide.
- Be able to examine a slide made from any of the above techniques.
- Be able explain what the various fecal examination techniques are best suited for and their problems (Table pg 5).
- Be able to explain how a specimen is collected and processed for parasitology, as well as the timing of repeat fecal exams.
- Have an appreciation of the general characteristics of the various phyla to which parasites belong (i.e. know how to determine if an organism is a protozoan, fluke, tapeworm, nematode or an arthropod).

At the Bench

A. Use of the microscope for fecal exams:

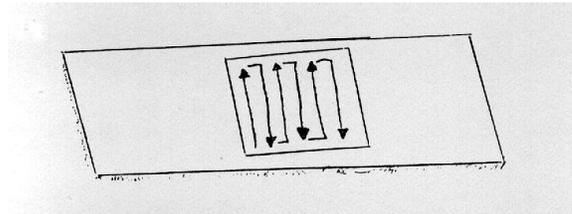
The following tips will help you adjust to using your microscope for the examination of fecal samples for parasites.

1. The first thing to remember is, unlike a histological section, a wet-mount of parasite eggs is three-dimensional and, therefore, you may find that you must continually adjust the focus to see objects at the bottom or top of the wet-mount.
2. Make sure you have the condenser iris diaphragm open so that there is **just enough** light to work with (the higher the aperture, the lower the contrast). When using the 4X and 10X objectives, the diaphragm should be almost closed; open it a little for use with the 40X objective and further for use with the oil lens.
3. The condenser should be moved to almost its top position (you should not be able to see the lamp filament). **Do not use the condenser to adjust the light level, use the diaphragm.**

B. Examining a wet-mount:

When examining a wet-mount for cysts and ova, start in one corner of the coverslip using your 10X objective and cover the slide in overlapping fields (see diagram #1). Use your 40X lens to examine any suspicious objects, and after you have completed the examination, repeat about 1/4 of it using the 40X objective to find the smaller cysts. Note that the addition of a drop of iodine to the sample will stain many eggs and cysts increasing their contrast.

Diagram #1.



C. Fecal examination techniques:

In today's lab you should do the following 4 techniques, making use of the samples of dog feces at your place. (These fecal samples contain eggs of nematode parasites.) **Record your results (# of eggs per coverslip) on the DATA SHEET (pg 16) and enter your data into the web site [<http://cal.vet.upenn.edu/projects/parasit06/website/datasheetlab1.htm>] by noon Friday.**

1. **Passive Saturated salt flotation** (use sample "A") - There are numerous devices for doing this type of flotation now in use in local veterinary hospitals. Several manufacturers have donated devices for your use and we will be using them throughout the course in order to allow you to become familiar with each type. - see instructions on Pgs. 6 and 7.

2. **Zinc Sulfate Centrifugal Flotation Technique** (use sample “A”) - see instructions on Pg. 8.

3. **Direct Wet Mount** (use sample “A”) - see instructions on Pg. 5.

In a future laboratory you will learn how to count the number of parasite eggs per gram of feces using the McMaster slide, which is in your slide box. The use of the Baermann apparatus for recovering larval nematodes from feces is demonstrated in today’s laboratory, you will be using the technique in a future lab.

The methods for examining feces covered in this laboratory are also covered in Foreyt’s “Veterinary Parasitology Reference Manual” pp. 1 - 10, and in Zajac and Conboy (2006) “Veterinary Clinical Parasitology, 7th Ed.” pp. 3 - 24 (as well as in earlier editions).

