Microbial Discovery Activity

Quantification of *Escherichia coli* Contamination in Water

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Intended Audience

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Activity</th>
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<td>K-4</td>
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<td>5-8</td>
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<tr>
<td>9-12</td>
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Activity Characteristics

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Activity</th>
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<tr>
<td>Classroom setting</td>
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<tr>
<td>Requires special equipment</td>
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<td>Uses hands-on manipulatives</td>
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<td>Requires mathematical skills</td>
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<td>Can be performed individually</td>
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<td>Requires group work</td>
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<td>Requires more than one class period (45 minutes)</td>
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<tr>
<td>Appropriate for students with special needs</td>
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Introduction

Description

This lab was designed to introduce high school students to using unique enzymes as a method of identifying organisms, namely *E. coli*, in water.

Abstract

The lab exercise assumes basic knowledge of prokaryotes (structure, function, metabolism, and respiration) and the functions and limitations of enzymes. Building upon this knowledge and using guided prompts, students brainstorm how to create an agar on which only coliform bacteria will grow and how to differentiate between *Escherichia coli* and other coliforms based on their enzymes. Finally, students filter surface water and place it on media that differentiates *E. coli* from other coliforms using an enzyme unique to *E. coli*. The resulting data are used to determine if the water meets the Minnesota state standards for safe swimming or drinking water.

Core Themes Addressed

<table>
<thead>
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<td>Microorganisms and Humans</td>
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<td>Microorganisms and the Environment</td>
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<td>Microbial Evolution and Diversity</td>
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<tr>
<td>Other – Common properties of life; cellular components</td>
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Interdisciplinary Themes Addressed

English:
Students will be expected to turn in a lab write-up summarizing their analyses, understanding of how the agar helps us differentiate between *E. coli* and other coliforms, and concluding whether the body of water is safe for swimming.

Chemistry:
Students will be expected to understand that enzymes speed up chemical reactions.

Mathematics:
Students will be expected to estimate the number of *E. coli* and coliform colonies using various methods, including counting a section of their filter paper and multiplying it by how many sections of that size appear on the filter.

Environmental Science:
Students will be expected to understand how health officials determine if water is potable or clean enough for recreational use.
Keywords

Bacteria, *E. coli*, Coliform, Environmental sample, Lactose, Water quality, Enzyme, Petri dish, Filtration, Catalyst, Bioremediation

Learning Objectives

1. Students will utilize the unique enzymatic properties of bacteria to brainstorm a design for agar media that will allow for the quantification of *E. coli* and coliform bacteria.
2. Students will differentiate and enumerate colonies of *E. coli* and coliforms on agar media.
3. Students will interpret water quality standards for freshwater in terms of numbers of colonies of *E. coli* allowed per sample of water.

National Science Education Standards Addressed

9-12 Science as Inquiry—Content Standard A:
-Design and Conduct Scientific Investigations
This activity addresses the standard by guiding students through the process of developing a method for differentiating *E. coli* from other coliforms. After we have worked together to understand the method, we use it to determine if our water samples are safe to swim in.

9-12 Physical Science—Content Standard B:
-Chemical Reactions
This activity addresses the standard by helping students to apply what they have learned about enzymes being biological catalysts and that all organisms have to make chemical reactions occur at a speed useful to a living organism. Students will be given information about an enzyme unique to *E. coli* and be asked to use it as a way to differentiate between *E. coli* and other coliforms.

9-12 Life Science—Content Standard C:
-The Cell
This activity addresses the standard by utilizing what students have learned about different types of bacterial cells being living organisms with unique qualities, just as exhibited on macroscopic species of animals with which they are more familiar. They will use their knowledge of the prokaryotic cell to understand how the cells of *E. coli* and other coliforms are similar, yet they are different based upon their different DNA.

9-12 Science in Personal and Social Perspectives
-Environmental Quality
This activity addresses the standard by studying *E. coli* in a larger context of surface water quality. Students are designing an experiment, performing an experiment, and analyzing data in order to determine if their water sample meets the Minnesota requirements for safe swimming water.
Student Prior Knowledge

Students should have a strong basic knowledge of prokaryotic cells, enzymes and water quality before beginning this activity. These topics are also reviewed in the student handouts included with this activity.

