Bacteria & Water Quality Testing Basics

HUDSON RIVER PK
RIVER PROJECT

Background Information: How do scientists monitor disease-causing bacteria in NYC waterways?

Part 1: What are pathogens and why are scientists monitoring them?

Pathogens are **bacteria**, **viruses**, or other **microorganisms** that can cause disease. There are many kinds of pathogens and they can be found almost everywhere on Earth. For example, a group of viruses called rhinoviruses are often responsible for the common cold; *Streptococcus* bacteria cause strep throat; and if you've ever had athlete's foot, it was caused by **fungi** called dermatophytes. The good news is, even though pathogens are everywhere, your **immune system** is designed to fight lots of different microorganisms that can make you sick, so just because you come into contact with a pathogen, that doesn't mean you will necessarily become ill. That's why you don't always get sick when friends or family members that you spend time around are sick.

However, if you are exposed to a high number of pathogens, or if the pathogens you are exposed to are particularly good at defending themselves against your immune system, there is a better chance you will become sick. That is why it is always best to avoid close contact with people who are ill (because we know pathogens are hosted in their bodies while they are sick) and to avoid situations where you are more likely to encounter dangerous pathogens (this is why we don't eat undercooked meat, which may contain *E. coli*, a bacteria that can cause severe stomach problems).

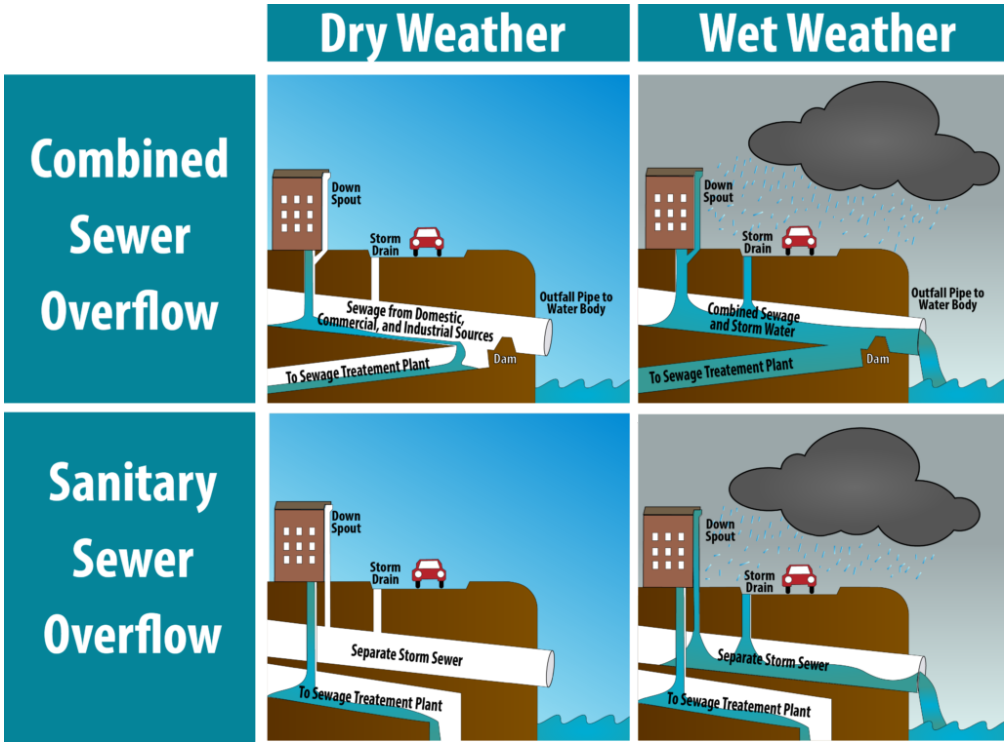
Although there are pathogens everywhere, it is important to try to prevent large numbers of pathogens, especially dangerous pathogens, from entering places where they might infect people or animals. That is why we **filter** our drinking water, have designated places for trash, and use a **sewage system**. However, in some cases, we cannot completely prevent pathogens from entering our environment, so we keep ourselves safe by tracking or **monitoring** where the bacteria are so we can do our best to avoid them, eliminate them, or decrease their entry into the environment.