Collection and Processing of Samples for Parasitology

A. Feces

1. Collection

- a. Ideally, feces should be processed as soon after passage from the animal as possible.
- b. Feces should be collected in airtight containers to prevent desiccation. When collecting horse or ruminant feces that might be in transit for a while before reaching the lab, collect it in a “zip lock” plastic bag and carefully remove as much air as possible before sealing it. This will keep it anaerobic and prevent the eggs in it from developing and possibly hatching.
- c. If the processing of a fecal specimen must be delayed, it may be:
 - I. Refrigerated (but not frozen) for several days (not recommended for samples with live larvae that you intend to examine using the Baermann technique).
 - II. Fixed, e.g., 10% formalin (5% formalin-saline is better for protozoal cysts). Add fixative to feces at a ratio 3:1 (v:v) and mix well. (Do not fix samples intended for use with the Baermann technique.)
 - III. Horse and ruminant fecal sample collected in a zip lock bag as described in “1b” can remain at room temperature for up to 5 days.
- d. If an animal has been treated with antidiarrheal preparations containing bismuth or kaolin, mineral oil, oral contrast material (barium) for radiology (all of these materials float) or antibiotics, then parasites may be difficult or impossible to find. Therefore, repeat fecal exam 5-10 days after treatment withdrawal.

2. Processing

a. First, examine the feces for blood and other clinical signs, then examine the inside of container for tapeworm segments (which are motile and may move away from the fecal mass).

b. Many techniques have been devised to increase the likelihood that parasites will be detected in a particular sample of feces. The merits and limitations of representative fecal processing techniques are summarized in the table on page 5. Step-by-step directions for performing the various methods are on the following pages.

3. Repeat fecal exams are suggested in the following situations:

a. Clinical signs suggest parasitism, but initial fecal exam was negative. (The infection may be pre-patent or just patent with low numbers of the diagnostic stage. One fecal exam will find about 72% of infections, while 3 fecal exams will find > 95% of infections.) Repeat in 2 or 3 days. Repeat for a total of 3 times within 7 to 10 days; if no parasites are seen after 3 examinations it is likely the animal is not infected.

b. Following specific therapy of a parasitic infection, have owner submit a fecal specimen 1 - 2 weeks following the last administration of drug. (This is late enough that all eggs and cysts will have been cleared from the gut, but, for most parasites, too early for reinfection to be showing up.)

Demonstrations

Checklist material

Parasites from various groups of invertebrates are shown in the demonstrations. Be sure you are able to put an unknown parasite into one of the following: Protozoa, Nematoda, Trematoda, Cestoda, Acanthocephala, Insecta, Arachnidia.

COMPARISON OF FECAL EXAMINATION TECHNIQUES

Technique	Best Used For:	Problems
Zinc Sulfate Centrifugal Flotation	First choice for standard fecal examinations. Only technique for <i>Giardia</i> cysts and best technique for <i>Trichuris</i> eggs. Will, in most cases, recover nematode larvae.	Trematode, Pseudophyllidean tapeworm and <i>Physaloptera</i> eggs may not always float. Nematode larvae may be crenated and the Baermann technique may be required for a positive identification. Protozoal trophozoites will usually be too crenated to identify.
Passive flotation: Saturated sucrose, saturated salt (sodium chloride or sodium nitrate), or zinc sulfate	Standard technique used in many veterinary clinics. Will miss most <i>Giardia</i> cases and many of the mild whipworm cases.	All the problems mentioned above, plus: Nematode larvae and <i>Giardia</i> cysts may be crenated beyond recognition. Commercial devices allow examination of only a small amount of feces.
Ethyl acetate sedimentation	Best technique for examining samples with a large amount of fat in them.	May take a long time to examine the resulting sediment if not combined with one of the above flotation techniques.
Baermann Technique	Best technique for recovering live nematode larvae for identification.	Takes a minimum of an hour to run and will recover only live nematode larvae. Samples with only a few larvae in them may have to be run overnight.
Direct Wet Mount	Least useful technique. Should be used only on liquid feces to look for protozoal trophozoites. Used as an adjunct to one of the fecal flotation techniques. Also a useful adjunct test when combined with a staining technique.	Examines only a small amount of feces and takes a very long time to examine the sample properly.

Techniques

Direct Wet Mount Fecal Exam

1. Place a **small** amount of feces on a microscope slide.
2. Add a drop of liquid to the feces and mix thoroughly. The type of liquid added depends on what you hope to accomplish with the technique. If you are examining a liquid fecal sample for the presence of protozoan trophozoites (live active protozoa) then use saline (if any extra liquid is needed). If you are looking for helminth eggs and protozoan cysts in a small sample (bird droppings, rectal smear, etc) then either water or iodine may be used.
3. Cover with a cover slip. Move the cover slip around until it lays flat. You should be able to read through the smear (light from the microscope must be able to pass through the sample in order for you to examine it).
4. Examine the slide using the 10X objective, and then go over it with the 40X objective.

