Subjects/Target Grades

Science and Social Studies
Grades 7-12

Duration/ Location

75-90 minutes plus additional time for incubation
Classroom and outdoor setting

Materials

Per class

- Indicator Bacteria teacher resource
- Monitoring for *E. coli* in Water Using Petrifilm™ teacher resource
- Interpreting Coliscan Pour Plates™ teacher resource.

Per small group

- *E. coli* monitoring kits

Lesson Three Explain: Managing Pathogens-

page 11 from lesson 3

Activity Overview

Students investigate local water quality by testing for indicator bacteria.

Lesson Procedure

1) Pose the question: *How can we tell if there are pathogens in the water?*

2) Direct counting and identification of all the different types of pathogens is expensive and time consuming. Introduce the idea of monitoring for pathogens through use of indicator bacteria such as fecal coliform bacteria and *Escherichia coli (E. coli)*. The *Indicator Bacteria* teacher resource diagram illustrates that fecal coliform bacteria are a subset of coliform bacteria and *E. coli* bacteria are a subset of fecal coliform bacteria.

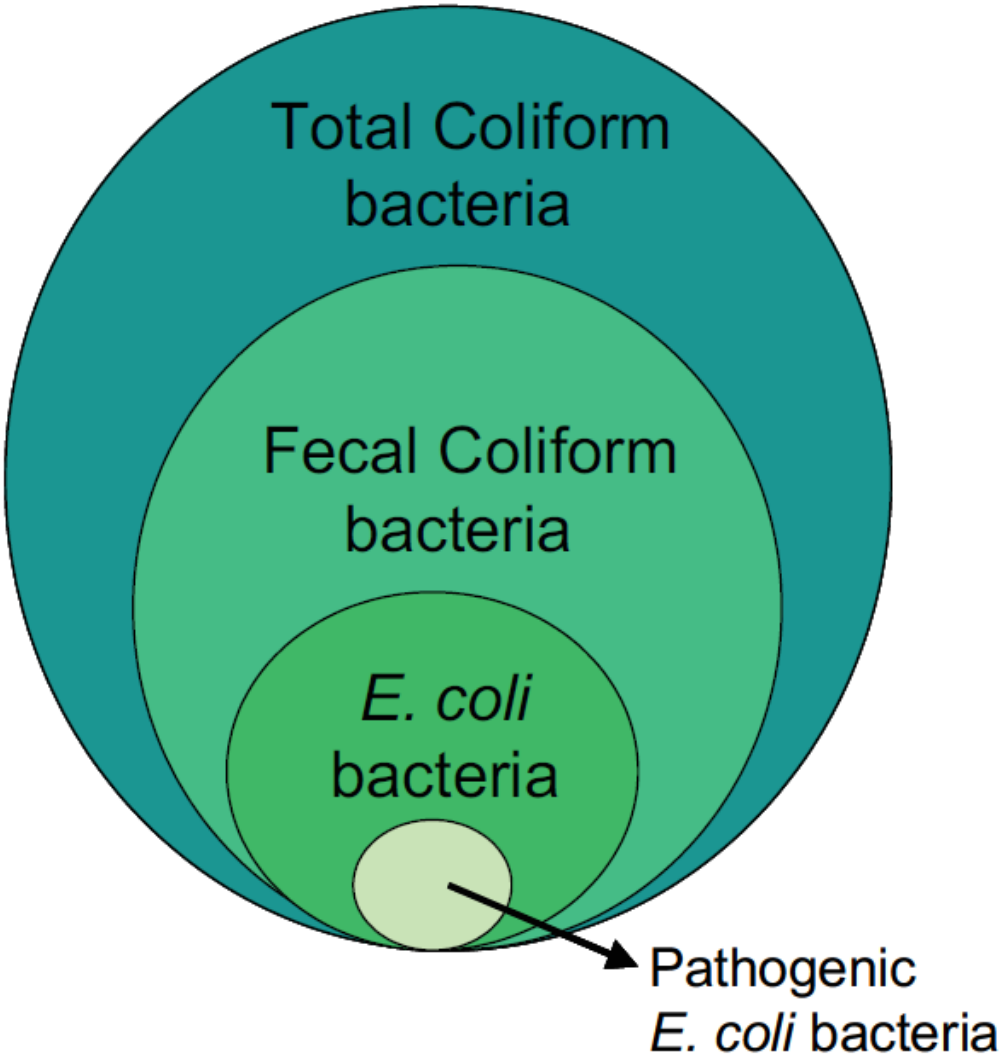
3) Engage students in water quality monitoring for bacteria. **Take appropriate precautions to protect student health.** This is especially important if there is a chance that the stream, pond, or river being studied for other water quality parameters has bacterial contamination. It would not be wise to sample a stream with heavy contamination without precautions such as wearing gloves and avoiding direct contact with water.

