# Fecal Coliform

**Note:** preheat incubator to 44.5º centigrade. Sterilize grab bottle, the top funnel of the filtration unit and the sample syringe in autoclave or boil for 5 minutes.

1. Collect water samples using sterilized bottles. (500ml or more….widemouthed bottle).

2. Wipe all work surfaces with a disinfectant. Wash your hands. Wear gloves.

3. Start with all equipment sterilized (boiled for 5 minutes of autoclaved), including filtration units, tweezers and syringes.

4. Label 4 pre-sterilized petri dish with the date, sample # (control, 1, 2 or 3) the volume of the sample (50ml or 100ml), and sample location on the frosted part.

**Examples:**

| | |
|---|---|
| 10/17/13 | 10/17/13 |
| Control | #1 |
| Nisqually River @ 6th Ave | Nisqually River @ 6th Ave |
| 100 ml | 100 ml |

5. Open the dish, being careful not to touch the pad with your fingers. Unscrew the neck of the broth ampoule and drain the broth onto the pad. Put the top back on the dish and set it aside.

6. Sanitize the forceps by dipping them in alcohol and then burning them off with a flame (matches or lighter).

7. Unscrew the top half of the filtration system and place a sterile filter paper on top of the membrane with the forceps, grid side up. (Be careful not to grab the light blue backing with the paper grid!)

8. Screw the top half of the filtration system onto the bottom half. Do not overtighten as the filter may tear.

9. Add enough deionized water to dampen the filter.

10. Fill the syringe to the 50 ml mark with deionized/distilled water (control) or sample water (for replicate #1, 2 and 3) and empty filled syringe through the hole in the top of the filtration unit. Do this twice for a 100 ml sample. *(Note: Most sites will use 100ml sample water, unless otherwise noted!)*

11. Make sure the filtration system is level. Using the suction pump, draw water through the filter. Swirl the system gently as you work the pump. This action reduces the number of bacteria adhering to the upper filtration system. Pump until the filter appears dry.

12. Re-sanitize the forceps.

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13. Unscrew the top half of the filtration unit and carefully remove the filter with the sanitized forceps.

14. Open the top of the petri dish and slide the filter across and into the dish with the grid side up. (Hold the petri dish as close as possible to the filtration system.) Replace the lid.

15. Empty the water into the hazardous water bucket. Repeat so there are 4 completed petri dishes for each water quality sample site (control, #1, #2, #3)

16. Invert the dish as you put in incubator (this allows condensation to rise, enabling you to see through top of dish). Incubate for 24 hours, plus or minus 2 hours.

17. Sterilize filtration units and syringes between each site's sample water.

18. Wash your hands.

## The following day…..

19. Count the colonies that are blue/purple/pink. Do <u>not</u> count yellow, brown, white or tan colonies. (Remember to multiply be necessary factor. For example, if you filtered 50ml of river water, multiply by 2 to get the number of colonies per 100 ml.)

20. Autoclave petri dishes or seal in a ziplock and throw away!

21. Wash your hands!