Because this technique examines only a very small amount of feces, it should only be used in the following circumstances:

- a. Liquid feces where protozoan trophozoites may be present.
- b. Fecal samples where the amount of feces obtained is too small to handle with any other technique.
- c. As an adjunct to a flotation technique where you are looking for eggs that do not float. (In this case you probably would be better off running an ethyl acetate sedimentation and then examining the resultant pellet using the direct wet mount method.)

Note: Circumstances "b" and "c" occur frequently when dealing with small fish, birds, amphibians and reptiles and thus the direct wet mount has some utility in dealing with fecal samples from these animals.

The OVASSAY PLUS Kits used in today's lab were a gift from Synbiotics Corp., San Diego, CA.

How to Collect a stool sample

1. Remove insert. Note the little cup-like area which forms the bottom of the insert.
2. Fill small end by pressing in stool. Loose stools may need to be scooped into the small end of insert.
3. Place insert back into the device. Close the lid of cap. Write pet's name on the cap and return loaded device to your veterinarian.

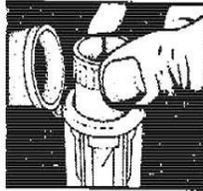
OVASSAY Plus Analyzing the Fecal Sample

1. With the filled insert in place, add OVASSAY Plus Zinc Sulfate Solution until fluid level reaches about half way up the device.
2. Mix thoroughly by rotating the insert. This separates eggs and cysts from fecal sample.
3. With the bottom edge of the cap, apply pressure on the insert until it is firmly seated in the device.
4. Carefully lift the device to the brim with flotation solution. Form a meniscus.
5. Place a cover slip on the meniscus for 5 minutes.
6. Then transfer cover slip to slide for microscopic examination. Close the cap tightly for easy disposal.

SYNBIOTICS CORPORATION

Instructions

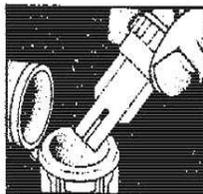
How to collect a stool sample with your Fecalyzer®



1. Lift cap and remove green insert.



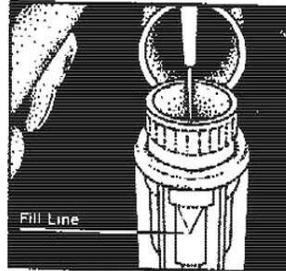
2. Push small end of green insert into stool sample. (If stool sample is loose, scoop into small end of green insert.)



3. Replace green insert and close cap. Return to your veterinarian.

EVSCO PHARMACEUTICALS
Affiliate of HGL, Inc. Buena, NJ (USA) 08010

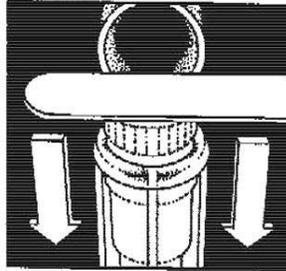
Laboratory Instructions



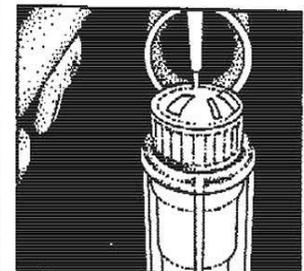
1. Lift cap but do not remove green insert. Fill green vial with FECASOL® Flotation Medium to the tip of the arrow embossed on side of vial.



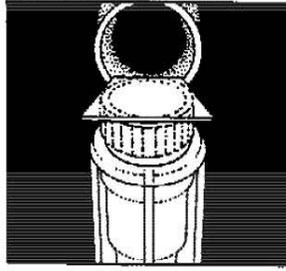
2. Rotate green insert vial back and forth to separate ova from fecal sample. Mix thoroughly.



