Water Quality Testing for Enterococcus 2-Day PD

Day 1

10:00-10:15 (15 min) Intro - teachers' names, what they teach, why they are attending PD
10:15-11:15 (60 min) Sampling - 5 samples along Pier 40; General water quality
11:15-11:35 (20 min) Wetlab tour
11:35-12:15 (40 min) Presentation
12:15-12:25 (10 min) Idexx pipetting practice
12:25-12:55 (30 min) Idexx
12:55-1:30 (35 min) Lunch
1:30-1:40 (10 min) Hyserve pipetting practice
1:40-2:10 (30 min) Hyserve
2:10-2:40 (30 min) Analyze prepared samples
2:40-3:00 (20 min) Questions, review

Day 2

11:00-11:40 (40 min) Intro to data sources; relationships between CSOs and oxygen, nutrients, etc
11:40-12:40 (60 min) Graphing/data analysis

1. MPN vs Rainfall, 2018: Pier 101, Prospect Park Lake, Gowanus Caroll
2. MPN vs Rainfall, 2017: Coney Island Creek-21st St, Canarsie Pier
3. MPN vs DO, 2017: Dutch Kills Head, Dutch Kills Mouth, English Kills
4. MPN vs. Rainfall, 2018: Peter Sharp Boathouse, W 172nd St, Pier 40, Stuyvesant Cove
5. MPN 2012-2019: Gowanus 2nd, Gowanus Carroll

12:40-1:15 (35 min) Lunch
1:15-1:35 (40 min) Analyze Idexx and Hyserve, discuss results
1:35-2:35 (60 min) Finish graphing, present
2:35-3:00 (25 min) Surveys, wrap-up

Water Quality Resource List

Data Banks

1. **New York City Water Tail Association - Citizens' Water Quality Testing Program**
   Enterococcus data for ~70 NYC sites, 2011-2019
   Recorded daily rainfall at Central Park (NOAA)
   https://www.nycwatertrail.org/water_quality.html

2. **Newtown Creek Alliance**
   Enterococcus, dissolved oxygen, etc at several sites in Newtown Creek, 2016-2018
   Recorded daily rainfall at LGA (NOAA) and Newtown Creek (Weather Underground)
   http://www.newtowncreekalliance.org/water-quality-sampling/

Current and Historical Environmental Conditions

1. **Weather Underground**
   Site-specific precipitation and other weather data
   https://www.wunderground.com/

2. **Hudson River Environmental Conditions Observing System**
   Real time precipitation, dissolved oxygen, etc. at Pier 84 and upriver sites
   https://www.hrecos.org/ >> https://ny.water.usgs.gov/maps/hrecos/

Maps

1. **Open Sewer Atlas**
   Water quality, CSO-shed, and sewershed maps for NYC (2016)
   https://openseweratlas.tumblr.com/maps

2. **HabitatMap - Where does my toilet flush to?**
   Detailed sewershed map (also houses NYS watershed map, NYC garbage map, etc)
   http://habitatmap.org/markers?colors=0_3_1&lat=40.705705&lng=-73.978195&maps=212_213_192&nogrp=1&t=terrain&z=10

3. **OASIS NYC**
   CSO sites, land use, land cover, etc, in NYC
   http://www.oasisnyc.net/map.aspx

4. **Newtown Creek Aerators**
   Sites where NYCDEP have installed mechanical aerators in Newtown Creek
   http://www.newtowncreekalliance.org/wp-content/uploads/2015/01/Screen-shot-2015-02-11-at-11.02.06-AM.png

Background Reading & Water Quality Reports

1. **SWIM (Stormwater Infrastructure Matters) Coalition**
   Factsheets, reports, and advocacy information on NYC stormwater infrastructure
   https://www.swimmablenyc.org/

2. **NYCDEP Harbor Water Quality Report, 2017**
   NYC water quality
   https://www1.nyc.gov/assets/dep/downloads/pdf/water/nyc-waterways/harbor-water-quality-report/2017-new-york-harbor-water-quality-report.pdf

3. **Riverkeeper - How's the Water?, 2017**
   Hudson River Estuary water quality report
   https://www.riverkeeper.org/wp-content/uploads/2017/11/Riverkeeper_WQReport_2017_final-1.pdf

