Coliscan Easygel Procedure

Sampling

Sampling Safety:

When possible, sample in teams of at least two people in case a sampler is injured or needs assistance. Sampling along bridges is convenient but can be the most dangerous spot to sample from. Only sample at a bridge site if it is the only option and the bridge is wide enough to accommodate pedestrians and traffic. Park your vehicle a safe distance from the bridge and have the car's hazard signal on. Use a volunteer to spot incoming traffic while another volunteer samples. Do not sample on bridge that has a no loitering or no fishing sign posted.

When sampling, use gloves or have alcohol sanitizer available to keep hands clean as the water may be contaminated due to sewage.

If sampling along a streamside or in the stream, do not sample when water levels are dangerously high, such as after a major rainstorm. Do not wade into water that is swift flowing or unusually high. Sampling may occur where it is open to the public such as a public boat ramp and parks. If sampling occurs on private property, obtain landowner permission before selecting the site.

If sampling by boat, sample teams must wear a Personal Flotation Device (PFD) and are either knowledgeable in operating the boat or the boat is operated by a person with such knowledge/training.

Remember, it is not worth sacrificing your safety for a sample. If you feel uncomfortable with a sampling site, look for another location that you are more comfortable with.

General Comments:

When sampling streams and rivers, it is critical to obtain a **representative sample**. This means that the water sample should be obtained from the main flow of the water body. In small streams (assuming it is safe), it is best to wade into the main flow of the stream. Collecting the sample from bridges using a sampling bucket is best for larger or deeper streams provided the bridge is safe to sample from. Prior to sampling, remember to remove the bottle of Coliscan media from the freezer and allow it to thaw completely before the plating step.

Method 1 sampling using a bucket:

1. Lower a clean (not sterile) bucket from a bridge using rope and partially fill the bucket.

2. Retrieve the bucket and swirl the sample water and dump the contents away from the sample site.

3. Lower the bucket once more and fill part way with a water sample. Try not to collect excessive sediment, mud, or other debris in the bucket.

4. Retrieve the bucket.

5. Using a U motion, fill the sample bottle by moving it through the water from one side of the bucket to the other in such a way to prevent water from near the hand from entering the bottle. Fill the bottle about ½ to ¾ full.

6. Cap the bottle and label it with the station ID using a waterproof pen. For split samples, use -1 or -2 at the end of the station ID value to note which split sample it is.

7. Fill out the field sheet with the necessary information.

8. Immediately place the sample bottle on ice for transport to the plating area.

Method 2 sampling directly in the stream:

1. Walk (or use a boat to move) upstream a short distance with minimal disturbance of the sediment.

2. Facing upstream, open the sample bottle.

3. Carefully fill a sterile bottle by submerging it below the surface as you move the bottle **away** from your body in an **upstream** direction. This method ensures any bacteria from hands or boots (or boat) does not enter the bottle. Avoid letting mud and other debris enter the sample bottle. Fill the bottle about ½ to ¾ full.

4. Cap the bottle and label it with the station ID using a waterproof pen. For split samples, use -1 or -2 at the end of the station ID value to note which split sample it is.

5. Fill out the field sheet with the necessary information

6. Immediately place the sample bottle on ice for transport to the plating area.

Coliscan Sample Plating and Analysis

Plating

1. The day before sampling occurs, remove the number of Coliscan Easygel media bottles from the freezer and to allow overnight thawing. If this does not occur before the samples arrive, the media may be thawed in a bowl of warm tap water. DO NOT place bottles in boiling water or in the microwave as it will destroy the media.

2. Label the bottom (smaller, taller piece) of the Petri dish provided in the Coliscan Easygel kit using a permanent marker. It is best to label the dishes using small lettering on the outer rim of the dish. The minimum information needed should be the site ID number, sample volume, and replicate number (if needed).

3. Retrieve the sample bottles from the cooler or refrigerator. Samples should not be frozen and must be tested with 24 hours of collection.

