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# Flint River GREEN Testing at the Stream

## MATERIALS
- LaMotte Test Kits
- Cooler with Coliscan
- Water
- Lab Bottles (TS and FC)
- Safety Goggles (1/person)
- Nitrile Gloves (2/person)
- Hazardous Waste Container
- Trash Bags
- Foil
- Boots or waders

## VOCABULARY
- Algae
- Aquatic
- Data
- Habitat
- Hazardous Waste
- Meniscus
- mg/L
- Parts Per Million (ppm)
- Photosynthesis
- Q-Value
- Run-Off
- Sediment
- Stormwater
- Surface Water
- Tributary
- Vegetation
- Water Quality Index (WQI)
- Watershed

## WATER QUALITY STANDARDS

### DRINKING WATER:
Where is your drinking water coming from?
Under the Safe Drinking Water Act, the EPA sets standards for drinking water quality from public drinking water supplies.

### SURFACE WATER:
The EPA and State of Michigan sets limits for each type of water quality test. If you find that your data is not within the standards, notify your mentor or FRWC immediately.

## WHAT DO THESE TESTS MEASURE?
Students will find that each test is somehow connected to one or more other tests. These connecting concepts will help students to identify patterns in their data which will contribute to being able to generate claims for suggested courses of action using evidence and reasoning. Other connecting concepts will help students link each test to other areas of investigation and link this project to other subject areas.

## EVIDENCE FOR DECREASES IN TEST MEASURE?
Each water quality test comes with its own set of both man-made and natural causes for changes in water quality. This section provides questions that help students think about what might cause these changes.

## EVIDENCE FOR INCREASES IN TEST MEASURE?
Students should look at areas surrounding the site through electronic resources and on-the-ground observations to answer these questions. These answers can help build claims, evidence, and reasoning for analysis.

## WEB LINKS
- Flint River GREEN website: [www.FlintRiverGREEN.org](http://www.FlintRiverGREEN.org) for past years’ data and resource links
- Flint River Watershed Coalition website: [www.FlintRiver.org](http://www.FlintRiver.org) for watershed maps and more
- Case Study: The Flint Water Crisis—What Happened and Why: [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5353852/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5353852/)
Flint River GREEN Data Collection:
It is extremely important that the data collected for this project be as high quality as possible. Some of this data is used to satisfy National Pollutant Discharge Elimination System (NPDES) storm water requirements and is being used by other policy makers to make decisions. Additionally, if a water quality problem is identified and action plan is developed for a civic approach to resolve or minimize the problem, the data should also be as accurate as possible.

**Use Standard Chemistry Procedures and Safety:**
These tests use small measurements and hazardous chemicals. Be sure to always follow safe and precise procedures.

- ALWAYS wear safety goggles and gloves when handling materials
- Work over a overflow container/tarp to prevent spills and wipe up your area after testing
- Understand what a meniscus is and use this to measure your samples
- When adding drops of chemicals, hold the bottle upside down and squeeze gently. Holding the bottle sideways or squeezing the bottle too much will add extra chemical to your sample.

**Standard Water Sample Collection Procedures:**
**Bucket Method:** Your group may find that dropping a bucket on a rope from a bridge or sending one person into the stream to collect a bucket of water may be safest and easiest for most tests (including pH, nitrates, turbidity, phosphates, fecal coliform, and total solids. If you do this, be sure to first rinse the bucket with stream water. It is highly recommended that the Temperature, Dissolved Oxygen, and Biochemical Oxygen Demand test samples are collected directly in the stream unless deemed unsafe.

**In-Stream Collection:** SAFETY NOTE: Do not stand in water above your ankles if wearing boots or above your knees if you are wearing waders. DO NOT EVER enter flooded streams.

1. Enter the stream downstream of your sample location with your bucket or sample bottle, wearing gloves and waders or boots.
2. Begin walking upstream aiming to stand as close to the middle of the main stream flow as possible. Move slowly allowing the sediment to wash behind you (sediment stirred up from the bottom could otherwise cause inaccurate readings).
3. Rinse your bucket or collection bottle with stream water and discard away from your sample location.
4. Collect your sample while facing upstream. Be sure to collect from the middle of the stream flow, in the middle of the water column—not just the surface and not too close to the bottom.

**Hazardous Waste:**
All treated samples, chemicals and containers should be disposed of in designated hazardous waste containers and disposed of following MSDS recommendations which can be found at [http://www.lamotte.com/en/support/sds-search](http://www.lamotte.com/en/support/sds-search). TIP: Your local waste authority or municipality likely has regular hazardous waste disposal days throughout the year. Containers should be labeled with all potential chemical components before being dropped off at events.

*Be sure to practice all of your tests in the classroom and if possible outdoors in a pond or ditch before going to the stream.*

*If you feel like you made an error at any point, you should re-do the test.*

*Always ask yourself if the result makes sense. If it does not make sense, talk with your mentor.*

*If you are conducting the same test multiple times (or if multiple groups do the same test), be sure to sample as closely to the same time on the same day as possible. Most test results can be averaged, except a mode (most common value) should be reported if doing multiple tests for pH and phosphates.*
### Flint River GREEN: pH

<table>
<thead>
<tr>
<th>MATERIALS</th>
<th>VOCABULARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaMotte pH Test Kit</td>
<td>Acidic</td>
</tr>
<tr>
<td>Gloves (2/person)</td>
<td>Leaching</td>
</tr>
<tr>
<td>Safety Goggles (1/person)</td>
<td>Basic (alkaline)</td>
</tr>
<tr>
<td></td>
<td>Logarithmic Scale</td>
</tr>
<tr>
<td></td>
<td>Acid Rain</td>
</tr>
<tr>
<td></td>
<td>Storm Outfall</td>
</tr>
<tr>
<td></td>
<td>Stream Bed Geology</td>
</tr>
</tbody>
</table>

**MATERIALS**

- LaMotte pH Test Kit
- Gloves (2/person)
- Safety Goggles (1/person)

**VOCABULARY**

- Acidic
- Basic (alkaline)
- Leaching
- Logarithmic Scale
- Solubility
- Acid Rain
- Storm Outfall
- Stream Bed Geology

<table>
<thead>
<tr>
<th>WATER QUALITY STANDARDS</th>
<th>DRINKING WATER: 6.5—8.5</th>
<th>SURFACE WATER: 6.5—9</th>
</tr>
</thead>
</table>

**WATER QUALITY STANDARDS**

- Neutral: 6.5—8.5
- Basic (alkaline): 6.5—9

**WHAT DOES THIS TEST MEASURE?**

- pH measures how acidic, neutral or basic (alkaline) water is. The pH scale goes from 0 - 14. Most life does well around the number 7. The further the water is in either direction from 7, the greater stress is upon living things. Strong acids, like “battery acid”, would have pH around 1 while a strong base like bleach would have a pH near 12. pH is a logarithmic scale, which means something with a pH of 6 is 10x as acidic as a liquid with a pH of 7. A liquid with a pH of 5 is 100x as acidic as a liquid with a pH of 7. The pH depends on may factors such as stream vegetation, stream bed geology and the presence of water pollutants.

**LOOK FOR THESE CAUSES**

- EVIDENCE FOR DECREASES IN pH (acidic)?
  - Have we recently had Acid Rain
  - Is fertilizer runoff possible nearby or upstream?
  - What types of industrial pollution might occur nearby or upstream?

- EVIDENCE FOR INCREASES IN pH (basic)?
  - What types of industrial pollution might occur nearby or upstream?
  - What natural minerals might be leaching (limestone increases pH)?
  - Is it possible there are any soaps/detergents?
  - Are there any recent fires that produced ash?

