

# **NEW INNOVATIONS FOR CHANGING REGULATIONS**

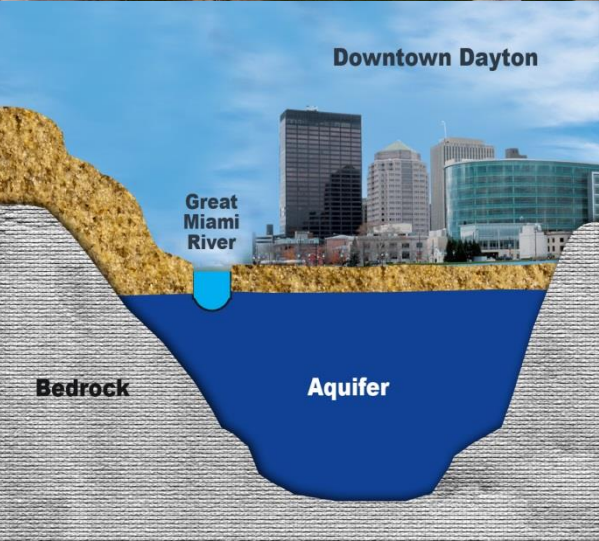
**RACHEL EASTER**

**WATER BACTERIOLOGIST CHEMIST**

**DIVISION OF WATER SUPPLY & TREATMENT**

**CITY OF DAYTON**





# BACKGROUND

- **The City of Dayton Water Department serves 400,000 people in the City of Dayton and surrounding Montgomery County.**
- **Pump 60-80 MGD.**
- **Two water treatment plants- both lime softening.**
- **Ground water under the influence of surface water.**
- **Lime Reclamation Facility**

# OVERVIEW

## Regulation, Laboratory Procedures and Innovation surrounding:

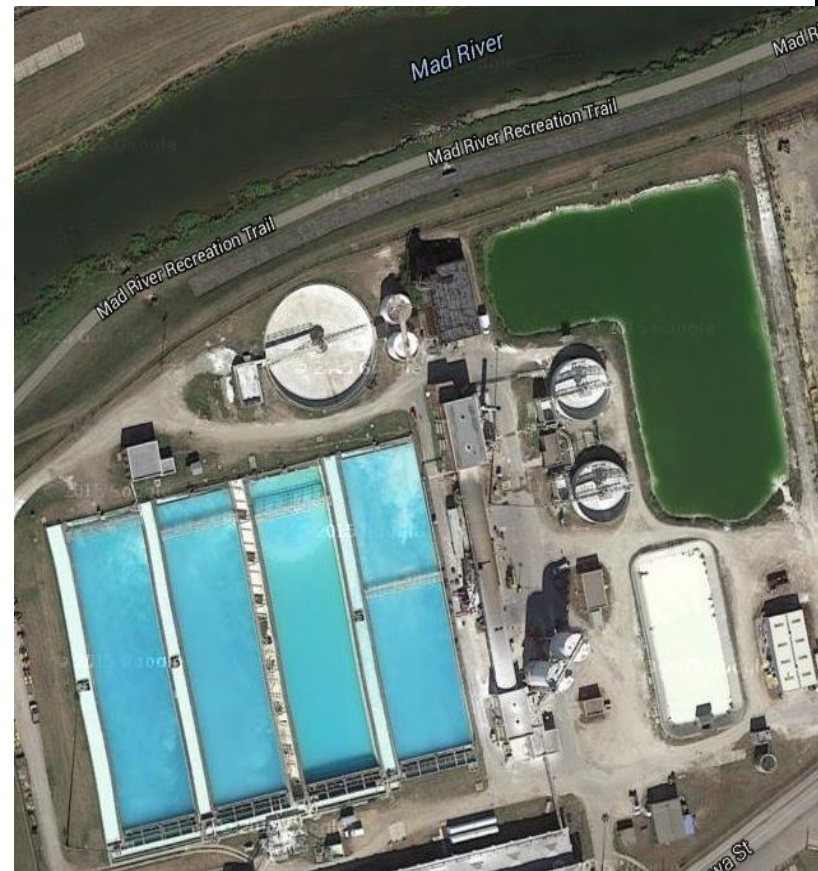
- National Pollutant Discharge Elimination System (NPDES)
- Heterotrophic Plate Count (HPC)
- Revised Total Coliform Rule (RTCR) and Long Term 2 Enhanced Surface Water Treatment Rule (LT2)
- Harmful Algal Blooms (HAB)

