



Stream Steward E. coli Sampling Instructions

Field Sampling Materials

Defrosted Coliscan Easygel nutrient bottles	Gloves (latex or nitrile); put on fresh pair each time you sample
Clear waste bag ; For used pipette and gloves	Pipettes (disposable); Use new one for each sample
Pencil and permanent marker	Purple cooler bag(s) with freezer packs ; Store nutrient bottles in after sampling. Do not store bottles longer than 5 hours before inoculation.
Sample ladle and sampling stick (extends to 22 ft); To take water sample from stream	Thermometer
E. coli Binder	Clipboard and sample forms

Materials Preparation at Pickup Facility

1. Make sure you pick up all field sampling materials in the list above before leaving to do field testing.
2. The Coliscan Easygel Nutrient bottles are located in the freezer. On unusually warm days, or if you will not be able to place samples in the incubator within the 5 hour time window, also include a freezer pack in your purple cooler bag.

Field Sampling Procedure

1. Make sure Coliscan Easygel nutrient bottle is **defrosted**. If the bottle is still containing ice, hold it in your hand for a few minutes, or put in your pocket. Do not shake the bottle.
2. Use thermometer to **measure air** (first site only) and **water temperature** (all sites). Give thermometer at least 2 minutes to adjust to temperature between readings.
3. Mark the top of the cap of the Coliscan Easygel nutrient bottle with **sample#** and **site#** on the cap. Record the information on the **sample form**.
4. Wearing gloves, **triple rinse** the **sample ladle AND/OR the 5mL pipette** with stream water. Use a new pipette and pair of gloves for each sample!
5. Obtain a **water sample** from just below stream surface. Keep particles out of sample.
6. Use a disposable pipette to measure **5mL** of sample water.
7. Pour measured 5mL of sample water into the labeled **nutrient bottle**.
8. Screw nutrient bottle lid back on. Discard pipette and gloves in the included **waste bag** after each sample. Use new disposable pipette and new gloves for next sample.
9. Put nutrient bottle with added sample water into **purple cooler bag**. Bring back to the lab or designated work area within 5 hours.
10. Fill out your **sample form** with sample #, site #, date, time, your initials, temperature (both air and water), weather conditions, observations, wildlife etc.

Incubation Steps

at Enumclaw Wastewater Treatment Plant Lab or other work area

1. **Petri dish** (*lid and bottom, specifically for Coliscan Easygel*)

Fill in the sample information (sample number, site number, date/time, your initials) on the pre-printed label, affix label to top of Petri dish near the edge.
2. Wearing gloves, pour the mixture out of nutrient bottle gently (try not to get air bubbles into it) into the *smaller diameter Petri dish bottom* (this has the agar growing medium).
3. Place the *larger lid* onto the Petri dish bottom. Keep the dish flat to avoid getting liquid onto the sides or lid.
4. Using two small pieces of tape, carefully tape the lid onto the bottom dish. Affix the label to the lid of the Petri dish. If necessary, very gently swirl nutrient liquid around the Petri dish for even coverage. Don't splash liquid on the sides or the lid.
5. Place Petri dish without tilting or jarring it into the heated incubator. The sample will "set" in the agar growing medium after an hour. Incubator should *ideally* be 90°F to 95°F if it can be set this accurately, but it is *not required to be exact*.
6. On the sample form note the time you placed sample into the incubator.
7. Check incubated samples approximately 48 hours after placing into incubator.
8. Fill out E. coli data sheet which will be used for pictures later.
9. Record results on E. coli Data Recording Sheet and the Data Entry Sheet. Look for dark blue or purple colonies in the Petri dishes. Record as either the exact number or "too numerous to count" if the dish is practically full of E. coli.
10. Take pictures of E. coli colonies with the plate sitting on the E. coli Data Recording Sheet.
11. Enter results onto Data Sheet. Please take note of any extensive E. coli growth on your results. If possible, please enter your findings in the online form (<http://kcdstreamstewards.wordpress.com/data-entry-form/e-coli-data-entry-form/> Password is BoiseCreek). Please notify KCD when your testing is completed. KCD will contact King County to notify of potential urgent water quality problem.