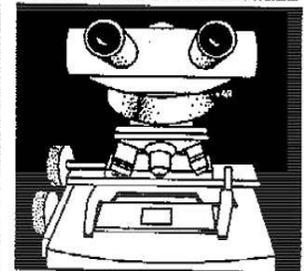
3. Seat green insert vial firmly in place with tongue depressor (or with thumbs).



4. Fill holder completely to form a meniscus with additional FECASOL Flotation Medium.



5. Float 22mm cover slip on meniscus for 15-20 minutes.



6. Transfer cover slip to slide for microscopic examination at 100X magnification.

7. Close cap and dispose of FECALYZER® to prevent cross-contamination.

The Fecalyzer units used in this lab were donated by EVSCO Pharmaceuticals, Buena, NJ (a division of Vétoquinol).

ZINC SULFATE CENTRIFUGAL FLOTATION METHOD

1. Fill a 15 ml centrifuge tube with ZnSO₄ solution (1.18 specific gravity)¹ and pour into a glass dish or plastic specimen cup.
2. Using a tongue depressor, push the feces (2 to 5 grams, a piece the size of a large grape) through the strainer into the ZnSO₄ solution in the dish. **TIPS: 1. The sieve must be in the liquid in order for the feces to be passed through. 2. The more feces you use, the more likely you will be able to find eggs which are present in low numbers.**
3. Using a funnel, pour the ZnSO₄-fecal mixture back into the centrifuge tube.
4. Centrifuge for 2 min at high speed (1500 - 2000 rpm).
5. Using a headed-rod or loop, remove a sample from the surface of the solution and place on a microscope slide. (Make 2 or 3 dips with the rod or loop to get enough material to examine, you want the equivalent of a large drop on the slide.) Add a drop of iodine² (to stain the cysts and ova) and a coverslip. Examine at 10X and then part of slide at 40X.

TIP: To increase the sensitivity of this technique either use more feces or do the following:

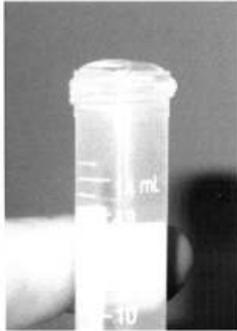
After removing the tube from the centrifuge, fill the tube with ZnSO₄ to just over the top of the tube, place a coverslip over the top of the tube and wait 10 min. Place a drop of iodine on a slide and place the coverslip onto the drop of iodine and examine at 10X. This modification also allows you to skip using the loop or headed rod to obtain your sample, and thus may be easier to do at a veterinary practice. OR place a cover slip on the tube before centrifuging (see hints on the next page).

TIP: If the sample contains a large amount of fat or other material that floats in water, you may want to wash the sample before doing the flotation. To do this, start at step 1 but use water instead of ZnSO₄. When you centrifuge the water-fecal mixture, the eggs, being heavier than water, will sink but the fat and other material will float. After centrifugation pour off the supernatant, add the ZnSO₄ solution and mix well. Centrifuge as in step 4 and examine as in step 5. OR use the ethyl acetate sedimentation technique to get rid of the fat.

1. **ZnSO₄ solution** (1.18 sp. Gr.) is made by adding 386 grams of ZnSO₄ to 1 liter of water. The mixture should be checked with a hydrometer and adjusted to 1.18. The ZnSO₄ solution should be stored tightly capped to prevent evaporation (and the resulting change in the specific gravity of the solution).
2. **Iodine solution:** 10 gms Potassium Iodide (KI) added to 1 liter of distilled H₂O. Shake to dissolve. Add 10 gms of Iodine (I₂) to the above solution. Allow to stand over-night with stirring, at this time you may still have Iodine crystals at the bottom, this is OK, just leave them there. This solution will stain (and kill) most parasite eggs and cysts (coccidial oocysts are an exception, they do not take in the iodine).

Helpful Hints for Fecal Floatation

Many veterinary customers are using the Straight 8 series for fecal floatation. The ova recovery rate is much higher using the centrifuge method where a coverslip is placed directly onto the top of a tube while spinning. The ova, being lighter than the floatation solution, are forced upward onto the glass of the coverslip. After spinning, simply remove the coverslip and place directly onto your microscope slide.