4) An example of a relatively inexpensive way to screen for bacteria is Petrifilm™. This method is described in *Monitoring for E. coli in Water Using Petrifilm™* teacher resource. Another method is described in the *Interpreting Coliscan Pour Plates™* teacher resource. Other methods are certainly available as described in *Citizens Monitoring Bacteria: A training manual for monitoring E. coli and Safe Waters and Healthy Waters: A guide for citizen groups on bacterial monitoring in local waterways* as annotated in the supplemental materials section of this lesson.

Vocabulary Terms

Indicator bacteria Members of two bacteria groups, coliforms and fecal streptococci, are used as indicators of possible sewage contamination because they are commonly found in human and animal feces. The EPA recommends *E. coli* as the best indicator of health risk from water contact in recreational waters

Indicator Bacteria



Fecal coliform bacteria which include E. coli are part of a larger group of colifom bacteria.

Source: Citizens Monitoring Bacteria: A training manual for monitoring *E. coli*: http://blog.uvm.edu/kstepenu/files/2016/09/Final_ecoli_06c1.pdf

Standard Operating Procedure for R-CARD® E. Coli Rapid Test Method Adapted from Roth BioScience, LLC:

The simple R-Card® method can be used to screen lakes and streams for *Escherichia coli* bacteria. This is essentially a semi-quantitative presence/absence method successfully used in programs such as Florida Lakewatch and volunteer monitoring in Michigan. One milliliter of water is inoculated on a gel medium card and incubated at 35°C for 24 hours. After 24 hours, colonies are counted. Blue/teal colonies are *E. coli* bacteria. For one mL of sample, each blue/teal colony represents 100 *E. coli* colonies/100 mL. The U.S. Environmental Protection Agency recommends that the geometric mean of *E. coli* be no more than 126 *E. coli* colonies per 100 mL in 5 samples over a 30-day period.

Specific Methods:

1. At least two hours before sampling, turn on and set the incubator to 35°C. Check the thermometer in the chamber to insure that the interior temperature is 35°C before placing any samples in the incubator.

2. Write the date and sample number in small print on the bottom of the card. Wear gloves and open the top portion (film) or use sterile forceps (Photo 1).

