# Watershed Watchdogs

## Assessing Water Quality

### Water Quality Testing Instructions


*For some tests, you will need the Kit Code, which is located on the bottom right hand side of the box lid. Example: Kit 3354*

# Table of Contents

# Safety Precautions

Read all instructions and note precautions before performing the test procedure.

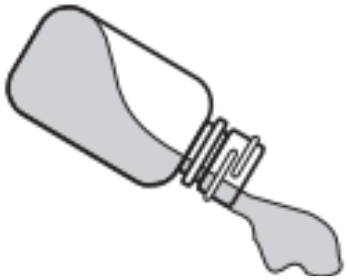

Store the test kit in a cool dry area.

Avoid contact between reagent chemicals and skin, eyes, nose, and mouth.


Use test tube caps or stoppers, not your fingers, to cover tubes during shaking or mixing.

Thoroughly rinse test tubes before and after each test.

Wipe up any reagent chemical spills immediately.



Avoid prolonged exposure of equipment and reagents to direct sunlight. Protect reagents from extremes of temperature.

Tightly close all containers immediately after use.

Do not interchange caps from containers.

# DISSOLVED OXYGEN

## WHY DOES DISSOLVED OXYGEN MATTER?

Aquatic organisms, like fish and bacteria, rely on oxygen for respiration. Oxygen enters water easily from the atmosphere, especially in streams where there are riffles (rapids) and is also produced by aquatic plants through photosynthesis. The amount of dissolved oxygen in water can be affected by warming temperatures, as warm water holds less oxygen than cold water. Additionally, excess nutrients that cause excessive plant growth results in less oxygen due to respiration and decomposition.

## STEPS:

### Part 1: Collect the Water Sample

1. Take the glass water sampling bottle with the black cap and rinse the bottle in the sample water.

2. Tightly cap the bottle and submerge to desired depth.

3. With the bottle under water, remove the cap and allow the bottle to fill.

4. Tap the sides of the bottle to dislodge any air bubbles.

5. Place the cap back on while the bottle is still under water.

6. Take the bottle back to your group. If there are air bubbles, repeat steps 2-6 to remove them.

**IMPORTANT NOTE:**

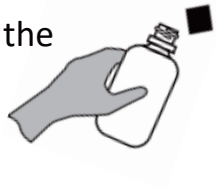
**Read all instructions for Part 2 before proceeding.**
This is important because any time you open the bottle, atmospheric oxygen is added.
This should be avoided as much as possible.

DISSOLVED OXYGEN continued:

## Part 2: Adding the Reagents (the chemicals to test for DO)

*Be careful not to introduce air into the sample during this section.*

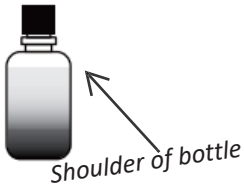
1. Remove the cap from the bottle.



2. **Immediately** add 8 drops of Manganous Sulfate Solution and 8 drops of Alkaline Potatssium Iodide Azide.

3. Cap the bottle and mix by inverting several times. A precipitate will form.

4. Allow the precipitate to settle below the shoulder of the bottle.




5. After precipitate settles, add 8 drops of Sulfuric Acid.

6. Cap and gently invert the bottle again to mix until the precipitate and the reagent have dissolved.

At this point the solution in your water sample bottle is “fixed”, meaning that contact with air will not affect your results. If the precipitate has not totally dissolved after two minutes, check with an instructor before going to the next step.


Check with your instructor

## Part 3: Titration

**Read this first:** Titration means adding solution a drop at a time to produce a measurable change. We like to call this the "drop and swirl method". Using your titrator, you will add one drop of solution to the fixed sample in the capped titration tube and then give the tube a gentle swirl. Keep doing this until you get the result you are looking for. You *must* keep the extra solution (liquid) in the titrator when you are done; you will need it later! The reading on the titrator at the end of Part 3 is your ppm reading.