**Michigan soils tend to be high in calcium which prevents lakes and streams from undergoing rapid changes in pH.**

**The pH of water determines the solubility and biological availability of nutrients (phosphorus, nitrogen, and carbon) and heavy metals (lead, copper, cadmium, etc.).** For example, heavy metals tend to be more toxic at lower pH (acidic) because they are more soluble here.

**WEB LINKS**

Flint River GREEN: pH

For Test Kit A: Octet Comparator - see photo (right):
1. Check to be sure everyone is wearing gloves and goggles.
2. Inventory the supplies in your test kit using next page.
3. Fill a test tube to the 5 mL line with sample water.
4. Add 10 drops of Wide Range pH Indicator.
5. Cap and mix gently.
6. Insert test tube into the comparator
7. Match sample color to a color standard and record as pH: __________
8. Dispose of your sample and rinse the test tube into the hazardous waste container.
9. Calculate a Q-Value on the pH Chart. Q-Value: _______
10. Check the Q-Value by entering your pH data at http://www.flintrivergreen.org/add-info/add-data/

For Test Kit B: Octa-Slide Viewer - see photo (left):
1. Check to be sure everyone is wearing gloves and goggles.
2. Inventory the supplies in your test kit using next page.
3. Fill a test tube to the 10 mL line with sample water.
4. Add 10 drops of Wide Range pH Indicator.
5. Cap and mix gently.
6. Insert test tube and the pH Octa-Slide 2 Bar into the viewer.
7. Match sample color to a color standard and record as pH: _______
8. Dispose of your sample and rinse the test tube into the hazardous waste container.
9. Calculate a Q-Value on the pH Chart. Q-Value: _______
10. Check the Q-Value by entering your pH data at http://www.flintrivergreen.org/add-info/add-data/

WHAT TO WATCH OUT FOR

• Be sure the glass is clean prior to testing, that nothing is left from previous samples.
• Take care to slowly add and count the drops of indicator to the sample. If you add more than 10 drops or if you lose count, dispose of your sample in a hazardous waste container and re-do the test.
• Be sure not to sample to close to a storm outfall. Ask a mentor for help if you are unsure.
• Do not disturb the stream bed while taking a sample. Also, do not take your sample too close to the surface or too close to the stream banks. The best place to take a sample is from the middle of the stream.
• It’s a good idea to perform a colorblindness test prior to viewing color scales such as pH. There are many free versions online.
Kit A: Octet Comparator pH Kit

Contents
a. Octet Comparator (pH 3.0-6.5) - [2193]
b. Octet Comparator (pH 7.0-10.5) - [2196]
c. (x2) Test Tube, Glass, 5mL w/cap - [0230]
d. Wide Range Indicator - [2218-G]

Kit B: Octa-Slide pH Kit

Contents
a. Octa-Slide 2 Viewer - [1101]
b. Octa-Slide Bar (pH 3.0-6.5) - [2193-01]
c. Octa-Slide Bar (pH 7.0-10.5) - [2196-01]
d. (x2) Test Tube, Square, Plastic, w/cap 2.5-5-10mL - [0106]
e. Wide Range Indicator - [2218-G]
# Flint River GREEN: Temperature & Weather

## Materials
- Calibrated Thermometer \(^{\circ}C\)
- Stopwatch or Timer
- Waders or Rubber Boots
- Calculator

## Vocabulary
- Stream Reach
- Coldwater Stream
- Warmwater Stream
- Metabolic Rates
- Industrial Pollution
- Impervious Surfaces

## Materials

<table>
<thead>
<tr>
<th>Water Quality Standards</th>
<th>Drinking Water:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No drinking water standards</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surface Water:</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5°F temperature difference in stream reach</td>
</tr>
</tbody>
</table>

## Water Temperature

Water temperature determines what kind of fish or other organisms will live in a habitat. Water temperature influences many systems such as the amount of oxygen that can be dissolved in the water, the rate of photosynthesis by aquatic plants, the metabolic rates of aquatic organisms, and the sensitivity of organisms to toxic wastes, parasites and diseases. Some animals can only live in cool water, like trout. This test will also measure the change in water temperature between two points to help identify any stream reaches that undergo large temperature changes that could be caused by thermal pollution.

## Evidence for Decreases in Temperature?

- Is there a lot of shade covering your stream at your sample location? How much shade occurs upstream?

## Evidence for Increases in Temperature?

- Is there industrial pollution upstream?
- Is there runoff from impervious surfaces?
- Do you see evidence of soil erosion nearby (more sediment in the water will cause it to heat up)?

## Coldwater Streams

- \(^{\circ}F\) have an average monthly temperature at or below the following: J:38 F:38 M:43 A:54 M:65 J:68 J:68 A:68 S:63 0:56 N:48 D:40.

## Warmwater Streams

- \(^{\circ}F\) have an average monthly temperature at or above the following: J:41 F:40 M:50 A:63 M:76 J:84 J:85 A:85 S:79 0:68 N:55 D:43

- Dark surfaces and particles heat up because dark colors absorb the sun’s rays.
- Water temperature fluctuates between day and night and over seasons and years.
- Colder water can hold more dissolved oxygen.

## Web Links
- USGS Water Science School: [https://water.usgs.gov/edu/temperature.html](https://water.usgs.gov/edu/temperature.html)
Flint River GREEN: Temperature & Weather

1. Record a weather report:
   - Date: ________________  Time: ____________ am/pm
   - Location: _________________________________________________________________
   - Weather: _________________________________________________________________
   - Type of precipitation in the last 24 hours: rain snow sleet hail none
   - Amount of precipitation in the last 24 hours: ________________________________
   - [Link](https://www.enviroweather.msu.edu/run.php?stn=flt&mod=w_rr&da1=20&mo1=10&da2=22&mo2=10&yr=2019&mc=531&ds=cd)
   - Additional notes about site: ______________________________________________

2. Circle what shading conditions best describes your location for this test:
   - direct sunlight  shaded  partial shade

3. Enter the stream wearing gloves and waders or boots. Aim to stand as close to the middle of the main stream flow as possible. 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.

4. Lower the thermometer 4” below the surface of the water. Wait 2 minutes.

5. Record your measurement in °C below.

6. Repeat this measurement 3 times: 1. ____ °C  2. ____ °C  3. ____ °C

7. Calculate an average: (Test 1 + Test 2 + Test 3)÷3 = ____ °C at sample site.

8. Report this value to the Dissolved Oxygen testing team.

9. Repeat this test approximately 1 mile upstream in similar shading conditions as soon as possible.
   - (Your teacher or mentor may also do this for you if that area is not accessible to students): ____ °C upstream.