Part 2: Why are there pathogens in our waterways?

New York City has a **combined sewer system**, as do several other cities throughout the U.S. This means that all sewage water--any water that goes down the drain when we wash our hands, do the dishes, shower, do the laundry, etc.--*combines* with **stormwater** that flows into the gutters and enters the same set of pipes that lead to **wastewater treatment plants**. At these plants, the combined sewage and storm water is filtered and treated with chemicals to kill any pathogens in it, then released into nearby waterways. Although this water may contain some chemicals that could be harmful to the environment, generally, the water is considered clean and safe to release into waterways.

However, some of the pipes in combined sewer systems lead directly to oceans, rivers, and streams, where sewage may flow out before it is processed at wastewater treatment plants. This is because when it rains or snows, the stormwater that enters the combined sewage system, when combined with a city's regular volume of sewage water, can be more water than the pipes can hold. The water has to go somewhere, and of course, we don't want it to back up into

homes through drains. Instead, any water above the amount that our wastewater treatment plants can hold overflows directly into waterways without being treated. That means that in NYC, raw sewage--the water that goes down your toilets and showers and sinks--goes directly into the Hudson River, East River, and other **open waters** all around the city. This is called a **combined sewer overflow**, or **CSO**.



Source: Sewerequipment.com

Unfortunately, CSOs are fairly common in NYC because our combined sewer system was built over 100 years, so it was designed to hold sewage water for a much smaller population than we have today in NYC. Think about it: more people use more water, and that water goes into roughly the same amount of pipes, so overflows are going to happen a lot more often. Today, about 20 minutes of medium rain can be enough for overflows to occur.

There are over 450 **CSO outfalls**, pipes that lead from the sewer system into waterways, throughout NYC. (Visit Open Sewer Atlas to view the locations of the outfalls and information about each CSO.) As you've probably already figured out, sewage water flowing into the waterways introduces a lot of pathogens into the environment. Like we discussed above, when you have a high concentration of bacteria in one area, it can become a lot more dangerous. That's the bad news about NYC's sewer system. But here's some good news:

1. Although our original sewer system was constructed over a century ago, many improvements have been made since then, such as adding new wastewater treatment plants to the system. Today, NYC has 14 plants throughout the five boroughs and the New York City Department of Environmental Protection is continuously working to increase the efficiency and capacity of the sewer system and decrease CSOs.

2. Despite combined sewer overflows, it's actually safe to swim in most NYC waterbodies most of the time. Remember, pathogens are usually most harmful in high concentrations. While you definitely shouldn't go swimming in an affected waterbody right after an overflow occurs, most of the harmful bacteria from sewage dies in open water within a couple of days. We'll talk more about this later in the lesson.

3. There are ways for people to find out when it's safe to come into contact with waterways where CSOs occur including pathogen monitoring! Using data from pathogen monitoring studies, people can find out when and where sewage is entering the water, how much bacteria is present in a certain area, and if it's safe to swim, kayak, fish, or do any other activity in or on the water.

Part 3: How do scientists monitor pathogens from CSOs?

There are a few different ways scientists can monitor pathogens that enter waterways from combined sewer overflows. We're going to focus on a method used in NYC by the Citizens' Water Quality Testing Program (CWQTP). CWQTP was founded by the NYC Water Trail Association, a group of local kayakers and rowers, and Hudson River Park's River Project, an organization that performs research and education on the Hudson River. They had three key questions when they set off to begin their study of sewage pathogens in NYC:

> **KEY QUESTION #1**: When CSOs occur (usually after it rains), is it safe to be in contact with the water in NYC waterbodies for activities like kayaking, fishing, or research?
>
> **KEY QUESTION #2:** If you take water samples in different waterbodies or different parts of one waterbody at the same time, will you find the same numbers of pathogens in all samples? In other words, can you test for pathogens at one site and know based on those results if all nearby water is safe for contact?
>
> **KEY QUESTION #3:** How long do pathogens live or stay in one area after a CSO occurs?

