**Lindgren funnel trap fluid filtration protocol**

BIL EDRR pathogens, Garnas Lab

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*Materials*

*Filtration*

* Vacuum filtration assembly (Fisher FB3001000 and XX1014700)
  + Funnel and pedestal (i.e., parts that directly contact samples) should be bleach cleaned and autoclaved
* Sterile serological pipette and pipettor or bulb
* Autoclaved nanopure filtered DI water (FDIW) (100 ml per sample)
* Autoclaved 1 um glass microfiber filters (Fisher AP1504700)
* Autoclaved coin envelopes
* Autoclaved 100 ml graduated cylinder
* Forceps
* Bunsen burner or alcohol lamp
* 95% ethanol (for flaming)
* 10% bleach in tub for decontamination of filtration assembly (make fresh daily)

*Filter processing*

* Autoclaved watch glasses
* Surgical scissors
* Forceps (larger forceps work well for handling watch glasses and smaller work well for handling filters)
* Bunsen burner or alcohol lamp
* 95% ethanol (for flaming)
* Zymo ZymoBiomics lysis tubes (0.1 and 0.5 mm matrix)

*Preparation of filtration setup*

* The filtration funnel(s) and pedestal(s) need to be fully decontaminated between samples, as they are in direct contact with sample material.
* Plan to decontaminate enough filter setups for the number of samples that will be run (i.e., one funnel/pedestal pair per sample); this can be done at the end of the day as well to prepare for filtrations the next day.
* Start by rinsing the funnel/pedestal with DI water, then submerge in 10% bleach for 15 minutes, then rinse again with DI water to remove bleach.
* Wrap the top of the pedestal (part that is on contact with the filter) and the openings of the funnel with aluminum foil (to protect from contamination after autoclaving
* Autoclave for a minimum of 20-minute instrument cycle (e.g., P11 20/10) or may also be autoclaved with liquids on a liquid cycle.

*Filtration procedure*

1. Thaw the required Lindgren trap funnel fluid samples and sanitize bench space with 70% ethanol or 10% bleach.
2. Assemble the filtration setup by setting the stopper with base into the mouth of the vacuum flask and connecting the hose to the flask and vacuum.
3. Flame the forceps and place a filter centered on the base, then place the funnel on top of the filter and clamp to the base with the aluminum clamp.
4. Turn on the vacuum.
5. Using a new sterile serological pipette draw the desired volume and then dispense onto the filter.
   1. We are currently testing volumes for reproducibility of species detection and reliability of DNA extraction/PCR.
6. Measure 100 ml of autoclaved FDIW using the sterile graduated cylinder and then pour over the filter.
7. Turn off the vacuum and carefully remove the clamp and funnel.
8. Flame the forceps, remove the filter, and place directly into a labeled sterilized coin envelope.
   1. **If testing multiple volumes from the same sample** repeat steps 4-10 with the remaining desired volumes from the same sample. It is not necessary to decontaminate the filtration setup or change pipettes between volumes from the same sample but be sure to keep the pipette contamination-free by placing back in the wrapper between pipetting.
9. Store the coin envelope in the freezer in a bag or cryobox.
10. Remove the glass base from the vacuum flask and wash the glass base and the funnel with soap and water, then rinse thoroughly with DI water.
11. Decontaminate the glass base and funnel by submerging in DI water for 30 minutes before filtering the next sample.

*Filter processing procedure*

1. Sanitize the Laminar flow hood or appropriate bench space with 70% ethanol or 10% bleach.
2. Flame forceps and place one sterile watch glass on the work surface.
3. Retrieve a filter in coin envelope from the freezer and label one lysis (bead beating) tube with the sample ID on the side of the tube.
4. Flame the forceps and remove the filter from the coin envelope, placing on the watch glass.
5. Flame forceps and surgical scissors. Using the sterile forceps and scissors, cut the filter paper into quarters, and then cut each quarter into small (~1-2 mm wide) strips. For example, see **figure 1**.
6. Using forceps, carefully pack the cut-up filter paper into the lysis tube. The paper can be packed densely into the tube (it should all fit!)
7. Store the packed tube in a cryobox or tube rack in the freezer until ready for DNA extraction.

**Figure 1.** Filter cutting template. Cut the filter into quarters, then cut each quarter into strips following the pattern outlined in steps 1-3.



Figure 1. Filter cutting template. Cut the filter into quarters, then cut each quarter into strips following the pattern outlined in steps 1-3.