Pre-field protocol, in the lab

Water bottle & Metaprobe sampling, Researchers/Tour Operators

1. Clean working surfaces with 10% bleach solution
2. Bring in cleaned cool box
3. Retrieve cool packs from freezer, wipe them with 10% bleach solution, and place in the cool box.
4. Count and pack equipment and consumables as per the Field packing list - if in the storage cupboard, bring these into the lab to sanitise exteriors and pack into the cool box.
5. Exit the lab with the cool box locked and ready
6. Retrieve the protocols, emergency contact sheet, and project description sheets from the storage cupboard

Metaprobe sampling, Citizen Scientists

1. Clean working surfaces with 10% bleach solution
2. Bring in sampling event bag (or box)
3. Retrieve, count and pack equipment and consumables as per the Field packing list - if in the storage cupboard, bring these into the lab to sanitise exteriors before packing.
4. Retrieve the Citizen Science materials packet, which should include: What's in my Bag? Protocols, Protocols for Kids, Who to Contact sheet, and project description sheet

Post-field protocol, in the lab

Water bottle sampling

Consumables needed *for one sampling event*:

- 2 Stervex filters, 0.22um
- 2 luer lock 50mL syringes
- 1 luer slip 1mL syringe
- 2 whirl pak sample bags 58mL
- 2 combistoppers
- approx. .67mL of ATL buffer
- pump
- flask with spout (Buchner flask)

- tubing to connect flask to pump and flask to Sterivex
- 2 funnels
- chemistry stand

1. Clean working surfaces with 10% bleach solution
2. Collect consumables, do not extract the ATL buffer at this stage.
3. Put gloves on, and remove three 1L water bottles (WB) - 2 treatment (WBT), and 1 control (WBC) - from the cool box.
4. Set up pumping equipment (see photo), such that the pump is connected by tubing to the Buchner flask spout. The top of the flash is plugged with a tube flowing through. This tube connects to the bottom of the Sterivex. The Sterivex is locked onto the syringe end of the 50mL Luer Lock Syringe, which is held vertical in the air by the chemistry stand. Finally, a funnel is abive and slightly in the 50mL syringe to allow for easier pouring into the syringe.
5. First you will filter the control (distilled water).
6. Wearing gloves, open the WBC and slowly pour approx 40mL into the funnel. Turn the pump on, starting at a low value, and turning it up after you've ensured th ar is flowing through and there aren't any leaks (these would be obvious quickly).
7. Continue to slowly pour water form the WBC into the funnel until none is left in the bottle. Wait for pump to pull all water through the Sterivex.
8. When all the water has been pulled through, remove the tubing from the bottom of the Stervex filter. Untwist the filter from the syringe.
9. Using a cigarette lighter, heat the outlet (narrow end) of the Sterivex just untl it melts. Gently press and twist against a hard metal surface to permentenly seal this end (see image).

10. Prepare sample label. Record the following information on each label using gel pen: sample ID and total mL filtered (WBT=2L, WBC=1L)
11. Change gloves and repeat steps # - # for all samples in the sampling event.
12. Change gloves, and add preseravtive ATL buffer to all samples in succesion. Fill a new 1mL luer slip syringe to the 0.7mL mark.
13. Insert the syringe into the Sterivex luer inlet (wide end) and slowly inject half of the contents of the syringe (367 µL = 0.37 mL). Before removing the syringe, tap

the Sterivex to ensure the buffer drains from the neck of the Sterivex. Because of the pressure, sometimes the buffer comes straight back out, so be careful of that.

14. One syringe of ATL buffer will do one sample (i.e. one Sterivex from 2 WBT and one Sterivex from the control). **Never** put the syringe back into the ATL vial. Only take buffer once per Luer Slip syringe.
15. Use a combistopper to seal the luer inlet (wide end) of the Sterivex. Shake to mix the ATL buffer all over.
16. Place all sealed Sterivex from one sampling event in one Whirl-Pak bag. Label the bag with the event ID and the sample IDs
17. Freeze as soon as possible for long term storage.
18. Clean all equipment with 10% bleach solution. When sterile and dry replace into storage.

Metaprobe sampling

1. Clean working surfaces with 10% bleach solution
2. Wearing disposible medical gloves, remove the small sample bag with the three 50mL tubes from the cool box.
3. Put sample bag into the freezer.