

PIJAC Pet Amphibian Trade Microbe Surveillance Pilot Study

Instructions for sampling

randomizing/selecting tanks

Instructional video here: <https://youtu.be/vTG-VeHI4JI>

Rational: We want to get an unbiased, representative sample from your facility. We therefore need to make sure that a random selection of tanks or containers are sampled.

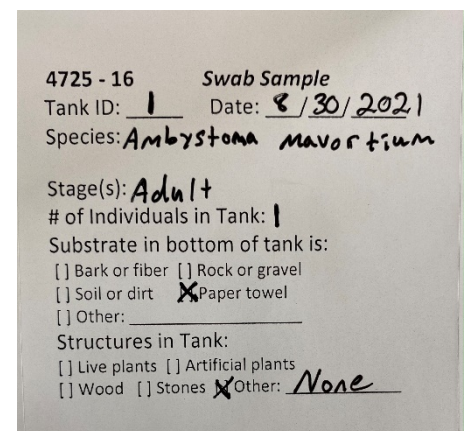
1. Go to: <https://brunnerlab.shinyapps.io/RandomNumGenerator/>
2. Enter the number of tanks in your facility (and optionally, the starting and ending number, if you already have a numbering scheme)
 - It does not matter how *you* number your tanks, just so that they are numbered in some sequential way
3. Click “Generate!”, and then print out list
4. Sample the tanks *in the order of this list*
 - You will likely not get to the end of the list with the materials we have provided. That is OK. Just go until you run out of materials or you can’t stand this exercise any more... We’ll take whatever you can give
 - You may run out of swabs before you run out of filters, or vice versa. This is OK, too. Just move on to the next tank in the list and sample it if you can

Swabbing individual animals from a tank (terrestrial housing)

Instructional video here: <https://youtu.be/L02EBWJsYDM>

Overview: We want to swab the skin of *up to* five individuals per tank. If there is one animal, swab that one. If there are 30, sample the first five you catch. If you run out of supplies, sample as many as you can.

1. Wear new gloves for each tank. You do not need to change gloves between individuals within a tank
 - If you have sweaty or clammy hands, you may wish to double-glove to make it easier to change the outer pair of gloves between tanks
2. Fill out the label on the WhirlPak bag (**please use a fine tip permanent marker as pens will smudge**)
 - Tank ID (to relate results back to a tank)
 - Species (Latin binomial, please)
 - Life stage(s) (Larva vs. adult)
 - Number of individuals in the tank
 - Type of substrate in the tank (bark or fiber, soil or dirt, paper towel, or other)
3. Swab the first animal
 - Open the swab
 - Hold the animal firmly so that it will not escape



4725 - 16 Swab Sample
Tank ID: 1 Date: 8/30/2021
Species: Ambystoma mavortium
Stage(s): Adult
of Individuals in Tank: 1
Substrate in bottom of tank is:
 Bark or fiber Rock or gravel
 Soil or dirt Paper towel
 Other: _____
Structures in Tank:
 Live plants Artificial plants
 Wood Stones Other: None

Figure 1. Example label for swab bag

- It can be helpful to place the animal in a sandwich bag, as seen in *Figure 2*, to limit its movement. If you decide to do this, use a clean bag for each animal.
 - Run the swab along the animal’s skin, pushing just enough to bend the swab-handle
 - Be sure to roll the swab against the motion of movement
 - Follow this pattern:
 - 5 times on dorsal surface/back
 - 5 times on ventral surface/belly
 - 5 times on each foot
 - 5 times on inguinal region/drink patch/around the cloaca
 - Place swab in the holder to air dry for a few minutes (*Figure 3*)
4. Repeat until all animals swabbed (up to five individuals)
 - Do not sample the same animal twice, please. Set aside those animals you have swabbed until you are finished
 5. Place all swabs in the labelled WhirlPak bag, swab side down
 6. Add three desiccant packs
 7. Roll up end of bag and twist twist-ties at ends to seal the bag
 8. Move on to the next tank or container on the list



Figure 2. Salamander in bag being swabbed

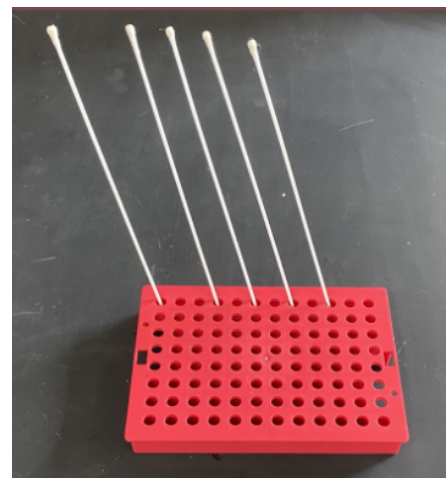


Figure 3. Example of swabs drying in provided rack. Make sure that swabs from different tanks don't touch each other if drying at the same time.

Sampling environmental DNA (eDNA) from water (Aquatic housing)

Instructional video here: <https://youtu.be/JcfBY2JnGJ0>

Overview: We want to collect DNA-bearing materials (skin sheds, mucous, crud, etc.) from the water housing animals. We want a single eDNA sample per tank. Filtering more water is “better,” but please do not break the filter (or your fingers!) trying to push too much through. We will take what we can get. That being said, please tell us how much water was pushed through the filter and the overall volume in the tank.