To answer these Key Questions, CWQTP first had to choose a pathogen to monitor. There are many kinds of pathogens that can be found in sewage and scientists monitor different pathogens using different methods, so the CWQTP founders used the following criteria to help them narrow the options down to a specific pathogen:

1. The pathogen is found in sewage, but not normally found in the environment. That way, if tests show the pathogen is present in the environment, scientists can reasonably conclude that it came from a combined sewer overflow.

2. Previous studies have shown that when the pathogen is present in waterbodies, people who swim in those waterbodies tend to get sick.

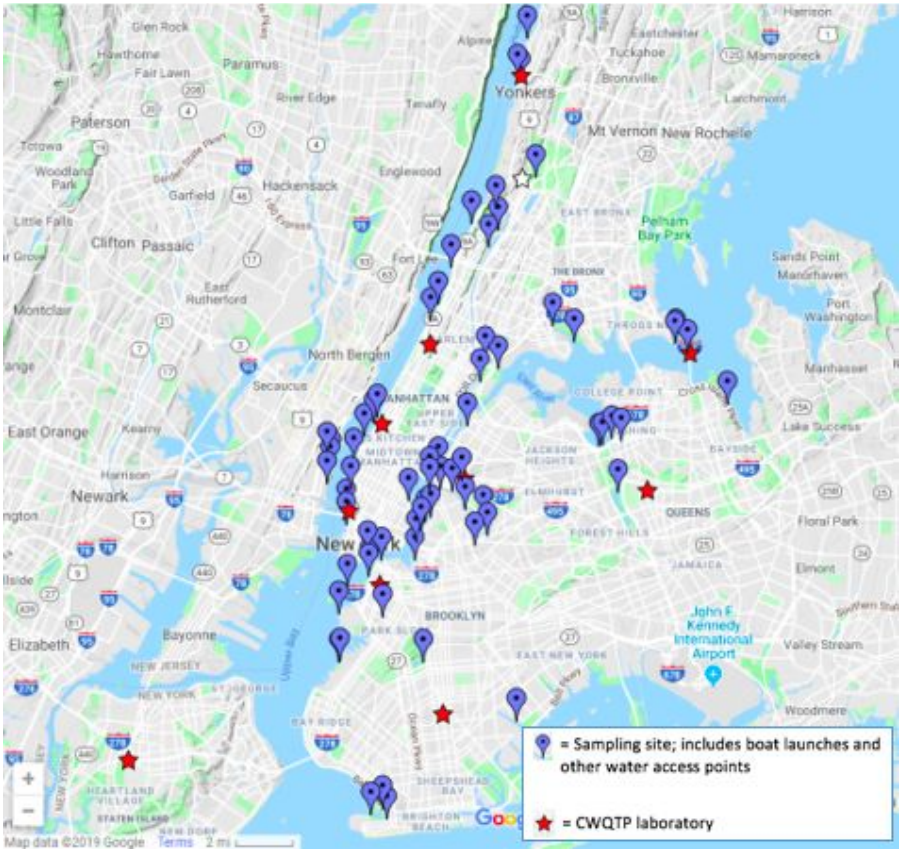
3. It is relatively easy and inexpensive to test for the presence of that pathogen and results can be obtained in 24 hours or less.

Based on those criteria, CWQTP chose to monitor **Enterococcus** (pronounced *enter-o-cox-us*) bacteria to determine if waterways were safe to come into contact with. Enterococci (plural of

Bacteria & Water Quality Testing Basics

Enterococcus; pronounced *enter-o-cox-eye*) are a group of bacteria that are found in the feces of warm-blooded animals. Since most animals that live in NYC waterways, such fish and invertebrates, are not warm-blooded, their feces don't contain Enterococci. Therefore, if high concentrations of Enterococci are found in these waterways, it is highly likely it came from human feces in sewage water. Additionally, even though Enterococci do not usually cause illness in humans, previous studies have shown that when Enterococci are present in waterbodies, people who swim in the water tend to get sick. Scientists determined that when Enterococci are present in a waterbody, it usually means that other sewage-associated pathogens that are more harmful, but may be harder for scientists to test for, are also present. For this reason, Enterococci are sometimes called **fecal-indicator bacteria** because they indicate the presence of human feces in the environment.

