# Flint River GREEN: Biochemical Oxygen Demand (BOD)

## Materials
- LaMotte Dissolved Oxygen Kit
- BOD Sample Bottle
- Foil
- Safety Goggles (1/person)
- Nitrile Gloves (2/person)
- Overflow Container
- Paper Towel
- Waders or Boots

## Vocabulary
- Biochemical Oxygen Demand (BOD)
- Plankton
- Organic Waste
- Food Processing Plants
- Macroinvertebrates
- Food Web

## Water Quality Standards

<table>
<thead>
<tr>
<th>DRINKING WATER:</th>
<th>SURFACE WATER:</th>
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<tbody>
<tr>
<td>- No drinking water standards</td>
<td>- No state standards for BOD</td>
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<td></td>
<td>- Good BOD is ( \leq 3 ) ppm (mg/L), Moderate is 4-8 ppm</td>
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<td>- Facilities that discharge into streams have permitted standards (City of Flint Wastewater Treatment Plant has a BOD permit for 9-24 mg/L).</td>
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## Biochemical Oxygen Demand (BOD)

BOD measures how much oxygen is being used by plankton (or microscopic plants and animals) in the sample. During the five days (in the dark), living organisms use oxygen to survive. When little oxygen is removed during the five days that indicates there are very few organisms using the oxygen. Organic waste can come from manure, decomposing plants, discharge from food processing plants, or agricultural runoff. High BOD can also come from natural sources, such as runoff from swamps.

## Evidence for Decreased BOD?
- Is there little or no organic waste present? If so, there won't be as many bacteria present using up oxygen to decompose it.

## Evidence for Increased BOD?
- Is there organic waste such as human or animal feces, decomposing plant matter (like leaves or grass clippings), food waste, or agricultural runoff nearby?
- Are there natural sources such as runoff from swamps?

## Connecting Concepts
- Nitrates and phosphates in a body of water can contribute to high BOD levels. Nitrates and phosphates are plant nutrients that cause plant life and algae to grow quickly. When plants grow quickly, they also die quickly. This contributes to the organic waste in the water, which is then decomposed by bacteria.
- At high BOD levels, organisms such as macroinvertebrates that are more tolerant of lower dissolved oxygen may appear and become numerous. Organisms that need higher oxygen levels will NOT survive which would heavily impact the ecosystem and food web.

## Web Links
- Video (2 min): University of Wyoming Extension—Decomposition: [https://youtu.be/GzH_FVgE3C8](https://youtu.be/GzH_FVgE3C8)
- Video (5 min): Chesapeake Bay Program—Plankton: [https://www.chesapeakebay.net/discover/ecosystem/plankton](https://www.chesapeakebay.net/discover/ecosystem/plankton)
Part 1 of 3 (at stream):
1. Inventory your test kit supplies—check to be sure everyone has gloves and goggles on first!
2. Enter the stream wearing gloves and waders or boots. Aim to stand as close to the middle of the main stream flow as possible and away from vegetation. Try not to kick up excess sediment from the bottom of the stream while you are working. SAFETY NOTE: Do not stand in water above your ankles if wearing boots or above your knees if you are wearing waders. DO NOT enter flooded streams.
3. Rinse the glass sample bottle with stream water, then empty the bottle and cap it.
4. While the cap is on the bottle, submerge the bottle completely (≥ 4”), then remove the cap and allow the bottle to fill with water.
5. While the bottle is still under water, tip the bottle sideways, tapping it until all of the air bubbles escape.
6. Cap the bottle while it is still under the surface of the water.
7. Remove the bottle and tip it upside down to be sure there are no air bubbles left inside. If there are air bubbles, repeat steps 3-7.
8. Cover immediately with foil and store away from sunlight at 20°C/68°F for 5 days.

