## COMMENTS ON PERFORMANCE OF FECAL IMMUNOASSAYS

Some comments that may be helpful in performing various immunoassay formats are provided to assist you in test performance and/or result interpretation. **It is very important to read the kit information sheet before use.** Currently, fecal immunoassays are available for *Giardia lamblia,* the *Entamoeba histolytica/E. dispar* group*, Entamoeba histolytica,* and *Cryptosporidium* spp*...* Development of reagents is also ongoing for *Dientamoeba fragilis* and the microsporidia. Some reagents are available in the research setting for *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, but they are not FDA approved. Based on the published literature, fecal immunoassays are more sensitive and specific than the routine Ova and Parasite (O&P) examination; this is particularly true for *Giardia lamblia*. However, unlike the O&P exam that facilitates the recovery of many different parasites, the fecal immunoassays are limited to one or two organisms only. The fecal immunoassays are also more sensitive than the special stains (modified acid-fast stains) for the coccidia (*Cryptosporidium* spp.)

Fresh specimens can be stored at 2-8°C and should be tested within 48 h or they should be frozen at -20 to -70°C (freezing not acceptable for FA method – freeze/thaw cycle will damage organisms). Stool specimens preserved in 10% formalin, MF, or SAF fixatives may be refrigerated (2-8°C) or stored at room temperature (20-25°C) and should be tested within 2 months. Stool specimens submitted in Cary Blair transport medium (or equivalent) should be refrigerated or frozen and tested within 1 week after collection. Fecal specimens that have been preserved in fixatives containing PVA are not acceptable for testing.

To enhance the sensitivity of the FA procedure, it is recommended that testing be performed on centrifuged stool (500 X *g* for 10 min).

**Enzyme immunoassays (antigen detection, no centrifugation recommended); the antigen will be found in the top fluid layer of the stool collection vial**

1. Remember to thoroughly rinse the wells according to the instructions; do not eliminate any of the rinse steps. Make sure each well receives the total number of rinses required.
2. Make sure the stream of buffer goes directly into the wells. Use a wash bottle with a small opening, so you have to squeeze the bottle to get the fluid to squirt directly into the wells.
3. When the directions tell you to "slap" the tray down onto some paper towels to remove the last rinse fluid, make sure you slap it several times. Don't be too gentle; the cups will not fall out of the holder.
4. Prior to adding the last reagents, the wells should be empty of rinse buffer (not dry, but empty of excess fluid).
5. **Note**: If you shake the specimen vial prior to testing, allow the vial contents to settle out for several minutes. Adding too much particulate stool to the wells will interfere with testing.

**Fluorescence (visual identification of the organisms)**

1. Since you will be looking for the actual organisms (cysts of *Giardia* and/or oocysts of *Cryptosporidium*), this test should be performed on centrifuged stool (500 X *g* for 10 min) to increase the sensitivity.
2. Remember to thin out the smear; it is important to make sure the slides are thoroughly dry before adding reagents. The slides can be placed in a 35ºC incubator for about 30 minutes to an hour to make sure they are dry prior to processing. If the material on the wells is too thick, it may not dry thoroughly and may fall off of the glass. It is better to let them dry longer rather than too short a time. A heat block is NOT recommended for this purpose.
3. <u>Gently</u> rinse the reagents from the wells; do not squirt directly into the wells, but allow the rinse fluid to flow over the wells.
4. Remember that not all clinical specimens will provide the 3+ to 4+ fluorescence that we often see in the positive control. Also, from time to time, you may see fluorescing bacteria and/or some yeast in certain patient specimens. This is not that common, but the shapes can be distinguished from *Giardia* cysts and/or *Cryptosporidium* oocysts.
5. The intensity of the fluorescence may vary, depending on the filters. If the fluorescent microscope dual filter system is used, it demonstrates both the yellow-green fluorescence and the red-orange counterstain and both *Giardia* and *Cryptosporidium* may not appear quite as bright as when using the yellow-green filter only. Both approaches are acceptable and may reflect personal laboratory preferences. However, remember that when the single FITC (yellow green) filter is used alone, some artifact materials may also appear to fluoresce more brightly,

while the artifact material might not be seen when both filters (FITC and counterstain) are used. Artifact material may fluoresce a dull color without the bright outlines seen around the *Giardia* cysts and *Cryptosporidium* oocysts that can be seen using both filter systems.

**Both filters = less fluorescence intensity, less visible artifacts**

**Single FITC filter = brighter fluorescence, more visible artifacts**

6. Make sure to examine the edges of the wells. Sometimes in a light infection, the edges may contain organisms, while in the middle of the well the organisms may be a bit more difficult to detect (thick area).

**Lateral Flow Cartridges (antigen detection, no centrifugation recommended); the antigen will be found in the top fluid layer of the stool collection vial**

1. If the stool is too thick, the addition of reagents will not thin it out enough. If the specimen poured into the well remains too thick, the fluid will not flow up the membrane. If your specimens arrive in fixative and there is no fluid at the top of the vial overlaying the stool, this means the vial may have been overfilled with stool. These specimens will have to be diluted with the appropriate diluent before testing.
2. It is always important to see the control line indicated as positive all the way across the membrane, not just at the edges.
3. A positive test result may be much lighter than the control line; this is normal.
4. At the cutoff time to read the result, any color at all visible in the test area should be interpreted as a positive.
5. Do not read/interpret the results after the time indicated in the directions; you may get a false positive result.
6. **Note**: If you shake the specimen vial prior to testing, allow the vial contents to settle out for several minutes. Adding too much particulate stool to the wells will interfere with testing.

References:


1. **Garcia, L.S. and R.Y. Shimizu**. 1997. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J. Clin. Microbiol*. **35:**1526-1529

2. **Garcia, LS and R.Y. Shimizu**. 2000. Detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens using the ColorPAC combination rapid solid-phase qualitative immunochromatographic assay. *J. Clin. Microbiol*. **38**: 1267-1268.

3. **Hanson, K. L., and C. P. Cartwright**. 2001. Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia lamblia. J. Clin. Microbiol*. **39**:474-477.

4. **Isenberg, H.D.** (ed.), 2004. *Clinical Microbiology Procedures Handbook*, 2nd ed. ASM Press, Washington, D.C., Parasitology Section in Vol 2 of 3 vols.

5. **Wilson, M., and P.M. Schantz**. 2000. Parasitic immunodiagnosis. In. Strickland, G.T. (ed), *Hunter's Tropical Medicine and Emerging Infectious Diseases*, 8th ed. W.B. Saunders Co., Philadelphia, pages 1117-1122.