Teacher Background Information

Bacteria are single-celled organisms. All bacteria are prokaryotes, meaning they do not have many of the internal cell parts that animal and plant cells have. For example, they do not have a nucleus, but they do have DNA and ribosomes (tiny working units that use the instructions “written” in DNA to create many, many different proteins). This gives bacteria the ability to produce their own proteins, which quickly fold into a delicate and unique three-dimensional structure that gives it its function or job within the cell or body. Enzymes are proteins that have an active site or location where specific chemicals known as substrates undergo chemical reactions more readily than elsewhere. The enzyme pocket is specially designed to fit a particular chemical compound. Once the chemical reaches the pocket, the chemical’s three-dimensional structure is altered in such a way that bonds are broken and formed much more easily than without the enzyme. Since the enzyme has the perfect pocket to fit only a specific chemical, it is said the substrate fits the enzyme like a lock in a key. If the enzyme structure is changed, it loses or decreases its ability to increase the rate of chemical reactions. For example, if the enzyme is heated, the increased kinetic energy of the particles of the aqueous enzyme suspension will alter or denature the protein structure of an enzyme. In situations of non-ideal pH (each enzyme has an ideal pH range in which it works effectively), the hydrogen bonds that stabilize the three-dimensional structure of the enzyme are disrupted and the enzyme function deteriorates. In the case of the enzyme we will be using to identify \textit{E. coli}, it is stable in the conditions provided in the experiment.

One way to differentiate between the many species of bacteria is to determine the specific enzymes a bacterium produces. This has been an especially useful technique for identifying bacteria that contaminate water. One particular bacterium, \textit{Escherichia coli}, is a common inhabitant of mammalian and avian digestive tracts and is shed in feces. Most strains of \textit{E. coli} are harmless, but some can lead to serious gastrointestinal sickness. In either case, the presence of \textit{E. coli} in water may indicate that the water has been contaminated with fecal material and may contain a variety of other types of fecal associated pathogens (other bacteria, viruses and parasites) and therefore is unsafe for use. The Environmental Protection Agency has put forth guidelines for monitoring the quality of freshwater by measuring \textit{E. coli} levels. Current standards for freshwater recreational use (e.g., swimming) are that \textit{E. coli} levels should not exceed 235 \textit{E. coli} per 100 ml of water. (Much stricter standards of \textit{E. coli} are
required for water to be considered potable.) In this experiment, students will explore one method of quantitating the number of bacteria found in a local water source and then determine how many of these bacteria are *E. coli* organisms. Students will filter water samples to trap bacteria and then allow a subset of these bacteria (called coliforms) to grow by adding a nutrient broth containing lactose. *E. coli* will be differentiated from the other coliform bacteria because the nutrient broth also has a chemical that causes *E. coli* colonies to be blue. The broth turns *E. coli* colonies blue by utilizing an enzyme found in *E. coli* (but not other coliforms), namely β-glucuronidase. The broth contains a chemical compound (X-Gluc) that is broken down by the enzyme into parts, one of which is blue. Further explanation of the test is provided in student handout part 2.

After the experiment and lab write-up, a discussion can be built about the ways enzymes have been utilized by humans, such as bioremediation of oil spills by microorganisms (bacteria eat oil as food, cleaning up the spill for humans), cleaning of water for drinking by microorganisms in water treatment facilities, and looking at similarities in the genetic code for ubiquitous enzymes, such as cytochrome C or hemoglobin, to make evolutionary classification of organisms (if two organisms have a very similar enzyme, chances are they are more related by evolution).

**Class Time**

**A few days before the experiment:** Student handouts part 1 and part 2 can be used sequentially to begin the discussion of the *E. coli* experiment in class and can be assigned as homework. Also, students will need to volunteer to bring in water samples from nearby surfaces of water on experiment day.

**Experiment day:** 1 day (plates will be in an incubator for 24 hours)

**Analysis Day:** 1 day (lab report can be finished and typed up at home, due a few days later)

**Teacher Preparation Time and Materials/Equipment**

Preferred group size is three students per petri dish sample. Each group will need one petri dish and pad, one 0.45-µm filter, and one m-ColiBlue24 ampule. The groups will share the filtration system (filter holder, hand vacuum pump, and 500-mL filtration flask) and ampule breaker.