1. Fill the titration tube to the 20 mL line with the fixed sample. Cap the tube.

2. Depress the plunger of the Tritator.

3. Fully insert the Titrator into the plug in the top of the Sodium Thiosulfate, solution.

4. Invert the bottle and withdraw the plunger until the large ring on the plunger gets to 0.0. You will have 10 ppm of Sodium Thiosulfate solution in the Titrator.

**STOP:** If small air bubbles appear in the Titrator, it may be because the plunger was not fully depressed before withdrawing. Give the Titrator a flick to move air bubbles to the tip. Keep the Titrator stuck in the Sodium Thiosulfate bottle, and squirt the solution back into the bottle to release any air bubbles. At this point, your sample solution will look yellow-brown. If it appears to look like a very pale yellow, skip to step 8.

5. Remove the Titrator and insert the tip into the opening of the titration tube cap.

6. Add **one drop** at a time, **swirling** the solution each time. Keep going until the solution changes from a yellow-brown color and into a pale yellow.

7. Carefully remove the Titrator and cap from the titrator tube. **Do not** disturb Titrator plunger, you will need the solution in it for later!

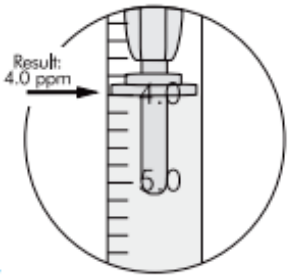
8. Add 8 drops of Starch Indicator Solution. The sample should turn blue.

## Part 3: Titration continued

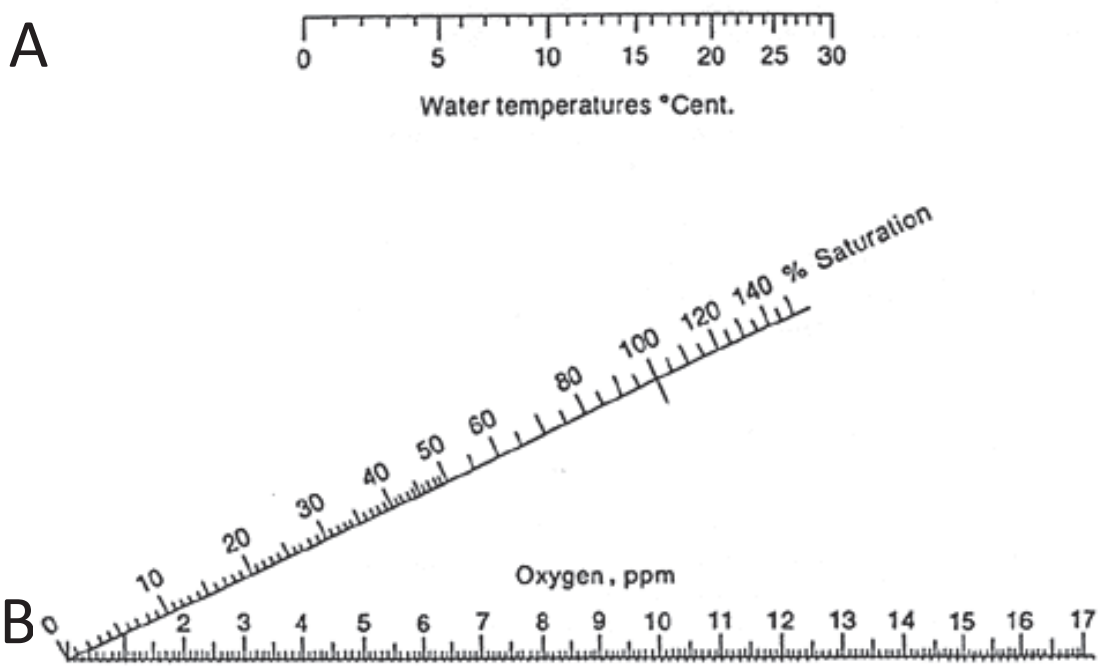
9. Cap the titration tube. Insert the tip of the titrator into the opening of the titration tube cap.

10. Continue titrating until the blue color disappears and the solution becomes colorless. **STOP HERE.** If a color change has not occurred, refill the Titrator and continue the titration.