Bulging Meniscus

Note: Fecal Floatation solution is much heavier than water, and must be used for balancing. Using a water tube will cause the centrifuge to spin off-balance, causing coverslips to break or damage to the centrifuge.

Using fecal floatation solution in both the sample tube and the balance tube, fill the tube to the top with a slight bulging meniscus over the top. Then place a coverslip onto the top of the tube, and tap it down with your finger to seat it on the rim.

It is fine to have a small bubble beneath the coverslip, but make sure that mostly liquid is contacting the coverslip.

Place the tube into the centrifuge



Tap down coverslip



Incorrect

Make sure that the coverslip is turned so that the edge is parallel to the edge of the rotor.

If the corner of the coverslip is pointing towards the center nut, then it will contact the flat metal portion of the rotor when spinning horizontal and it will fall off.



Correct

Jorgensen Labs

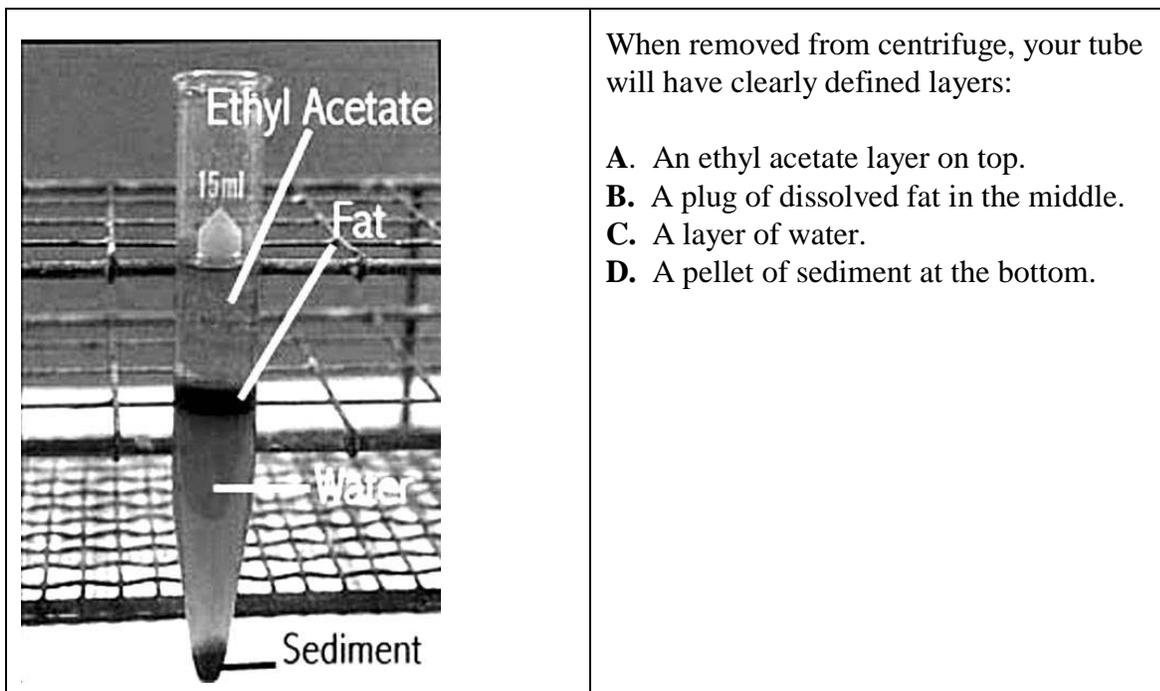
1450 Van Buren Ave. - Loveland, CO 80538
800-525-5614 - www.jorvet.com - email: info@jorvet.com

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Be careful using this technique - if one coverslip comes off or if it breaks (use the heavier coverslips for this method) it may throw the centrifuge out of balance and cause the coverslips on the other tubes to come off.