4. Mix the water sample in the sample bottle by shaking or swirling for several seconds.

5. Transfer the desired volume (0.5 – 5.0 milliliters) to a bottle of Coliscan medium using a sterile pipette. Do not reuse the pipette for other sample bottles. Only work with one sample at a time to avoid confusing bottles.

6. Gently swirl the bottle of Coliscan media for several seconds so that it mixes with the sample water. Do not shake the bottle as this will cause the medium to foam and makes reading the colonies difficult during the counting phase.

7. Open the labeled Petri dish and pour the entire contents of the bottle into bottom portion of the Petri dish. It is important to perform this step on a level surface so the solution forms an even layer across the plate. DO NOT leave the Petri dish interior open to the air for longer than necessary as mold and other contaminates can enter and contaminate the plate.

8. If needed, gently swirl the Petri dish so the solution of Coliscan media and sample water covers the entire plate. Allow the solution to solidify (approximately 60 - 90 minutes) prior to incubation. Once solidified, taping both side of the Petri dish closed will help avoid lids accidently falling off.

Incubation

Incubate the Petri dishes **upside down** for 24 – 36 hours at 35° - 40° Celsius. This is approximately 95° - 105° F. If no incubator is available, place the Petri dishes in the safest, warmest spot area possible away from direct sunlight. If incubating at room temperature, plates may need to incubate for 48 to 72 hours.

Data Analysis (Scoring)
Use white or graph paper as a background to make identifications easier. If there are a large number of colonies (>100), drawing quadrants on the paper can help to count colonies.

1. Count the number of dark blue to royal purple colonies on each plate and record this number in field sheet. Do not count teal colored or pink – dark red colonies or colonies colored anything other than dark blue to royal purple.

2. If more than 60 *E.coli* colonies are counted, report the result as if the plate contained 60 colonies with the greater than (>) sign on the field sheet. If an accurate estimate of the number of *E.coli* is desired, perform one of the following steps.

    a. Using the drawn quadrant, count the *E.coli* colonies that are more than 50% inside each quadrant and add the values together. If there are more than 30 or so *E.coli* in a quadrant, count the colonies in a random quadrant and multiply the value by four. Record this value and proceed to step 3.

    b. If there is an extremely high number of *E.coli* seen and an accurate estimate is desired, place the Petri dish over a piece of graph paper.

        i. Calculate the surface area of the graph paper covered by the Petri dish by counting the number of squares across the fattest portion of the Petri dish bottom. Divide this value by 2 to get the radius (r) and use the formula: 3.142 * (r * r). For example, if the radius is 5 the result is- 3.142 * (5 *5) or 3.142 * 25 = 78.55 squares.

        ii. Count the number of *E.coli* that is over 50% contained in 10 random squares and divide this value by 10 to get an average value per square.

        iii. Multiply this average *E.coli* colony per square by the calculated surface area found in step i. Record this value and proceed to step 3.

3. Calculate the number of *E.coli* cells per 100 milliliters and record on the data form. Use the following formula: **(# *E.coli* colonies counted / ml sample size) x 100**

Disposal of Waste
1. Dispose used pipettes and sample bottles in as regular trash. They are also recyclable.

2. Rinse empty bottles of Coliscan medium one to three times with tap water and dispose as normal trash. (This is to wash out all of the media to prevent pathogens from growing.)

3. Wipe down the area where plates were prepared or counted with a sanitizing liquid. Lysol® wipes work well. Avoid performing the Coliscan test where food is present or prepared.

4. After recording the results on the field sheets, open and place dishes in a 1 gallon plastic Ziploc™ style bag and pour two to four ounces of bleach or rubbing alcohol into the bag and seal the bag shut. Shake bag for 30 seconds and dispose as normal trash.