Related News Articles and Other Sources

1. *City's Pumping Air Into Polluted Newtown Creek has Critics Worried*. City Limits, 2018
   https://citylimits.org/2018/08/08/citys-pumping-air-into-polluted-newtown-creek-has-critics-worried/

2. *A Park to Sop Up Pollutants Before They Flow Into the Gowanus Canal*. NYTimes, 2015
   https://www.nytimes.com/2015/12/16/nyregion/sponge-park-in-brooklyn-to-treatpolluted-waters-of-gowanus-canal.html

3. *Beach Haven Apartments Fined $400,000 for Dumping Raw Sewage Into Coney Island Creek*. Bklyner, 2017.
   https://bklyner.com/beach-haven-apartments-fined-400000-for-dumping-raw-sewage-into-coney-island-creek/

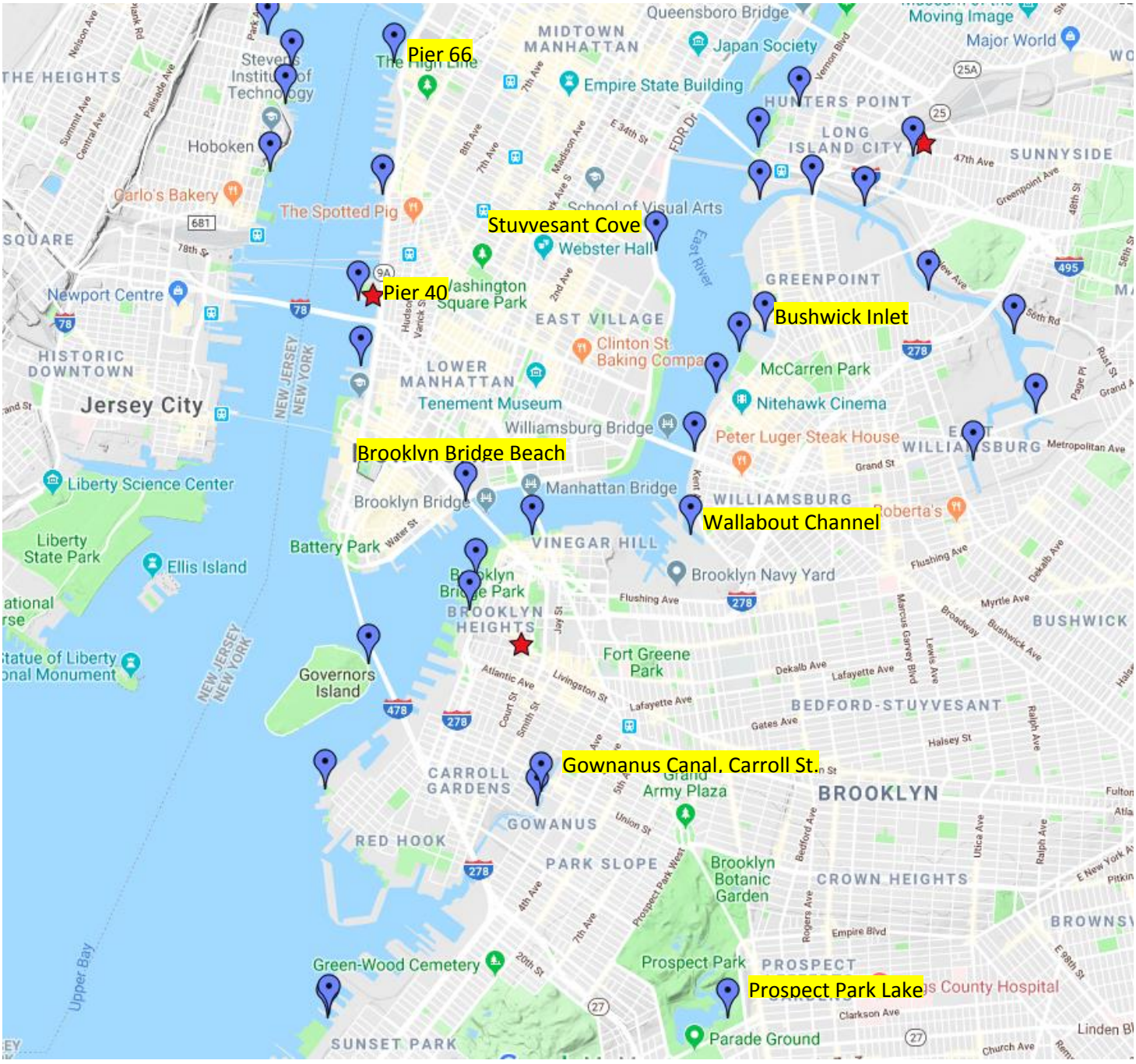
Further Reading

1. **Enterococci as Indicators of Environmental Fecal Contamination**
   Boehm AB, Sassoubre LM. *Enterococci as Indicators of Environmental Fecal Contamination.* 2014 Feb 5. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Inrmary; 2014.
   https://www.ncbi.nlm.nih.gov/pubmed/24649503

2. **Volunteer Water Quality Monitoring - Presenting Bacteria Data Effectively**
   http://blog.uvm.edu/kstepenu/files/2016/11/Bact-Present_XV.pdf