11. The ppm of Sodium Thiosulfate used corresponds with the ppm or mg/L of dissolved oxygen. Record the result for amount used on your data sheet. Discard all solutions in the chemical waste container and rinse everything thoroughly.




## Part 4: Determining Percent Saturation




With the thermometer on the side pocket of your kit, find the water temperature for scale A. Submerge the thermometer 4 inches below the water surface. Hold it in place for about 2 minutes and read the temperature in Celcius while it is still in the water. Next, find the amount of titrant (ppm) you used on scale B. Use a straight edge to line up the two results to get the % saturation result in the middle. Record result and circle yes or no for acceptable on your data sheet.

## WHAT DO THESE NUMBERS MEAN?

| | | |
|---|---|---|
| >100%-80% saturation of dissolved oxygen | is | Sufficient/Acceptable |
| 79-60% saturation of dissolved oxygen | is | Stressful/Unacceptable |
| <60% saturation of dissolved oxygen | is | Fatal/Unacceptable |

# TURBIDITY

## WHY DOES TURBIDITY MATTER?

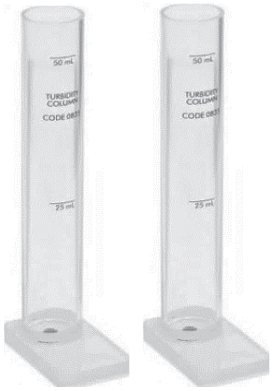
Turbidity is a measure of how cloudy water is. Turbid (cloudy) water contains suspended solids such as soil particles (clay, silt, fine sand), plankton, algae, and microorganisms. These materials are typically in the size range of 0.004 mm (clay) to 1.0 mm (sand), large enough to block some of the light rays and reduce the amount of light that can pass through the water. The higher the turbidity, the less light passes through to the plants living under water. Turbidity is measured in JTUs (Jackson Turbidity Units). Readings are made by using a standard turbidity reagent to match the turbidity of a water sample. If turbidity is present, it will interfere with the passage of light through the column of liquid.

## STEPS:

1. Fill the turbidity tube labeled "sample" to the 50 mL line with sample water. If the black dot on the bottom of the tube is not visible when looking down through the column of liquid, pour out a sufficient amount of the test sample so that the tube is filled to the 25 mL. If not, leave it at 50 mL.

2. Fill the second turbidity tube labeled "standard" with clear tap or distilled water that is equal to the amount of sample being measured.

3. Place the two tubes side by side and note the difference in clarity. If the black dot is equally clear in both tubes, the turbidity is zero. If the black dot in the sample tube is less clear than it is in the standard tube, proceed to the next steps.



*Turbidity Tubes*


*Standard Turbidity Reagent*

TURBIDITY continued:

If the dot is fuzzy or less clear in the sample tube than it is in the standard tube, there is some turbidity in the water. In the next steps, you will be adding reagent.

**You must keep track of how many times you add reagent. Remember, you are matching the cloudiness of the water, not the color.**

4. Shake the Standard Turbidity Reagent vigorously. Add 0.5 mL to the standard tube. Use the stirring rod to stir content of both tubes to equally distribute turbid particles. Stop and check for amount of turbidity by looking down through the solution at the black dot. If the dot in the standard tube and sample tube are the same, stop the test here. If the dot on the sample is still fuzzy or less clear, continue to add Standard Turbidity Reagent in 0.5 mL increments to the standard tube, mixing after each addition until the turbidity equals that of the sample.

5. Each 0.5 mL addition to the 50 mL size sample is equal to 5 JTUs. If a 25 mL sample size was used, each 0.5 is addition is equal to 10 JTUs. See the table below to find your final result and mark it on your data sheet. Then, determine if the reading is in the acceptable range and circle yes or no on your data sheet.