ETHYL ACETATE SEDIMENTATION
(For Fat Extraction Prior to ZnSO₄ Centrifugal Flotation)

1. Pass a grape-sized piece of feces through a sieve into about 9 ml of water and pour into a 15 ml centrifuge tube.
2. Add about 3 ml of ethyl acetate, plug the tube with a rubber stopper and shake the tube vigorously. **CAUTION:** Test materials before placing Ethyl Acetate into them. This solvent will dissolve many types of plastic!! The white plastic centrifuge tubes used in the lab are OK, but clear hard plastic tubes and the disposable polystyrene cups will dissolve.
3. Remove the rubber stopper and centrifuge the tube (1500-2500 rpm) for 1 to 2 minutes.
4. Using a stick, "ring" the plug of fat at the water - ethyl acetate interface (the plug adheres to the side of the tube and must be detached before the liquid contents of the tube can be poured off).
5. Pour off the supernatant, being careful to leave the pellet at the bottom of the tube intact. (Flush the ethyl acetate down the sink with plenty of water.)
6. Resuspend the pellet in distilled water, centrifuge, then pour off the water (this removes any leftover fat and ethyl acetate). Resuspend the pellet in flotation solution, centrifuge again, and remove the material from the top of the float to examine for eggs (see ZnSO₄ technique on Lab 1 Pg. 9).



THE BAERMANN TECHNIQUE

In 1917, while working in Java, the Dutch physician Dr. Baermann developed a simple method for isolating nematodes from soil. Today veterinarians use his method for the extraction of live larval stages of nematode parasites from feces.

Technique (modified for use in the office lab):

- 1) Place a sieve in a custard dish or other similar container.
- 2) Spread about 10 grams of feces* on a piece of tissue paper and place it into the sieve.
- 3) Place warm** water in the custard dish until it just covers the feces, taking care not to disrupt the feces.
- 4) Allow to sit for at least one hour.†
- 5) Lift off sieve.
- 6) Pour liquid into a 50 ml centrifuge tube.
- 7) Let sit for 20 minutes.
- 8) Using a Pasteur pipet, remove a drop of the sediment at the bottom of the tube and place it on a microscope slide for examination. (Be careful not to resuspend the sediment before you take a sample from it.)

* Use **fresh** feces - refrigeration may kill *Strongyloides stercoralis* larvae.

** This technique makes use of two characteristics of parasitic larval nematode behavior:

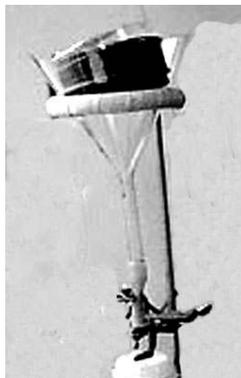
- 1) The warmer it is, the more active the larva (up to a point, you don't want to cook them!; 37 to 40° C is as warm as you want to get), and, in addition, some larvae are thermotaxtic and will move towards the warmer water under the filter paper.
- 2) Most parasitic larval nematodes are poor swimmers.

Therefore, the following events take place when the sieve is placed in the water:

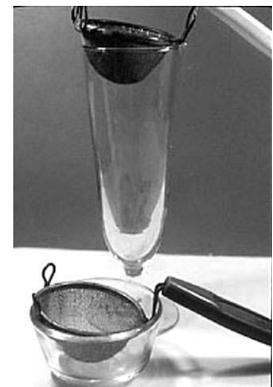
The larvae will be moving around in a random fashion and within any given time interval some of them will migrate through the tissue and fall into the water. Because they can't swim they sink to the bottom and over time a number accumulate there. The more active the larvae are (i.e. the warmer the water) the greater the number of larvae that accumulate at the bottom in a given time interval.

† The longer you wait, the more larvae will fall to the bottom of the dish, but with time, the fecal sample breaks down and begins to pass through the tissue leading to an accumulation of sediment along with the larvae.

Baermann apparatus



Modified Baermann apparatuses



STOLL EGG COUNTING TECHNIQUE

A method for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. The advantage of this technique is that it requires no specialized equipment, the disadvantage is the counting takes a long time because of the amount of extra (non-egg) material on the slides.