# **NPDES**

**NATIONAL POLLUTANT DISCHARGE ELIMINATION  
SYSTEM**

# NPDES REGULATION

- **National Pollutant Discharge Elimination System is a set of effluent limitations for discharging pollutants to natural waterways.<sup>2</sup>**
- **Permit in place to ensure safety of the wildlife where the water is being discharged.**
- **The City of Dayton has an NPDES permit to discharge from the L-shaped Lagoon to the Mad River.**





# NPDES REGULATION (CONT.)

- **Per City of Dayton's permit, the amount of Total Chlorine and Total Solids discharged to the river is strictly limited.**
- **The amount of Chlorine discharged to the river cannot exceed 0.038 mg/L.**
- **The amount of total solids discharged to the river cannot exceed 45 mg/L at any point and cannot average more than 30 mg/L solids.**



# NPDES LAB PROCEDURE

- **USEPA Standard Method 4500-Cl G for drinking water analysis uses DPD powder pillows and a colorimeter to quantify chlorine.**
- **This method can measure Cl from 0.02-2.00 mg/L.**
- **This process takes approximately three minutes.**



# NPDES LAB PROCEDURE (CONT.)

- A 10mL portion of sample is added to a the vial.
- The vial is placed in the colorimeter and blanked.
- Add the total chlorine DPD packet to the sample.
- Wait three minutes, the sample will turn from clear to pink.
- Read the sample in the colorimeter.
- The stronger the pink color, the greater the chlorine
- The colorimeter will give a Cl value, in mg/L to two decimal places.





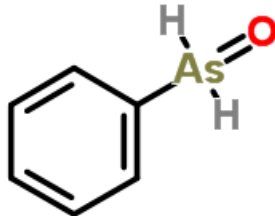
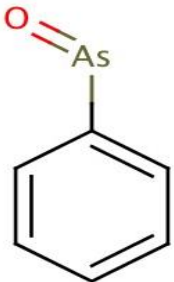
# NPDES INNOVATION

- For the purposes of testing the CI from the L-shaped lagoon, for the NPDES permit, the City of Dayton has moved away from the DPD method to an amperometric titration method.
- The Hach TitraLab AT1000 uses a forward titration to detect a CI range of 0.003-5.00 mg/L.



# NPDES INNOVATION (CONT.)

- Based on Standard Method 4500-Cl D, the Titralab AT1000 uses potassium iodide, and a pH buffer to titrate a chlorine sample with phenylarsine oxide (PAO).
- The end point is determined by a Pt-Pt Electrode
- The chemical reaction at work is:



# NPDES INNOVATION PROCEDURE

- **Titralab AT1000 for Total Chlorine Analysis:**
  - Measure out 200 mL of samples
  - Insert probes and tubing into sample
  - Add potassium iodide powder
  - Wait for results



# NPDES OLD PROCEDURE VS INNOVATION

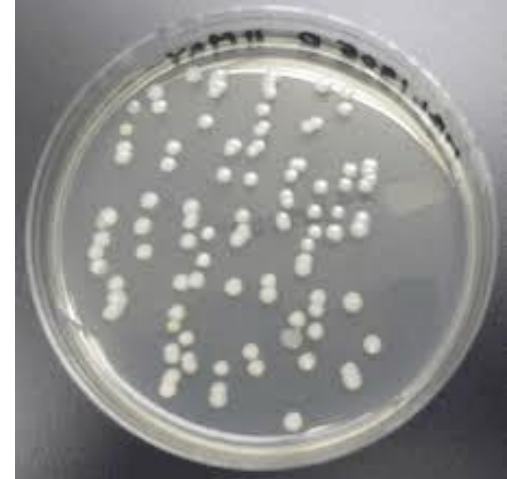
- **Hach Colometric DPD method has a magnesium interference.**
- **The L-shaped lagoon contains magnesium from lime residuals.**
- **Amperometric titration method can account for magnesium interference.**