## Turbidity Test Results

| Number of Measured Additions | Reagent added mL | 50 mL Graduation JTU | Secchi Disk Comparison cm | 25 mL Gradation JTU | Secchi Disk Comparison cm |
|---|---|---|---|---|---|
| 1 | 0.5 | 5 | 120 | 10 | 90 |
| 2 | 1.0 | 10 | 90 | 20 | 30 |
| 3 | 1.5 | 15 | 60 | 30 | 25 |
| 4 | 2.0 | 20 | 30 | 40 | 20 |
| 5 | 2.5 | 25 | 28 | 50 | 15 |
| 6 | 3.0 | 30 | 25 | 60 | 13 |
| 7 | 3.5 | 35 | 23 | 70 | 10 |
| 8 | 4.0 | 40 | 20 | 80 | 7.5 |
| 9 | 4.5 | 45 | 18 | 90 | 5 |
| 10 | 5.0 | 50 | 15 | 100 | 2.5 |
| 15 | 7.5 | 75 | 9 | 150 | |
| 20 | 10.0 | 100 | 2.5 | 200 | |

## WHAT DO THESE NUMBERS MEAN?

| | |
|---|---|
| 0-7 JTU | is a good, clean, healthy ecosystem/acceptable |
| 8-10 JTU | is slightly polluted/acceptable |
| >10 JTU | is polluted/unacceptable |

# PHOSPHATES Kit 3121-02

## WHY DO PHOSPHATES MATTER?

Phosphorus is essential for life. When phosphorus combines with four oxygen atoms, it forms a phosphate ion. Phosphate that is not combined in any molecules in plants or animals, making it available for reaction, is called “orthophosphate.” Natural water bodies contain low levels of orthophosphates, which become a growth-limiting factor for producers, as they compete for the amount available. However, human impacts such as fertilizer use, climate change, and waste disposal causes excess phosphate levels in water, fostering algal blooms, also known as eutrophication. As the algae decays, they deplete oxygen in the water. This test allows extremely faint colors to be matched to color standards by viewing the reaction.

## STEPS:

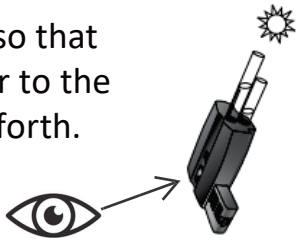
1. Fill a test tube to 10 ml line with sample water. Place this tube in the rear hole on top of the Low Range Comparator.

2. Fill the other test tube to 10 mL line with sample water. Use the 1.0 mL pipet to add 1.0 mL of Phosphate Acid Reagent to this tube. Cap and shake to mix.

3. To the same tube, use the 0.1g spoon to add one level measure of Phosphate Reducing Reagent. Cap and mix until dissolved.

4. Place test tube in front hole. Set timer for 5 minutes and wait. [While you are waiting, fill out the remaining parts of your data sheet.]

5. Remove any test tube caps and angle the Octa-Slider Viewer so that light is shining through the test tubes. Match the sample color to the color standard on the Octa-Slide by moving the bar back and forth.



When you find a matching color, record the number on the Octa-Slide on your data sheet. This is your phosphate level. Using the information below, determine if this reading is in the acceptable range. Circle yes or no on your data sheet.

## WHAT DO THESE NUMBERS MEAN?

0 - 0.306 mg/L of orthophosphates is acceptable

>0.306mg/L of orthophosphates causes eutrophication/is unacceptable

# NITRATES Kit 3354-01

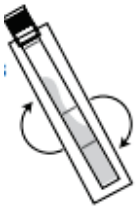
## WHY DO NITRATES MATTER?

Plants and animals rely on nitrogen for proteins and nucleic acids. Animals obtain it from food, while plants absorb it from soil and water, including nitrates from organic matter decomposition. Excess nitrogen in streams caused by climate change, fertilizer use, septic tank leaks, livestock manure, and fossil fuel burning can foster algal blooms that reduce dissolved oxygen levels as algae decays. Higher nitrate levels can impede oxygen transport in human bloodstreams.

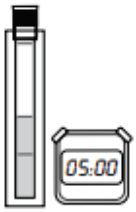
## STEPS:

1. Fill the test tube to the 5 ml line with the sample water.
   Add one Nitrate #1 tablet.
   Cap and mix until tablet dissolves.