Scientists in the CWQTP network test for Enterococci in water samples from over 70 sites in NYC (different areas of different waterbodies). Because CWQTP aims to determine if combined sewer overflows affect all sites the same way (**Key Question #2**), it is important to take all of the samples on the same day, around the same time of day, so scientists can directly compare results from all 70 sites. You can imagine that it is difficult for a group of scientists to be in 70 different places at once; this is where **community scientists** help out. Community scientists are volunteers from the community--people just like you--who contribute to science projects. In the case of CWQTP, these community scientists take weekly samples from May to October from local waterbodies and deliver them to CWQTP partner labs to be tested for Enterococcus.



Map showing 2019 Citizens' Water Quality Testing Program sampling sites and partner laboratories.

Bacteria & Water Quality Testing Basics

HUDSON RIVER PK
RIVER PROJECT

Part 4: Laboratory Process:

Once the scientists receive the water samples from the different sites, they test them for the presence of Enterococci using the **IDEXX Enterolert** method. In this method, a chemical food that only Enterococci bacteria will eat, or **metabolize**, is added to each sample. This chemical was designed so that when the Enterococci digest the chemical, it breaks down into a particle that glows, or **fluoresces**, under UV light. After the food is added to the water samples, it is placed in an incubator at 41 degrees Celsius (98 degrees Fahrenheit, standard human body temperature) the temperature at which Enterococci grow most quickly to incubate for 24 hours.

If Enterococci are in the water samples, they will grow in the incubator, using the chemical food as energy, and they will multiply. As the Enterococci multiply, they will eat more and more of the food, and therefore produce more of the fluorescent particles. After 24 hours, the scientists take the samples out of the incubator and look at them under a UV light. If the samples fluoresce (glow) blue, the scientists know that Enterococci are present. Remember, *only* Enterococci can produce the blue fluorescent particle using the chemical the scientists mixed into the samples, so even if there are other pathogens in the sample (such as bacteria that would normally be found in river water), those pathogens won't make the sample fluoresce blue.

In order to determine if water contains enough bacteria that contact with that water could cause disease, scientists must be able to count the number of bacteria in a sample. In the Enterolert method, bacteria are able to be counted indirectly based on how much the sample fluoresces. Below is an image of the tray in which bacteria are grown using the Enterolert method. In the previous paragraph, we learned that chemical food is added to each water sample and then the sample is incubated. Scientists do an extra step before incubation if they want to count the bacteria in the sample, as opposed to just determining if bacteria are present or absent in the sample. After adding the food and letting it dissolve in the sample, scientists pour the sample into a **Quanti-Tray** (see image to the right) and then uses a device called a **Quanti-Tray Sealer** to seal the foil back to the plastic tray.

Once the tray is sealed, small quantities of the sample are trapped in each of the 97 large and small wells, or pockets, of the tray. After the sample is incubated for 24 hours, the tray is viewed under UV light. Keep in mind, the Enterococci were floating around the sample water that was poured into the tray. If there were just a few tiny bacteria cells, they might have only ended up in one or two of the wells before the tray was sealed. If there were hundreds of Enterococci in the original sample, they might have been distributed to many of the wells, where they multiplied in the incubator. Depending on how many Enterococci were in the original water sample, a corresponding number of wells will fluoresce under the UV light. The laboratory supply company, IDEXX, that created the Enterolert method, provides calculations based on their research that can be used to determine the *most probable number of bacteria* that were a 100 mL water sample.

Bacteria & Water Quality Testing Basics

HUDSON RIVER PK
RIVER PROJECT

Part 5 - What can scientists learn from the most probable number of bacteria in a water sample?

Scientists use the term “most probable number” (MPN) of bacteria to express a very close estimation of the number of individual bacteria cells that were in a sample based on the results of their testing. As we learned earlier, pathogens usually are more likely to make a person sick if there are more of them present in the environment around that person. So, the higher the MPN of disease-causing bacteria in a water sample, the greater the chance that people will get sick if they swim in the waterbody that sample came from.