1. Weigh out 3 grams of feces.
2. Measure out 42 ml of water and place it into a dish. Using a tongue depressor, push the 3 grams of feces through a sieve into the water. Lift the sieve and hold over the dish. Push out any remaining water from the feces.
3. While stirring the water-feces mixture, take 0.15 ml of the suspension and spread over 2 slides. Cover each slide with a long coverslip (or 2 regular size coverslips).
4. Examine both slides for worm eggs, **the total number of eggs counted X 100 represents the number of eggs per gram of feces.**
5. **The mathematics:** 0.15 ml is 1/300 of 45 ml (42 ml water and 3 gm feces) so the number of eggs in 0.15 ml X 100 is equal to 1/3 of the total number of eggs in the original 3 grams and thus equal to eggs per gram (**EPG**).

McMASTER EGG COUNTING TECHNIQUE

This is another method for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. The advantage of this method is it is quick as the eggs are floated free of debris before counting, the disadvantages are you must use a special counting chamber and it has a detection limit of 100 EPG (unless multiple counts are done on the same sample or more feces is added to the same volume of flotation solution).

1. Weigh out 2 grams of feces.
2. Pass the feces through a sieve into a dish containing 60 ml of ZnSO₄ or saturated salt solution. Lift the sieve and hold over the dish. Push out any remaining solution from the feces.
3. While mixing vigorously (you may want to put the solution into a flask to prevent spillage) take a sample of the mixture with a pipette and transfer it to one of the chambers of the McMaster slide. Repeat the procedure and fill the other chamber.
4. Wait 30 sec, then count the total number of eggs under both of the etched areas on the slide. Use your 10X objective (first check to see that this objective can be swung into place without hitting the slide, if it hits the slide, count with the 4X lens). Focus first on the etched lines of the grid, then go down a tiny bit, the eggs will be floating just below the top of the chamber. **Multiply the total number of eggs in the 2 chambers by 100, this is the eggs per gram (EPG).**
5. **The mathematics:** The volume under the etched area of each chamber is 0.15 ml (the etched area is 1 cm X 1 cm and the chamber is 0.15 cm deep) so the total volume examined is 0.3 ml. This is 1/200 of 60 ml. Since you started with 2 gms of feces and then multiplied by 100, the final result is **eggs per gram** of feces.

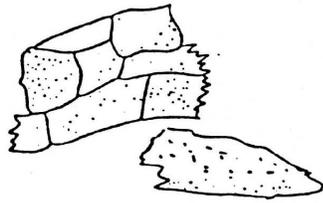
Modified Wisconsin Sugar Flotation Method

This method of determining the EPG is probably the most commonly used method. (First used by the University of Wisconsin's Parasitology Laboratory, it is a modification of the Stoll technique.) It is the most accurate as it counts all the eggs in 3 grams of feces and because it is a flotation method it has little debris to interfere with the count. However, if the EPG is high, there may be too many eggs to count.

1. Fill a 15 ml test tube with 10 ml of Sheather's* solution.
2. Weigh 3 grams of feces and place into a cup.
3. Pour the Sheather's* solution from the test tube into the cup and mix well.
4. Place a funnel into the test tube, place a strainer into the funnel and pour the fecal-sugar solution mixture through the strainer into the test tube. Using a tongue depressor, squeeze the liquid out of the feces that is left in the strainer.
5. Centrifuge the tube for 2 to 4 minutes.
6. Fill the tube to just over the top with more Sheather's solution and place a cover slip onto the meniscus.
7. Let sit for about 5 minutes, then remove the cover slip and place on a slide.
8. Examine the entire cover slip and count the number of eggs that you find.
9. The number of eggs counted is the number per 3 grams of feces, so divide by 3 to find the EPG.

* Sheather's Solution: Add 454 gm (1 lb) of table sugar to 355 ml of very hot water. Stir until dissolved and allow to cool. This solution will grow mold if left out, so keep refrigerated and use quickly. Some people add 6 ml of formaldehyde to the solution to preserve it.