2. Add one Nitrate #2 CTA tablet to the test tube. Cap and immediately slide test tube into silver protective sleeve. Mix for 2 minutes to dissolve the tablet.
   Note: If missing a sleeve, borrow from another group or keep the test tube out of the sun.



3. Leave test tube inside the silver protective sleeve. Set a timer and wait 5 minutes. [While waiting, fill out the remaining parts of your data sheet.]
   After 5 minutes, remove the tube from the protective sleeve.
   Note: If missing a sleeve, leave test tube inside kit box to keep out of the sun.




4. Insert the tube into the Octa-Slide 2 Viewer. Hold the viewer so that non-direct light enters through the back. Match sample color to a color standard.

When you find a matching color, multiply the corresponding number by 4.4 and record the result on your data sheet. This is your nitrate level. Using the information below, determine whether this reading is in the acceptable range, and circle yes or no on your data sheet.

## WHAT DO THESE NUMBERS MEAN?

0-4.4 mg/L is acceptable, healthy amount of nitrogen

>4.4 mg/L is unacceptable, potential for eutrophication

# CHANGE IN TEMPERATURE

## WHY DOES TEMPERATURE MATTER?

Water temperature changes within a river's segments, called reaches, impact various chemical aspects of the water. Human activities contribute to these temperature shifts by altering stream bank vegetation, removing tree canopy that provides shade, impounding water with dams, and releasing heated water from industrial sources like power plants. Additionally, runoff from surfaces such as roads and parking lots, which flows into storm drains and eventually into the river, serves as a significant source of both heated and contaminated water.

## STEPS:

Find the thermometer in your kit on a small side pocket.

1. At the site where you are performing the water quality tests, but away from the shore, submerge the thermometer about 4 inches below the surface. Do not let it touch the bottom of the stream and hold it tightly.
2. Hold in place for about two minutes, until it remains constant, and read the temperature while the thermometer is still under water.
3. Record the temperature on your data sheet in degrees Celsius.
4. Immediately go 10 meters upstream from your original test site and repeat this procedure. If you are unsure where to test, ask your educator. Try and find a location that has similar habitat to where you took the first test- i.e. similar water depth, sunlight, and water speed.
5. Record the temperature on your data sheet in degrees Celcius

Determine the temperature change for that “reach” using the following equation:

**Temperature downstream - temperature upstream = temperature change**
**(minus) (equals)**

Using the information below, determine whether range, and circle yes or no on your data sheet.

## WHAT DO THESE NUMBERS MEAN?

| | |
|---|---|
| A change of 0-5 degrees Celsius | is acceptable |
| A change of greater than 5 degrees Celsius | is unacceptable, indicator of thermal pollution |

