# Standard Operating Procedure

# for IDDE Catchment Investigation Procedure

**Table of Contents:**

**Purpose…………………………………………………………………………………………………………………………………1**

**Materials Needed………………………………………………………………………………………………….………………1**

**Sampling Procedure……………………………………………………………………………………………………………….4**

**Conduct a field test using the YSI Pro Plus sonde……………………………………………………………………5**

**Lab Test Procedure for *E. Coli* / *Enterococci*……………………………………………………………………………7**

**IDEXX 51 Well Quanti-Tray MPN Table……………………………………………………………………..……………8**

**Surfactant Screening………………………………………………………………………………………………………………9**

**Lab Procedure for Total Nitrogen…………………………………………………………………………………………10**

**Threshold Conditions………………………………………………………………………………………………..…………12**

**User Manuals………………………………………………………………………………………………………………………14**

**Survey123 Template……………………………………………………………………………………………………….15-18**

## Purpose

This document outlines the protocols for sample collection, use of field kits, storage and conveyance of samples, field data collection and storage, and catchment investigation in accordance with the City of Dover, NH Illicit Discharge Detection and Elimination (IDDE) Plan.

## Sample Collection Requirements

* All samples are to be collected during ‘dry weather conditions’ which is defined as less than 0.1 inches of rainfall over the previous 24-hour period and no significant snow melt is occurring.
* Prioritized outfalls are to be sampled first. Priority and ranking are determined according to the predetermined ‘Outfall Assessment and Priority Ranking Procedure’ outlined in the permit [Section 2.3.4.7] when dry weather discharge is observed at an outfall location. For purposes of this procedure dry weather discharge is considered any discharge from the outfall or interconnection that exceeds 50 gallons per day or 0.035 gallons per minute. Effectively what this means is that flow should be sufficient to collect the sample volume required within 5 minutes.
* All sampling personnel are to don proper personal protective equipment (PPE) during the sampling procedure (gloves, proper footwear, pants, etc.)

## Materials Needed

## Sampling (field)

* About 300 ml of water sample for all analyses.
* The *E. coli* sample should be taken separately.
* 3- [120 ml sterile sampling bottles](https://www.coleparmer.com/i/disposable-sterile-sampling-vials-screw-top-caps-pc-120-ml-100-cs/0604582?PubID=UX&persist=true&ip=no&gclid=EAIaIQobChMIkZ6a2vvY8gIVy_bICh0FXgp3EAQYAyABEgKMIvD_BwE).
* YSI sonde with appropriate sensors.
* Millipore Sterivex Sterile Filter Unit
* Medex M/F luer lock plug
* BD 50 mL syringe
* Permanent marker for labeling.

## Surfactant Screening (lab)

* Untreated (non-bleached) paper for sample collection and testing. Standard [yellow Post-It Notes](https://www.amazon.com/Post-Americas-Favorite-Sticky-Canary/dp/B00006JNNE/ref=sr_1_6?dchild=1&keywords=post+it+notes&qid=1610646289&sr=8-6) are perfect as they are small, inexpensive, and most importantly, they do not contain optical brighteners and fluoresce under UV light.
* [365 nm UV flashlight](https://www.amazon.com/Alonefire-Flashlight-Rechargeable-Ultraviolet-Blacklight/dp/B08B64B7PP/ref=sr_1_19?crid=186VFEM0RID6N&dchild=1&keywords=365nm+flashlight&qid=1610645808&sprefix=365+nm%2Ctools%2C166&sr=8-19) (“black” light). This flashlight, for example, is 365 nm, rechargeable, and includes UV safety glasses. Note: many UV flashlights are 395 nm and may work but less effectively.
* *Optional but suggested:* UV enhancing safety glasses to protect eyes for prolonged exposure to UV light and to enhance the contrast for analysis.