Part 2 of 3 (at school 5 days after collection):
1. Inventory your test kit supplies—check to be sure everyone is wearing gloves and goggles first!
2. Work over a container and make sure no air is added to the sample while adding chemicals.
3. Remove the foil and the cap from the bottle. Add 8 drops of Manganese Sulfate Solution AND 8 drops of Alkaline Potassium Iodide Azide.
4. Cap the bottle and invert several times. A precipitate will form.
5. After the precipitate settles below the shoulder of the bottle, remove the cap and add 8 drops of Sulfuric Acid, 1:1. SAFETY NOTE: This chemical may burn if it gets on your skin, even through your clothes. Make sure you wash any skin contact with this chemical immediately with soap and water.
6. Cap the bottle and wipe off any chemical overflow.
7. Mix by gently inverting several times. DO NOT shake the bottle. Your sample should turn orange.
8. Continue inverting until the precipitate has dissolved (5-10 minutes). Use this time to read and understand part 3 of these instructions.
9. The sample is now fixed and Part 3 of the test may be completed the next day if short on time.
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**Part 3 of 3 (at school):**

1. Gloves and Goggles! Fill the **titration tube** to the **20mL** line with the fixed sample from Part 2.
2. After shaking the bottle, add **8 drops** of Starch Indicator Solution and cap the titration tube. Your sample should turn blue. **NOTE:** This step usually comes later in the instructions that come with the LaMotte kit. Instead, it is easier and accepted to add the Starch Indicator Solution here.
3. Depress the plunger of the Titrator completely.
4. Insert the tip of the Titrator into the hole in the plug on the top of the Sodium Thiosulfate 0.025N titrating solution.
5. Invert the bottle once the Titrator has been inserted and slowly withdraw the plunger until the widest ring on the plunger lines up with the zero (0) line on the scale. **NOTE:** If small air bubbles appear in the Titrator, get rid of them by partially filling the Titrator and then pumping the titration solution back into the container. Repeat until the air bubbles disappear.
6. Turn the bottle upright and remove the Titrator. **SAFETY NOTE:** The Titrator is very sensitive. Be sure everyone in your group is wearing goggles and keep the Titrator pointed away from others during this test.
7. Insert the tip of the Titrator into the opening of the titration tube cap.
8. Slowly depress the plunger to dispense the titrating solution, stopping at each whole number and swirling the titration tube until the color begins to fade.
9. When the color begins to fade, add smaller amounts of the titrating solution as you continue (stop at each subdivision on the Titrator scale to swirl). You want to pinpoint the exact reading on the scale that the solution turns colorless. **TIP:** View reaction over a white piece of paper.
10. If you dispense all of the titrating solution before your sample turns colorless, fill the Titrator again and continue the titration. If this occurs you will need to add 10 to your final reading.
11. Once your sample is colorless, read where the widest ring meets the scale on the Titrator to the nearest subdivision (each subdivision is 0.2). If you feel you added too much titrating solution, you can re-do the test with the rest of your fixed sample form Part 2.
12. Final Reading: _____ (+ 10 if you re-filled the Titrator) = ________ Parts Per Million (ppm)
13. Record your result, clean up, and dispose of your chemicals in a hazardous waste container.
14. Subtract your final reading here from the final reading of your dissolved oxygen test at the stream to get your Biochemical Oxygen Demand (BOD): _____ DO(1) - _____ DO(2) = _____ ppm BOD
15. Use this BOD value with the BOD Q-Value chart to calculate your Q-Value: _______ (ppm=mg/L).

**WHAT TO WATCH OUT FOR**

- Make sure to remove the air bubbles from the Titrator when adding the Sodium Thiosulfate.
- Make sure to add 10 to your final reading if you refilled the Titrator.
- Be sure to subtract the reading you got on this test from the reading that was calculated on the dissolved oxygen test.
- Try to take the temperature reading from the stream as close as possible to the time that you did Parts 1 & 2.
For BOD, use Dissolved Oxygen Kit

*You will also need foil or tape to cover the Water Sampling Bottle for 5 Days Prior to Testing

**Contents**

a. Manganous Sulfate Solution - [4167-G]
b. Alkaline Potassium Iodide Azide - [7166-G]
c. Sulfuric Acid, 1:1 - [6141WT-G]
d. Sodium Thiosulfate, 0.025N - [4169-H]
e. Starch Indicator Solution - [4170WT-G]
f. Direct Reading Titrator - [0377]
g. Test Tube, 5-10-12.9-15.20.25mL, glass - [0608]