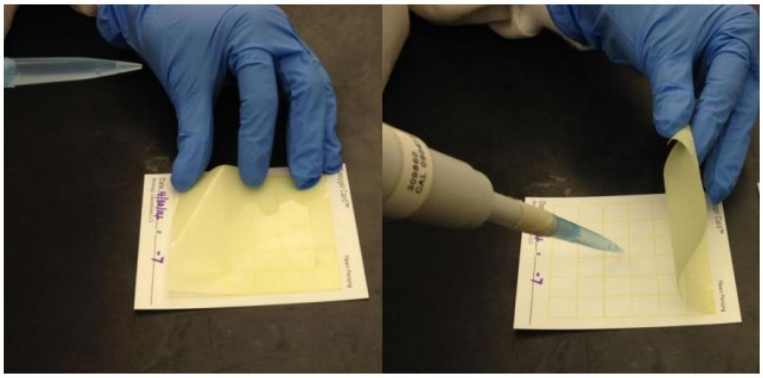


Photo 1. Open the film

Photo 2. Lift the film

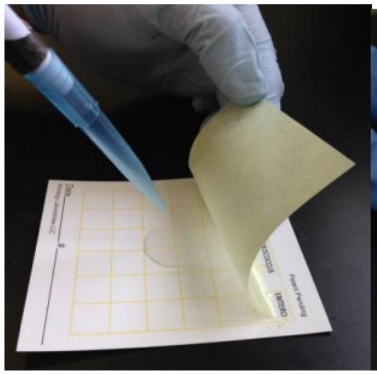


Photo 3. Pipette 1 mL sample

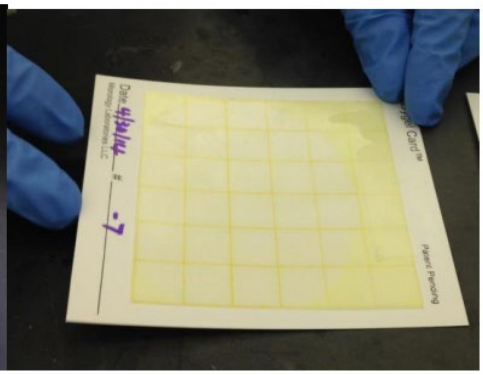


Photo 4. Cover with the top film

Photos from: https://cdn.shopify.com/s/files/1/0511/7152/4758/files/R-CARD_E._Coli_-_Operating_Procedure_5e17a121-e576-4c3f-9747-20b912665b1c.pdf?v=1661878104

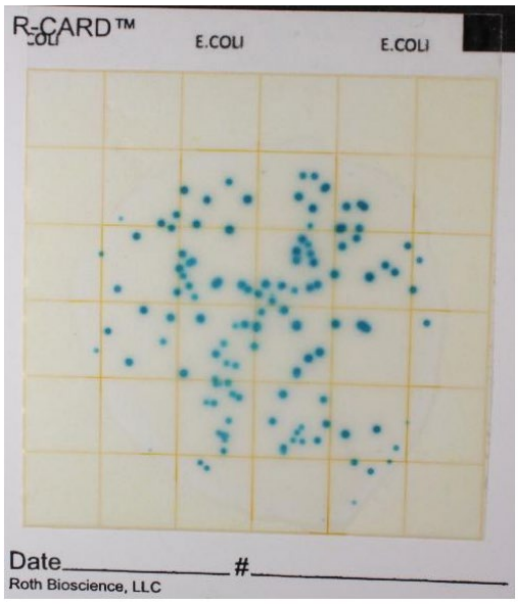
3. Using a pipette with a sterile tip or a sterile dropper, withdraw one milliliter of water from the sample. Dispense the one milliliter sample onto the center of the card (Photos 2-3).

4. Slowly roll the top film down onto the sample to prevent air bubbles. Wait 1 min to allow liquid to spread automatically. There is no need to use a spreader (Photo 4). Note: Some samples do not automatically spread in as large an area as may be wanted, but it is a simple matter to encourage spreading by gently applying pressure on the top after it is lowered on the inoculum.

5. Incubate at 35±0.5°C for 15-24 hrs (no more 24 hrs).

6. Count the number of colonies detected by green/teal colonies present on the card between 15-24 hr incubation and record as the number of E. coli/volume of sample for that test.

7. After counting the bacteria, use a dropper to place 1 mL of bleach on the Petrifilm. Place in a biohazard bag or sealed plastic bag and dispose of properly.



Green/turquoise colonies are counted as *E. coli*.

