Protocol for maintenance *N. brasiliensis (Nb)* life cycle:

- Infect 8-10 BALB/c mice via **subcutaneous** injection with 500 L3 larvae. (Immuno-deficient mouse strains on a BALB/c background can also be used for increased larval yields, but avoid repeated passaging through immune-deficient strains)

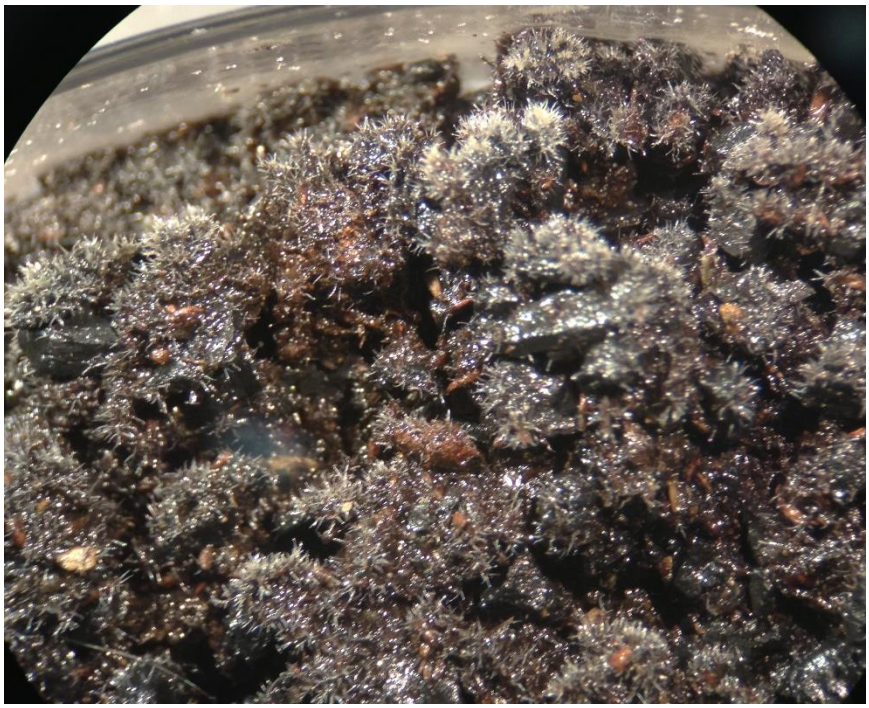
- At day 7 after infection, sacrifice the mice via cervical dislocation and collect the feces from the large intestine as well as the contents of the caecum in a 50mL tube of ~40mL warm PBS.

- Take equal volumes of autoclaved activated filter carbon and autoclaved soil/sphagnum moss (a 50mL conical tube of each) and thoroughly mix these contents together with the warm PBS+feces mixture with a spatula in a 1000mL beaker.

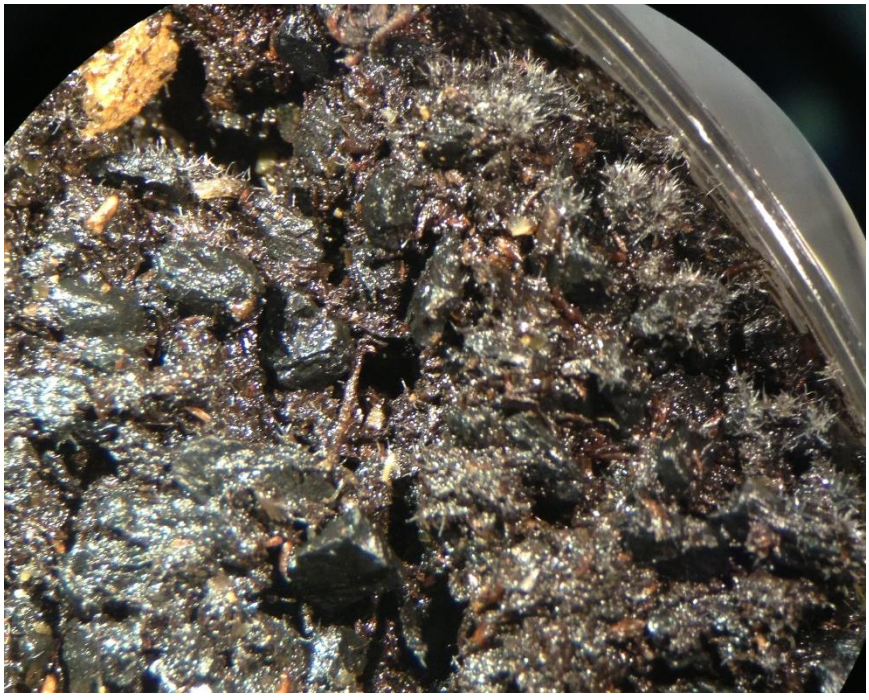
- Take a 100x25 petri dish and cut a kimwipe into a circle that fits the diameter of the bottom of the petri dish.

- Carefully add the mixture in the beaker into the petri dish with a spatula and ensure that the mixture is evenly distributed. The culture should be moist, but not so much that there is noticeable liquid at the bottom of the dish.

- Cover the plate and let it sit for at 7 days at room temperature. At day 7, there should be a lot of larvae visible in the culture if viewed under a dissecting scope with external light source. (If done correctly, the culture plate should show sufficient density with Nb L3 larvae – see pictures below) **Be sure to hydrate the culture every week with a few mL of sterile distilled water to ensure that the culture doesn't dry out.**



Nb culture plate with high density of L3 larvae



Nb culture plate with low density of L3 larvae