# HPC

**HETEROTROPHIC PLATE COUNT**

# HPC REGULATION

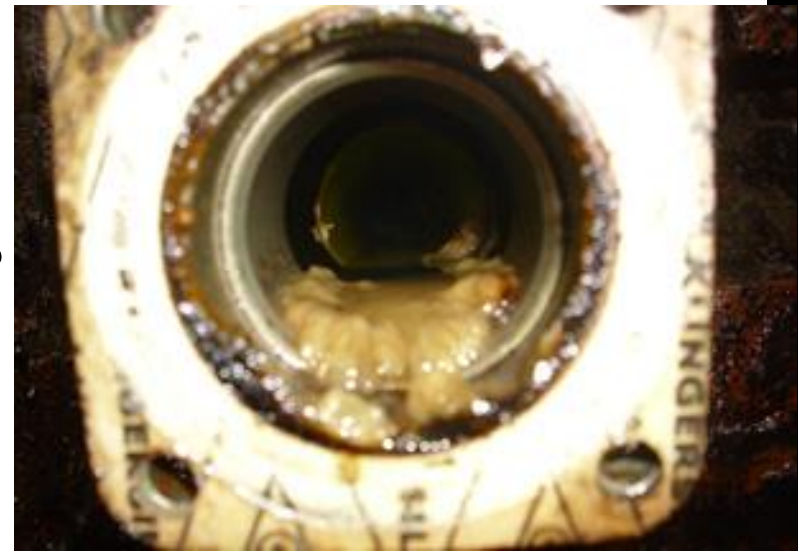


- **HPC is standard analytical procedure to measure a of variety of bacteria commonly found in water.**
- **These bacteria are naturally present in the environment.**
- **HPC has no MCL. They are merely an indicator of the maintenance of the water system.<sup>1</sup>**



# HPC REGULATION (CONT.)

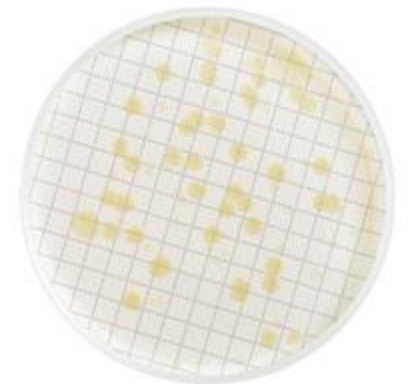
- A high heterotrophic plate counts indicates biofilm build up in the distribution system.
- Although no MCL exists, treatment techniques should aim to maintain HPC below 500 CFU/mL.<sup>5</sup>
- For water utilities, the finished water should contain less than 10 CFU/mL.<sup>5</sup>
- There are various methods that can be used to measure HPCs.



# HPC LAB PROCEDURE

## MEMBRANE FILTRATION METHOD

- According to standard method 9215B, HPCs are determined via membrane filtration method.
- A 100mL sample is filtered, plated on R2A agar and incubated for 48 hours.
- After 48 hours, the colonies are counted.
- The results are reported as colonies per 100mL.
- Some samples must be diluted as to not have a result of “too numerous to count” (aka  $\geq 200$  colonies per plate).



# HPC LAB PROCEDURE

## IDEXX METHOD

- IDEXX provides a **SimPlate** method for Heterotrophic plate counts, this is based on the same principles as standard method 9215B.
- **SimPlate** uses a multiple enzyme technology medium.<sup>3</sup>



# HPC LAB PROCEDURE

## IDEXX METHOD (CONT.)

- This method works on the premise that waterborne bacteria will metabolize an enzyme substrate and produce a specific (fluorescent) signal.
- The plate is filled with sample and incubated for 48 hours.
- The number of wells that fluoresce convert to a most probable number.<sup>3</sup>





# HPC INNOVATION LUMINULTRA

- **The Luminultra method utilizes a photometer to measure the light produced from ATP contained in bacteria.<sup>6</sup>**
- **For drinking water, the intra-cellular ATP of living biological cells are measured to obtain a microbial count equivalent.<sup>6</sup>**

# HPC INNOVATION LUMINULTRA (CONT.)

- Results given in minutes.
- Portable set up.
- Reports results for all bacteria present.
- Long shelf life for consumables.
- Complex procedure.





