Harvest of *Brugia* microfilariae from gerbil peritoneal wash by density gradient centrifugation

1. Fill a 10 mL syringe with sterile PBS, inject into peritoneal cavity of euthanized jird (18 mL if using anesthetized jird). Massage the abdomen and withdraw fluid. Alternately, you can insert a transfer pipet through an abdominal incision. Transfer peritoneal wash to a 15 mL conical tube.

2. Centrifuge in tabletop centrifuge for 10 minutes at 2,000 rpm, 10°C. Aspirate supernatant and resuspend sediment in 5 mL PBS.

3. Put 5 mL lymphocyte separation media (like HistoPaque) into a 15 mL conical tube. Overlay the mf suspension over it gently. Centrifuge again for 15 minutes. After the spin you will see the mf at the bottom of the tube. Carefully aspirate supernatant, including cell layer at interface of the PBS and the cushion.

4. Wash sediment twice with PBS at same speed. 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)

5. Resuspend the mf in 10 mL PBS. Count 10 μL of a 1:100 dilution using the light microscope (no cover slip needed). Do this in triplicate and obtain a mean mf count for that dilution. Meanwhile, pellet the mf and resuspend in 1 mL of PBS. Transfer to a microfuge tube and spin at 5,000 x g. Remove all supernatant and flash freeze pellet in liquid nitrogen or dry ice/ethanol (make sure you use an appropriate marker that won't come off).

These mf should be fine for RNA isolations or antigen preps. The number and viability depend on the age of the infection and history of previous harvests in that gerbil. You should expect to see >95% motile mf with occasional eggs and very few gerbil cells.

Yield:

$$\frac{\text{mean \# mf counted}}{\text{10 ul of 1:500}} \times (5 \times 10^5) = \text{total \# mf in 10 mL}$$

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