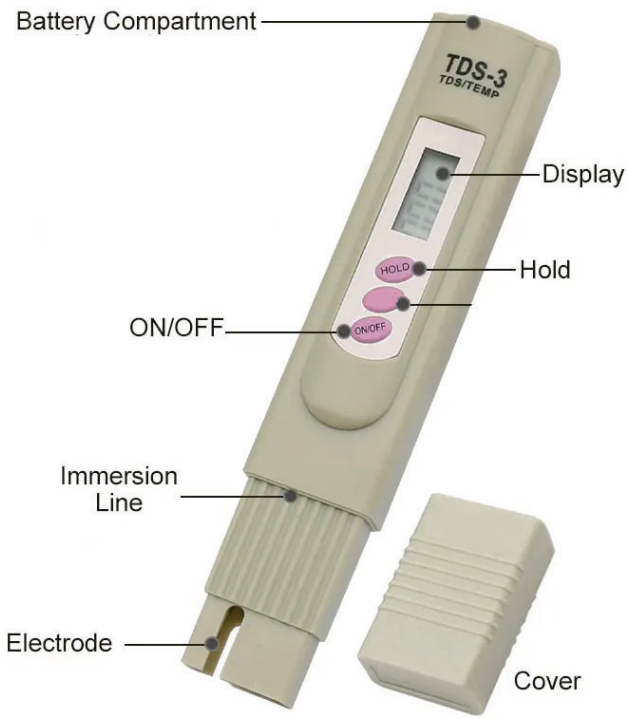
# TOTAL DISSOLVED SOLIDS

## WHY DO TOTAL DISSOLVED SOLIDS MATTER?

Bodies of water contain varying amounts of dissolved materials, measured as Total Dissolved Solids (TDS). TDS sources include runoff from urban areas carrying salts, fertilizers, and other materials. Rainwater has minimal dissolved content (<10 ppm TDS), while rivers usually range from 100 to 2,000 ppm. Municipal water systems aim for <500 ppm TDS for drinking water. Elevated TDS levels give water a mineral taste and may induce a laxative effect. For this parameter, the water sample is tested in place. This means the meter is placed in the actual body of water, not a test tube.

## STEPS:

1. Remove the cover from the bottom of the TDS meter.
2. Press the ON/OFF button once to turn the meter on. When you are sure the meter is on, dip the meter's electrode (the uncapped end) into the water sample up to the immersion line. Hold the electrode in the sample for 10 seconds to stabilize the reading.
3. Press the HOLD button to hold the reading. Remove the meter from the water.
4. Record the ppm reading on the display on your data sheet.
5. Press the ON/OFF button once to turn the meter off. The display will be blank when it is off.
6. Rinse the electrode with distilled or tap water provided by your instructor. Put the cover back on the meter.
7. Use the guidelines below to determine whether the total dissolved solids is in the acceptable range, and circle yes or no on your data sheet.



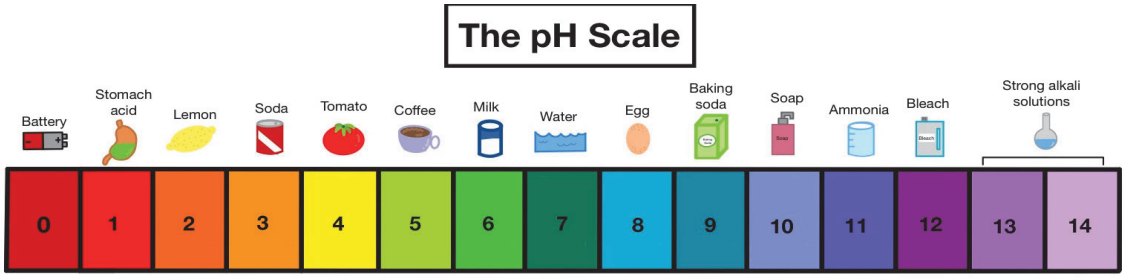

## WHAT DO THESE NUMBERS MEAN?

| | |
|---|---|
| < 500 ppm TDS | is acceptable |
| >500 ppm TDS | is polluted/unacceptable |

# pH Kit 5858-01

## WHY DOES pH MATTER?

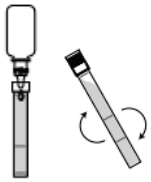
The pH test is one of the most common variables used in water quality testing. pH ranges from 0-14, with 7 considered neutral, 14 most basic, and 0 most acidic. What we call pH is really a ratio of hydrogen ion (H+) to hydroxide ions (OH-) in the sample. Changes in pH can come from climate change, coal mining, and gases from power plants. The chart below shows the pH of common substances, and the chart at the end of some aquatic organisms.