## Total Nitrogen (lab)

* Hach digester (DRB 200)
* Hach Spectrophotometer (DR1900, DR2800, DR3900)
* Total Kjeldahl Nitrogen (TKN) TNT 880 test kit
* Nitrate TNT 836 test kit
* 1-2 mL pipet(s)
* Distilled water
* Chem wipes
* At least 10 mL of water sample for all analyses

## Field Kits

* Field kits will contain:
  + YSI handheld sampling device and appropriate sensors
  + Sample collection containers
  + Nitrile gloves
  + Data collection tablet/iPad
* Field kit maintenance:
  + After each day of sampling, all field kit supplies are to be replenished and sampling equipment is to be properly cleaned.
    - Refer to **YSI Pro Plus User Manual Page 58** for maintenance procedure.

## Sample Storage and Conveyance

* *E. coli/ Enterococcus* samples are to be tested upon return from the field. Maximum holding time is 6 hours. (per EPA CFR Title 40, Chapter 1, Subchapter D, part 136) (per NEMI 9221 A, B, C, F).
* The maximum hold time for all samples will be 6 hours.
* The Nitrate TNT 836 test should be run no later than 3 hours after sampling for most accurate results. Store in a cool location. See the manual for instructions on preservation beyond this time period.
* Samples will be transported and held at 34-39°F (1-4⁰ C) until testing occurs.
  + This is most simply done using a cooler containing ice packs. Do not allow the ice packs to come into direct contact with the sample containers.
  + A liquid thermometer should be kept in the cooler to allow for easy temperature monitoring. \*We recommend affixing the thermometer to the inside of the cooler.

## Field Data Collection and Storage

* All field data will be collected using the Survey123, or similar data collection software on a tablet/iPad. See attachment.
* In the event that a tablet is unavailable, all the information will be collected on a paper form containing the same information.
* Collected information will include:
  + Outfall number
  + Outfall location
  + Date and time of sampling
  + Weather conditions
  + Time since last rainfall (hours)
  + Amount of last rainfall (inches)
  + Receiving water
  + Shape, dimensions, physical condition, and material of outfall pipe
  + Spatial location (coordinates provided by Survey123 or similar application)
* Data collected on the YSI handheld device can be uploaded into a spreadsheet and connected to the data point in the Survey123 map.
  + Refer to **YSI Pro Plus User Manual Pages 52-53** for data management procedure

