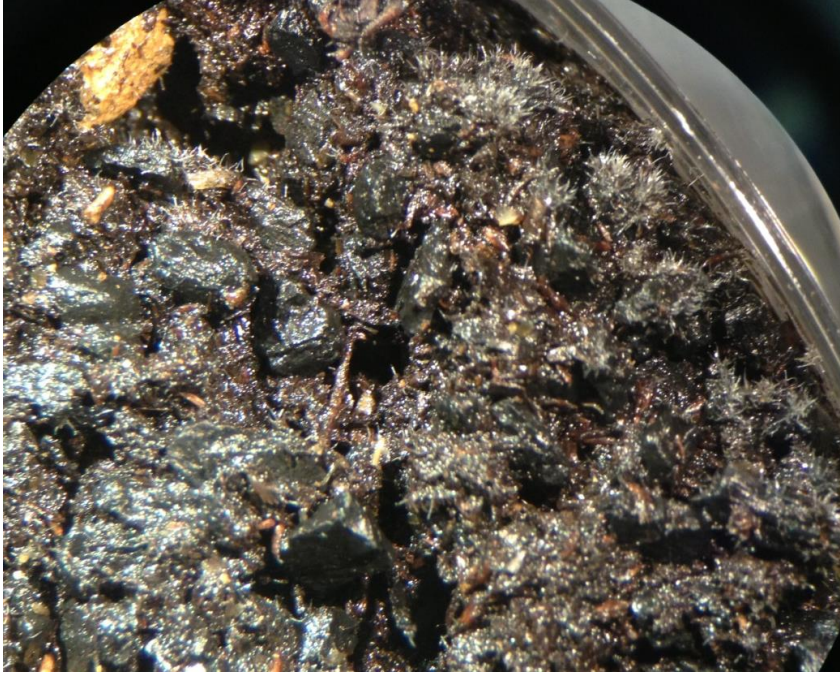


N. brasiliensis (Nb) isolation protocol:

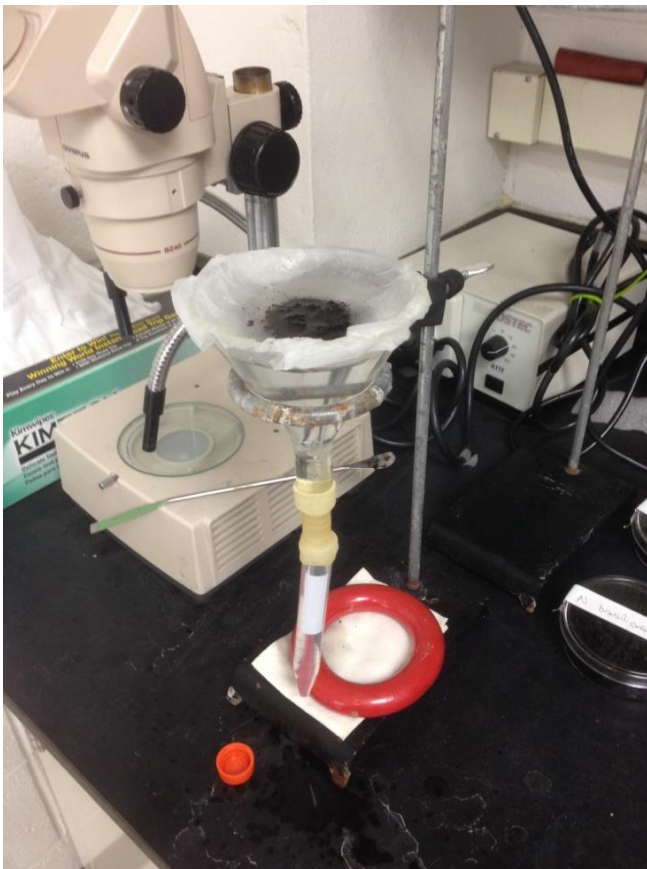
- Nb L3 larvae should be visible under a dissecting scope (Picture 1). Set up a small Baermann apparatus – connect a glass funnel to a 15mL conical tube with rubber tubing and attach to a ring stand (Picture 2). Pour warm (37°C) PBS into the funnel so that the liquid is about a half-inch from the top. Lightly flick the rubber tubing to ensure that there are no air bubbles in the tubing. (**Note:** We add antibiotics to the PBS in our Baermann during the isolation – 400ug/ml Neomycin Sulfate (Gibco) and 400 U Penicillin & 400ug/ml Streptomycin (Cellgro))
- Place a kimwipe over the top of the funnel so that it rests on top of the warm PBS. Ensure the ends of the kimwipe adhere to the rim of the funnel and that there are no air bubbles under the kimwipe. Fold the edges of the kimwipe over on the top the rim of the funnel so PBS doesn't drip down the side.
- Gently add small portions of the Nb culture plate (that have visible larvae) on top of the kimwipe with a clean spatula. Nb larvae should be visible migrating through the kimwipe after addition of the culture portions. It takes roughly 1.5 hrs for the larvae to get to the bottom of the conical tube. (**Note:** Larvae may also get stuck on the sides of the funnel during isolation and not make it into the conical tube. After most of the larvae have collected at the bottom, carefully remove the kimwipe and use an aspirating pipette to collect these larvae for maximum recovery)
- Clamp the tubing and remove the 15mL conical tube from the Baermann funnel. Spin the larvae down for **3 minutes at 1000 rpm**, wash 2X with warm PBS, count and standardize to 5000 L3/mL (or other desired concentration).

Culture maintenance:

Hydrate the Nb culture once a week by pipetting ~5mL sterile distilled water over the culture area. Keep culture dish covered and store at room temperature.



Picture 1 - Nb L3 larvae viewed under dissection scope (with external light source)



Picture 2 - Small Baermann setup