Purchase the following from Hach Company (www.hach.com):

Note—This is a 2+ year supply, but is the lowest volume sold. Some purchases are one time only. We suggest partnering with other schools within your district or a local college or university for assistance in borrowing or purchasing appropriate equipment. Additionally, one could contact the U.S. Environmental Protection Agency (EPA) or local water quality testing centers to request donations of expired media or supplies.

1) m-ColiBlue24® Broth, Glass Ampules, pk/20 (Note—keep refrigerated)
   Product #: 2608420
   USD Price: $45.39
2) Filter, Membrane, Pore Size 0.45 µm, Diameter 25 mm, 100/pk
   Product #: 2514101
3) Petri Dish with Pad, 9x50 mm, pk/100 (Pall)
Product #: 1471799
USD Price: $50.45

4) Ampule Breakers, Media Ampules for 2-mL PourRite ampules (1 time purchase)
Product #: 2484600
USD Price: $13.95

5) Filter Holder, Magnetic (1 time purchase)
Product #: 1352900
USD Price: $242.00

6) Pump, Vacuum, Hand-operated (1 time purchase)
Product #: 1428300
USD Price: $89.59

Additional supplies needed: sterile forceps, ethanol burners or ethanol to dip sterilize the forceps, lab squirt/squeeze bottle of sterile water, 500 mL filtration flask with side opening for vacuum hose.

The experiment requires incubation at 35°C for 24 hours.

Methods

After using student handout parts 1 and 2 to introduce the complex way they will use enzymes to enumerate *E. coli* versus other coliforms, ask students to volunteer to bring in water samples from an area of surface water of their interest. When asking students to bring in water samples, ask them to collect them in a clean container and store them in a refrigerator until brought to school to slow down bacterial growth. We would like to count the number of bacteria as they were in the water source. Additionally ask the students to drop the water samples off to you before school and store them in a refrigerator or cooler with ice for the same reasons.
1. Obtain a sterile petri dish and absorbent pad.

Note: Do not touch the pad or the inside of the petri dish with your fingers.

2. Invert 1 ampule two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth by using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

Note: Be careful with the broken glass from the ampule. Place in discard container.

3. At the membrane filtration apparatus, use sterile forceps (shake alcohol off and allow to dry before using). Place a membrane filter, grid side up, on top of the filter assembly. Attach the funnel.

4. Shake the experimental water sample vigorously to mix. Pour 50 mL of sample into the funnel, measuring on the side of the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times for 2 to 3 seconds with sterile water. Vacuum filter after each rinse.

Notes:
Step 1—Sterile means without living organisms (such as bacteria or mold) contaminating your materials. Your materials should arrive sterilized and wrapped in plastic. Open the packaging as close to the experiment as possible and be sure to securely seal any unused petri dishes with absorbent pads in the plastic sterilized bag in which they were delivered. Our hands contain plenty of bacteria, so it is important not to contaminate our sterilized petri dishes with bacteria from non-water sources by only touching the outside of the petri dish and lid.

Step 3—Alcohol is used to kill the bacteria on the forceps (just as it is commonly used in hand sanitizer to sterilize your hands). Be sure the forceps are dry, so areas of the filter do not become saturated with alcohol, killing any bacteria that may land on that area.

Step 4—I suggest measuring your water sample using a graduated cylinder, which is rinsed thoroughly between each sample. We flush the sidewalls of the container to assure that nearly all of our bacteria from the water sample reach the filter and will be included in our experiment and to clean the apparatus for the next student group. This step is essential to avoid cross-contamination between samples and should be done with careful supervision, so as not to be overlooked.
5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.

6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid. (Note: Bacteria will not grow if there is not contact with the media—have all students double check for bubbles between the filter and the media.)

7. Place the petri dish in the incubator pad side down/grid side of filter up and incubate at 35 ± 0.5 °C for 24 hours. (Note: This is the opposite of how to position most solid agar plates in an incubator.)

8. Remove the petri dish from the incubator and examine the filters for colony growth. Any colony (red or blue) that grows consists of coliform bacteria. Count red and blue colonies using the equations below to indicate total coliforms. Blue colonies specifically indicate E. coli. See note below.

Step 7 — If an incubator is not available, a homemade incubator can be constructed using a light bulb or heating pad in an enclosed chamber. It may also be possible to leave plates at ambient temperature for a longer period to allow colony formation, although we have not tested this.