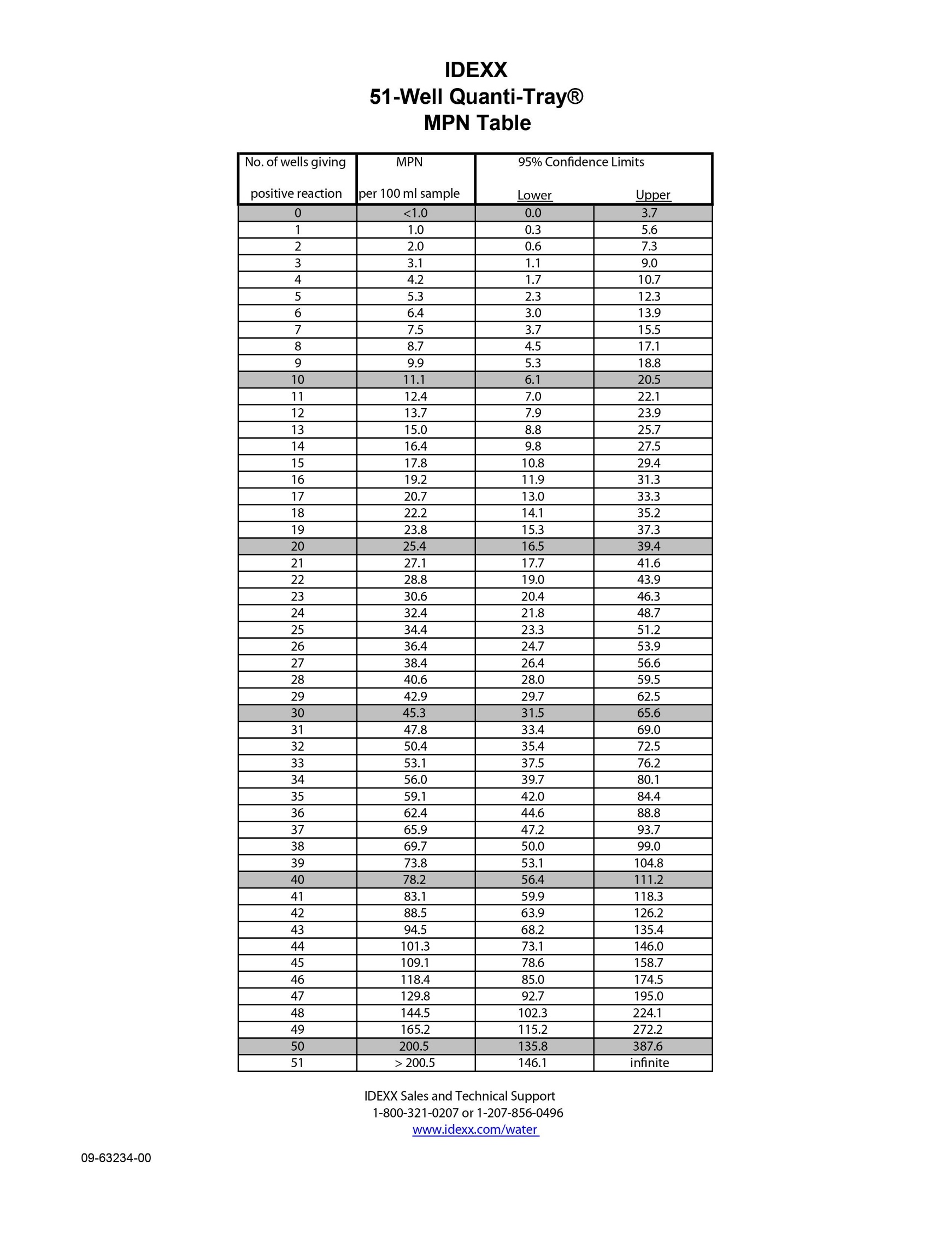
1. **Sampling Procedure**

*Note: this protocol is written assuming you will collect samples in the field, label, store on ice, and process the samples back at a field lab. You can also take samples with the YSI Pro Plus in the flowing water stream.*

Locate outfall to be sampled. If the outfall is inaccessible, follow the stormwater drainage map to the next accessible upstream access point for sampling.

* 1. Document outfall number and complete the field data collection form using the tablet/iPad Survey 123 form, or similar application/form (template form included as an appendix).
  2. To take the Genomic sample, fill the 50 mL syringe with water from the outfall
     1. Screw the Sterivex filter unit onto the front of the syringe, push the water through
     2. Repeat 10 times or until no more water will pass through the filter unit
     3. Cap each filter side with two medex M/F lock plugs and freeze until processed
  3. To start, Label your sterile *E. coli* (freshwater) or *Enterococci* (coastal) bottle and two additional sample bottles/containers with the date, time, and name of outfall/ location.
  4. Fill each sample bottle separately.
  5. Take the sample bottle and unscrew the top.
     1. Hold the bottle cap facing down so nothing can get onto the underside of the lid.
     2. Do NOT touch the underside of the lid to avoid transferring bacteria.
     3. Do NOT set it down on the ground because something could get on it.
     4. By following these steps, you greatly reduce the chance of bacteria getting into the bottle, so you will not get a false positive.
  6. Fill the bottle from the outfall making sure to not get any sand, sediment, or debris in the container.
     1. *A very important note: the* E. coli/enterococci *samples must be run within six hours of them being taken. Make sure to plan enough time to run all the bacteria samples in the lab when done sampling.*
  7. Next, take your two other sample bottles/containers and fill them the same way but making sure to fill them to the 200 mL mark.