THE RIVER PROJECT

2019 Citizens' Water Quality Testing Program
Partial Site Map



Credit: New York City Water Trail Association

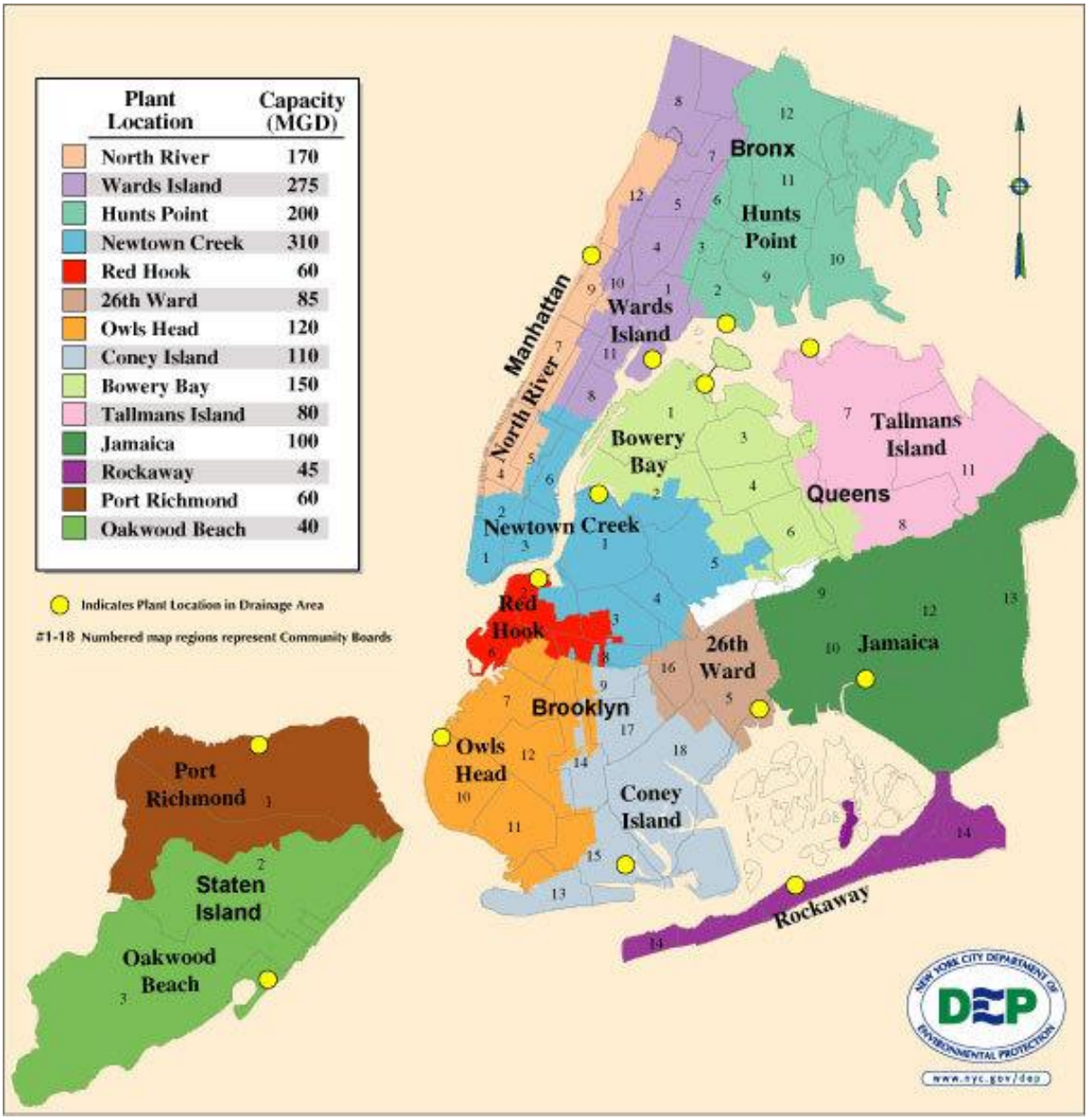
THE RIVER PROJECT

Map of NYC Combined Sewer Outfalls

Credit: Open Sewer Atlas

THE RIVER PROJECT

NYC “Sewershed” Map



Credit: Habitat Maps

THE RIVER PROJECT

Name_____ Date_____

Central Park Rainfall (inches), Source: NOAA

9/6/2019	0.32
9/7/2019	0.02
9/8/2019	0
9/9/2019	Trace
9/10/2019	0.01
9/11/2019	0
9/12/2019	0.17

NYC DOH Enterococcus Standards (for swimming)
Green: <35 MPN--acceptable
Yellow: 35-104 MPN--unacceptable if levels persist
Red: >104 MPN--unacceptable

Sample Date: 9/12/2019

Site	Large Wells	Small Wells	MPN
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 Late			

MPN = most probable number of Enterococci/100mL water

Sources

2019 Citizens' Water Quality Testing, New York City Water Trail Associate
https://www.nycwatertrail.org/water_quality.html

Wet Weather Map (Combined Sewer Outfalls), Open Sewer Atlas
https://openseweratlas.tumblr.com/wetweathermap

Where does my toilet flush to? (Sewershed Map), Habitat Maps
http://habitatmap.org/markers?marker_id=144-newtown-creek-sewage-treatment-facility

What is a CSO?, Akron Waterways Renewed!
http://www.akronwaterwaysrenewed.com/about-us/combined-sewer-overflow-cso-explained.aspx

HyServe Compact Dry ETC Enterococcus Testing Protocol

Precautions and Notes:

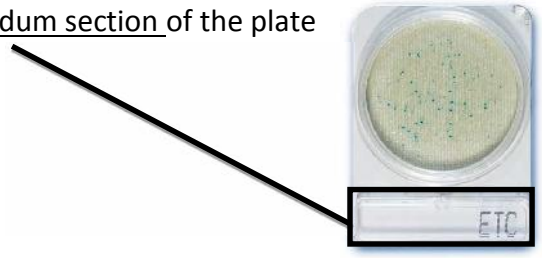
The HyServe Compact Dry ETC count plate contains *Enterococcus* growth media that is light yellow in color. This media is food for *enterococci* to grow into colonies that can be visibly seen so that they can be counted and quantified. While the media itself does not pose a threat to the user, it is **imperative** that one does not touch the media at any time otherwise the test will be compromised.

The units used for bacteria monitoring is MPN/100mL or CFU/100mL and are somewhat interchangeable. MPN stands for "Most Probable Number" and CFU stands for "Colony Forming Units". CFU is literally the number of colonies you see (say on a plate), while MPN is the probability of seeing that number of colonies **if** the same water sample was tested on a plate. In the case of the HyServe Compact Dry ETC Plates, the units are CFU/100mL since plates are used. Idexx does not involve plates and so they use the units MPN/100mL. In general, units should always be noted in any test so that the reader knows what he/she is dealing with.

Since only 1 mL of water sample will be used for the HyServe Compact Dry ETC plates, the number of colonies will need to be multiplied by 100 in order to standardize the units to other types of bacteria tests that use a full 100mL.

Directions:

1. Write the Date, Time and Site Name in the memorandum section of the plate



2. When you are ready to sample, open the disposable plastic pipette and depress the top bulb portion to expel some of the air.