So how do scientists calculate the most probably number of bacteria? Bacteria are very, very tine single-celled organisms. You cannot count individual bacterial cells with the naked eye or even under a microscope. This makes is very difficult and sometimes impossible to count all the bacteria in a sample. One way scientists count bacteria is by growing bacteria in a petri dish on a gel-like substance that contains food to help certain bacteria grow on its surface. (Please note: You can use the **“Monitoring Bacteria in Your Home”** lesson to grow bacteria yourself using a similar method.) This is prepared in a way so as to make sure the bacteria cells are spread out on the surface of the plate. Scientists can determine how many bacteria were in an original sample based on the way the bacteria have multiplied on the petri dish.

The scientists who developed the IDEXX Enterolert method]

Government agencies use this scientific concept to determine safety standards for waterbody use. Waterbody use is generally divided into two categories: recreational activities that involve primary waterbody contact, including swimming, or secondary contact, including kayaking, wading, fishing, or any activity in which only a person’s limbs are in contact with the water. The chart below shows the New York City Department of Health standards for swimming (primary contact) based on most probable number of Enterococcus bacteria in a 100 ml water sample. (Note that there are many more Enterococci in the entire waterbody. The chart shows the number of bacteria that would be found in 100 ml of water from a waterbody--the entire volume of the waterbody is much, much greater, so there could millions or billions of bacteria cells in the entire area of water. The sample is *representative* of the whole.)

Citizens' Water Quality Testing Program Protocols

HUDSON RIVER PK RIVER PROJECT

Advanced Methods in Restoration Science for High School Teachers

Hudson River Park River Project

NATIONAL SCIENCE FOUNDATION ITEST DRL 1759006 (CDO0006463)

Curriculum and Community Enterprise for Restoration of a Keystone Species in New York Harbor

---

Project Background:

The Citizens' Water Quality Testing Program (CWQTP) began formally in 2012 after a short pilot in 2011. The program operates during the 20 week boating season to test water recreation sites not tested by others. Results are sent out to those interested weekly during the program, and all data is available to the public on the NYCWTA website throughout the year. Our goal is to create baseline data for the public that can help predict bacterial concentrations per unit effluent. Water samples are collected by volunteers and brought to partner labs to be processed. All volunteer water samplers are trained at TRP at the beginning of the season. All samples are processed using IDEXX Enterolert to measure levels of *Enterococci,* a common fecal indicator bacteria. New York City Department of Health Enterococcus standards for swimming are used:

- MPN <35 = acceptable for swimming
- MPN between 35 and 104 = unacceptable if level persists
- MPN >104 = unacceptable for swimming

Sampling Protocols:

1. Make sure to bring a sterilized jar, black plastic bag, and small insulated lunchbox, ideally with icepack.
2. Other water quality parameters may be taken when collecting your sample so you may also want to bring a hydrometer, thermometer, secchi disk, and small sampling jar for a dissolved oxygen sample. Remember to bring small bottles of manganous sulfide, alkaline potassium iodide azide, and sulfuric acid with you so that the sample can be fixed in the field.
3. When taking the sample, keep your sterilized jar capped until just above the surface and immerse it to desired depth (6 inches/to the wrist).
    a. Let it fill nearly all the way, **allowing for a small pocket of air.**
4. Cap the bottle.
5. Properly label each sample, especially if you have duplicates or blanks.

   a. Date
   b. Time
   c. Location
6. Place the sample into a black plastic bag and it into your insulated box ASAP with the following information.
7. Deliver sample to designated cooler/lab fridge ASAP.

Idexx Enterolert Laboratory Protocols:

1. To prepare a sample for the incubator, first make sure that the lab is properly disinfected. If there is anything inside the lab that isn't supposed to be, remove it. A disinfecting check-list is posted on the door of the lab, make sure that all tasks are done before you start preparing the samples.
   a. Disinfections 1x per week; usu. Wednesday (day before processing).
   b. All tables and other objects should be wiped down with disinfectant wipes/ bleach dilution and double checked with the UV light for any fluorescence.
      i. Table surface
      ii. Supply containers
      iii. Sealer
      iv. Incubator
      v. Quanti-tray insert
      vi. Pipette bulb
      vii. Sharpies
      viii. Door knob (both sides)
   c. The floor should be mopped semi frequently (not required every week)
2. Preheat the incubator to 41°Celsius (41±0.5C° is acceptable)
   a. It is already set, so simply press and hold the power button (lowest in the row) until the screen displays temperature.
   b. **This should be done a few hours prior to allow warm-up time**
   c. Incubator may be left on for several days during the week if additional processing is required, but should be turned off for the weekend.
   d. Make sure incubator reads 41±0.5C°
3. Samples should be transferred to the lab fridge
   a. **Samples must be processed within 6-8hrs of being taken**
4. Clipboard, pencil, and data sheet should be brought into lab
5. Supply containers should be restocked:
   a. Sterile water pop-tops (100mL vessels with 90mLs water)
   b. Sterile pipettes
   c. Quanti-trays
   d. Enterolert reagent
   e. Nitrile gloves
6. Wash your hands thoroughly, up to the elbows.
   a. Dry with paper towel; do not touch anything en route to lab.
   b. Immediately put on rubber gloves
7. Turn on Quanti-tray sealer
   a. It will take a few minutes to warm up

   b. Green light will turn on when ready
8. Arrange sample vessels chronologically by time taken
   a. Write site names and sampling times on data sheet
      i. Randomly select one site to perform a split duplicate; denote this with two line items each with the site name, then followed by 1 or 2.
      ii. Add a line for Control sample last
9. Tally the total number of samples to be processed (including split and control)
10. Add Enterolert reagent to that number of pop tops
   a. Unwrap plastic seal on pop top, then dispose
   b. Gently open pop top and place it on table, taking care not to touch the inside of the vessel, only the tab on lid.
   c. Break off Enterolert reagent pack,
   d. Break individual Enterolert capsule away from self
      i. Pour reagent into pop top, then dispose
   e. Close pop top
   f. Swirl until reagent is completely dissolved
      i. No visible particulate should remain
      ii. Solution will be pale yellow
      iii. NOTE this may be done two at a time; one in each hand
11. A sterile pipette must be used for each sample
   a. Grip pipette bag and gently shake to make it possible to remove a single pipette without touching others
   b. Fit bulb over end
   c. **Sample vessels should always be inverted once or twice before pipetting**
   d. Open sample and pop top
   e. Pipette 10ml of water into pop top. **Make sure the pipette does not touch anything, including the sides or lips of either vessel.**
   f. Close pop top
   g. Remove used pipette from bulb and dispose
12. Pour sample/reagent mixture from pop top into a Quanti-tray
   a. Remove single Quanti-tray from bag without touching other
   b. Gently press fist into top back of Quanti-tray, just under the tab
   c. Gently pull tab to open Quanti-tray
   d. **Sample/reagent mixtures should always be inverted once or twice before transferring to Quanti-tray**
   e. Pour sample/reagent mixture from pop top into Quanti-tray
      i. Careful not to spill!
   f. Tap/agitate bottom of Quanti-tray to remove all air bubbles
13. Gripping from the bottom with one hand, press on the back of the Quanti-tray with fingers while tilting near-horizontally, other hand on the top
   a. This is attempting to get as many wells filled with liquid as possible to ensure a viable sample
   b. If more than a few drops escape, the sample must be discarded and made anew.

Citizens' Water Quality Testing Program Protocols

14. Carefully place horizontal Quanti-tray into rubber insert
15. Feed Quanti-tray with insert into the sealer
    a. You will have to push a little at first, but stop once the sealer "takes" the tray
16. Remove sealed Quanti-tray and insert from back of sealer
17. Inspect Quanti-tray to ensure ALL wells have liquid in them, if not, sample must be discarded and made anew
18. Inspect both Quanti-tray and insert to ensure there were no leaks
    a. If small leak did occur, wipe insert down with disinfectant wipe and let dry
19. Write down the site name and time incubated (date optional) on back of Quanti-tray with sharpie
    a. Careful not to press hard and puncture backing
20. Place labeled Quanti-tray into incubator and close

<u>Performing a blank/control sample:</u>

- Treat a blank sample exactly the same as any other, just use sterile water from a pop top in place of any sample water. It will still need to reagent and to be place properly in the Quanti-tray as per above.

