Standard Centrifugation Fecal Examination Technique

Supplies:

Fecal collection canister (with fresh feces)
Disposable scoopula/smoothie straw
Disposable test tube with lid
Test tube rack
Funnel
Cheesecloth (double layer)
Clean coverslip, microscope slide & microscope
Sugar floatation solution* in a glass bottle** with lid
Disposable plastic pipette

Figure 1. Fecal collection canister with attached lid and "liner."

Instructions:

1. Collect approximately 2-5 g of feces (2-5 cc).
2. Partially remove the blue plastic "liner" of the fecal collection canister, see Figure 1. You may need to use a disposable scoopula (smoothie straw) to keep feces in the collection canister, scraping fecal debris out of the plastic "liner".
3. Add sugar flotation solution* to the fecal collection tube (approx. half full) using the disposable pipette. Be careful not to fecal contaminate the pipette or the bottle of sugar solution.
4. Using disposable scoopula, mix feces with the added flotation solution to make a slurry.
5. Set up filter apparatus as shown in Figure 2 (disposable test tube, test tube rack, funnel and cheesecloth). SAVE the test tube lid for later.
6. Pour slurry from fecal collection canister into the filter apparatus (Fig. 2). Use the pipette and sugar floatation solution to rinse out fecal collection canister into filter apparatus. Use scoopula to agitate the solution in the cheesecloth so it goes into the test tube.
7. Dispose of cheesecloth, fecal collection canister and (blue) liner. Remove funnel, placing it onto paper towel.
8. Add additional sugar floatation solution to the disposable test tube until filled to 13-14 mL.
9. Cap the disposable test tube and place into BALANCED centrifuge.
10. Centrifuge*** at 1200 rpm (280 x *g*) for **5 minutes.**
11. Remove tube and let stand in test tube rack. Take off cap and set it aside for later.
12. Without disturbing the solution below, add additional sugar flotation solution to the tube until a slight positive meniscus forms as shown in Figure 3.
13. Allow to stand undisturbed for **10 minutes.**
14. Place a coverslip on the tube. Allow it to stand an additional **10 minutes undisturbed.**
15. Remove the coverslip (being careful not to allow sugar solution to drip onto the top of the coverslip; make sure your fingers do not transfer sugar solution to the top, either). Add the coverslip (sugar side down) to a microscope slide.
16. Systematically examine the entire area under the coverslip at 10X magnification as shown in Figure 4. Note that allowing the slide to sit for an extended length of time or overnight will result in osmotic distortion or accumulation of sugar crystals rendering diagnosis nearly impossible.
17. If you find a suspected fecal parasite/egg, compare to the chart. You may wish to use the 40X objective lens to confirm. However, most parasites can be identified at 10X objective lens. *Giardia* cysts and *Cryptosporidium* oocytes may require 40X objective lens (400X total magnification) and possibly even oil immersion.

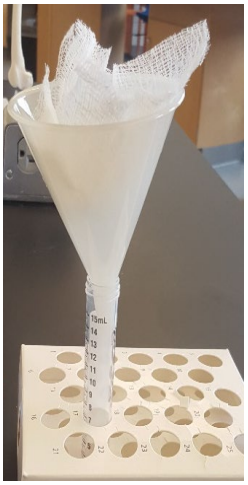

Figure 2. Filter apparatus assembly

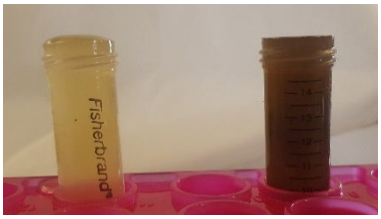

Figure 3. Tube on left has positive meniscus. Tube on right does not.

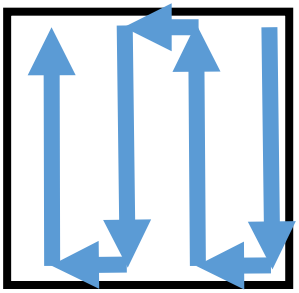

Figure 4. Square represents the coverslip. Pattern for systematic examination of slide.

***If sugar flotation solution is unavailable,** sodium nitrate solution (specific gravity 1.20) can be used with less time sitting undisturbed (it is not as viscous as sugar solution). However, sodium nitrate has a greater likelihood of osmotic distortion.

****Glass bottle with lid and disposable pipette** are convenient for this lab. Sugar solution **must** be kept in closed container to keep concentration accurate. Squeeze bottles of sugar solution are not recommended. The sugar solution will "gum up" the squeeze bottle straw and be useless for the next lab.

*****If centrifuge is unavailable** for use with sugar floatation solution, gravity may be used as long as the tube is allowed to stand longer than the protocol above. Omit steps 8-11. Allow 20 minutes with the coverslip sitting on top of the tube (instead of steps 13-14).

Procedure modified from Comparison of Common Fecal Floatation Techniques for the Recovery of Parasite Eggs and Oocysts.
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