# HPC LUMINULTRA SOP

- **Calibrate Luminometer.**
- **Mix sample for homogeneity.**
- **Remove plunger from 60 mL syringe, and attach filter.**
- **Pour into plunger sample**
- **Push sample volume through filter and into waste a rate of 3-5mL per second.**
- **Stop before air is pushed through filter, ensure filter remains wet.**



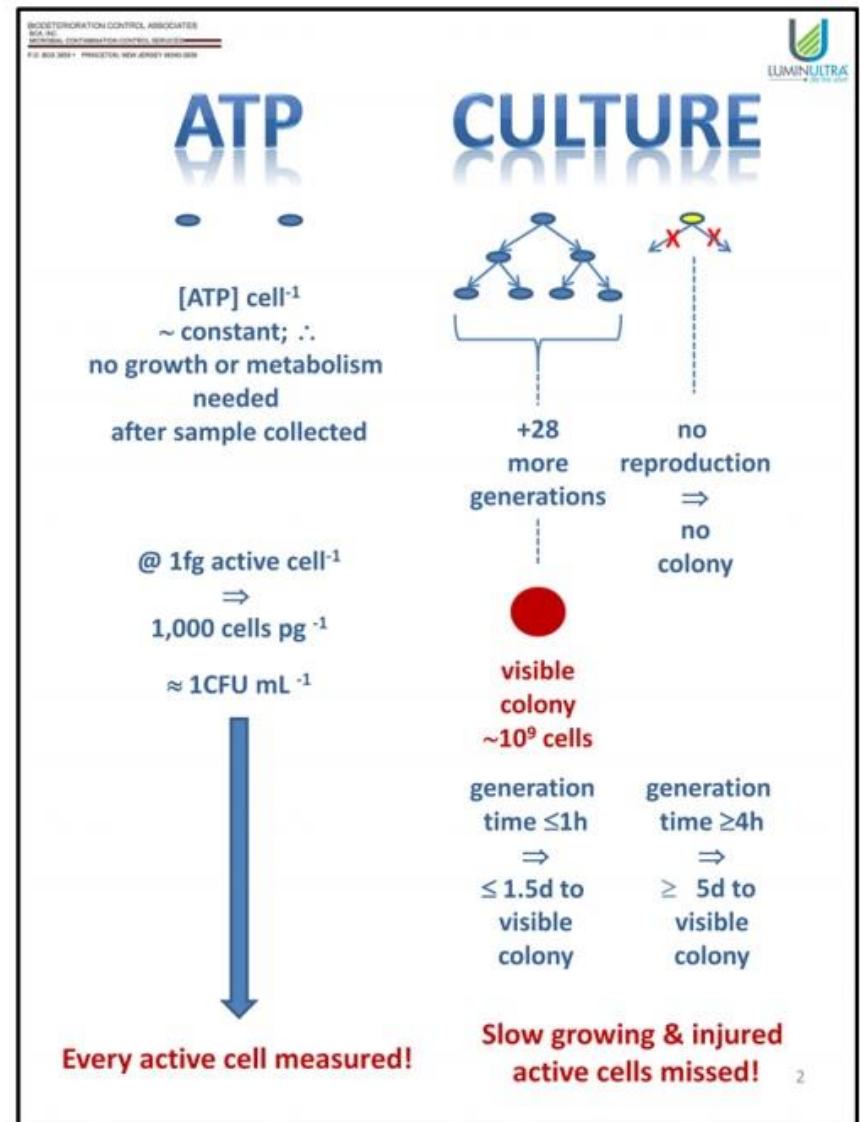
# HPC LUMINULTRA SOP (CONT.)

- Detach filter, and remove plunger.
- Re-attach filter and add 1mL of UltraLyse 7.
- Push Ultralyse entirely through filter, to dry, into an UltraLute dilution tube.
- Cap and invert 3x.
- Pipette 100 $\mu$ L of that into an assay tube and add 100 $\mu$ L Luminase.
- Insert assay tube and results are given instantly.



# HPC PROCEDURE VS INNOVATION

- Membrane filter method: sensitive, time consuming, inexpensive
- IDEXX method: unreliable, time consuming, moderate expense
- Luminultra method: portable, less time, expensive, extensive procedure