Procedure adapted from Roth BioScience, LLC:
https://cdn.shopify.com/s/files/1/0511/7152/4758/files/R-CARD_E._Coli_-_Operating_Procedure_5e17a121-e576-4c3f-9747-20b912665b1c.pdf?v=1661878104

R-CARD® E. Coli Rapid Test Information: https://www.rothbioscience.com/collections/r-card-ecoli

INTERPRETING COLISCAN® POUR PLATES

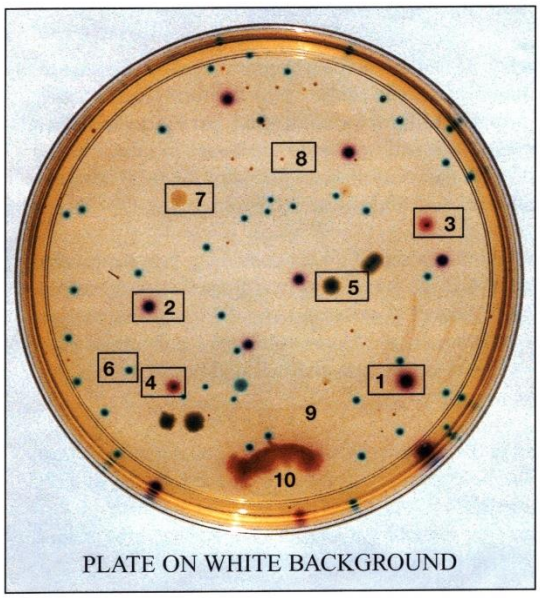
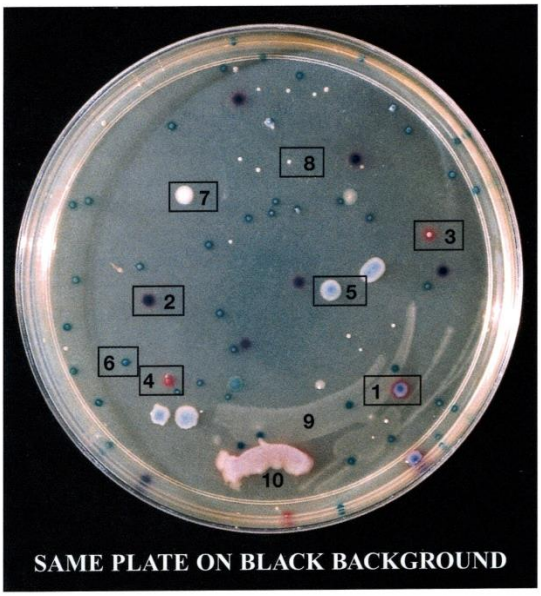


PLATE ON WHITE BACKGROUND



SAME PLATE ON BLACK BACKGROUND

Explanation of colony types (24-48 hrs. incubation)

1. purple surface colony (hazy halo)
2. purple submerged colony
3. pink surface colony
4. pink submerged
5. blue-green surface colony (white halo)
6. blue-green submerged colony
7. white surface colony
8. white submerged colony
9. white spreader on plate bottom
10. pink spreader on surface

Note that submerged colonies are smaller than the same type growing on the exposed surface and color and appearance are different when viewed over different backgrounds.
No's. 1 & 2 are typical *E. coli* (fecal coliform) colonies which produce both galactosidase and glucuronidase and are purple due to the combination of the pink and blue-green chromagens that indicate the presence of the respective enzymes.

No's. 3 & 4 are typical general coliforms (Genera *Citrobacter, Enterobacter, Klebsiella*) which produce galactosidase and are therefore a pink colony color.

No's. 5 & 6 are characteristic of less common bacteria that produce glucuronidase only and are therefore a blue-green colony color.

No's. 7 & 8 are characteristic of bacteria that produce neither galactosidase nor glucuronidase and therefore are a white or colorless colony.

No's. 9 & 10 are spreaders and can each be counted as only one colony.

Bacteria that appear like No's. 5, 6, 7, 8 & 9 are likely members of the family Enterobacteriaceae, but are not technically coliforms because they don't produce the characteristic enzyme pattern. However, these types include such important genera as *Proteus, Salmonella and Shigella* and should not be ignored as insignificant.

MICROLOGY LABORATORIES, LLC., P.O. BOX 340, GOSHEN, IN 46526
PHONE: 219-533-3351 ▪ FAX: 219-533-3370
https://www.micrologylabs.com