

E. Coli analysis using Coliscan Easygel method

Before you go out for sampling, TURN ON THE INCUBATOR. This will help to attain a stable temperature. Set it to '3' (approximately) on the temperature setting knob.

SAMPLING

It is important that the sterile bottle remains sterile; therefore, **DO NOT RINSE and DO NOT ALLOW ANYTHING OTHER THAN RIVER WATER TO CONTACT THE MOUTH END OF THE STERILE BOTTLE OR THE INSIDE OR BOTTOM OF THE CAP.**

Step by step directions are provided below:

1. Place two thick elastic bands around the one liter sample bottle permanently mounted on your sampling pole.
2. Place the sterile bottle, capped end up (do not unscrew the cap yet), securely within the elastic bands so that it is securely affixed to the one liter sample bottle.
3. Once the capped bottle is securely held by the rubber bands, unscrew the cap. **Important:** To avoid contamination of the sterile bottle or cap, hold the bottle near the bottom and the cap near its top edge. If you set the cap down, make sure its open side faces up to prevent contamination. **DO NOT TOUCH THE INSIDE OF THE LID OR THE LIP OF THE BOTTLE.**
4. Using the sample pole, plunge the uncapped sterile bottle into the water to about 1 foot below the surface and allow the sterile bottle to fill (a small amount of air in the bottle is fine).
5. Once filled, remove the sampling pole from the river, re-cap the sterile bottle carefully. **DO NOT TOUCH THE INSIDE OF THE LID OR THE LIP OF THE BOTTLE.**
6. Remove the capped sterile bottle from the sampling pole and bottle, put it in a sealable bag and immediately place it in the cooler with ice. Before analysis, temperature of the sample must be kept between 4-6°C (*no freezing*) for a maximum of 6 hours.

ANALYSIS

- 1) Check incubator temperature for stabilization. It should read 32-37°C. If it is not reading within the range, adjust the temperature from the temperature setting knob and wait (10-12 minutes) for it to stabilize.
- 2) Analysis should be done indoors. A clean bench top should be used. Please note that pieces of equipment like sample containers, petri dishes and pipets are sterilized. Dirty hands (oil, dust, hand moisturizer, etc.), dust in the air or on the bench top could be some of the common sources of contamination.
- 3) Fill the information on the 'E. Coli data sheet'. Label the petri dishes with a permanent marker. **DO NOT invert the petri dishes. DO NOT take the lid off of the petri dish while labeling it.** Write down the following info on the lids of the petri dish:
 - site name (One analysis per site No replicates!)
 - date
- 4) Transfer 3ml of the water from the sample container into a bottle of Coliscan Easygel. **Swirl** (gentle; no vigorous shaking in order to avoid unnecessary air bubbles) the bottle to mix sample into the Easygel.
- 5) Pour the mixture into the correctly labeled Petri dish placed on the flat clean surface/bench top. Place the lids back on the petri dish as soon as possible. Make sure that the entire base of the dish is covered with the liquid. **Avoid splashing the liquid over on the side of the dish or on the lid (this could be tricky!).**
- 6) Incubate the petri dish/dishes at 35°C (= 95°F) for 24 hrs. Record the Incubation Start Time as well as the Start Temperature (displayed on the incubator) on the data sheet.

COUNTING THE COLONIES

- 1) After 24-30 hrs of incubation, take the petri dishes out of the incubator and record the Incubation End Time as well as the End Temperature (displayed on the incubator) on the data sheet.
- 2) Remove the top cover of the petri dish in a way that the **accumulated moisture does not fall on the media** and disintegrate one or more of the colonies.
- 3) Count all the PURPLE colonies on the petri dish (disregard any light-blue, blue-green or white colonies). Record the info on the 'E. coli data sheet'. **Be very careful when differentiating between pink and purple!**
- 4) Count the PINK colonies and report the info on the data sheet.

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Notes:

- a) *E. coli is one specific type of coliform bacteria. In simple terms, PURPLE color corresponds to one odd member (E. coli) of a large PINK family of coliform bacteria.*
- b) *PURPLE colonies correspond to E. coli population only and PINK + PURPLE correspond to total coliform population.*
- c) *If the water was relatively clean, it is very much possible that you may encounter very few (or none) PURPLE colonies.*

CALCULATIONS

E. coli population count is reported as CFU/100 ml. CFU stands for 'colony forming units'. The calculations for converting the colony count to CFU/100 ml of sample are as follows:

- a) Divide 100 by the number of ml of the sample used (you used 3ml of the sample in step #4 under analysis).
- b) Multiply the count for number of PURPLE colonies by the result obtained in a). This will give you the actual E. coli count in 'CFU/100 ml' units. For example, for a 3 ml sample, $100/3 = 33.3$. If you counted 2 PURPLE colonies, it will equal to 66.6 CFU of E. coli/100 ml of water sample. Report 66.6 under the E. coli column on the datasheet.

DISPOSAL

- 1) After recording all the data, place 5ml of undiluted bleach onto the surface of the solidified Easygel present in the petri dish, replace cover. Allow it to sit for 5 minutes.
- 2) Place the petri dish in a sealable water tight bag. Recycle all the plastics (pipettes, petri dishes, bottles).

STORAGE

- 1) **Easygel: Should be stored in the freezer!!**
- 2) Petri dishes and the pipettes: To be stored in a clean environment at room temperature.
- 3) Bleach: Store with other household chemicals.