Step 8 — Using a dissecting microscope to view your petri dishes is optional, as most colonies are visible. However, sometimes only the center of a colony will be colored. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as E. coli. If a student’s water is particularly dense with coliform bacteria, you can suggest that the student either count an “average”-looking grid square and estimate the number of E. coli on the plate or dilute the sample in half (by adding only 25 mL of the sample of water) and multiply the number of coliform and E. coli colonies found by two.

Ask your students to record their data in a data table, such as provided in student handout part 2. Some sample sites will have none or very limited number (less than 10 colonies) of coliform bacteria. Moderately contaminated sites will have a number of colonies between 10 colonies and your local EPA limit on safe swimming waters (in Minnesota, this level is 235 colonies of E. coli per 100 mL). Highly contaminated sites will exceed the safe level of contamination for your area.

Students are often too quick to state “too numerous to count” (TNTC) once they see the numerous colonies of bacteria on their filter. Encourage them to select a small area of the grid to count that has an “average” amount of colonies. Use this to estimate the total number of E. coli and coliform colonies on the whole plate by using multiplication, and use this as evidence that the colonies are actually TNTC.
After students analyze the data, have them turn in a lab write-up. Requirements for one type of write-up are found in student handout part 3, or the teacher can use his or her own lab report format.

Delivery

We suggest beginning the activity using student handout parts 1 and 2 to engage the students in how this EPA-approved analytical method was developed to test for levels of *E. coli* in water samples. Further engage the students by asking them to bring in water samples from lakes where they swim or that look clean enough to drink. Since students know the land area around these samples, they can make more specific conclusions as to the source of *E. coli* contamination if it is found. Analysis day can also be used as a day to begin their lab reports and so using computers this day is a possibility.

Safety Issues

All microbiological samples should be treated as a potential biohazard. Even environmental samples can contain harmful bacteria and care should be taken when these bacteria are isolated and grown to large cell density on petri plates. Care should be taken to protect yourself and students from exposure to large amounts of coliform bacteria. Unless condensation on the lid is a problem for viewing colonies, tape the petri plates shut to prevent accidental exposure when viewing and counting colonies. If the lid must be removed, stress care and caution to the students. Wash hands frequently or use gloves while plating and handling. Disinfect the workspace before and after the experiment using alcohol or a 10% bleach solution (100 mL bleach diluted in a total of 1,000 mL water). After the experiment, soak the petri dishes with coliform bacteria in a 10% bleach solution to kill all bacteria before disposing of used petri dishes and filters in normal trash.

It is helpful to demonstrate the breaking of a glass ampule so students anticipate the “popping” sound of glass breaking and will position their hands correctly so the ampule is not dropped after opening. Students will need to break a glass ampule in their hand to open the sterile broth. Ask them to cup the underside of their ampule in one hand and squeeze the ampule breaker with the other. Encourage the students to avoid contamination of the broth with their hands by having another student help them remove the ampule breaker. Have a broken glass waste container ready for the broken ampules from m-ColiBlue24 broth.

Field Testing

This laboratory was run as a conclusion to a biochemistry unit with 80 sophomore-level high school students enrolled in biology classes.

Assessment and Evaluation of Activity

Student learning can be assessed by evaluating student answers to questions in handouts 1 and 2 and through the lab write-up. The following is the rubric that was used to evaluate the student lab write-up. Modify the following rubric to fit your classroom.
The following is an example of an actual student lab report produced for this activity.

E. coli Presence in a Local Water Sample Experiment

Data:

<table>
<thead>
<tr>
<th># of Coliform Colonies</th>
<th>Observations</th>
<th># of E. coli Colonies</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 colonies</td>
<td>There were 42 big, dark red colonies of coliforms, but there was also many, many small, faint, red dots that were difficult to see that were not included in the count.</td>
<td>10 colonies</td>
<td>One colony was really big, the others were small and hard to see.</td>
</tr>
</tbody>
</table>

Analysis:

- Coliform colonies per 100mL = (coliform colonies counted/mL of original sample filtered) * 100
  42 + 10 = 52
  52 / 50 = 1.04
  1.04 * 100 = 104 colonies per 100mL

- E. coli colonies per 100mL = (E. coli colonies counted/mL of original sample filtered) * 100
  10 / 50 = 0.2
  0.2 * 100 = 20 colonies per 100mL