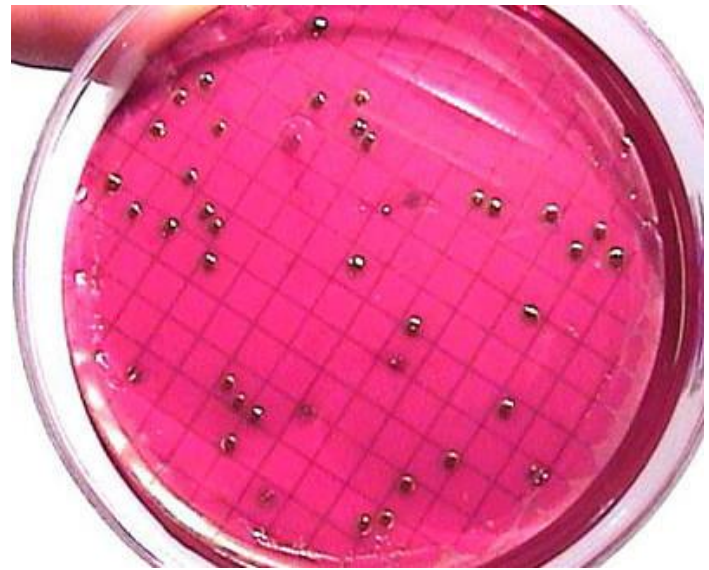


# **LT2**

**LONG TERM 2 ENHANCED WATER  
TREATMENT RULE**

# REGULATION LT2 RULE

- **The LT2 rule builds upon the Safe Drinking Water Act.**
- **Rule was added to monitor *Cryptosporidium*.<sup>9</sup>**
- **Crypto is resistant to disinfection.**



# PROCEDURE LT2 RULE: MONTHLY COLLECTION

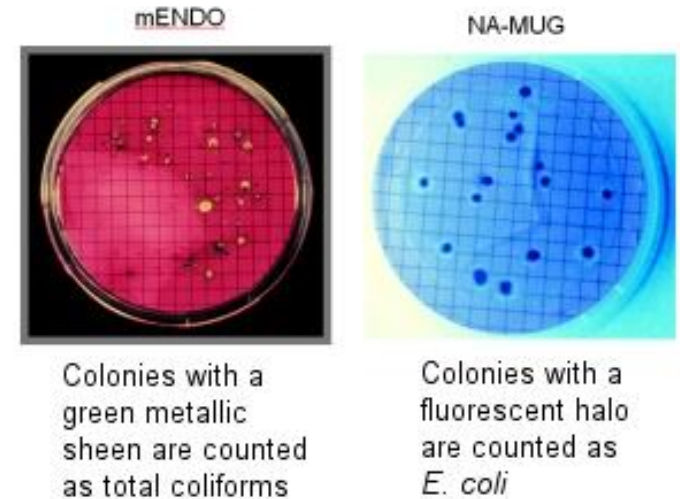
- **Samples are collected at raw water inlet.**
- **Line is flushed for 2-3 min.**
- **Envirochek filter is connected to apparatus.**
- **At least 10 L of water is filtered through.**
- **Both ends of filter are stoppered, enclosing the remaining amount of water inside.**





# INNOVATION LT2 RULE: TESTING

- A raw water bacteria sample is collected
- Sample is analyzed via membrane filter method on mENDO agar and incubated for 22-24 hours.
- Colonies are counted.
- Filter is then re-plated on to nutrient agar with MUG for four hours.
- Colonies that fluoresce are counted as E. Coli.



# PROCEDURE LT2 ANNUAL COLLECTION & TESTING

## Matrix Spike:

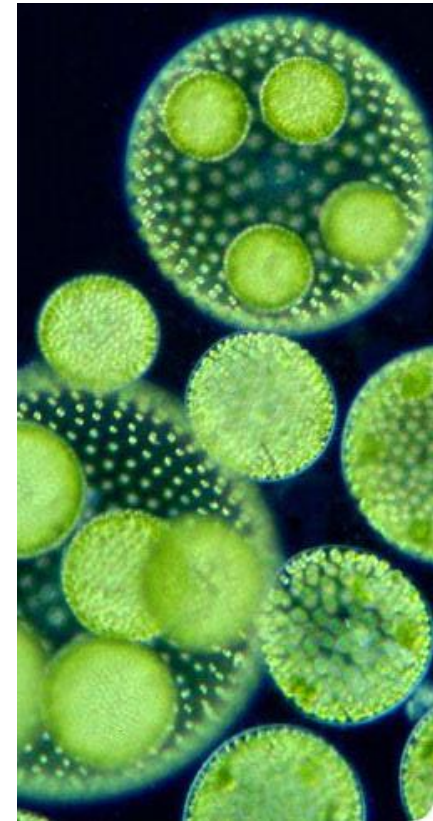
A 10 L bulk sample of raw water is collected in a cubitainer and sent to a Crypto lab for a matrix spike analysis. This is collected once, every twenty samples.<sup>10</sup>



