Water Quality Kit

Teacher's Guide

Background:

Water is the universal, essential solvent. Without water, living systems could not function, and life as we know it would cease to exist. Water assumes a more vital role in life than even food. If an organism were deprived of food, it would survive far longer than if deprived of water.

A microbiologically pure water source is something that most North Americans and Europeans take for granted. Unfortunately, many developing countries do not have the luxury of pure, safe drinking water. In those countries, water must be boiled before it can be drunk safely.

Microbiologically pure water is essential to both human and animal health. Because water possesses excellent buffering capabilities, it can become a haven for diverse types of microorganisms--from the relatively harmless ferrobacteria, to potential pathogens such as *Pseudomonas aeruginosa* (infections), *Salmonella typhi* (typhoid), *Shigella dysenteriae* (dysentery), and *Vibrio cholorae* (cholera), and some strains of *Escherichia coli* (dysentery).

Water can become contaminated in many ways. In fact, a low level of bacteria is present in most water sources. Such contamination, however, is natural and usually harmless. Contamination resulting from improper disposal of human fecal material constitutes a far greater risk to human health.

Human intestinal bacteria fall into the general group of bacteria knows as coliforms. Technically, coliforms are described as gram-negative, lactose-fermenting rods. Although their primary habitat is a mammalian host, some are able to thrive in soil and water. *Escherichia* and *Enterobacter* species are among the most commonly encountered types of coliforms. *E. coli* is the standard indicator bacterium that is used to indicate fecal contamination of water sources. This is due to the fact that it is only found in the intestinal tract of mammals, and because it is able to be recovered from water samples with relative ease in comparison with other fecal coliforms.

In the United States and Canada, the bactericidal properties of chlorine are commonly used to limit the number of bacteria in our water supply. Additionally, well-designed sewage disposal and treatment programs help to keep the water free from contamination. Municipal water supplies are constantly monitored to assure the effectiveness of their systems.

Water testing, however, is in no way a one-dimensional activity. Full-body-contact water, such as lakes, rivers and oceans, is monitored during the summer months to ensure that it is safe for swimmers. (For further information on acceptable coliform levels see Appendix I.) Water testing is common in the food and dairy industry to certify that the water used in canning and cooling processes doesn't present a potential microbiological hazard. Water quality monitoring is also used in many high-technology industries to prevent bacterial contamination from interfering with the operation of delicate electronic machinery.

The Coliscan **Easygel** medium in this kit is a new formulation for water testing. It contains a sugar linked to a dye which, when acted on by the enzyme b-galactosidase (produced by coliforms including *E. coli*), turns the colony a red color. Similarly, there is a second sugar linked to a different dye which, when acted on by the enzyme b-glucuronidase (produced only by *E. coli*), turns the *E. coli* colony a blue color. Because E. coli produces both b-galactosidase and b-glucuronidase, the colony grows with a purple color (red + blue). The combination of these two dyes makes possible the unique ability to use one test to quantify and differentiate both coliforms and *E. coli*. (When counting coliforms, the purple *E. coli* colonies must also be added with the red colonies because *E. coli* is a member of the coliform group)

Experiment Design:

This kit is designed to acquaint students on a fundamental level with two different types of media, microorganisms, and techniques that can be used in water testing.

Each group of students will first collect a water sample, then plate it out on Total Count **Easygel** and Coliscan **Easygel** media. After incubation, counts will be made to determine aerobic plate counts and coliform/*E.coli* counts in the collected samples.

Materials:

1. 15 units Total Count (SPC, Standard Plate Count) **Easygel**
2. 15 units Coliscan **Easygel** (FREEZE Coliscan BOTTLES ONLY UPON ARRIVAL, THAW DAY BEFORE USE.)
3. 30 **Easygel** pretreated petri dishes
4. 15 sterile sample tubes
5. 15 sterile 3 mL droppers
6. Teacher's Guide
7. Student Worksheet (Make 1 copy for each student)

Procedure for Teachers:

Day 1 of the experiment:

1. Use sterile sample tubes to collect 15 water samples from a variety of sources such as local rivers (both upstream and downstream from sewage treatment plants if available), lakes, ponds, aquariums, ditches, water fountains, tap water, wells, etc. You may choose to either collect the samples yourself or allow your students to select their own sources (this may increase their curiosity). **Water samples that are to be kept longer than 1 hour prior to plating should be kept cold (refrigeration or on ice, but not frozen) from the time they are collected until the time when they are plated.** (For additional information on collecting samples see Appendix II.)
2. Divide students into seven groups. Provide each group with 4 pretreated petri dishes, 2 bottles of Total Count (TC) **Easygel**, 2 bottles Coliscan **Easygel** and 2 sterile 3 mL droppers. You may use the 2 extra tests to run your own samples or save them as back-ups for the students.
3. Instruct students to label the dishes with their names, the date, their water source and the medium type (i.e. TC 1, TC 2, Coliscan 1 and Coliscan 2). A permanent marker or wax pencil will work.
4. Instruct the students to sterilely transfer water from the sample tubes into the bottles of Total Count **Easygel** and Coliscan **Easygel**, consulting Table I to determine proper inoculation amounts. Students should swirl the bottles to distribute the inoculum and then pour the medium/inoculum mixtures into the correctly labeled petri dishes. Gently swirl and rock the poured dish until the entire dish is covered with liquid (but be careful not to splash over the side or on the lid).

Table I: Inoculation of Easygel

Water Sources	Medium Type	
	Total Count	Coliscan
Environmental: rivers, lakes, ponds, ditches, aquariums.	0.1 to 0.5 mL (3-15 drops)	1.0 to 5.0 mL
Drinking Water: well water, municipal water, etc.	1.0 to 3.0 mL	5.0 mL

5. The dishes may be placed right-side-up directly into a level incubator or warm spot in the room while still liquid. Solidification will occur in approximately 45 minutes.
6. Incubate at 35˚ C (95˚ F) for 24 hours. (For additional information on incubating dishes see Appendix III.)

Day 2 of the experiment:

7. Remove dishes from the incubator and return to student groups. Students should now:
    a. Count all the colonies on the Total Count dish report results in terms of colonies/mL of water.
    b. Count all the purple colonies on the Coliscan dish (disregard any light blue or white colonies), and report the results in terms of *E. coli*/mL of water.
    c. Count all the red, pink and purple colonies on the Coliscan dish (disregard any light blue or white colonies) and report the results in terms of coliforms/mL of water

(For additional information on interpreting results see Appendix IV.)

8. Dishes may be incubated an additional 24 hrs if the students would like to see how they look after a total of 48 hours incubation. Dispose used petri dishes properly (See Appendix V).

Study Questions:

1. How would you assess the purity of the water you tested? Would you be willing to drink this water? Why or why not? Would you be willing to swim in it? Why or why not?
    Students may use the information in Appendix I or their own impression of what constitutes a safe level of microbe to formulate their answers.

2. Why is a larger inoculum used for the Coliscan medium than for the Total Count? Why would *E. coli* turn red if the Coliscan did not contain the blue dye?
    Because coliforms normally constitute only a small percentage of the microbial population in a given sample, the amount of inoculum must be increased in order to have enough coliforms on the dish. *E. coli* is a type of coliform, and therefore has the same enzyme (b-galactosidase) as other coliforms. This enzyme causes the red color, and if there were no other dye involved in the Coliscan medium, *E. coli* would only be red. With both dyes in the medium, the red plus the blue colors make *E. coli* a purple color.

3. Why should drinking water be relatively free of coliform bacteria?
    If coliforms are present in water, the chances of fecal contamination are high. Water contaminated by feces is a health risk, due to pathogenic microorganisms that may be found in feces, such as *Shigella*, *Salmonella*, and *Vibrio* *spp*.

4. Which is more indicative of fecal contamination, red or purple colonies on the Coliscan medium? Why?
    While the red dye in Coliscan selects for coliforms that may be of either fecal and non-fecal origin, the blue dye selects specifically for *E. coli*--a coliform of exclusively fecal origin. Therefore, the purple color is more indicative of fecal contamination.

Appendix I: Acceptable Levels for Bacteria in Water

Water Source	Maximum permissible coliform count (Colonies/100 mL)
Municipal drinking water	4
Shellfishing water	70
Recreational water	200

Appendix II: Collecting Water Samples.

Water samples may be collected from a wide variety of sources. In addition to the potential sources already mentioned, you may wish to demonstrate the effect of heat on microorganisms by comparing water before and after it has been boiled, the effect of sanitizers such as bleach or you may wish to test the microbiological purity of your distilled (or deionized) water source. Care should be taken when collecting water samples to assure that they are not contaminated through contact with your skin. For example, if you are collecting a samples from a stream, you hand should be down-stream from the mouth of the bottle as much as possible.