Conclusion:
The purpose of this lab was to discover how many E. coli colonies were present in a 50mL sample of water from a local source. The water was from the St. Croix River. Agencies are concerned with the level of E. coli in community water sources because E. coli that is ingested causes intestinal illness that is extremely harmful to humans. E. coli is an indicator of unhealthy fecal contamination. The bacteria media that we used to grow the coliform bacteria had an inhibitor in it that prevented other types of bacteria from growing. The media also had lactose in it which coliform can survive on solely, but most other bacteria can’t. A way to distinguish the coliform bacteria colonies from the E. coli colonies is the E. coli bacteria turns blue, the coliform red. E. coli bacteria turns blue because it produces a unique enzyme called B-glucuronidase. The bacteria media had a specific substrate in it that when it reacts with the enzyme in E. coli, the colony turns blue. The water from the St. Croix had 104 coliform colonies per 100mL of water. Twenty of the colonies were E. coli, the rest were other coliform bacteria colonies. Therefore, the water in the St. Croix is safe to swim in, but not safe to drink. Some possible sources of error for this experiment are the bacteria media could have been contaminated, perhaps from a human hand, and the water sample could have come from a dirtier or cleaner part of the river. To improve this experiment it would be...
good to take samples from different parts of the river to see if the results were similar. For good further research an experiment on the *E. coli* levels of other rivers in Minnesota could be conducted to compare results.

**Supplementary Materials**

Results of this experiment could be used to create student presentations by water sample site. Modifications of this experiment could include the following: use the data to discuss the properties of bacteria (metabolism of food sources, such as lactose, single-celled life, etc.), use data to discuss environmental water quality from select sites in your area or drinking water sources. Additionally, the background information surrounding the EPA standards for drinking or swimming water in Minnesota could be modified to the parameters for your area.

**Suggested Resources**

Prokaryotes:
http://www.biology.arizona.edu/cell_bio/tutorials/pev/page2.html
http://www.earthlife.net/prokaryotes/welcome.html
Coliforms, *E. coli* and water testing:
Beach closings:
Search your local news stations for beaches that have been closed due to high levels of *E. coli*

**References**


Introduction

Soon you will be using what you already know about enzymes to understand one application of them. You will use the activity of the β-glucuronidase enzyme, which is an enzyme specific to the bacterium *E. coli*, to detect *E. coli* presence in water samples from the community.

What do you already know about the subject? Answer the following questions:

1. What is a bacterium (plural: bacteria)?

2. Are bacteria bad, good or both? Explain.

3. Name three places you think bacteria live.

4. Do you think we have any bacteria on or inside of us? Explain.

5. Explain where your intestine (also known as the bowel) is located inside your body.

6. What is *E. coli*? List anything you know about it.
Procedure

You will be filtering water samples from local sources by using filter paper that will trap very small, suspended particles, such as bacteria. You will try to grow the bacteria from the filters in our lab. When scientists want to grow bacteria in a lab, they grow them on a nutrient agar—similar to a nutritious gelatin or broth that only certain bacteria like to eat.

Your job is to design a nutrient agar that will allow a group of bacteria, called coliforms, to grow. *E. coli* is a type of coliform, but there are many other types of coliforms as well. So you must also design a way to differentiate *E. coli* from other coliforms. Use the following facts and guiding questions to design your agar. This actual question has been explored by many companies—one of which we will use in our experiment! Now go ahead and think like a scientist!

**Facts to use when designing your nutrient agar:**

- Coliforms can use lactose (a sugar) as their sole source of energy, whereas most other bacteria do not.
- Certain inhibitors (chemical poisons) can be added that inhibit non-coliform bacteria from growing.
- *E. coli* has β-glucuronidase (an enzyme) and the other coliforms do not. The substrate of the enzyme is a molecule that undergoes a color-changing reaction using the enzyme as a catalyst. The substrate molecule becomes dark blue after β-glucuronidase does its reaction.

**DESIGN YOUR NUTRIENT AGAR**

What should be present (or should be absent) in the agar we use so that only coliforms grow?

How will we be able to tell if the coliforms that are growing are *E. coli* or just other coliform bacteria? Explain how you can identify *E. coli* colonies, being as specific as you can.
Student Handout Part 2:
Quantification of Escherichia coli
Contamination in Water

Read the following excerpts of an article describing how scientists have developed an agar to differentiate E. coli from other coliforms using enzymes, hopefully just like you described!

