Fecal Diagnostic Testing: Why It Still Matters

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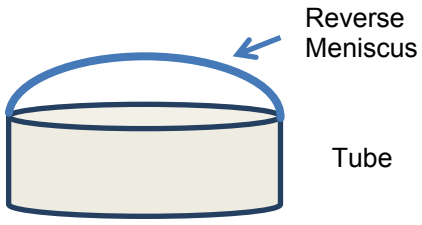
It is human nature to want to play with the newest and greatest "toys." As the science of veterinary medicine has advanced, we, as veterinarians, have tended to follow the trend of newer is better. However, there is one very basic, tried and true, diagnostic procedure that is in my opinion is still better than any new technology, and that is the fecal float. The float is the "bread and butter" diagnostic technique for diagnosis of intestinal parasites. For this reason it is important to understand how a float works, and in what situations it should be utilized.
As a disclaimer, these proceedings are not meant to be an all-inclusive summary of how to do a fecal, but rather to serve as an adjunct to previously published procedures that are detailed in Georgis' Parasitology for Veterinarians (9th ed.) by Dwight D. Bowman.

First, there are four main fecal diagnostic techniques: the fecal float, the direct, sedimentation and the Baermann. The fecal float is the most widely used of these techniques and therefore, is the technique on which we will focus our attention. Fecal floats are not all created equal. There are different solutions, as well as the debate as whether to perform a passive float or to use a centrifuge. However, there are some basics that should be common to all techniques, regardless of which method or solution that you choose.

1) More is better:
The basis of the fecal float is that we can isolate or concentrate the eggs in order to diagnose an infection. It would follow that the more material or feces with which you begin, the more likely that you will be able to detect eggs, or oocysts. Now, literally, insert the fecal loop. While this is an excellent device for obtaining feces from a dog or cat, it will rarely provide enough feces. The Companion Animal Parasite Council (CAPC) recommends that you obtain at least one gram (preferably 2-3) of feces to perform a fecal float. The easiest way is to ask the owner to bring the feces to the appointment with them. However, if that does not happen, obviously, you have to make do with what feces you can obtain.

2) Filter, Filter, Filter:
It is generally recommended that you mix the feces with ~10 ml of your solution of choice. This amount of fluid can be varied. The main point is that you mix and break up the feces, in order to try to equally distribute any parasite eggs or ova through the solution. After mixing, the solution is then poured into the appropriate container through cheesecloth, in order to remove any remaining large particle matter. The solution is then poured into a "cup" or centrifuge tube depending on whether you are performing a passive or centrifugal float. After you pour the solution into the tube, there should be a reverse meniscus as shown below:

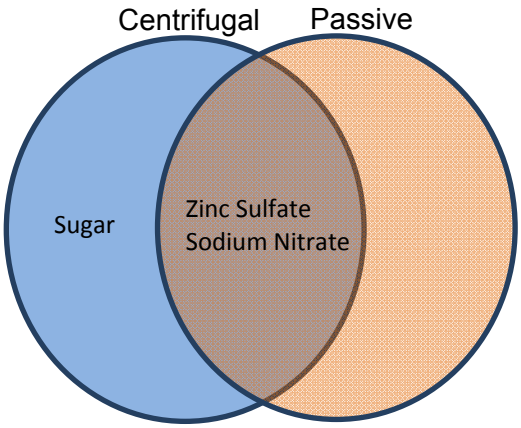


3) Quicker is not always better…
More often than not, flotations are much shorter than the desired time. You need time for the solution to do its job, so to speak. Passive floats should sit for 15 minutes, while centrifugal floats should spin for 10 minutes. If you take less time, you may have gotten clients out the door quicker, but could have made a diagnosis of "No parasites seen," when there were actually parasites present.

Is there only one right way?
CAPC, as well as most veterinary parasitologists, recommend that the best method for performing fecal flotation

is the centrifugal float, using a sucrose-based solution. This procedure is by far the most sensitive, and you will be much more likely to diagnose infections using this method. However, while this is the preferred method (the data supports this), there is more than one way to perform a fecal. The two big areas of debate are passive versus centrifugal float, and the type of solution to use. Not all solutions can be used with both methods:

This is summarized below in Figure 1:



Types of solutions:

Let's start our discussion with the different types of solutions that can be used for flotation. The float is based on the physical property of specific gravity, which we normally think about in terms of urine and an annoying concept in chemistry. Basically, if the specific gravity of an object, such as an egg, is less than that of the solution, the egg will 'float.' The higher the specific gravity or 'heavier' the solution, the 'heavier' an egg or ova that can be floated.

All solutions are not created equal. There are two basic types of solutions: salt and sugar. Each has its own pros and cons. Sugar is considered the best choice because of the relatively higher specific gravity, which means the solution can "float" the heaviest eggs. The three salt solutions that are used are: zinc sulfate, sodium nitrate, and sodium chloride. As zinc sulfate and sodium nitrate are used most commonly, we will focus our discussion on these.

Sugar or Sucrose (aka Sheather's sugar solution): Each solution has its pros and cons. Sugar is considered the best solution because it can float the "heaviest" eggs, including whipworm (*Trichuris vulpis*) eggs, which can be difficult to diagnose. In other words, sugar provides the most sensitivity. If the sugar solution has a high enough specific gravity then sometimes heavier eggs, such as spirurid, fluke, and tapeworm eggs can be floated. If not using a sugar solution, then one would have to use the sedimentation technique, in order to diagnose these eggs.