## STEPS:

1. Fill a test tube to the 10 ml line with the sample water.

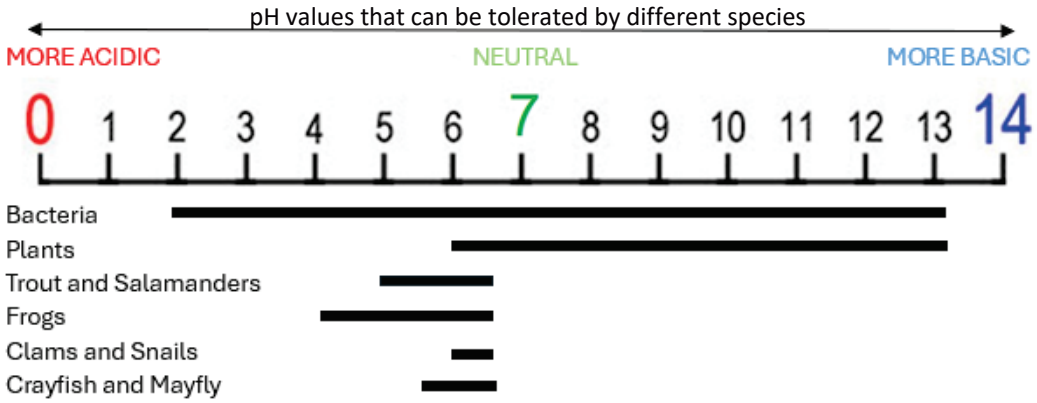
2. Add 10 drops of the Wide Range pH Indicator. Cap and mix.



3. Insert the test tube into the Viewer.

4. Use the colored bars to match the sample color to a color standard. Hold the Viewer so that non-direct light enters the back.

Record your pH result on your data sheet. Using the information below, determine whether this reading is in the acceptable range and circle yes or no on your data sheet.




## WHAT DO THESE NUMBERS MEAN?

pH of 6.5-9 is ideal and acceptable

pH of 4-6 is stressful, but acceptable

pH of 10-11.5 is stressful, not acceptable

pH of 0-3 is fatal, not acceptable

pH of 12-14 is fatal, not acceptable

# FECAL COLIFORM

## WHY DOES FECAL COLIFORM MATTER?

Fecal coliform bacteria (most commonly Escherichia coli, abbreviated E. coli) are found naturally in the lower intestine of many vertebrates, including humans. They are not found in water unless intestinal wastes (feces) have contaminated the water, so their presence in water is a reliable indicator of fecal contamination.

Fecal coliform bacteria do not usually cause disease, but many other types of organisms present in sewage do. It is much easier to test for E. coli than for all the other possible types of fecal coliform. Therefore, E. coli can be used to warn us about the possible presence of those other pathogenic (disease-causing) organisms.

## STEPS:

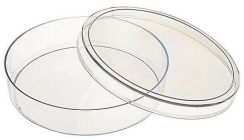
### Part 1 - In the Field - Setting up the Petri Dish

1. Use a sterile pipette to add 3 mL of sample water to the Coliscan bottle.
2. Tightly cap the Coliscan and water mixture and gently swirl. **Do not shake.** Shaking will cause foam to form, making the plate difficult to read.
3. Pour the Coliscan and water mix into a pre-treated Petri dish on a level surface You want the mixture to cover the bottom surface of the Petri dish.
4. Tape the Petri dish shut. Label with the date and time of sampling.
5. Cover and set the Petri dish on a level surface until the liquid forms a gel, about 30-45 minutes.
6. Have your teacher take the Petri dish back to the classroom and follow instructions on Part 2.



3 mL of sample water


Add 3 mL to bottle



Pour mixture to Petri dish

FECAL COLIFORM continued:

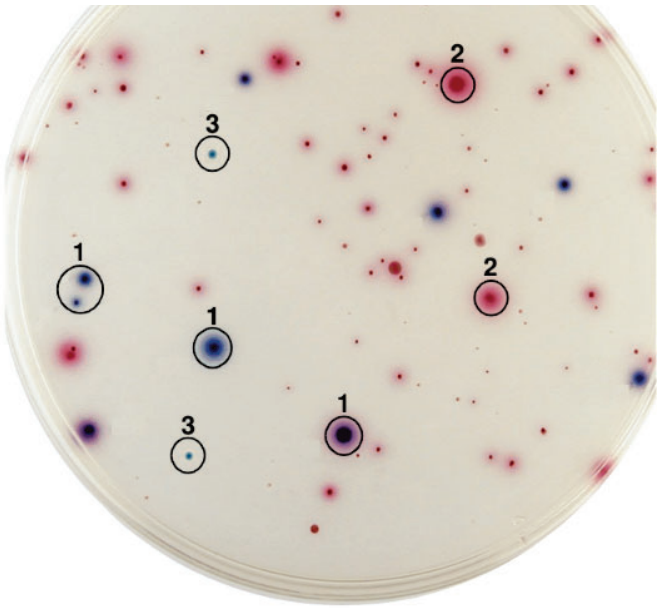
## Part 2 - In the Classroom - Reading and Calculating Results