10. Subtract the upstream from downstream temperature:
    - ____ (downstream) - ____ (upstream) =________°C Temperature Change (ΔT:°C)

11. Use the ΔT:°C to calculate a Q-Value on the Temperature Change Chart. Q-Value: ______


**WHAT TO WATCH OUT FOR**
- Take your reading in the main flow of the stream. Keep the thermometer below the surface, but not touching the bottom of the stream.
- Hold your thermometer in the stream for no less than 2 minutes, then read your thermometer immediately after pulling it from the water.
- Take the temperature at both locations under similar amounts of shade.
- Take the upstream temperature as soon as you can after the test site temperature. As the day heats up, so does the stream.
- Use the change in temperature between the two locations to determine the Q-Value.
- Make sure you are using Celsius: °C = (°F - 32.0)÷2.80  °F = (°C x 1.80)+32.0
Flint River GREEN: Temperature

Chart 5: Change in Temperature ($\Delta T$, °C) Test Results

$\Delta T$: °C
Flint River GREEN: Total Solids

**MATERIALS**
- Empty Water Bottle (or other bottle that holds > 250 mL)
- Cooler
- Gloves (2/person)
- Safety Goggles (1/person)

**VOCABULARY**
- Total Solids (TS)
- Total Dissolved Solids (TDS)
- Total Suspended Solids (TSS)
- Heavy Metals

**WATER QUALITY STANDARDS**

<table>
<thead>
<tr>
<th>DRINKING WATER:</th>
<th>SURFACE WATER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg/L Total Dissolved Solids</td>
<td>No standards for surface water in Michigan</td>
</tr>
</tbody>
</table>

Water is often referred to as the universal solvent because so many substances can dissolve in it. As water moves, it picks up a variety of solid materials that can become suspended or dissolved in the water or can settle out farther downstream. **Total Solids (TS)** is a measure of a combination of **total suspended solids (TSS)** and **total dissolved solids (TDS)**. Total suspended solids are substances in water that will not settle at the bottom of a container not in motion, but can be captured by a filter. Total dissolved solids are substances that are dissolved in water. If a solid is heavy enough to settle at the bottom of a container not in motion (such as leaves, soil, and sewage), it is neither TSS or TDS, but is still included in the total solids measurement. High concentrations of total solids may contribute to unpleasant taste in drinking water and affect other water quality measures. This test is done at a professional lab. Students collect the sample.

**WEB LINKS**

**WHAT DOES THIS TEST MEASURE?**

**连接概念**
- Water is often referred to as the universal solvent because so many substances can dissolve in it. As water moves, it picks up a variety of solid materials that can become suspended or dissolved in the water or can settle out farther downstream. **Total Solids (TS)** is a measure of a combination of **total suspended solids (TSS)** and **total dissolved solids (TDS)**. Total suspended solids are substances in water that will not settle at the bottom of a container not in motion, but can be captured by a filter. Total dissolved solids are substances that are dissolved in water. If a solid is heavy enough to settle at the bottom of a container not in motion (such as leaves, soil, and sewage), it is neither TSS or TDS, but is still included in the total solids measurement. High concentrations of total solids may contribute to unpleasant taste in drinking water and affect other water quality measures. This test is done at a professional lab. Students collect the sample.

**LOOK FOR THESE CAUSES**
- EVIDENCE FOR DECREASES IN TOTAL SOLIDS?
  - Are riparian zones vegetated and healthy?
  - Have nearby communities reduced road salt and chemical usage?
  - Are sewer and septic systems maintained?
  - Is the aquatic food web balanced?

- EVIDENCE FOR INCREASES IN TOTAL SOLIDS?
  - Is there evidence for erosion/excess soil?
  - Is there industrial waste/sewage entering stream?
  - Is there potential for excess salts/minerals?
  - Is there excess phytoplankton/algae growing?
  - Are there decaying plants and animals?
  - Are bottom feeding fish present such as carp?

- High concentrations of total solids can increase turbidity and water temperature.
- High concentrations of total solids can decrease photosynthesis by reducing the amount of light accessible to aquatic plants.
- High concentrations of total solids can increase the binding of sediment with toxic compounds and heavy metals.
Flint River GREEN: Total Solids

1. Check to be sure everyone is wearing gloves and goggles.
2. Inventory: You will need a cooler, a marker/pen, and an empty and rinsed water bottle (or other glass or plastic container that will hold 250 mL or more). A typical water bottle holds 500 mL.
3. Label your container with “Total Solids” along with the date, time, your school, teacher name, and site name (stream name and place).
4. Enter the stream with your bottle, while wearing gloves and waders or boots following standard water sample collection procedures.
5. Fill your collection bottle with at least 250 mL of stream water following standard water sample collection procedures.
6. Cap your sample and immediately place in a cooler. Transfer the sample to a refrigerator as soon as possible. Sample must be kept at or under 6°C (42.8°F).
7. Your teacher or mentor will help get your sample to the lab within 2 days for testing where they will measure, dry and weigh your sample. Currently the City of Flint processes the total solids samples and your teacher will receive a result in 1-2 weeks: TS: ________ mg/L
8. Use the TS mg/L to calculate a Q-Value on the Total Solids Chart. Q-Value: ________
9. Check your Q-Value by entering your total solids data at http://www.flintrivergreen.org/add-info/add-data/

### WHAT TO WATCH OUT FOR

- Make sure your label is filled out and remains clear to read.
- Be sure to collect the water sample following standard collection procedures so that you do not introduce extra sediment and solids into the sample.
- Be sure to keep your sample refrigerated at all times.
### Flint River GREEN: Turbidity

#### MATERIALS
- LaMotte Turbidity Test Kit
- Bottled or Clear Tap Water
- Gloves (2/person)
- Safety Goggles (1/person)

#### VOCABULARY
- Jackson Turbidity Units (JTU)
- Erosion
- Turbidity
- Waste Water Discharge
- Designated Use
- Urban Runoff
- Clarity
- Suspended Solids
- Light Penetration
- Base Flow

#### WATER QUALITY STANDARDS
- **DRINKING WATER:**
  - State of MI: 0.5 JTU
  - Most drinking water providers strive for even better at 0.1 JTU
- **SURFACE WATER:**
  - There is no state standard except to say that turbidity should not occur in any unnatural quantity which is or may become harmful to any designated use.

Turbidity is a physical measure of the clarity of water: the greater the turbidity, the less clear the water. Increased amounts of suspended solids in water will reduce the light penetration into that water. A recent rain or runoff event can also greatly affect turbidity. If you have a turbidity reading of over 25 Jackson Turbidity Units (JTU), try to explain why this is the case by looking at the surrounding landscape and checking recent weather reports. Turbidity can be caused by materials such as clay, silt, fine particles of inorganic and organic matter, algae, and plankton. This test compares the turbidity of stream sample water to that of clear water.

#### WHAT DOES THIS TEST MEASURE?

#### EVIDENCE FOR DECREASES IN TURBIDITY?
- Have there been periods of low flow (base flow)/reduced stormwater run-off?
- Are there industry-specific best management practices (BMPs) in place such as vegetated shorelines and buffer strips?

#### EVIDENCE FOR INCREASES IN TURBIDITY?
- Is there soil erosion?
- Is there waste water discharge upstream?
- Any recent urban runoff from rain/flooding?
- Are there bottom-feeding fish (like carp)?
- Do you see algal growth?
- Has there been recent flooding?