Use the following definitions to help you read the article:

- **Intestinal flora**: microscopic organisms (microorganisms) that live in your intestine
- **Host**: the organism that supports the life of another organism (feeds it, provides shelter, etc.)
- **Pathogen**: any disease causing agent, especially a microorganism
- **Opportunistic pathogens**: microorganisms that cause disease when the host is already weakened, but not when it is strong and well.
- **Fecal contamination**: evidence of feces (poop) has been found (has contaminated) a substance
- **Colony of bacteria**: one bacterium that has “cloned” itself to make a spot of bacteria we can see on a plate of agar (bacteria normally reproduce by cloning themselves). It can be assumed that the many, many bacteria in the colony all originated from one bacterium.

_History of E. coli and why it is a fecal contamination indicator (1)_

*Escherichia coli*, originally known as _Bacterium coli_ commune, was identified in 1885 by the German pediatrician, Theodor Escherich. _E. coli_ is widely distributed in the intestine of humans and warm-blooded animals and part of the essential intestinal flora that maintains the physiology of the healthy host. _E. coli_ is a member of the family _Enterobacteriaceae_, including known pathogens such as _Salmonella_, _Shigella_, and _Yersinia_. Although most strains of _E. coli_ are not regarded as pathogens, they can be opportunistic pathogens that cause infections in immunocompromised hosts. There are also pathogenic strains of _E. coli_ that when ingested, causes gastrointestinal illness in healthy humans.

In 1892, Shardinger proposed the use of _E. coli_ as an indicator of fecal contamination. This was based on the premise that _E. coli_ is abundant in human and animal feces and not usually found in other places. Furthermore, since _E. coli_ could be easily detected by its ability to ferment lactose, it was easier to isolate than known gastrointestinal pathogens. Hence, the presence of _E. coli_ in food or water became accepted as an indicator of recent fecal contamination and the possible presence of pathogens. Although the concept of using _E. coli_ as an indirect indicator of health risk was sound, it was complicated in practice, due to the presence of other enteric bacteria like _Citrobacter_, _Klebsiella_ and _Enterobacter_ that can also ferment lactose and act similarly to _E. coli_ when they are grown in a lab, so that they are not easily distinguished. As a result, the term "coliform" was coined to describe this group of enteric bacteria. In 1914, the U.S. Public Health Service adopted counting coliforms as a more convenient measure of sanitation.

Although coliforms were easy to detect, their association with fecal contamination was questionable because some coliforms are found naturally in environmental samples. As a result, _E. coli_ has reemerged as an indicator, partly facilitated by the introduction of newer methods that can rapidly identify _E. coli_.

Currently, all 3 groups—coliforms, fecal coliforms, and _E. coli_—are used as indicators but in different kinds of tests. Detection of coliforms is used as an indicator of sanitary quality of water or as a general indicator of sanitary condition in the food-processing environment. Fecal coliforms remain the standard indicator of choice for shellfish and shellfish harvest waters; and _E. coli_ is used to indicate recent fecal contamination or unsanitary processing.
Almost all the methods used to detect *E. coli*, total coliforms or fecal coliforms are enumeration methods that are based on lactose fermentation.

**How Glucuronidase Is Used to Detect *E. coli***

*E. coli* is unique from most other coliforms in that it produces an enzyme called β-glucuronidase. Scientists have developed substrates for this enzyme that are chemically changed by the enzyme into a molecule that humans see as bright blue. Below, you will see that that the substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG or X-Gluc) is broken into parts by β-glucuronidase. The two resulting products are a double ringed structure and glucuronic acid. The double ringed structure undergoes a further reaction, attaching itself to another ring just like it. This end product is seen as blue to the human eye. Therefore, if scientists add this X-Gluc to their agar and a blue color is seen on or around a colony of bacteria, the colony had β-glucuronidase and was *E. coli*!!

1. *Peter Feng*, Stephen D. Weagant, Michael A. Grant. History of *E. coli* and why it is a fecal contamination indicator. BAM: Enumeration of *Escherichia coli* and the Coliform Bacteria, September 2002.