Field Data Sheet

Submit sample bottles and completed field data sheets to:

Site Name and #: _____ Sampler(s):_____

Sample Date: _____(mm/dd/yyyy) Time: _____(hh:mm military format)

GENERAL OBSERVATIONS AND WEATHER CONDITIONS: (circle most appropriate)

Weather Type: Sunny, Partly Cloudy, Overcast, Fog/Haze, Drizzle, Intermittent Rain, Rain, Snow

Rainfall: _____ INCHES past 24 hours

Water Color: Clear, Muddy, Oily, Foamy, Milky/Gray, Green, Brown, Black, Iridescent Sheen, Ice, Other_____

Water Odor: Natural, Gas/Oil, Chlorine, Rotten Egg, Sewage, Chemical, Other_____

Additional Comments (e.g. wind, recent events, wildlife, dead fish, debris, anything unusual):

_____

_____

_____

Was Coliscan sample collected: ☐ Yes ☐ No Sample placed on ice: ☐ Yes ☐ No

Split sample collected: ☐ Yes ☐ No

Total time Spent Monitoring: (Includes travel to and from monitoring site, equipment preparation, sample collection, and time spent filling out data sheet _____hours (Round to the nearest 15 min.)

Monitor Signature: _____ Date: _____

Coliscan Easygel Plating and Counting Results: Performed upon return from sample run

Incubation time: _____hours (to nearest hour) Incubation temp: ____. ___^{o} C (to nearest half degree)

Amount of water sample added to media bottle (max 5 ml): Do Split 2 if a split sample received

Sample 1: _____ml (A1) Split 2: _____ml (A2)

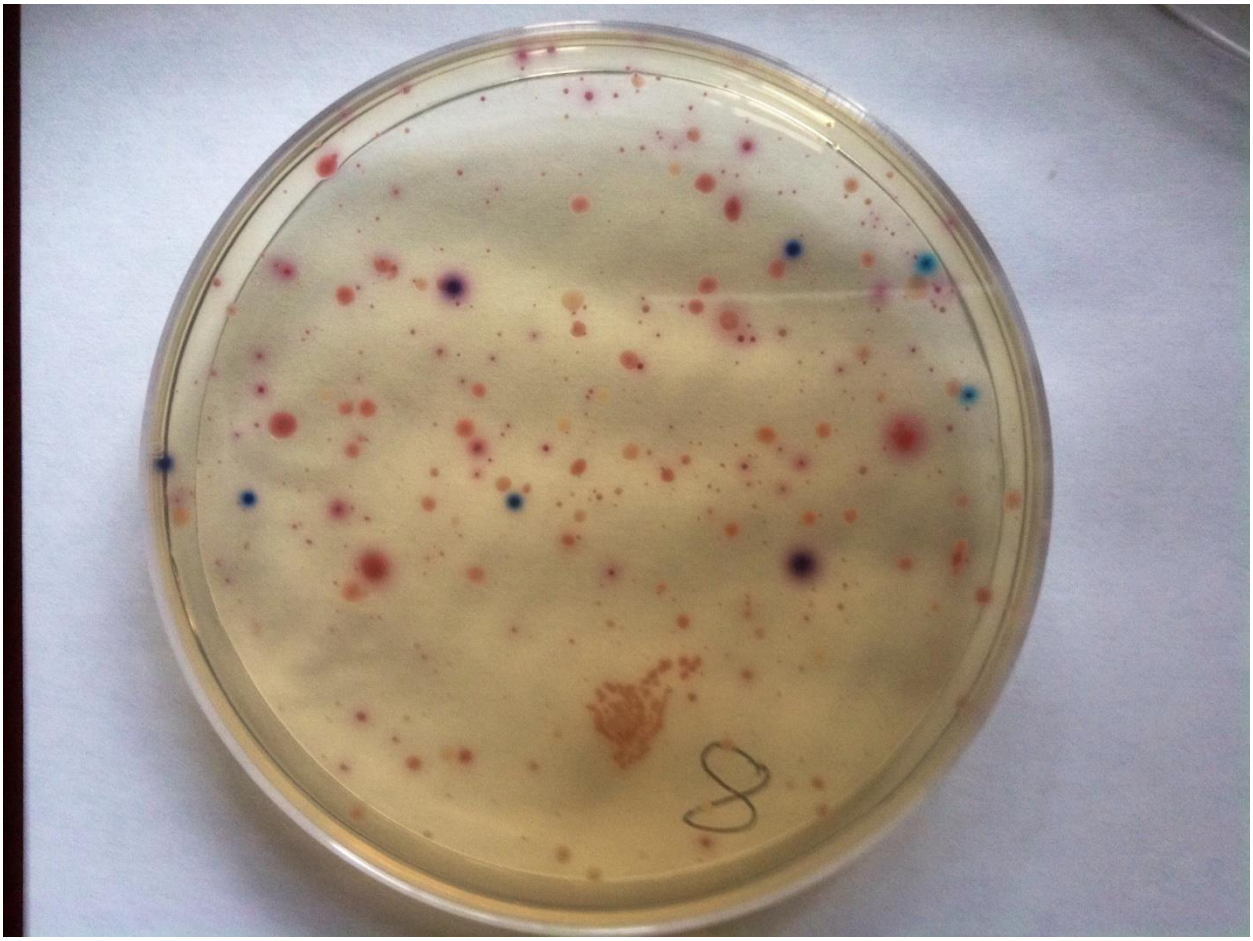
Total # of <u>royal purple or dark blue colonies</u> on plate:

Sample 1: _____(B1) Split 2: _____(B2)
Note: disregard any pink, blue-green or white colonies - these are not *E.coli* bacteria

To calculate the Total Colonies of *E.coli* bacteria per 100 ml of water:
1. Divide 100 by the ml of water used. 2. Multiply this quotient by the number of purple colonies counted

Sample 1: ([100 / A1] * B1) = _____CFU/100ml Split 2: ([100 / A2] * B2) = _____CFU/100ml
Note: Round up results that end in a fraction (ex. 33.3333 is reported 34). If zero colonies are counted, record result as if 1 colony was counted on the plate and use the less than (<) sign.

Coliscan Colony Guide



8 E. coli colonies rangeing from dark blue to royal purple. Note two clonies on the far right appear with a teal halo. The center is dark blue so is counted as E. coli.

If the colony appeared teal in color all the way through the colony, it would not be E. coli.

Pink/Red dots are coliform and generally not counted. Brownish/white clonies are other bacteria types not counted.

ESTIMATED COSTS FOR COLISCAN MONITORING

Start-up Costs per Monitoring Group

Item	Estimated Cost
Incubator*	$55.00
Cooler (6 quart)	$ 7.00
One-gallon zipper bags (40)**	$ 3.00
Bleach**	$ 1.50
Tape**	$ 1.50
Sharpie markers**	$ 4.00
TOTAL	**$72.00**

* The "Little Giant Still-Air Incubator", Model 9200 chicken egg incubator available at Southern States or similar agriculture stores. A thermometer is included. The optional fan kit is not necessary. The incubator easily holds 12 plates and can hold up to 50 if stacked in a staggered pattern to allow for air flow.

** Consumable supplies that monitoring groups could be expected to replace as needed. The Sharpie markers will insure that plates will be labeled clearly without loss of information. The bleach, tape, and zipper bags are required for safe handling and disposal of used plates.

Cost of consumable supplies*

Item	Estimated Cost
Coliscan Easygel (#25001) 10 tests	$24.86 ($19.86 if ordering more than 10 kits)
3 ml dropper (#DRP03) each	$00.23 each ($2.30 for 10 droppers)
30 ml bottle (#CLB30)	$00.26 each ($2.60 for 10 bottles)
Total	$24.76 - $29.76 per 10 samples

Or buy

Coliscan Easygel kit (10 Coliscan tests, 10 1 ml droppers, 10 sample bottles)	
#CWK10	$28.78

*****The best location to purchase the Coliscan test kits is to go to the manufacturer Micrology Labs (www.micrologylabs.com or 1-888-EASYGEL). If going to the website, click on the Water tab near the top of the page and then Shop Online. Coliscan media is under Easygel Media. CWK10 combo kit is under Water Testing. Bottles and pipettes is under Other Supplies