#### WEB LINKS
- Factsheet: US Global Change Research Program—2014 National Climate Assessment: Midwest Fact-

### CONNECTING CONCEPTS
- As light penetration decreases, so does photosynthesis by plants. This decreases food for herbivores and also results in decreases in oxygen production.
- High turbidity can make it difficult for predators that use sight to see and capture their prey. This explains why a rain event can also lead to bad fishing until the water clears back up.
- Sediment in the water also can carry phosphorus and other contaminants.
- The frequency and intensity of storms have increased in the Great Lakes region over more than 50 years.
- Factsheet: US Global Change Research Program—2014 National Climate Assessment: Midwest Fact-
1. Check to be sure everyone is wearing gloves and goggles.
2. Inventory the supplies in your turbidity test kit using the following page.
3. Fill one turbidity column (A.) to the 50 mL line with the stream sample water from an area of the stream that no one has walked around in yet. If you cannot see a dot at the bottom when looking down the water column, pour out the water until you reach the 25 mL line and note this for later.
4. Fill the second turbidity column (B.) with an amount of bottled or “clear” water equal to the amount in turbidity column A.
5. Place the tubes side by side and notice the difference in clarity by observing the black dot at the bottom of the turbidity columns. TIP: View over white paper to make comparison easier.
6. If both turbidity columns are equally clear, the turbidity is zero and you will not need to add any chemical; proceed to step 12. If the black dot at the bottom of turbidity column A appears more cloudy/hazy, proceed to step 7. TIP: use the edges of the black dot to compare cloudiness.
7. Shake the Standard Turbidity Reagent vigorously. DO NOT FORGET TO SHAKE IT UP.
8. Using the dropper, add 0.5mL of Standard Turbidity Reagent to turbidity column B (the clear water) then stir the tube to equally distribute turbid particles. Compare the columns.
9. Keep adding Standard Turbidity Reagent in 0.5mL intervals, stirring and comparing the columns until turbidity column B (clear water) is as cloudy/hazy as turbidity column A (stream sample water). Keep track of the number of additions of Turbidity Reagent: ______ # of 0.5 mL additions
10. Calculate and record the turbidity in Jackson Turbidity Units. If your samples are 50mL, each 0.5ml addition equals 5 JTU. If a 25mL sample is used, each 0.5L addition of Reagent equals 10 JTUs. Use the chart at the right: _______ JTU
11. Dispose of your samples and rinse the test tubes into the hazardous waste container.
12. Calculate a Q-Value on the Turbidity Chart. Q-Value: _______
13. Check the Q-Value by entering your pH data at http://www.flintrivergreen.org/add-info/add-data/

**WHAT TO WATCH OUT FOR**

- Retrieve your stream sample upstream from other students or from a part of the stream that no one has been walking around in yet.
- Be sure to add the Standard Turbidity Reagent to the “Clear” water (Column B) and NOT to the stream sample water (Column A)
- Make sure you shake up the Standard Turbidity Reagent before use.
- Be sure to use the stir stick to stir the water after each addition before comparing columns.
- Disregard the color of the water and focus only on the cloudiness or clarity.
Flint River GREEN: Turbidity

Turbidity Kit

Contents

a. Standard Turbidity Reagent - [7520-H]
b. (x2) Turbidity Columns - [0835]
c. Test Tube Brush - [0513]
d. Pipet, 0.5mL, plastic, w/cap
e. Plastic Stirring Rod - [1114]

Turbidity Q-Value Chart

Turbidity: NTU / JTU (JTU & NTU are interchangeable.)

Note: If turbidity is > 100.0, Q-Value = 5.0
Flint River GREEN: Nitrates

**MATERIALS**
- LaMotte Nitrate Test Kit
- Gloves
- Safety Goggles
- Stopwatch or Timer

**VOCABULARY**
- Nitrogen (N)
- Nitrate (NO₃⁻)
- Nitrogen Cycle
- Eutrophication
- Nitrate-Nitrogen (NO₃⁻-N)
- Buffer Strip

**MATERIALS**

**VOCABULARY**

**WATER QUALITY STANDARDS**

**DRINKING WATER:**
- < 10 mg/L nitrates

**SURFACE WATER:**
- < 20 mg/L nitrates

**WHAT DOES THIS TEST MEASURE?**

**LOOK FOR THESE CAUSES**

**EVIDENCE FOR DECREASES IN NITRATES?**
- Are there sustainable farming practices (such as soil testing to determine proper fertilizer application, buffer strips, crop rotation, cover crops, and organic farming)?
- Are riverbanks and riparian areas healthy?
- Are wells & sewer/septic systems maintained?

**EVIDENCE FOR INCREASES IN NITRATES?**
- Are there places that use fertilizers nearby?
- What sources of animal manure (pets, wildlife, farm animals) are nearby?
- Are there possible failing septic/sewer systems?
- Do you see decomposing septic/sewer systems?
- Are there sources of detergents nearby?

**CONNECTING CONCEPTS**

**Nitrogen** is the most abundant element in the Earth’s atmosphere, making up about 78% of the air around us. **Nitrates** are a form of nitrogen that all plants need to grow. Nitrogen is a gas and in order for it to be accessible to plants and other organisms, it must be converted into water-soluble forms such as nitrates during the **nitrogen cycle**. High levels of nitrates in surface water can lead to increased plant growth, which could cause **eutrophication**. If the stream is used as a drinking water supply, this could negatively affect the health of those drinking the water if it is not properly treated.

**WEB LINKS**

- **Video (4 min.)** - The Nitrogen Cycle. University of Southern California: [https://www.youtube.com/watch?v=PfqvACMyg68](https://www.youtube.com/watch?v=PfqvACMyg68)
- **Case Study Student Activity**—The Dead Zone: Ecology and Oceanography in the Gulf of Mexico. National Center for Case Study Teaching in Science: [http://sciencecases.lib.buffalo.edu/cs/files/dead_zone.pdf](http://sciencecases.lib.buffalo.edu/cs/files/dead_zone.pdf)
- **Video (8 min.)** - Ecosystems on the Edge: Nutrient Odyssey. Smithsonian. [https://ecosystemsontheedge.org/nutrient-odyssey/](https://ecosystemsontheedge.org/nutrient-odyssey/)

**pH** measures how acidic, neutral or basic (alkaline) water is. The pH scale goes from 0 - 14. Most life does well around the number 7. The further the water is in either direction from 7, the greater stress is upon living things. Strong acids, like “battery acid”, would have pH around 1, while a strong base like bleach would have a pH near 12. pH is a logarithmic scale, which means something with a pH of 6 is 10x as acidic as a liquid with a pH of 7. A liquid with a pH of 5 is 100x as acidic as a liquid with a pH of 7.

- Like phosphorous, excess nitrogen causes extra plant growth. When those plants die, they decompose. Decomposition uses up oxygen which affects how much oxygen is available for organisms in the water.
- A bag of fertilizer is labeled with three numbers. The first number indicates how much nitrogen is available for plants. A 100 pound bag of 12-0-0 fertilizer contains 12 pounds of nitrogen for plants.
- High nitrate levels in drinking water have been known to cause a potentially fatal blood disorder in infants called "blue-baby" syndrome (a reduction in the oxygen-carrying capacity of blood).
Test Kit A: Octet Comparator - see photo (above)

1. Check to be sure everyone is wearing gloves and goggles. **This test is very hazardous.**
2. Inventory the supplies in your test kit using the inventory list on the pages that follow.
3. Rinse and then fill a sample bottle with water from the stream using standard water quality testing collection procedures.
4. Fill a test tube to the 2.5 mL line with water from the sample bottle.
5. Add 2.5 mL mixed acid by filling the tube until the liquid reaches the 5 mL line using a dispenser cap or eye dropper. **Do not pour chemical directly from the bottle.**
6. Cap and mix gently. Wait 2 minutes.
7. Add 0.1 g of Nitrate Reducing Reagent using the 0.1 g spoon.
8. Cap and invert gently for 60 times in one minute.
9. Wait 10 minutes.
10. Insert test tube into the comparator and match the sample to a color standard.
11. Match sample color to a color standard and record as ppm Nitrate-Nitrogen (NO₃-N) : ______ ppm
12. Convert to Nitrate: multiply your result by 4.4: _______ ppm x 4.4 = ________mg/L Nitrate (NO₃)
13. Dispose of your sample and rinse the test tube into the hazardous waste container.
14. Calculate a Q-Value on the Nitrate Chart using your value from Step 13 (NO₃):  **Q-Value: _______**
15. Check the Q-Value by entering your Nitrate-Nitrogen (NO₃-N) data at [http://www.flintrivergreen.org/add-info/add-data/](http://www.flintrivergreen.org/add-info/add-data/)