**SUMMARY QUESTION:**

In the space below, please write a one-paragraph summary about how scientists test for *E. coli* as an indicator of fecal contamination. Please include any of the scientific methods that we are likely to use in our procedure, as we will be trying to identify *E. coli* from our water samples.
The State of Minnesota began requiring testing of *E. coli* as an indicator of fecal contamination in water in 1967. Currently, surface waters (lakes, streams, etc.) are required by Minnesota state law to be at or below a level of 235 colonies of *E. coli* per 100 mL to be considered safe for recreational use for humans (e.g., swimming). In contrast, the standards for water to be considered safe for drinking are much stricter. A federal government agency, the Environmental Protection Agency (EPA), has set a limit of zero *E. coli* found in 95% of drinking water samples taken per month. Consider the following table stating an estimate of times a month a potable source of water will be tested for the presence of *E. coli*.

<table>
<thead>
<tr>
<th>Amount of People Served by Water Source</th>
<th>Amount of Water Samples Tested Per Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 million or more</td>
<td>~450</td>
</tr>
<tr>
<td>1 million to 1,000 people</td>
<td>~5</td>
</tr>
<tr>
<td>25 to 1,000 people</td>
<td>~1</td>
</tr>
</tbody>
</table>

**Purpose of Experiment:** To enumerate (count the number of) *E. coli* colonies found in a sample of surface water (pond or stream).

**Procedure:**
1. Bring in a 50-ml sample of water from a local body of water.

2. Follow steps 1 to 8 in the protocol below to determine the number of coliform and *E. coli* bacteria in your sample.
1. Obtain a sterile petri dish and absorbent pad.

Note: Do not touch the pad or the inside of the petri dish.

2. Invert 1 ampule two or three times to mix broth. Break open an ampule of m-ColiBlue24 broth by using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

Note: Be careful with the broken glass from the ampule. Place in discard container.

3. At the membrane filtration apparatus, use sterile forceps (shake alcohol off and allow to dry). Place a membrane filter, grid side up, on top of the filter assembly. Attach the funnel.

4. Shake the experimental water sample vigorously to mix. Pour 50 mL of sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times for 2 to 3 seconds with sterile water. Vacuum filter after each rinse.

5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.

6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Place the petri dish in an incubator and incubate at 35 ± 0.5 °C for 24 hours.

8. Remove the petri dish from the incubator and examine the filters for colony growth (we won’t use microscopes). Any colony (red or blue) that grows consists of coliform bacteria. Count red and blue colonies by using equations below to indicate total coliforms. Blue colonies specifically indicate *E. coli*. See note below.
Note: Sometimes only the center of a colony will be colored. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as blue. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as *E. coli*.

3. Record your data and observations in Table 1.
   a. Coliform and *E. coli* density is reported as the number of colonies per 100 mL of sample.
      i. If growth covers the entire filtration area of the membrane or a portion of it, and colonies are not discrete, report results as “confluent growth with or without coliforms.”
      ii. If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “too numerous to count (TNTC).”

Table 1

<table>
<thead>
<tr>
<th>Number of Coliform Colonies</th>
<th>Notes/Observations</th>
<th>Number of <em>E. coli</em> colonies</th>
<th>Notes/Observations</th>
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4. Use the following formulas to calculate the number of coliform and *E. coli* bacteria per 100mL

Coliform colonies per 100 mL = \( \text{Coliform colonies counted} \times 100 \), m\(L \) of original sample filtered

\( E. coli \) colonies per 100 mL = \( \text{E. coli colonies counted} \times 100 \), m\(L \) of original sample filtered

5. Prepare a lab write-up for the experiment. Include the following components in your write-up:
   a. Data: Include a table with data from your experiment. Make sure your table is constructed properly.
   b. Analysis: Include the equation, as well as your calculations and other analysis.
c. Conclusion: Include the following information in a paragraph that should read like a book:

   i. Restate the purpose of the lab.
   ii. Explain why agencies are concerned with levels of *E. coli* in community water sources.
   iii. *Thoroughly* explain how the bacterial agar was created so that only coliform bacteria could grow **and** why *E. coli* colonies turn blue.
   iv. Summarize the data in a few sentences.
   v. Report possible sources of error in your procedure.
   vi. Recommend improvements to your procedure or ideas for further experiments building upon the ideas used in this experiment.
   vii. Recommend to a government environmental board whether the water your sample came from is safe for swimming or drinking.

References