Analyzing Enterolert Samples:

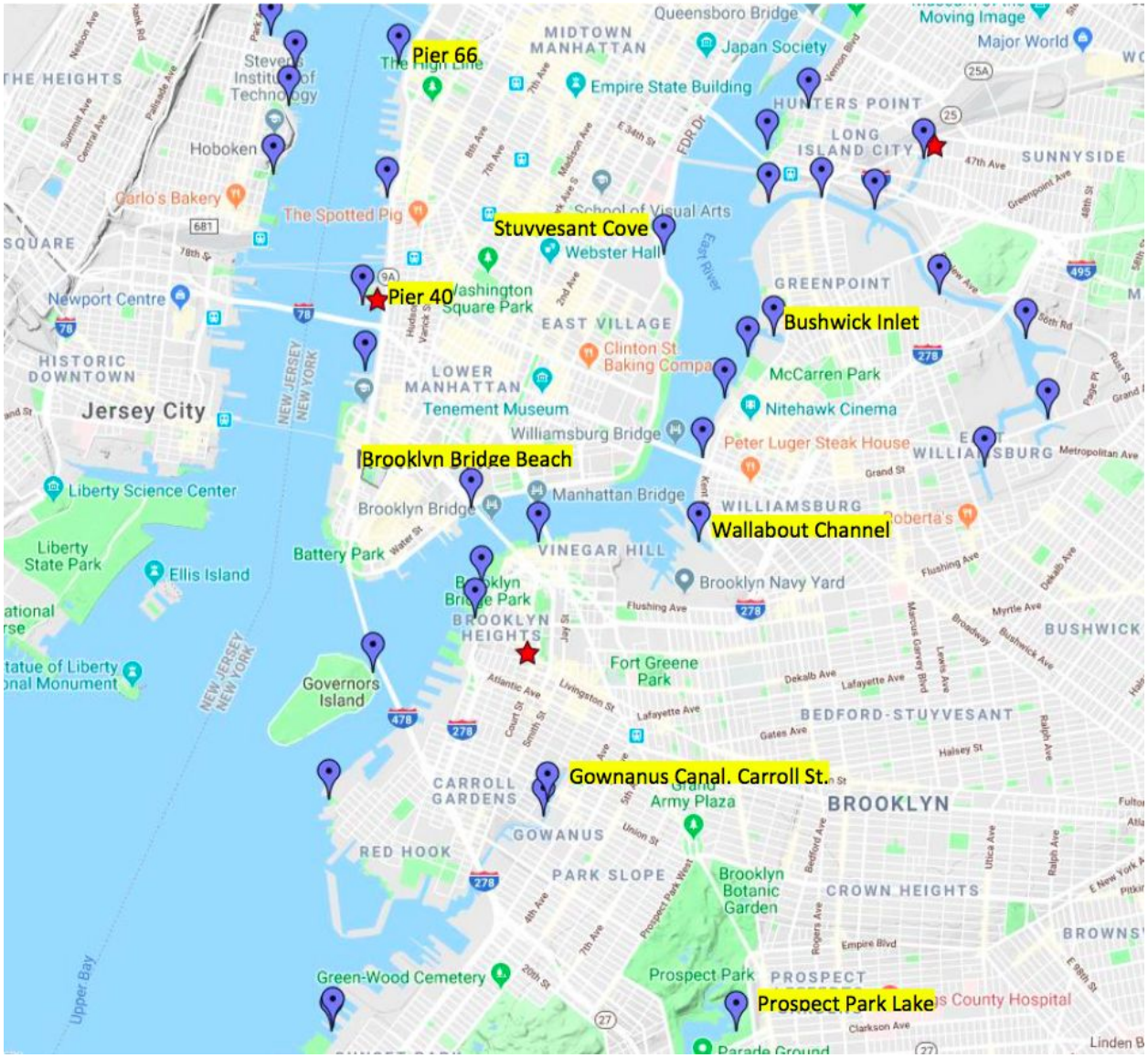
1. Wait at least 24, but no more than 28 hours before analyzing Quanti-Trays.
2. Turn off all of the lights in the back lab, shut the door, and close the curtain so that you're in complete darkness.
3. Turn on the 365nm UV light.
    a. Never direct at face of self or others.
4. Take one sample out of the incubator at a time.
5. Hold the UV light approximately 5 inches above the Quanti-tray and count only the **Blue Fluorescent** cells. These cells are positive for enterococcus.
    a. Make sure to count and record the small and large cells separately.
    b. All blue fluorescent cells should be counted, although some will be much more vibrant than others.
    c. Make sure to face light away from your eyes and skin.
    d. If difficult to tell whether a cell is fluorescing, view Quanti-tray through phone camera, fluorescence can sometimes be easier to spot through the lens.
    e. Do not count any color fluorescence other than blue