**WHAT TO WATCH OUT FOR**

- Add chemical to the test tube and not the sample bottle.
- Be sure to wait the full ten minutes!
- Remember that ppm is equal to mg/L.
- Be sure to convert to Nitrate before using the Q-value graph.
- When entering your field data on the WQI data sheet, enter your ppm Nitrate-Nitrogen (before you multiplied by 4.4).
- It’s a good idea to perform a colorblindness test prior to viewing color scales such as pH. There are many free versions online.
Flint River GREEN: Nitrates (kit B)

**Test Kit B (Octa-Slide Viewer):**
1. Check to be sure everyone is wearing gloves and goggles. **This test is very hazardous.**
2. Inventory the supplies in your test kit using the inventory list on the pages that follow.
3. Rinse and then fill a sample bottle with water from the stream using standard water quality testing collection procedures.
4. Fill a test tube to the **2.5 mL line** with water from the sample bottle.
5. Add 2.5 mL mixed acid by filling the tube until the liquid reaches the 5 mL line using a dispenser cap or eye dropper. **Do not pour chemical directly from the bottle.**
6. Cap and mix gently. Wait 2 minutes.
7. Add 0.1 g of Nitrate Reducing Reagent using the 0.1 g spoon.
8. Cap and invert gently for 60 times in one minute.
9. Wait 10 minutes.
10. Insert test tube and Octa-Slide Bar into the comparator and match the sample to a color standard.
11. Match sample color to a color standard and record as ppm **Nitrate-Nitrogen (NO₃-N) : ______ ppm**
12. Convert to Nitrate: multiply your result by 4.4: ________ ppm x 4.4 = ________ mg/L Nitrate (NO₃⁻)
13. Dispose of your sample and rinse the test tube into the hazardous waste container.
14. Calculate a Q-Value on the Nitrate Chart using your value from Step 13 (NO₃⁻): **Q-Value: ________**
15. Check the Q-Value by entering your Nitrate-Nitrogen (NO₃-N) data at [http://www.flintrivergreen.org/add-info/add-data/](http://www.flintrivergreen.org/add-info/add-data/)

**WHAT TO WATCH OUT FOR**
- Add chemical to the test tube and not the sample bottle.
- Be sure to wait the full ten minutes!
- Remember that ppm is equal to mg/L.
- Be sure to convert to Nitrate before using the Q-value graph.
- When entering your field data on the WQI data sheet, enter your ppm Nitrate-Nitrogen (before you multiplied by 4.4).
- It’s a good idea to perform a colorblindness test prior to viewing color scales such as pH. There are many free versions online.
Flint River GREEN: Nitrates

Kit A: Octet Comparator Nitrate Kit

Contents
a. Mixed Acid Reagent - [V-6278-H]
b. Nitrate Reducing Agent - [V-6279-C]
c. Eye Dropper OR White Dispenser Cap - [0692]
d. Spoon, 0.1g, plastic - [0699]
e. (x2) Test Tubes, 2.5 & 5.0mL, glass, w/caps - [0820]
f. Bottle, Water Sample - [0688]
g. Nitrate-N Comparator, 0.25-10.0ppm - [3109]

Kit B: Octa-Slide Nitrate Kit

Contents
a. Mixed Acid Reagent - [V-6278-H]
b. Nitrate Reducing Agent - [V-6279-C]
c. Eye Dropper OR White Dispenser cap - [0692]
d. Spoon, 0.1g, plastic - [0699]
e. (x2) Test Tube, 2.5-10mL, plastic, w/caps - [0106]
f. Bottle, Water Sample - [0688]
g. Nitrate-N Octa-Slide 2 Bar, 0.25-10.0ppm - [3109-01]
h. Octa-Slide 2 Viewer - [1101]

## Flint River GREEN: Phosphates

### MATERIALS
- LaMotte Phosphate Test Kit
- Lab Phosphate Bottle (labeled)
- Water Sample Collection Bottle (can be an empty water bottle rinsed)
- Gloves
- Safety Goggles
- Cooler
- Stopwatch or Timer

### Vocabulary
- Phosphorous
- Phosphate
- Orthophosphate
- Total Phosphate (TP)
- Algal Blooms
- Harmful Algal Blooms (HAB)
- Saginaw Bay Watershed

### Materials

<table>
<thead>
<tr>
<th>Water Quality Standards</th>
<th>Drinking Water:</th>
<th>Surface Water (Total Phosphates):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>• Within a lake or reservoir: &lt; 0.025 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Streams that discharge into lake or reservoir: &lt; 0.05 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Streams not flowing into lake or reservoir: &lt; 0.1 mg/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What Does This Test Measure?</th>
</tr>
</thead>
</table>
| Phosphorous is an element that makes up about 12% of the earth’s crust. It is an important plant nutrient that helps with root and flower development. Too much phosphorous contributes to eutrophication.

Phosphorous is found in many forms. The tests you will use require an understanding of a few of these: Phosphorous almost always occurs in water in the form of various phosphates. These are broken into three groups: orthophosphates, condensed phosphates, and organically bound phosphates. Together, these three groups determine Total Phosphates (TP). Orthophosphates are easily tested for and help to estimate the amount of phosphorus available for algae and plant growth because it is the form most often used by plants and animals. Water quality professionals generally speak about phosphorous by referring to total phosphates. Testing for total phosphates is complex, so you will send samples to a lab for this result. |

<table>
<thead>
<tr>
<th>Evidence for Decreases in Phosphates?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Is runoff controlled/slowed?</td>
</tr>
<tr>
<td>• Are riparian areas maintained and healthy?</td>
</tr>
<tr>
<td>• Look for sustainable agricultural practices.</td>
</tr>
<tr>
<td>• Are residents using fertilizers w/o phosphates</td>
</tr>
<tr>
<td>• Are leaves/grasses kept out of storm drains?</td>
</tr>
<tr>
<td>• Is erosion controlled at construction sites?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evidence for Increases in Phosphates?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Is there soil erosion?</td>
</tr>
<tr>
<td>• Are there excess fertilizers reaching the stream?</td>
</tr>
<tr>
<td>• Is there excess manure from pets, farms, and wildlife? Is there sewage entering the stream?</td>
</tr>
<tr>
<td>• Is there decomposing plant material?</td>
</tr>
<tr>
<td>• Are there detergents entering the stream?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Look for These Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A bag of fertilizer is labeled with three numbers. The middle number indicates how much phosphorous is available for plants. A 100 pound bag of 12-10-10 fertilizer contains 10 pounds of phosphorous for plants.</td>
</tr>
<tr>
<td>Like nitrogen, excess phosphorous causes extra plant growth. When those plants die, they decompose. Decomposition uses up oxygen which affects how much oxygen is available for organisms in the water.</td>
</tr>
<tr>
<td>Too much phosphorous can lead to algal blooms. These can be toxic or contain toxic bacteria that are harmful to humans and animals. Lake Erie is noted for recent harmful algal blooms affecting drinking water.</td>
</tr>
<tr>
<td>The Saginaw Bay Watershed geography is similar to the Western Lake Erie Basin and faces similar issues.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Connecting Concepts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Video (2 min) - Nutrient Pollution. EPA. <a href="https://www.epa.gov/nutrientpollution/problem">https://www.epa.gov/nutrientpollution/problem</a></td>
</tr>
<tr>
<td>Video (6 min.)—Lake Erie Algal Blooms: <a href="http://lakeeriealgae.com/">http://lakeeriealgae.com/</a></td>
</tr>
</tbody>
</table>
THIS PROCEDURE REQUIRES TWO TESTS:

TEST #1: A sample to send to the lab to test for Total Phosphates (which cannot be done at the stream). The results from the lab are the only results that should be included in your final WQI score.

TEST #2: The Phosphate test kit that you will do at the stream (which only tests for Orthophosphates). This will help you begin to analyze your data while you wait for Total Phosphate results (which could be up to two weeks). **NOTE:** Orthophosphates do not have a Q-Value Chart, but Total Phosphates do.