Appendix III: Incubation.

If possible, Total Count and Coliscan dishes should be incubated in an incubator set at 35˚ C for 24 hours. If an incubator is not available, dishes may be kept at room temperature. However, bacterial growth will be slower and results may take 48 hours. If possible, wrap the petri dishes in a towel and place in a warm spot. Be careful, however, that the temperature does not exceed 37˚C.

Appendix IV: Interpreting Results.

Total Count: Bacteria colonies will grow as distinct, white-to-yellow masses. Fungal colonies will grow as fuzzy, filamentous clumps. Colonies that grow under the surface will generally be more compact.

Coliscan: General coliform colonies will grow as circular, smooth-edged, pink to red masses. Occasional non-lactose fermenters will grow as colorless to white colonies. *E. coli* colonies will grow with a purple color rather than a simple blue due to the combination of red + blue colors. Sky-blue colonies should not be counted as either general coliforms or as *E. coli*. Coliforms that grow on Coliscan may be of either fecal or non-fecal origin. *E. coli* is always of fecal origin, and therefore indicates fecal contamination.

Appendix V: Waste Disposal.

Because the materials used in this kit can grow living cultures, they should be sterilized before they are disposed. If an autoclave is available, heat at 15 lbs. pressure for 15 minutes. If an autoclave is not available, several other methods will suffice:

1. Place dishes in a pressure cooker and cook at 15 lbs. for 15 minutes. (This is the same as autoclaving.)

2. Place dishes in an oven-proof bag, close it with a small hole for steam to escape, and heat in an oven at 300˚ F for 45 minutes.

3. Place dishes in a large pan, cover with water and boil for 45 minutes.

After sterilizing, dishes may be safely disposed of in the trash.

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Student Worksheet

Day 1 of the experiment:

Each group of students will receive the following: 4 pretreated petri dishes, 2 bottles of Total Count (TC) **Easygel**, 2 bottles Coliscan **Easygel**, 2 sterile 3 mL droppers and a water sample (or a collecting tube for you to collect your own water sample. Follow your teacher's instructions).

1. Label your petri dishes with the following: names, date, water sample source and the medium type (TC 1, TC 2, Coliscan 1 and Coliscan 2).
2. After consulting Table I to determine the proper amount of inoculum, use the sterile dropper pipet to measure the correct amount of water. For example, if you need 1.0 mL, you will draw water into the pipet until the water reaches the 1.0 mark on the side of the pipet. Lift the tip out of the water and draw the water the rest of the way into the pipet bulb.
3. After measuring the correct amount of water, open a bottle of TC and deposit the water into the bottle. Replace the cap, and swirl gently. Remove the cap and pour into the correct petri dish. Label the dish with the amount of water you added.
4. Repeat steps 2 and 3 for the other Total Count bottle and the Coliscan bottles. Follow your teacher's instructions for incubation procedure.

Day 2 of the experiment:

5. Remove dishes from the incubator and:
   a. Count all the colonies on the Total Count dish report results in terms of colonies/mL of water.
   b. Count all the purple colonies on the Coliscan dish (disregard any light blue or white colonies), and report the results in terms of *E. coli*/mL of water.
   c. Count all the red, pink and purple colonies on the Coliscan dish (disregard any light blue or white colonies) and report the results in terms of coliforms/mL of water
6. Dispose of your materials according to your teacher's instructions.

Table I: Inoculation Amount

Water Source	Medium Type	
	Total Count	Coliscan
River, lake, pond, etc.	0.1 to 0.5 mL (3-5 drops)	1.0 to 5.0 mL
Drinking water	1.0 to 3.0 mL	5.0 mL

Table II: Results

Medium Type	Colonies / mL of water	
Total Count	1	
	2	
Coliscan	Red+Purple (coliforms)	Purple *(E. coli)*
	1	
	2	

Study Questions:

1. How would you assess the purity of the water you tested? Would you be willing to drink this water? Why or why not? Would you be willing to swim in this water? Explain.

2. Why is a larger inoculum used for the Coliscan medium than for the Total Count? Why would *E. coli* turn red if the Coliscan did not contain the blue dye?

3. Why should drinking water be relatively free of coliform bacteria?

4. Which is more indicative of fecal contamination, red or purple colonies on the Coliscan medium? Why?