1. **Conduct a field test using the YSI Pro Plus sonde**
   1. Turn on the meter by pressing the green circle button.
   2. When the screen comes on, it should show at least a temperature reading in degrees Celsius or Fahrenheit depending on your chosen options, a barometric pressure reading in mmhg, and a specific conductivity reading in us/cm.
   3. The end of the cord attached to the meter should have a small metal port that is covered with a red cap. It is important to leave the red cap on until a probe is inserted into the port. The cap will keep water and debris from entering the hole and damaging or destroying the sensor port.
   4. The next step is to make sure that both the conductivity and salinity results are being displayed. To turn these on is a two-step process.
      * First you click the probe button and then select setup. Look at the row that says conductivity and see if it says on or off next to it. If it says on, then you are all set but if it says off, click on conductivity. The next screen has a box that just be clicked to turn on conductivity. Click the box and it will turn it on. Then press escape to return to the main screen where the results are displayed.
      * Probe 🡪 Setup 🡪 Conductivity 🡪 Escape to return to main screen.
      * To turn on salinity, click the probe button. Then scroll down to display. From there scroll down to conductivity and click it. Then scroll down to salinity and click that as well. In this screen you will see three options. You want to scroll down to ppt and click that. Then press the escape button which will take you back to the main menu.
      * Probe 🡪 Display 🡪 Conductivity 🡪 Salinity 🡪 PPT🡪 Escape
   5. Attach the ammonia (NH3) sensor to the probe by removing the red cap at the end and screwing the sensor into it. Caution, nothing should be forced in or screwed in with abnormal tightness. This could strip or damage the connections and ruin the instrument. If properly aligned the sensor should easily be installed and fall into alignment without much effort.
      * Then click the probe button and choose setup.
      * Scroll down to ISE1 and then choose NH3. Then press the escape button which will take you back to the main menu.
      * Probe🡪 Setup🡪 ISE1🡪 NH3🡪 Escape
   6. The YSI screen should now have the following units displayed.
      * The temperature in degrees Celsius/fahrenheit
      * The barometric pressure in mmHg
      * The conductivity in us/cm
      * The salinity in ppt
      * The ammonia in mg/L
   7. If there are multiple dashed lines or strange symbols through some of the result areas, don’t worry about it for now. It just means that nothing is detected yet. If dashed lines or strange symbols are not replaced by real numbers when you start sampling, then there is a problem. If symbols are not replaced, start by repeating step 2d and try again.
   8. Now take the end of the cord with the sensor and submerge it in one of the water sample bottles. The important things to note here are that the sensor is fully submerged and that the two holes near where the cord begins are fully submerged as well. You may have to jiggle the sensor to keep refreshing the sample within the probe. If the water level is too shallow such that the probe cannot be fully submerged, then you will have to add more sample water.
   9. It may take a few minutes for all of the readings on the YSI screen to stabilize. If the numbers keep jumping back and forth within a small range, you can take the average or one of the numbers it keeps stopping on.
   10. Record the ammonia, specific conductivity, salinity, and temperature on your tablet or field sheet making sure to note the units for each parameter.
   11. Once you are done sampling with the YSI meter, take off the metal cage, and then rinse the whole sensor area with DI or distilled water. Make sure to get water into the two holes at the top of the probe.
   12. You have gathered all the data and samples you will need for this outfall. You can move onto the next outfall sample. Remember, the *E.coli* samples must be run within six hours of collection.
2. **Lab Test Procedure for *E. Coli* (*fresh water*) / *Enterococci* (*salt or brackish water*):**
3. In the lab, take your *E. coli* sample bottle and make sure your sample is 100 ml exactly. If you need to pour off some sample to get to 100 ml, be sure to shake the sample first.
4. One packet of reagent will then be added to the sample. The cap is then replaced, and a swirling motion is performed until the reagent is dissolved into the sample. The reagent will have a hard time dissolving into an ice-cold sample, so be sure to let it sit for a short time.
   * 1. For freshwater samples use Colilert, Brackish or saltwater samples will be run with Enterolert.
5. Prep your Quanti-tray by writing on the back the sample name, location or other indicator and the date and time the sample was run. Use a soft tip sharpie so that you do not break through to one of the wells.
6. Open the Quanti-tray by holding it in one hand and squeezing the sides, you can gently pull the tab if you need to. Be careful not to touch the inside of the tray. Gently tap the side to remove excess air bubbles.
7. The sample is then carefully poured into the tray and placed onto the rubber sealer mat.
8. The tray and sealer mat are then put through the sealer which should have been warming up for a few minutes. You will know it is ready when you have a green light.
9. Place the tray and the rubber mat with the backing of the Quanti-tray facing up and the opening of the Quanti-tray facing out.
10. Let the sealer grab the tray and pick it up a few seconds later from the back of the sealer.
11. Once the sample tray comes out of the sealer it must be incubated:
    * 1. For Colilert- put the Quanti-tray in the incubator at 95°F (35°C) +/- 0.5°C for 24 hours.
      2. For Enterolert- put the Quanti-tray in the incubator at 106°F (41°C) +/-0.5°C for 24 hours.
12. AFTER the 24-hour incubation period:
    * 1. Take tray out of incubator.
      2. If there is no color that indicates that no total coliforms are present.
      3. To check for *E. coli / enterococci* you will need to use a UV light to see how many wells fluoresce. Sometimes it is tricky to decide if what you are seeing is a positive. Use the comparator to help.
      4. Mark the number of wells with positive reaction on your datasheet.
      5. Calculate the Most Probable Number (MPN) using the IDEXX 51-Well Quanti-Tray MPN Table or the IDEXX MPN generator software found at: <https://www.idexx.com/en/water/resources/mpn-generator/>