1. Check to be sure everyone is wearing gloves and goggles.
2. Check to be sure you have an acid-treated lab phosphate bottle, a water sample collection bottle, AND a LaMotte Test Kit. Inventory the LaMotte Test Kit using the following pages.
3. Enter the stream with a water collection bottle (an empty water bottle will work). Wear gloves and waders or boots and follow standard water sample collection procedures.
4. Fill your water sample collection bottle following standard water sample collection procedures.

**TEST #1: Total Phosphate Lab Sample:**

1. Carefully remove the lid to the lab sample bottle (which is treated with sulfuric acid—do not dip the lab bottle in the stream!)
2. Pour your water sample from your collection bottle to the lab sample bottle.
3. Check to be sure the lab label is filled out with your school, teacher name, site name, time and date then place in cooler to be delivered to the lab later.

**TEST #2: Orthophosphate Student Test:**

Follow the test instructions on the next pages. Be sure to check which kit (A or B) you are using and follow the correct set of instructions.

**WHAT TO WATCH OUT FOR**

- During the Total Phosphate lab sample collection, be sure to pour water into the bottle and keep in a cooler always! Don’t submerge the lab bottle in the stream.
- During the Orthophosphate Test, add chemical to a test tube and not the water collection bottle.
- During the Orthophosphate Test, be sure to wait the full five minutes!
- Be sure to use only Total Phosphates on the Q-value graph. The Orthophosphates cannot be converted to Total Phosphates and there is no Q-Value chart for Orthophosphates.
- When entering your field data on the WQI data sheet, be sure to enter the result you received from the lab and NOT your orthophosphate result.
- While waiting for your lab results, you can still use Orthophosphate results to analyze your data. Your Total Phosphate result will likely be slightly higher.
- If using LaMotte Kit A, carefully follow the instructions for how to use the Axial Reader.
- If using LaMotte Kit B, be sure to compare the color standards through the test tubes and not from the side of the Comparator.
TEST #2: Orthophosphate Student Lab Sample:
(Kit A) Low Range Phosphate Kit w/Axial Reader (3121-01)
1. Be sure everyone is wearing gloves and goggles.
2. Fill a test tube to the 10 mL line with water from the collection bottle.
3. Use a 1.0 mL pipet to add 1.0 mL of Phosphate Acid Reagent to the test tube.
4. Cap and mix gently.
5. Use the 0.1 g spoon to add 0.1 g of Phosphate Reducing Reagent.
6. Cap and mix until dissolved. Wait 5 minutes.
7. Position the Comparator inside the Axial Reader so the mirror inside faces you.
8. Insert the Comparator with the numbers on the Comparator also facing you.
9. Insert the water ampule into the left, square hole in the Comparator (between 0 and 0.2).
10. Insert the sample test tube into the Axial Reader directly behind the water ampule.
11. Fill the remaining two test tubes to the 10 mL line with untreated sample water and insert them into the Axial Reader on either side of the treated sample.
12. Slide the Axial Reader upward until the top of it is level with the top of the Comparator.
13. Your test kit should now look like the photo on the right. Hold and tilt the test so that sunlight is directed down the treated sample to the mirror.
14. Begin by viewing your treated sample through the top, left, unlabeled window of the Comparator.
15. If your sample color is darker than the surrounding color standards, move your axial reader down to view your sample through the bottom, left, unlabeled window of the Comparator.
16. If your sample color is darker than these surrounding color standards, move the distilled water ampule and all three test tubes to the right side of the comparator and repeat the process, starting at the top.
17. Record your result as ppm Orthophosphate which is equal to mg/L Orthophosphate: _______ mg/L

Clean Up and Analysis:
1. Dispose of your samples and rinse the test tubes into the hazardous waste container.
2. Your Orthophosphate value from the LaMotte test is a good estimate of Total Phosphates. You should begin analyzing your data using the Orthophosphate reading while you wait for Total Phosphate results from the lab (which could be 2 weeks). **NOTE:** You can expect your Total Phosphate result to be at least the amount that you recorded for orthophosphates. It will likely be slightly higher; potentially up to 4 times higher.
3. Use your results to calculate a Q-Value on the Total Phosphate (TP) Chart: Q-Value: _______
4. Check the Q-Value by entering your Total Phosphate data at [http://www.flintrivergreen.org/add-info/add-data/](http://www.flintrivergreen.org/add-info/add-data/). Teachers: be sure to enter the GM Total Phosphate Score when submitting your data online.
**Orthophosphate Student Lab Sample:**
*(kit B) Low Range Phosphate Kit with color bar (3121-02):*

1. Be sure everyone is wearing gloves and goggles.
2. Fill a test tube to the 10 mL line with water from the collection bottle.
3. Use a 1.0 mL pipet to add 1.0 mL of Phosphate Acid Reagent to the test tube.
4. Cap and mix gently.
5. Use the 0.1 g spoon to add 0.1 g of Phosphate Reducing Reagent.
6. Cap and mix until dissolved. Wait 5 minutes.
7. While waiting, position the color slide bar into Insert test tube into the Comparator Viewer.
8. Insert the treated sample into the hole directly above the color slide bar in the Comparator Viewer.
9. Fill a second test tube to the 10 mL line with untreated sample water from the collection bottle.
10. Place this tube in the other hole (directly behind the test tube containing the treated sample).
11. Position the comparator so that light shines down through the test tubes. Tilt the comparator until you see the color standards and sample illuminated. (You will be comparing the standard to the color projected on the screen within the Comparator and not the actual sample in the tube).
12. Match the color of the reaction to the closest color standards by sliding the color slide bar while looking at the projection (within the top, square hole of the Comparator) and stopping when the projected color appears to match the color standard in view.
13. Read the numerical result from the color slide bar and record your result as ppm Orthophosphate which is equal to mg/L Orthophosphate: _______ mg/L

**Clean Up and Analysis:**

a. Dispose of your samples and rinse the test tubes into the hazardous waste container.
b. Your Orthophosphate value from the LaMotte test is a good estimate of Total Phosphates. You should begin analyzing your data using the Orthophosphate reading while you wait for Total Phosphate results from the lab (which could be 2 weeks). **NOTE:** You can expect your Total Phosphate result to be at least the amount that you recorded for orthophosphates. It will likely be slightly higher; potentially up to 4 times higher.
c. Use your results to calculate a Q-Value on the Total Phosphate (TP) Chart: **Q-Value:** _______
d. Check the Q-Value by entering your Total Phosphate data at [http://www.flintrivergreen.org/add-info/add-data/](http://www.flintrivergreen.org/add-info/add-data/). Teachers: be sure to enter the GM Total Phosphate Score when submitting your data online.
Kit A: Axial Reader Phosphate Kit

Contents:
- a. Phosphate Acid Reagent - [V-6282-G]
- b. Phosphate Reducing Agent - [V-6283-C]
- c. (x3) Test Tubes, 10mL, glass, w/caps - [0843]
- d. Pipet, 1.0mL, plastic - [0354]
- e. Spoon, 0.1g, plastic - [0699]
- f. Distilled Water Ampoule, 5mL - [2748]
- g. Phosphate Comparator, 0.0-2.0ppm - [3122]
- h. Axial Reader - [2071]

Kit B: Low Range Comparator Phosphate Kit

Contents:
- a. Phosphate Acid Reagent - [V-6282-G]
- b. Phosphate Reducing Agent - [V-6283-C]
- c. (x2) Test Tube, 10.0mL, glass, w/cap - [0843]
- d. Pipet, 1.0mL, plastic - [0354]
- e. Spoon, 0.1g, plastic - [0699]
- f. Low Range Comparator Viewer - [1102]
- g. Phosphate low Range Comparator Bar 0.0-2.0ppm - [3122-01]