    i. Yellow-green is most common of the possible, secondary fluorescence colors. This denotes non-target bacteria that can partially consume the reagent.

6. Write down the number of large and small wells fluorescing on the back of Quanti-tray.
7. Repeat until all samples have been analyzed under UV.
8. **Make sure to write this information on data sheet.**
9. When finished, make sure to turn off the incubator and clean up after yourself.
    a. Quanti-trays and vessels should be saved for at least 1 week before sterilization and disposal.
10. Use the Most Probable Number (MPN) table taken from Standard Methods for the Examination of Water and Wastewater.
    a. Find the MPN value on the chart for each sample by finding the intersection of the rows and columns with the # of wells filled.
    b. Multiply number on chart by 10 (due to the 1:10 dilution)
        i. This gives final reading of MPN per 100mL

CLASSROOM ACTIVITY

Now it's your turn to analyze water samples from eight sites in NYC, shown on the map below.



Part 1. Below are pictures of Quanti-trays, each containing a sample taken from one of eight sites. The samples have been incubated for 24 hours and are shown under UV light. Count the large and small wells that are fluorescing (the rectangular well at the top counts as one large well). Use the MPN Table to calculate the most probable number of Enterococci in each sample. Click here for the MPN Table and instructions for how to use it. Record your data in the table below.

Sample Date: 9/5/2019

Site	Large Wells	Small Wells	MPN Enterococci

Pier 66, Hudson River			
Pier 40, Hudson River			
Bushwick Inlet, East River			
Stuyvesant Cove, East River			
Wallabout Channel (BNY), East River			
Brooklyn Bridge Beach, East River			
Gowanus Canal at Carroll St.			
Prospect Park Lake			

MPN = most probable number of Enterococci/100mL water

Central Park Rainfall (inches), Source: NOAA

8/30/2019	0
8/31/2019	0
9/1/2019	0
9/2/2019	0.3
9/3/2019	0
9/4/2019	0
9/5/2019	0

Analyze your data

1. Based on the Department of Health safety standards for Enterococci, are there any sites where the water quality was good enough for swimming on September 5, 2019?

1. Compare the MPN Enterococci for the eight sites. What factors might cause differences in MPN at different sites? Use the map above to help inform your answer.

1. Look at the Central Park Rainfall data for the week the water samples were taken. What inferences about the effect of rainfall on water quality at these sites can you draw from the information in the table?

Scientists often use outside sources of data or information to help them understand and analyze the data they collect. Open Sewer Atlas's Wet Weather Map is a great resource for data on CSOs in NYC. Click on the link and explore the map features. Each brown circle on the map indicates a combined sewer outfall. If you click on a brown circle, you can find out information about that outfall based on 2016 data, including the total volume of sewage overflow from that outfall that year, the number of overflow events that produced that volume of sewage, and the minimum amount of rain, in inches, that caused an overflow

from that outfall. Using the map above to help, find the sewer outfalls near the eight sites that you collected MPN data on.

1. Using data from Open Sewer Atlas, explain why the MPN Enterococci might have been different in samples taken from Pier 66 and Gowanus Canal at 2nd Street on September 5, 2019.
1. Find Prospect Park Lake on the Wet Weather Map. Do you notice anything about it that surprises you? What might be another source of Enterococcus bacteria in this lake? (There are hints in the photo of Prospect Park Lake below.)