1. **Lab Test Procedure for Surfactant Screening**
   1. All UV testing should be performed in a dark room for best results.
   2. Prepare two reference papers to provide a clear positive and negative reference for comparing to samples. Run one reference paper under tap water and apply a known surfactant with optical brighteners to the other (most laundry detergents work well). The positive reference should be confirmed to fluoresce (bright bluish glow) under UV light. Label papers as “Ref. Pos.” and “Ref. Neg.” or similar.
   3. Label a Post-It Note (or selected sampling paper) with the sample ID at the top of the paper.
   4. Note that samples that are dried fluoresce brighter than wet samples.
   5. Apply the sample to the paper and allow to dry for best results. Simply pouring some of the sample onto the paper is sufficient.
   6. Put on your safety glasses if you are using them and turn off the lights.
   7. Expose the sample paper and references to UV light.
   8. Determine if the sample is *positive*, *negative*, or *retest*.
      1. *Positive* – the sample will definitely fluoresce (glow) a bright bluish color and will resemble the positive laundry soap reference.
      2. *Negative* – the sample is dull with no glow and resembles the negative reference with tap water.
      3. *Retest* – may occur with some contamination and is not clearly positive or negative. Retest with the same sample or another sample may need to be collected.
   9. Turn on the lights, record the result, and dispose of the sample paper.
   10. Keep the positive and negative reference samples for future tests.
2. **Lab Test Prodecure for Total Nitrogen**

## Nitrate TNT 836 Test

## Carefully pipet 0.2 mL of water sample into vile from Nitrate TNT 836 test kit

## Carefully add 1.0 mL of Solution A to the vile (be sure to adequately clean the pipet or use a separate pipet)

## Close the vile

## Invert a few times until no more streaks can be seen in liquid

## Wait 15 minutes then thoroughly clean outside of vile with chem wipe

## Insert vile into cell holder of Hach spectrophotometer. Scan barcode on vile to select test, push READ. Record Nitrate reading.

## 

## Total Nitrogen TNT 826 Test

## Preheat the digester (DRB 200) to 120°C (248°F), select 30 minutes.

## Quickly add 1.3 mL of sample, 1.3 mL of solution A, and one tablet B to a reaction tube.

## Close tube immediately, do not invert.

## Place in the digester and heat for 30 minutes.

## Allow to cool to room temperature.

## Invert a few times.

## Carefully pipet 0.5 mL of digested sample into the test vial.

## Carefully add 0.2 mL of solution D.

## Invert a few times until no more streaks can be seen in liquid

## Wait 15 minutes then thoroughly clean outside of vile with chem wipe

## Insert vile into cell holder of Hach spectrophotometer. Scan barcode on vile to select test, push READ. Record TN reading.