1. Wear new gloves for each tank
 - If you have sweaty or clammy hands, you may wish to double-glove to make it easier to change the outer pair of gloves between tanks
2. Fill out the label on the WhirlPak bag (**please use a fine tip permanent marker as pens will smudge**)
 - Tank ID (so that you can relate results back to a tank)
 - Species (Latin binomial, please)
 - Life stage(s) (Larva vs. adult)
 - Number of individuals in the tank
 - Approximate volume of water in tank
 - Please provide units! (Metric is preferred, but we can convert)
 - Leave “Volume filtered (mL)” blank until after filtering
3. Open large (60 mL) syringe and filter from wrappers
 - keep the filter wrapper so you have a clean spot to place the filter
4. Filter water
 - Draw up 50 mL water (use gradations on syringe)
 - Screw filter onto filled syringe (*Figure 5*)
 - Push water through filter
 - Please do not filter water back into container
5. Repeat, filtering as much water as possible
 - Unscrew filter and put in a clean place (e.g., the filter’s wrapper)
 - Draw up an additional 50 mL water
 - Push water through filter
 - Be careful not to break the filter (*Figure 5*)
 - If water becomes very difficult to press through the filter it is likely clogged. Please stop filtering at this point
 - Write the volume of water pressed through the filter on the last line of the WhirlPak bag

4725 - 1 Filter Sample
Tank ID: 7 Date: 8/30/2021
Species: Agalychnis saltator
Stage(s): Adult, Larva
of individuals in tank: 2
Water volume in tank: 40L
Is water recirculated? (yes/no) []
[] One tank [] Multiple tanks
Is tank water filtered? (yes/no) []
[] Biofilter/sponge [] Charcoal
[] UV [] Other: _____
Volume filtered for sample (mL):

Figure 4. Example label for filter

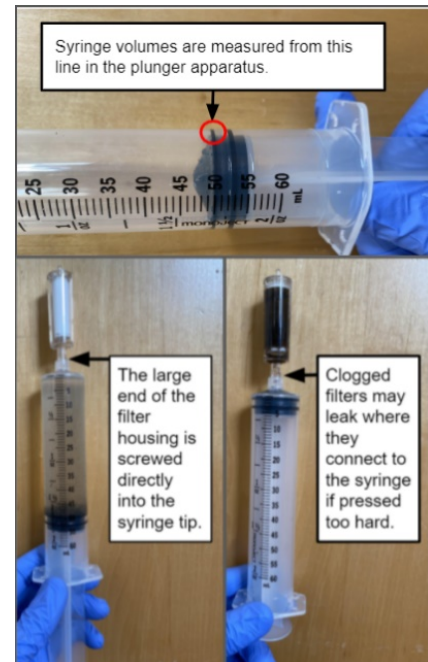


Figure 5. Instructions for syringe use.

6. Dry filter, two times
 - Unscrew syringe
 - Draw up ~50mL of air
 - Reattach syringe to filter
 - Push air through filter to remove excess water
 - Repeat
 - Remove large (60mL) syringe and discard
 - Tap the filter outlet on a clean paper towel to dry it

7. Seal filter outlet (*Figure 6, #1*)

- Take one of the red luer lock caps pre-filled with black polymer sealant, and press it over the filter's outlet

8. Add filter preservative (*Figure 6, #2*)

- Unscrew the luer lock cap from the small (3 mL), pre-filled syringe and secure the syringe to the filter
- Push the liquid from the small syringe gently into the filter housing with the sealed filter tip facing the ground
- The liquid should *just* fill the filter case

9. Fully seal the filter (*Figure 6, #3*)

- Remove the now empty 3mL syringe and dispose of it
- Acquire one of the red luer lock caps, without sealing polymer, and screw its larger end over the remaining filter opening

10. Place filled, sealed, filter in labelled WhirlPak bag

- Roll up end of bag and twist twist-ties at ends to seal the bag

11. Move on to the next tank or container on the list

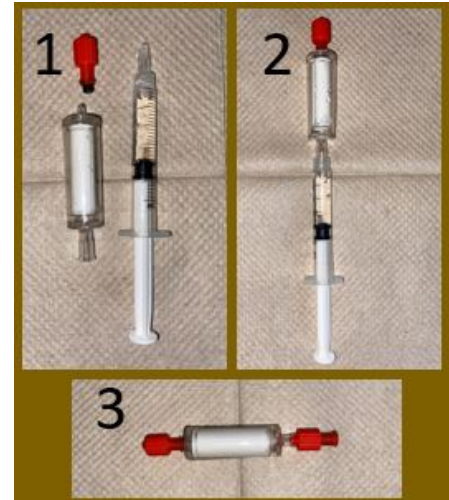


Figure 6. How to fill and seal the filter housing using the red cap with sealing polymer, 3mL syringe, and empty red cap

Preparing for shipping

- Create and write down a unique PIN (4-8 digits is fine) on the included sheet
 - Keep one copy and send the other to us
 - This is the only way you can retrieve your results, so *please be sure not to lose the PIN*
- Ensure all bags are closed and *labelled*
- Place all bagged samples in padded return envelope
- Make sure return label is firmly affixed
- Place in any outgoing mail drop

Questions?

- You can anonymously ask questions and see answers at the project website's FAQ (<https://onehealth.tennessee.edu/pijac-blog/>)
- If anonymity is not important, you can direct questions to the scientists or industry partners (<https://onehealth.tennessee.edu/pijac-contact/>)

- If you need additional supplies, please contact Josh Jones with PIJAC using the following: Email: josh@pijac.org, Phone: 202-452-1525

Thank you!