Total Phosphate Q-Value Chart

*Note: Use GM Lab Score to calculate

**Flint River GREEN: Fecal Coliform**

**MATERIALS**
- Coliscan Easygel (1 bottle/test)
- Treated Petri Dish (1/test)
- 1 to 5mL Test Tube/Sterile Pipette (can use clean test tube from other test kit)
- Sterile Water Sample Collection Bottle
- Cooler
- Gloves (2/person)
- Safety Goggles (1/person)
- Incubator (or access to 48 hrs of 68°F)

**VOCABULARY**
- Total Coliform (TC)
- Fecal Coliform (FC)
- Effluent
- Coliform Bacteria
- E. coli
- Feces
- Pathogenic

**WATER QUALITY STANDARDS**

<table>
<thead>
<tr>
<th>DRINKING WATER (colony/100 mL water):</th>
<th>SURFACE WATER (colonies/100mL water):</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 Total Coliform</td>
<td>Total Body Contact (swimming): 200 Fecal Coliform</td>
</tr>
<tr>
<td></td>
<td>Partial Body Contact (boating): 1000 Fecal Coliform</td>
</tr>
<tr>
<td></td>
<td>Treated Sewage Effluent: No more than 200 Fecal Coliform</td>
</tr>
</tbody>
</table>

**EVIDENCE FOR DECREASES IN COLIFORM?**
- Do local residents regularly test and maintain any nearby septic systems?
- Are nearby livestock kept away from streams?
- Is there natural vegetation alongside streams (filters pollutants from run-off)?
- Are manure and dog waste regularly removed or protected from rain and from surface run-off?

**EVIDENCE FOR INCREASES IN COLIFORM?**
- HUMAN: Have there been combined sewer system or sanitary sewer system overflows? Possible illegal storm drain connections/dumping, leaking sewer pipes, failing septic systems? Are there marinas, campgrounds, or other waste pump-out facilities?
- NON-HUMAN: What types of animals live nearby (wild/pets/farms)? Has there been recent flooding?

**WHAT DOES THIS TEST MEASURE?**

- Unlike other water quality testing parameters, Fecal Coliform are living organisms, meaning they are a bioindicator of water quality. Many macroinvertebrates are also bioindicators of stream health.
- Some contributing factors (such as manure and run-off) that increase coliform also affect other water quality parameters.

**WEB LINKS**
- Video—Plating Coliscan (8 min): [https://www.youtube.com/watch?v=g1jj6j-rEl](https://www.youtube.com/watch?v=g1jj6j-rEl)
- Video—Incubating and Counting with Coliscan (8 min): [https://www.youtube.com/watch?](https://www.youtube.com/watch?)
NOTE: This test does not come packaged in a typical test kit.

While preparing for this test, decide if you will be plating the petri dishes while back at school OR at the stream. It is usually easier to do so back at school if there is time, otherwise you will need to carefully hold the dishes flat until the gel sets (45 min.).

A.) If plating back at school: the collected water sample must immediately be placed on ice or in a cooler/refrigerator until used.

B.) If plating at the stream: be sure to keep the Easygel frozen until the day of the test, then keep it in a cooler to take to the stream until ready to use.

PART 1: COLLECTION
1. Enter the stream with a sterile water collection bottle (an empty water bottle will work), wearing gloves and waders or boots. Aim to stand as close to the middle of the main stream flow as possible and try not to stir up any bottom sediment. SAFETY NOTE: Do not stand in water above your ankles if wearing boots or above your knees if you are wearing waders. DO NOT EVER enter flooded streams. Your group may find that collecting a large bucket of water from a bridge or by sending just one person into the stream may be safest for this as well as other tests.

2. Fill your collection bottle using standard water sample collection procedures. **TIP:** The test for Total Solids also requires a sterile water bottle, needs to be kept refrigerated, and requires at least 250 mL (a water bottle typically holds 500 mL), so you may decide to collect a full water bottle and use this sample water for both tests.

3. If you are doing the rest of the test back at school, cap the sample and place it in a cooler until you are ready to continue later. If you are plating back at school, you are now finished with the streamside portion of this test. When you are ready to plate, continue with Step 4.

PART 2: PLATING
4. Check to be sure everyone is wearing gloves and goggles.

5. Inventory your supplies:
   - Treated petri dishes (1/test) - TIP: keep covered at all times except when pouring mixture and don’t touch the inside of the dish
   - Coliscan Easygel medium (in cooler, 1/test)
   - Sterile Water Collection Bottle with Sample Water (in cooler)
   - 1—5 mL test tube/graduated cylinder/sterile pipette

6. Choose an amount of sample water to add to the Easygel bottle (between 1 and 5 mL) and carefully pour or pipette this amount into the bottle in a sterile manner. The amount doesn’t matter as long as you remember how much you add. **NOTE:** If you are testing drinking water, always use 5mL.

7. Label the bottom (smaller, treated side) of your petri dish with the amount of water you used

8. Write down how much sample water you added to the Easygel bottle: ________ mL Water

9. Cap and swirl the bottle until the solution is completely mixed, then pour into the treated portion of a petri dish. Place the cover on your petri dish immediately and gently swirl until the entire bottom is covered.

10. Allow gel to solidify (30-45 min.) then flip dish upside down and incubate on a level surface following the directions below:
   - Using an incubator: 35°C (95°F) for 20—48 hours (do not incubate beyond 48 hours)
   - At Room Temperature: 20—23°C (68-74°F) for 48 hours or more (watch every 12 hours until some pink or purple colonies form, then wait an additional 24 hours to allow them to mature)
PART 3: COUNTING COLONIES

11. Using the timeline from Step 10 in Part 2, determine when you should begin counting your pink (coliforms) and/or purple colonies (E. coli or fecal coliform). Other colored colonies of bacteria may grow such as white, blue or green—you may disregard these colonies because they are not coliforms. If you are doing stream testing, proceed to step 12. If you are doing drinking water testing, proceed to step 16.

12. For stream testing, count only the purple (fecal coliform) colonies in your dish. *Tip: You may wish to place your dish over a piece of graph paper to help you count.*

13. Record your result: __________ fecal coliform colonies in dish

14. Convert the number of colonies to report results in “Fecal coliform per 100 mL of water” by following these steps:

   A. Divide 100 by the amount of sample water you added to the Easygel bottle in Step 6 (you should have recorded this number on Step 7 and labeled your petri dish with this same number): 100 ÷ ____ mL (from step 7) = ________ (multiplier)

   B. Use the multiplier result from above and multiply this by the number of fecal coliform colonies you counted in your petri dish:

      ________ (multiplier) x ________ colonies = ________ Fecal Coliform / 100 mL of water

      (For example, if you used 2.5 mL of sample water and counted 4 purple colonies of fecal coliform: 100 ÷ 2.5 = 40 and 40 x 4 = 160 Fecal Coliform per 100 mL of water)

15. Any reading over 200 Fecal Coliform is cause for concern. Contact your mentor or the FRWC immediately. Unless you are testing drinking water, proceed to step 19.

16. For drinking water testing, you will need to calculate total coliform and not just fecal coliform. For total coliform, count all of the pink AND purple colonies (not green, blue or white). *Tip: You may wish to place your dish over a piece of graph paper to help you count.*

17. Record your result: __________ total coliform colonies in dish

18. Multiply the number of total coliforms by 20 (because you should have used 5 mL of sample water if you are testing drinking water and 5 x 20 = 100) to convert to “total coliform per 100 mL of water”:

      ________ total coliform colonies in dish x 20 = ________ Total Coliform / 100 mL of water

19. DO NOT dispose of your petri dishes or Easygel Bottles in the trash. Ask your teacher what they would like you to do with them (Teachers: these can be disposed of safely once sealed in a Ziploc bag along with straight bleach or rubbing alcohol and mixed to ensure that all surfaces have been decontaminated).