## Total Kjedahl Nitrogen TNT 880 Test

## Preheat the digester (DRB 200) to 100°C (212°F), select 60 minutes.

## In quick succession, carefully add the following to reaction tube:

## 1.3 mL of water sample

## 1.3 mL of solution A

## 1 tablet B

## Immediately close reaction tube and do not invert

## Insert vial into the digester and heat vile for 60 minutes at 100°C

## Remove vial after step d) and wait for reaction tube to cool down to room temperature

## Add 1 MicroCap C

## Close reaction tube and invert a few times until all of the contents have been removed from MicroCap and no more streaks are seen

## Slowly pipet 0.5 mL of the digested sample into vile 1 (red label)

## Slowly pipet 0.2 mL of Solution D into vile 1 (red label)

## Immediately close vile 1 and invert a few times

## Slowly pipet 1.0 mL of undigested sample into vile 2 (green label)

## Slowly pipet 0.2 mL of solution D into vile 2 (green label)

## Immediately close vile 2 and invert a few times until no streaks are seen

## Set reaction timer to 15 minutes

## After 15 minutes thoroughly clean outside of vile 1 (red label) with chem wipe

## Insert vile 1 (red label) into cell holder of Hach spectrophotometer. Scan barcode on vile to select test, push READ. Record reading.

## Remove vile 1 (red label) from cell holder

## Thoroughly clean outside of vile 2 (green label) with chem wipe

## Insert vile 2 (green label) into cell holder of Hach spectrophotometer. Scan barcode on vile to select test, push READ.

## Record readings. Report the value of Total Nitrogen (TN) for the IDDE screening.

## *Note: If your spectrophotometer does not have the correct program after reading the barcode, you may update the machine’s software via a USB drive and an update from the Hach website. Do a web search for “Hach DR XXXX software update,” download the file for your instrument then read and follow the instructions.*

**Threshold conditions:**

The following are thresholds generally related to common water quality limits. Fecal Indicator Bacteria results that are above threshold conditions will require further catchment investigation. See catchment investigation procedure in the IDDE plan.

|  |  |  |  |
| --- | --- | --- | --- |
| **Test** | **EPA Benchmark** | **Concentration Levels Indicating Need for Further Investigation** | **Remarks** |
| *E.coli* | > 235 E. coli/100 mL | >4000 *E. coli*/100 mL | Undiluted wastewater will generally have *E. coli* levels an order of magnitude or more, higher than the EPA benchmark. Pet waste, wildlife sources and regrowth of bacteria in storm drains have been shown to contribute to elevated *E. coli* levels above the benchmark. |
| Ammonia | > 0.5 mg/L | >0.5 mg/L | In the absence of other wastewater indicators, follow-up investigation is performed when the ammonia concentration is 0.5 mg/L or higher. If other wastewater indicators are present, then a 0.25 mg/L benchmark is used. Decomposing vegetation under anoxic conditions can release ammonia to water, which can be misleading. |
| Surfactants | > 0.25 mg/L | Presence | Detection of low concentrations (0.1- 0.3 mg/L) of surfactants is common at stormwater outfalls. Most detections are not correlated with other wastewater indicators and do not lead to a definite source. These detections may be attributable to outdoor vehicle or building washing. |
| Total Chlorine | > Reporting Limit | >0.50 mg/L | The field test used for total chlorine analysis is sufficiently sensitive to detect municipal potable water sources diluted by groundwater or runoff approximately 3 to 10 fold, depending on the strength of the potable chlorine residual and type of chlorination used. Total chlorine is a decent indicator of treated drinking water leaks and potentially graywater sources, but may also be permitted non-stormwater discharges. If high levels are consistently identified in a sample without other wastewater indicators, such as bacteria or ammonia, then discussions with water utility should precede comprehensive investigation of drainage area. |
| Specific Conductance | N/A | >600 μS/cm | Specific conductance alone is not a reliable indicator of wastewater contamination. Road salt and metals from pipe corrosion often result in levels in the 1,000-5,000 μS/cm range. However, flows contaminated with wastewater generally have specific conductance above 600 μS/cm. Very high level (>5,000 μS/cm) may indicate an industrial illicit connection. |
| Total Phosphorus | N/A | >0.3 mg/l | Phosphorus alone is not a reliable indicator of wastewater sources. High levels of phosphorus may be present in stormwater discharges due to erosion in the drainage area or other natural sources. Treated drinking water may also be high in phosphorus to meet anti-corrosion requirements in drinking water distribution systems and may be identified during dry weather sampling if a water line flushing activity or other drinking water discharge is present in the storm drain system. |
| Total Nitrogen | N/A |  | Naturally occurring levels of nitrate and total nitrogen vary substantially across the country, and statistical analyses of water quality data suggest that appropriate reference levels range from 0.12 to 2.2 mg/L total N. |

