



PR017

G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

How Clean Is Our Water

Teacher's Handbook

(Cat. # BE-107)



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MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 30 students (six groups of five students.)

- 1 vial K12 Agar Stab
- 1 vial Sterile Water
- 2 vials Coliform Broth (for controls)
- 1 bottle Coliform Broth
- 30 Centrifuge Tubes (2ml)
- 10 Large Transfer Pipettes (sterile)

SPECIAL HANDLING INSTRUCTIONS

- All reagents can be stored at room temperature

ADDITIONAL EQUIPMENT

- UV Lamp or UV Light Box (optional)
- Incubator (optional)

TIME REQUIRED

- **Day 1:** 2 hours
- **Day 2:** 20 minutes

AIMS

- Test water samples for presence of bacteria.
- Use a colorimetric and fluorescent assay.
- Collect water samples.

BACKGROUND

Water purity is a major concern throughout the world and can result in deadly diseases. One major factor causing contaminated water is the presence of fecal matter in the water system that can carry diseases such as cholera. As a result, several tests are used to monitor water quality. One important test is the test for coliform bacteria. Coliform bacteria is common in soils, plants and animals and one specific group of coliform bacteria is found in the digestive tract of warm blooded animals and is known as the fecal coliform group.

Drinking of water contaminated with coliform bacteria may not directly lead to illness, but the presence of coliform bacteria is a strong indicator of the water being contaminated with fecal matter and therefore having a great chance of carrying other deadly contaminants.

The normal test for coliform bacteria is routinely a two-step process. First, the water is tested for total coliform bacteria. This test is based on the coliform bacteria expression of the β -D-galactosidase enzyme that cleaves the chromogenic substrate, X-gal, to produce a blue color. The second part of the test is to test for fecal coliform bacteria, in particularly *Escherichia coli*. *E. coli* makes a specific enzyme, β -D-glucuronidase, which cleaves a fluorescent substrate. When viewed under UV light the substrate glows bright blue, confirming the presence of *E. coli*.

This kit allows students to collect water samples and, using a single substrate, test for the presence of total coliform bacteria and *E. coli*.

PRE EXPERIMENT SET UP

Water Sample Collection.

1. Each student needs to collect a water sample. This can be done in advance to allow students to bring samples from home, from lakes, streams and ponds. Alternatively, the water collection can be done in advance as a supervised class.
2. Simply supply the group with a Centrifuge Tube (2ml) and have them fill the tube to a maximum of $\frac{1}{4}$ full, to the "0.5" mark, from the source and seal and bring to the class.

Positive and Negative Controls.

1. Use sterile techniques when handling controls to prevent cross contamination.
2. The morning of the experiment, label two vials of Coliform Broth with “E.coli” and “Sterile Water”.
The Sterile Water will act as the negative control and the E. coli is a positive control for both tests
3. Transfer 1 drop of Sterile Water to the “Sterile Water” labeled vial of Coliform Broth.
4. Pour about ¼ a vial of Sterile Water into the K12 Agar Stab. Seal and leave for 30 minutes at room temperature. Shake vigorously.
5. Transfer 1 drop of sterile water from K12 agar stab, which is an *E. coli* bacterial suspension, to the “E.coli” labeled vial of Coliform Broth.
6. Transfer both vials to a 37°C incubator or a warm area of the classroom at the same time as the students’ vials.

MATERIALS FOR EACH GROUP

- 6 2ml Collection Vials
- 1 bottle Coliform Broth with Sterile Transfer Pipettes (Shared with class)

PROCEDURE

1. Label the Collection Vials with your name and a brief description of the source. Record the name of the students in your group and the source of their water in the Results section.
2. Collect water samples from various sources as detailed by your teacher. When collecting water samples, fill the tubes no more than ¼ full, fill to the “0.5” mark.
3. Using aseptic techniques, use a sterile Transfer Pipette and fill the collection tubes approximately to the “1.5” mark with coliform broth.



Ensure that the transfer pipette does NOT come in to contact with your sample or the collection tube. Also ensure that the transfer pipette does NOT touch any other surface other than the inside of the Coliform Broth bottle. If this occurs, request a fresh sterile pipette.

4. Shake the Collection vial to mix.
5. Place the vial in a warm incubator or in a warm area of the classroom. Your teacher will show you where.
6. The vials are left overnight for the reaction to occur.
7. The next day, examine the reactions for a blue color and then examine with a UV lamp, if available, to visualize the fluorescence and record your observations in the result section.

RESULTS, ANALYSIS & ASSESSMENT

Group names and source of water:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Discuss your results below. Describe the presence of the blue color and the amount of fluorescence seen.

Discussions will vary depending on the samples collected.

Examine the controls provided by your teacher and compare to your samples. Discuss below:

Sterile Water Control has no color and no fluorescence. No coliform bacteria present.

E. coli Control has a blue color and fluorescence, E.coli bacteria present.

Compare the controls and your samples to the other groups. Discuss the differences in their water samples.

Discussions will vary depending on the samples collected.

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RESULTS, ANALYSIS & ASSESSMENT

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