OBJECTS SOMETIMES MISTAKEN FOR HELMINTH EGGS AND PROTOZOAN CYSTS



plant cells



plant hair



seed fibers



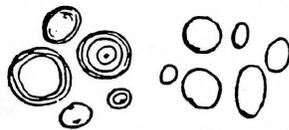
starch granules



protein particles



vegetable cells

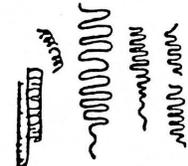


fat droplets - oil droplets

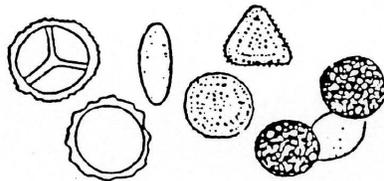


air bubbles

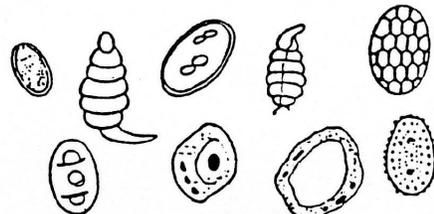
scratches on slide



plant spring cells



pollen grains



fungus and yeast spores

Blood

Collected for two basic procedures:

1. Smears - to detect protozoal and rickettsial infections (e.g., *Trypanosoma*, *Babesia*, *Anaplasma*). Smears must be fixed and stained to reveal organisms.
2. Concentration - to detect microfilaria (i.e., *Dirofilaria* and *Dipetalonema*)

If blood is not to be processed immediately upon removal from the patient, an anticoagulant must be added to the sample. Among those commonly used:

- a. Heparin - effect lasts only for a matter of hours
- b. EDTA - effect lasts several days

1. Procedure for making Blood smears (thin films):

- a. Clean slide by wiping with alcohol. Handle slides by edges only. (Any grease on the slide will cause the dried blood to flake off during staining).
- b. Place a **very small** drop of blood near the end of a slide.
- c. Place the end of another slide (the "spreader") on the sample slide so that the edge of the spreader is just ahead of the drop of blood.
- d. Holding the spreader at an angle of about 30° (relative to the sample slide), draw it back until its edge just touches the drop of blood. The blood will then run along the entire edge of the spreader slide
- e. Push the spreader briskly in one fluid motion completely across the sample slide. Note that the blood is being **dragged behind** the spreader, not pushed in front of it. (There is a video of this procedure on the lab's web site.)
- f. If the correct amount of blood was applied, the smear should end before the end of the slide, and the smear should end in a "feathered edge," a region where the blood cells are well separated.
- g. Air dry.
- h. Fixation and staining - various methods can be used. Normally a commercial staining kit is utilized following the manufacturer's instructions.

2. Procedures for concentration of blood (Knott and Filter tests):

see Laboratory #5.

LAB 1 DATA SHEET

This sheet is for your records and should remain in your lab manual. Enter your data into the web site (address will be given in lab) by tomorrow.

- 1] Count the number of nematode eggs that you find under the coverslip for each procedure.
- 2] Estimate the time it took to do the procedure (from when the feces was obtained until the egg count was recorded).

PROCEDURES FOR DOG FECAL SAMPLE

	Egg Counts	Time needed to do procedure
Passive Float (Saturated Salt) (in commercial device)	_____	_____
ZnSO₄ Centrifugal flotation	_____	_____
Direct Wet Mount	_____	_____

CHECKLIST OF OBJECTIVES FOR LABORATORY 1

1. Use of the microscope for examination of fecal floats.
2. Use of the commercial device (Advantages and disadvantages of the saturated salt flotation).
3. How to do a Zinc Sulfate flotation (advantages and disadvantages).
4. How to do an Ethyl Acetate sedimentation (advantages and disadvantages).
5. How to do a Direct Fecal Smear (advantages and disadvantages).
6. Demonstration of the Baermann technique. (Know how to run it and under what circumstances it is used).
6. Know when to use each of the fecal examination techniques (see Table 1, pg. 4)
- 7.
8. Answer the Review Question at the end of the demonstrations. (**Note: The review question is in the same form as the questions asked on the lab exams.** There will be a review question at the end of each lab's demonstrations to give you some practice before the actual exam. (You can find out the answer to the review question by looking on the back of the card the question is written on.)
9. Fill out the data sheet on the web site (<http://cal.vet.upenn.edu/paraav/forms/lab1data.htm>) with the results of each fecal examination that you ran. The results will be tabulated for the class and reported to you in the next lab.