Tips on stool processing and staining

Processing or staining step	Commentary and tips
Direct wet mount – water or saline	In general, organisms do not maintain their typical morphology in distilled water; even in saline, trophozoites may not maintain adequate morphology very long; even with viable organisms, motility may be difficult to see (lower microscope light intensity); direct wet mounts should be prepared on fresh (unpreserved) liquid or very soft stools only (more formed stools will usually not contain any trophozoites.
Direct wet mount – iodine	If the iodine is too dark, it may interfere with helminth egg detection; eggs will resemble debris and/or artifacts; internal protozoan morphology may be too dark; iodine should be the color of strong tea; iodine will kill the organisms, thus no motility will be seen
Concentration - sedimentation	Rinse solutions can be water, saline, or formalin (regular 5% or 10% solution); do not rinse too many times (the supernatant fluid does NOT have to be clear); the more you rinse, the more likely you are to lose organisms each time; although a cleaner sediment may be easier to read, too much rinsing will result in lost organisms; a dirtier sediment is preferred to maximize organism detection; do not make coverslip preparations too thick (thinner is preferable); EVERY centrifugation step should be at 500 x *g* for 10 min
Concentration – flotation	Rinse solutions can be water, saline, or formalin (regular 5% or 10% solution); do not rinse too many times (the supernatant fluid does NOT have to be clear); the more you rinse, the more likely you are to lose organisms each time; many labs use a single rinse or two at the most; it is important to use the correct specific gravity zinc sulfate (1.18 for non-formalin fixed material, 1.20 for specimens received in formalin); BOTH the surface film and sediment must be examined (not all parasites will float using the zinc sulfate method). Because both the surface film and sediment must be examined using this method, most laboratories use the sediment concentration method.
Permanent stained smear – smear preparation	The smear should be neither too thick or too thin (you should be able to read newsprint through the fecal smear); if using PVA preserved specimens, make sure the excess PVA is removed from the stool prior to smear preparation (absorb excess PVA from specimen by placing a bit of specimen onto paper towels – wait a few minutes – then prepare smears); smears should be thoroughly dry prior to staining (with the exception of using liquid Schaudinn's fixative); **formalin-fixed stool is not appropriate for permanent staining, although SAF can be used with iron-hematoxylin stain**

Permanent stained smear – staining	If specimen is preserved in a fixative containing mercuric chloride, it is mandatory that all mercury be removed prior to staining (iodine and subsequent alcohol steps); dehydration (moisture removal) must be complete or protozoan morphology will be difficult to see; the best dehydration occurs using absolute ethanol; if the “commercial” absolute alcohol is used (ethanol plus other alcohols), the dehydration is not as complete, so dishes containing this solution must be changed more often (at least weekly and every few days if staining more than a few slides at a time); xylene substitutes do not dehydrate as well as xylene (dishes must be changed more often); xylene substitutes take longer to dry, however, slides must be thoroughly dry prior to mounting with immersion oil; **formalin-fixed stool is not appropriate for permanent staining, although SAF can be used with iron-hematoxylin stain**