3. Insert the disposable plastic pipette into the sample of river water and very slowly release until the water line reaches the 1 milliliter (mL) mark.

While holding the pipette still slightly depressed with the correct volume (1 mL) at hand, take the pipette completely out of the water and then release it fully. All the water will then accumulate to the top bulb of the pipette.

4. Remove the plastic lid covering the growth media on the HyServe Dry ETC count plate and depress the bulb of the pipette to release the 1mL of water in the center of the plate. The water sample will diffuse automatically. Keep the pipette as you will use the same one later to sterilize the plate before disposal.

5. Put the plastic lid back on top of the plate and wait a minute for the medium to gel up. Then turn the capped plate upside down and incubate the plate at 35 ± 2 °C for 20-24 hours.

6. After 20-24 hours have elapsed, count the number of blue/blue green colored colonies that have formed on the plate:

    a. You should count all colonies formed, regardless of variation in size and shape or even if they are not quite blue/blue green in color. Bacteria other than Enterococcus are inhibited to grow and they do not form any colonies.
    b. Placing a white piece of paper under the plate may help you see the colonies more clearly for counting purposes.
    c. Also, the growth area is 20 cm^2. The back of the plate has a grid carved of 1 cm x 1 cm to make the colony counting easier. In case of any difficulty counting the colonies due to the large number of colonies grown, total viable count can be obtained by multiplying 20 by the number of colonies per 1cm^2 square counted from several squares.
    d. In cases where there are hundreds (100-300) of colonies, you could count the number of colonies using the method “c” above, or the old fashioned way. For values above 300 colonies (i.e. 30,000 CFU/100mL), it is beyond the enumeration capabilities of the plate. We then count that as “TNTC” which stands for “Too Numerous To Count.”

7. Take a close-up picture of a plate (the flower setting on your camera) so that we have a record of the plate and can refer back to it and re-count as needed.

8. When the plate have been counted, take the used pipette and drop 1mL of bleach on the plate. It can now be disposed of properly.

IDEXX Protocol for Enterococcus Testing

1. Incubators are preheated to 41°C and the IDEXX sealer is turned on.
2. Samples are transported to the lab in an opaque bag and stored in refrigerator.
3. Lab technicians should wash hands past wrists and avoid touching anything until inside lab. Wear gloves once inside lab.
4. The supplies for sample preparation are set out:
    a. Sterile pipettes
    b. Sterile mixing vessels
    c. Sterile water
    d. Quanti-trays
    e. Reagent
    f. Data sheet
5. When lab bench is prepared, samples are removed from refrigerator and organized by time from earliest to latest.
6. Mix one reagent packet into 90mL of sterile water by swirling the vessel. Make sure that the powder dissolves completely.
7. Pipette 10mL of the sample into the same mixing vessel, cap, and invert.
8. Open the Quanti-tray carefully and pour the sample into it. Make sure that there are no air bubbles in the small wells.
9. Place the Quanti-tray into the rubber mold and run them through the IDEXX sealer.
10. Label each Quanti-tray with the sample location and time of incubation and then put into the incubator.
11. On data sheet, record date, sample location, time of collection, time of incubation, time of analysis (day 2), number of large and small fluorescing cells (day 2), and Most Probable Number [MPN] (day 2).
12. On day two, 24-26 hours of incubation, at least two technicians count all large and small fluorescing cells, and calculate MPN using the IDEXX Quanti-tray/2000 MPN table and multiplying by ten.

Quality control protocol:

1. Mix one packet reagent into 90mL of sterile water by swirling the vessel. Make sure that the powder dissolves completely.
2. Pipette 10mL of the sterile water into the same mixing vessel, cap, and invert.
3. Follow steps 8 and 9 above.
4. Label the Quanti-tray with “Control”, time of incubation, and then put into incubator.
5. Follow steps 11 and 12 above.

IDEXX Enterolert Data Sheet

Date						
	Time sample		Time	Enterococci Results		
Site	taken	Incubated	Analyzed	Small	Large	MPN

Hyserve Compact Dry Data Sheet

Date				
Site	Time sample taken	Incubated	Time Analyzed	Entero CFU