Another pro of sugar solution is that it is relatively easy and cheap to make. Finally, sugar does not crystallize quickly, thus allowing you some time to read the slides. The cons of sugar include that *Giardia* cysts will become distorted in sugar solution, and, of course, that it is 'sticky.'

To make a sugar solution use the following steps:
Materials:
454 g granulated sugar (1 1b)
355 ml tap water (12 oz or 1 ½ cups)
6 ml full-strength (37%) formaldehyde (this is added to prevent mold growth).

Procedure:
1. Heat water to near boiling.
2. Add the granulated sugar, and stir until the sugar is dissolved.

3. Allow the mixture to cool to room temperature, and then add the formaldehyde.
4. Check the solution's specific gravity, and adjust it to 1.27 by adding water or sugar.

As a note, if you do not wish to add the formaldehyde, which prevents mold growth, you can store the solution in the refrigerator.

Salt solutions: There are a number of advantages with salt solutions. One is that they are significantly less 'messy,' than sugar solutions, and as indicated above, they can be used for both passive and centrifugal floats. Also, salt solutions are less likely to distort *Giardia* cysts. In the case of zinc sulfate, the solution can be easily made by dissolving 350 grams of zinc sulfate in 1000 ml of water, and then adjusting the specific gravity to 1.18. Also, Sodium Nitrate (aka Fecasol) can be purchased, and can have a range of specific gravities (1.18 to 1.30). The con of both of these solutions is that they are relatively more expensive than sugar. Also, they will both crystallize quicker than sugar, with zinc sulfate crystallizing quicker than sodium nitrate. You will need to check the specific gravity of salt solutions regularly, especially zinc sulfate. What you will notice is that over time crystals will form in the bottom of the plastic jug. Since the salt is no longer in solution, this means that you are basically performing fecal floats with water. You will get a lot of "negative" results, but not because you have great compliance from your clients. The final, and in my opinion the most important, 'con' is that due to the lower specific gravity of solutions, you will not be able to detect heavier eggs.

For your reference, listed below are the specific gravities of some common eggs and solutions:

Solution	specific gravity		Parasite	specific gravity
Sodium nitrate	1.18-1.20		*Toxocara canis*	1.09
Zinc sulfate	1.18-1.20		*Toxocara cati*	1.1
Sucrose	1.25-1.27		*Ancylostoma* spp.	1.06
Sodium chloride	1.18-1.20		*Trichuris vulpis*	1.15
			Taeniid-type ova	1.23
			Physaloptera spp.	1.24

Passive (or simple) versus centrifugation:
The debate about passive versus centrifugal floats is directly related to the debate about solutions. As stated above, sugar floats can only be performed with centrifuges. Thus, the same criticisms of sugar floats apply to centrifugal floats. Additionally, I hear from both current veterinarians and veterinary students that centrifugal floats take too much 'time.' Where one can set up multiple passive floats in an almost assembly line manner, you have to wait the required 10 minutes for the centrifuge to finish spinning another sample. You can, however, spin 6 samples at once. This inconvenience is more than outweighed by the increased sensitivity of the centrifugal float method. Remember, with the centrifuge you are not only floating "lighter" eggs in a "heavier" solution, but you are also applying centrifugal force. If you do not want to use sugar, remember you can still use zinc sulfate or sodium nitrate for a centrifugal float.

For centrifugation, there are 2 types of centrifuges that may have to use: the swinging bucket and the fixed-angle. The procedures for both are listed below:

Swinging bucket method:
1. Mix the feces and solution of choice.
2. Pour through cheesecloth or strainer into a centrifuge tube.
3. Place tube into a holder.
4. Add enough solution to form a reverse meniscus. Place into centrifuge.
5. Place a cover slip on top of the tube. Make sure that the coverslip is firmly "seated" on top of the tube.

6. Spin at 1,200 to 1,500 rpm for 10 minutes.
7. Place the coverslip on a slide and examine at x100 magnification (the x10 objective).

Fixed-angle centrifuge method:
1. Mix the feces and solution of choice.
2. Pour through cheesecloth or strainer into a centrifuge tube to within ½ to 1 inch from the top.
3. Place into centrifuge.
4. Spin at 1,200 to 1,500 rpm for 5 minutes.
5. Place tube into a holder.
6. Add enough solution to form a reverse meniscus.
7. Place a cover slip on top of the tube. Make sure that the coverslip is firmly "seated" on top of the tube.
8. Let stand for 10 minutes.
9. Place the coverslip on a slide and examine at x100 magnification (the x10 objective).

Both these methods work quite well, and I would encourage people to adopt the centrifugal float in their practice.

A few final notes on other fecal diagnostic procedures:
1) Direct smears: These should be the exception, not the rule, unless the other procedures are cost-prohibitive. The preferred method for identification of *Giardia* spp. trophozoites.
2) Sedimentation: This procedure should be done when you suspect a parasite infection (i.e. fluke eggs), where the eggs could be difficult to float using normal methods.
3) Baermann: In small animal practice, this method is used to diagnose the feline lungworm, *Aelurostrongylus abstrusus*.

In conclusion, the fecal float has stood the test of time as a diagnostic technique. It will most likely be used in veterinary practices for decades to come, which is exactly why everyone should learn to perform the technique correctly.