**USER MANUALS**

**Ammonia, Chlorine, Conductivity, Salinity, Temperature**

YSI Pro Plus: <https://www.ysi.com/File%20Library/Documents/Manuals/605596-YSI-ProPlus-User-Manual-RevD.pdf>

***E. coli/ Enterococci***

Quanti-Tray Sealer: <https://123.idexx.com/resource-library/water/quanti-tray-sealer-plusmanual-en.pdf>

Colilert: <https://www.idexx.com/files/colilert-procedure-en.pdf>

Enterolert: <https://www.idexx.com/files/enterolert-procedure-en.pdf>

***Total Nitrogen***

Hach Nitrate TNT 836: <https://www.hach.com/asset-get.download.jsa?id=19556239158>

Hach Total Kjeldahl Nitrogen (TKN) TNT 880: <https://www.hach.com/asset-get.download.jsa?id=19556239178>

**TABLES AND SOFTWARE**

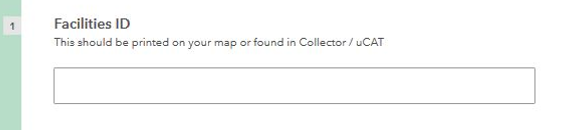
IDEXX 51-Well MPN table: <https://www.idexx.com/files/qt51mpntable.pdf>

IDEXX MPN generator software: <https://www.idexx.com/en/water/resources/mpn-generator/>

Appendix: Survey 123 questions

MS4 2020 Dry Weather Inspections

1. Facilities ID



1. Reason for Inspection
   1. Initial Inspection
   2. Follow-up Inspection
   3. Dry Run
   4. Other



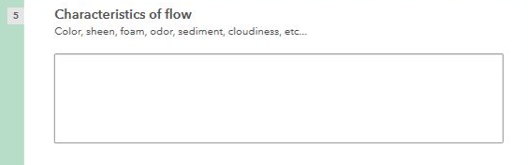
1. Is Outfall Accessible?
   1. Yes
   2. No



1. Dry Weather Flow?
   1. No – No signs of dry weather flow
   2. No – Signs of dry weather flow
   3. Yes – Is flowing
   4. Other



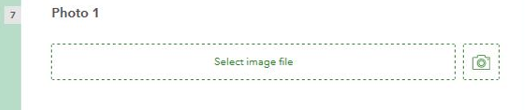
1. Characteristics of Flow
   1. Example – color sheen, foam, odor, sediment, cloudiness, etc.



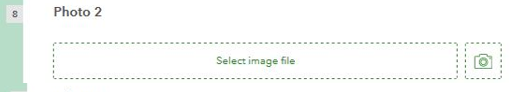
1. Characteristics of outfall
   1. Example – physical condition



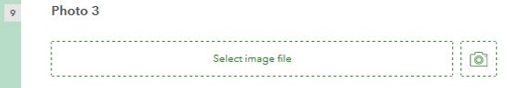
1. Photo 1



1. Photo 2



1. Photo 3



1. Photo 4



1. Photo 5



1. Notes



1. Date/Time



1. Inspector Name



1. Map Location

