Purification of *Brugia* microfilariae by filtration

MATERIALS:

Buffer of choice
5 or 10 mL syringes
syringe filter holders (such as 25 mm easy pressure from Pall, order number PN 4320)
25 mM membranes, 5 uM pore size (such as Millipore Isopore TMTP filter, order number TMTP02500)

1. Assemble syringe filter with prewetted membrane and O-ring. Remove the plunger from the syringe, and attach the syringe barrel to the filter.

2. Add the liquid containing your mf solution (peritoneal wash, blood, etc.) to the syringe, to a maximum of 1/10 the syringe capacity. Fill the rest of the syringe with buffer, and use the plunger to push the sample through the filter. Unscrew the syringe from the filter, then remove the plunger from the syringe barrel.

3. Reattach the syringe barrel to the filter, fill with buffer of choice, and use plunger to push sample through membrane. Repeat washing as necessary (typically washing twice will remove the majority of host cells and other contaminants).

Two strategies to recover mf from membrane:

4a) Centrifugation: Disassemble the syringe tip filter unit, and place membrane into a 15 mL conical tube. Rinse surface with 1 mL buffer, remove membrane from tube, and centrifuge tube to collect mf. If RBCs visible in pellet, lyse them in water by tapping the tube to disrupt pellet, then add 3 mL dH_20. After 15 seconds, add 12 mL PBS and wash. You may need to repeat this. (IMPORTANT NOTE: DO NOT USE WATER IF THE MF ARE TO BE USED IN MOSQUITO INFECTIVITY EXPERIMENTS)

4b) Floating: Disassemble the syringe tip filter unit, and place membrane face down into a puddle of buffer in a small watch glass. Use membrane forceps to gently agitate membrane, allowing mf to swim away. I routinely use 250 – 500 uL buffer in watch glass for this purpose.

Enumeration of mf: see 'HARVEST OF B. MALAYI MICROFILARIAE FROM GERBIL PERITONEUM USING DENSITY GRADIENT CENTRIFUGATION'

SHELLY MICHALSKI 8-28-09