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Modified McMaster Egg Counting For Quantitation of Nematode Eggs.

Fecal worm egg examination methods are based on the principle of differential density. In other words, parasite eggs sink in water, but they will float in various chemical solutions that are more dense than water (technically, they have a higher specific gravity) because the eggs are lighter than the fluid used as a floatation solution. The most inexpensive and easiest floatation solution to make is using table salt. One quart of floatation solution is sufficient for about 30 McMaster examinations.

The first step is to collect freshly passed feces that are uncontaminated by soil or bedding. The best way is to use a rubber glove and extract feces directly from the rectum. Alternatively, a feces can be picked up off the ground if done soon after deposited. The collection container should be labeled with the name (number) of the animal and the date of collection. Fresh samples work best, but accurate results can be obtained if the sample is kept refrigerated during the interim. If samples are not refrigerated the eggs will hatch within 12 to 24 hrs. Once hatched, they cannot be counted.

Materials:

Compound microscope

Scale

Saturated sodium chloride (table salt)*

50 ml centrifuge tube with screw cap. Note: tube should be marked with ml increments.

Tongue depressor

Pipet (1 ml syringe or eye dropper works well)

McMasters egg counting slide**

Paper towels

A fresh fecal sample should be collected and kept refrigerated until tested

*Saturated Sodium Chloride:

Table salt 1 pound box

Tap water 3 quarts

Heat in pan with stirring until boiling, then let cool at room temp. The solution will look cloudy and some material will precipitate - this is OK. Pour clear part of solution into a dispensing container of some kind.

Store at room temperature. Do not refrigerate as additional solute will precipitate.

Note: Fecal floatation solutions are also commercially available, but are significantly more expensive than using this recipe (although not high dollar).

**To order this slide, contact:

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Procedure:

1. Weigh out 2 grams of feces into a 50 ml centrifuge tube and fill to 30 ml with salt solution.
 - a. It is recommended to purchase a small scale and weigh feces, but if you do not have a scale you can still get a close estimation by putting 28 ml of salt solution into a 50 ml centrifuge tube first, and then adding feces until a volume of 30 ml is achieved.
2. Pour off approximately 25 ml of the salt solution into another small container keeping feces in the tube (can use tongue depressor).
3. Let soak for a few minutes and mix (soft feces) or break up (fecal pellets) with a tongue blade. .
4. Add back about ½ of the salt solution and mix well, breaking up any remaining feces as best as possible.
5. Add back the remaining salt solution and screw the cap back onto the tube.
6. Shake tube vigorously for about 1 minute to disrupt any remaining feces as much as possible.
7. Set tube aside for a few minutes to let bubbles dissipate.
8. Wet McMaster chamber with water and dry top and bottom on paper towels.
9. Rock (don't shake) tube several times to thoroughly mix solution without causing large air bubbles to form.
10. Immediately pipet (using 1 ml syringe or eye dropper) a sample of the suspension and fill both sides of counting chamber. Work quickly. If it takes more than a few seconds to load the first chamber, then mix fecal solution again and refill pipet before loading the second chamber.
11. Let stand for 1-2 minutes to allow eggs to float to top.
12. Count all eggs inside of grid areas (greater than 2 of egg inside grid) using low power (10x) objective. Focus on the top layer, which contains the very small air bubbles (small black circles, if numerous large air bubbles are visible, remove the fluid and refill).
13. Count only trichostrongyle/strongyle eggs (oval shaped, ~ 80-90 microns long). Do not count strongyloides (oval, ~ 50 microns long), tapeworm eggs (triangular/D-shaped) or coccidia (various sizes). Notations are made as to the presence of other species, but only the trichostrongyle/strongyle eggs are counted.
14. Once filled, the chambers can sit for no longer than 60 min before counting without causing problems. Longer than this and drying/crystal formation may begin.
15. Total egg count (both chambers) x 50 = EPG (eggs per gram).
 - a. Note: This is a dilution technique and theoretically this ratio of feces to flotation solution will not detect infections with less than 50 eggs per gram of feces (1 egg seen on slide), so it is not very accurate for samples with low numbers of eggs. On a practical level this is not important because from a clinical standpoint, slight differences in results when egg counts are low do not matter.

Notes:

Fairly soon after counting is complete thoroughly rinse out the McMaster chamber with warm running water. Doing so will keep the chamber clean and ready it to be used again. If fecal solution dries in the chamber do not soak in soapy water for long periods as this will cause the chamber to become cloudy. If the chamber gets dirty, soak for only a few minutes in water containing dish soap and then rinse completely with tap water. This is one method for performing a McMaster fecal egg count. Other different but similar protocols are routinely used in many labs, so you may see a slightly different procedure recommended elsewhere. The important thing is to use the same procedure each time.