1. Incubate in a warm place (29.5-35.0 C) for 24 hours or at room temperature for 48 hours. Results after 48 hours should not be counted.
2. After incubation, count bacteria colonies by looking at the Petri dish on a white sheet of paper, and then on a black sheet of paper for comparison.
3. Count only the **dark blue and purple colonies** that appear as fecal coliform.
4. All other colored colonies are members of the coliform group, but only the ones in dark blue and purple are fecal. The E.Coli are generally the fastest growing types of bacteria on this medium.
5. After 48 hours other types of bacteria may appear, which should not be regarded.
6. To determine the coliform colonies per 100 mL of water, divide 100 mL by the 3mL sample and then multiply that number by the number of coliform colonies o n your Petri dish. The equation is:
   (100/3) x the number of coliform colonies = colonies/100 mL
7. Record the number of colonies/100 mL on student data sheet.
8. Using the information below, determine whether this reading is in the acceptable range and circle yes or no on data sheet.

## WHAT DO THESE NUMBERS MEAN?

<200 colonies/100 mL is acceptable

>200 colonies/100 mL is polluted/unacceptable




1. E. *coli* (dark blue/purple)
2. Other Coliforms (pink/red)
3. Teal/Green colonies

# BIOCHEMICAL OXYGEN DEMAND (BOD)

## WHY DOES BOD MATTER?

Organisms that need oxygen to stay alive are called “aerobic.” When aerobic bacteria decompose aquatic organic matter, they use oxygen in the water. Biochemical Oxygen Demand (BOD) is a measure of how much oxygen these bacteria use in the aerobic oxidation of organic matter. Bacteria use oxygen dissolved in water to decompose excess algae, animal manure, and pollutants such as inadequately treated sewage.

## STEPS:

Ask your teacher if your class is completing the BOD test today. If so, they will provide you with your own water sample bottle.

Your goal in collecting the water sample is to make sure that there are no bubbles or air pockets in the sample bottle.

1. Take the water sample bottle provided by your teacher and rinse the bottle in the sample water.
2. Submerge the bottle (with lid attached) under water (your hand will get wet). Remove the cap while the bottle is under water, and tilt until it is completely filled. While the bottle is completely submerged, tap the sides of the bottle until all air bubbles are gone.
3. **Important**, while the bottle is still under water, put the cap back on the bottle. When you take the bottle out of the water, invert it (turn it upside down) with the cap on to check again to make sure no air bubbles are trapped inside. If there are any bubbles, repeat the process until you can fill the bottle all the way, with no bubbles.
4. Cover the bottle tightly with aluminum foil, label, and bring it back to school with you. Keep it wrapped in the aluminum foil, and store undisturbed in a dark place for five days. Ideally, the sealed BOD sample is placed in an incubator at 20°C ± 1°C for 5 days.

## BIOCHEMICAL OXYGEN DEMAND continued:

Then, using the 5-day old sample water, follow the test procedures for Dissolved Oxygen, and record the mg/L on your data sheet.

To determine BOD, subtract your result from the DO reading of the same-day sample:

**DO of same-day sample (mg/L) – DO of sample after 5 day incubation (mg/L) = BOD**

Using the information below, determine whether this reading is in the acceptable range, and circle yes or no on your data sheet.

## WHAT DO THESE NUMBERS MEAN?

< 5 mg/L difference is acceptable

6-29 mg/L difference is considered moderately polluted/unacceptable

> 30 mg/L difference is unsafe/unacceptable