20. For stream testing, calculate a Q-Value on the Fecal Coliform Chart. Q-Value: ________


**WHAT TO WATCH OUT FOR**

- Always keep the Coliscan Easygel frozen until testing day. Keep in a cooler on testing day.
- If you transport the sample back to school for mixing and plating, be sure to use a cooler.
- If you plate the sample at the stream, be sure to keep it covered and very level until you get it back to school, then be sure to place it in the incubator or on a register right away.
- Make sure to check the sample at 12 and 24 hour increments.
- Make sure you use a treated petri dish and not a regular one.
- Make sure to pour the mixture into the treated side of the dish.
- Use sterile procedures (i.e. don’t touch the inside) and introduce other bacteria to the sample.
- More detailed instructions: [https://www.micrologylabs.com/page/95/Instructions](https://www.micrologylabs.com/page/95/Instructions)
Fecal Coliform Test

Materials Needed to Perform the Test

a. Coliscan® Easygel® Solution
b. Easygel® Pretreated Petri Dish
c. Collection Bottle (nonspecific)
d. Pipet or Test Tube (must be able to measure 1-5mL of sample, can be taken from another test kit if is cleaned prior to use.)

Fecal Coliform Q-Value Chart

Note: if $FC > 10^5$, $Q = 2.0$
Aquatic animals need oxygen to live just like land animals do. In order for oxygen to be made available for most aquatic organisms, it must be dissolved in water. Higher dissolved oxygen is usually better. We typically find the greatest biodiversity in waters with high levels of dissolved oxygen. This test measures the amount of oxygen in water and is paired with a temperature reading to determine the percent saturation (the amount of dissolved oxygen in the water sample compared to the maximum possible amount that could be present at the same temperature).
**Flint River GREEN: Dissolved Oxygen**

**Part 1 of 3 (at stream):**
1. Inventory your test kit supplies—check to be sure everyone is wearing gloves and goggles 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.

**Part 2 of 3 (at stream):**
1. Make sure everyone is wearing safety goggles and gloves.
2. Work over a container and make sure no air is added to the sample while adding chemicals.
3. Remove the cap from the bottle and add 8 drops of Manganous 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). While waiting for your precipitate to dissolve, collect the Biochemical Oxygen Demand (BOD) sample. If a different group is assigned to the BOD test, 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 at school if short on time.

**WHAT TO WATCH OUT FOR**
- This is the most complex test to complete. A very common mistake is caused when a sample is not taken directly from the stream. Do no collect a bucket of water to take your sample from if it is avoidable. Extra splashing in the bucket or from pouring one bucket into another can introduce extra oxygen from the atmosphere and give you a false reading.
- Make sure you get all air bubbles out of the bottle while it is still submerged in the stream.
- Work over an overflow container. Have paper towel ready to clean up any spill over.
Flint River GREEN: Dissolved Oxygen

**Part 3 of 3 (at stream or back 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 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. **TIP:** View reaction over a white paper.
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.
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 from 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. Use your final reading in ppm along with the temperature (°C) on the Percent Saturation chart to calculate percent saturation of dissolved oxygen for your sample site: ______ % Saturation
15. Use your % Saturation to find your Q-Value on the Temperature Change Chart. **Q-Value:** ______
16. Check the Q-Value by adding the temp. data at [http://www.flintrivergreen.org/add-info/add-data/](http://www.flintrivergreen.org/add-info/add-data/)

**WHAT TO WATCH OUT FOR**

- Make sure to remove the air bubbles from the Titrator when adding the Sodium Thiosulfate.
- Make sure you used the stream temperature in Celsius when calculating percent saturation.
- Be sure to use % Saturation to determine the Q-value, NOT ppm oxygen.
- Make sure to add 10 to you final reading if you refilled the Titrator.
- Try to get the temperature reading from the stream as close to the time that you did Parts 1 & 2 as possible.
Flint River GREEN: Dissolved Oxygen

% Saturation:

On the chart at the right, draw a straight line from the temperature at your site (°C) to your Dissolved Oxygen reading (mg/L). The percent saturation can be read where your line crosses the diagonal line.

There is also a fairly complicated equation you can use to determine percent saturation available at http://www.waterontheweb.org/under/waterquality/oxygen.html.

LaMotte 5860-01 Dissolved Oxygen Test Kit Percent Saturation Chart
Dissolved Oxygen Kit

Contents

a. Manganese 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]
h. Water Sampling Bottle, 60mL, glass - [0688-DO]
**Flint River GREEN: Biochemical Oxygen Demand (BOD)**

### MATERIALS
- LaMotte Dissolved Oxygen Kit
- Biochemical Oxygen Demand (BOD)
- BOD Sample Bottle
- Plankton
- Foil
- Organic Waste
- Safety Goggles (1/person)
- Food Processing Plants
- Nitrile Gloves (2/person)
- Macroinvertebrates
- Overflow Container
- Food Web
- Paper Towel
- Waders or Boots

### WATER QUALITY STANDARDS

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<thead>
<tr>
<th>DRINKING WATER:</th>
<th>SURFACE WATER:</th>
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<tr>
<td>- No drinking water standards</td>
<td>- No state standards for BOD</td>
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<tr>
<td>- Good BOD is ( \leq 3 ) ppm (mg/L), Moderate is 4-8 ppm</td>
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<tr>
<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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</tbody>
</table>

### WHAT DOES THIS TEST MEASURE?

- 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.

### 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

**EVIDENCE FOR DECREASED BOD?**

- 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/ecsysteam/plankton](https://www.chesapeakebay.net/discover/ecsysteam/plankton)
Flint River GREEN: Biochemical Oxygen Demand (BOD)

**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 Manganous 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.

**WHAT TO WATCH OUT FOR**
- A very common mistake is caused when a sample is not taken directly from the stream. Do not collect a bucket of water to take your sample from if it is avoidable. Extra splashing in the bucket or from pouring one bucket into another can introduce extra oxygen from the atmosphere and give you a false reading.
- Make sure you get all air bubbles out of the bottle while it is still submerged in the stream.
- Store the covered bottle away from sunlight for 5 days before adding any chemical.
- Be careful not to introduce air bubbles by adding chemicals too quickly.
Flint River GREEN: Biochemical Oxygen Demand (BOD)

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).
16. Check the Q-Value by adding your BOD data at http://www.flintrivergreen.org/add-info/add-data/
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]

Chart 4: 5-Day Biochemical Oxygen Demand (BOD₅) Tests

Note: if BOD₅>30.0, Q=2.0
Electronic links to all of the following resources can be found at the Flint River GREEN website under the Links tab:


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<td>Flint, MI</td>
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<td>Water Quality</td>
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<td>Video (2 min.)</td>
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<td>Biochemical Oxygen Demand</td>
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<tr>
<td>37. Light Penetration</td>
<td>81. Vegetation</td>
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<td>38. Logarithmic Scale</td>
<td>82. Warmwater Stream</td>
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<td>40. Meniscus</td>
<td>84. Water Quality Index (WQI)</td>
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<tr>
<td>41. Metabolic Rates</td>
<td>85. Watershed</td>
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<td>42. mg/L</td>
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<tr>
<td>43. Nitrate (NO₃⁻)</td>
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<td>44. Nitrate-Nitrogen (NO₃⁻-N)</td>
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