# PROCEDURE LT2 ANNUAL TESTING (CONT.)

## Microscopic Particulate Analysis:

100 L of water sent through an MPA filter over 12-24 hours. This is sent out for analysis, for: particulate debris, protozoans, algae and other organisms.<sup>11</sup>

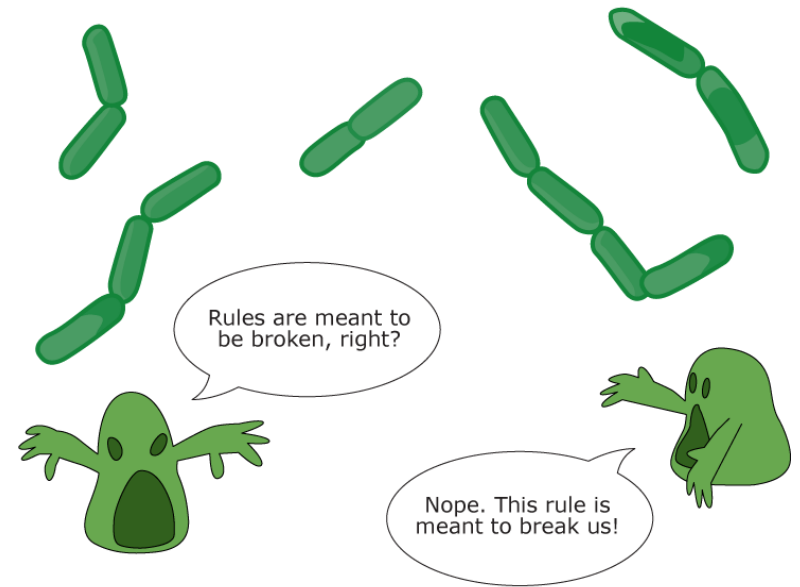


# **RTCR**

**REVISED TOTAL COLIFORM RULE**

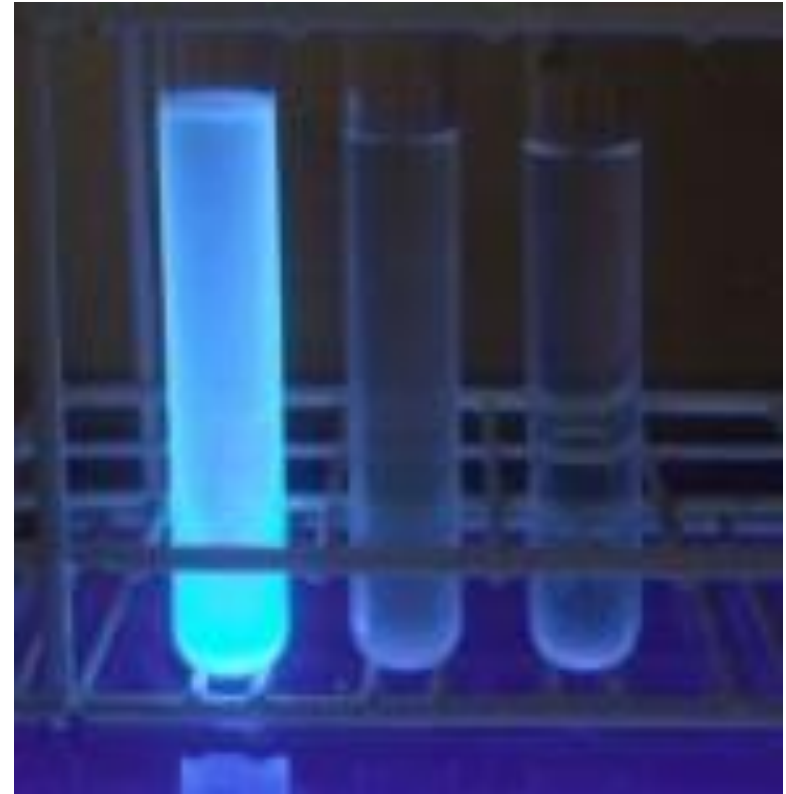
# REVISED TOTAL COLIFORM RULE REGULATION

- Final revisions made 2/2014.
- In effect 4/2016.
- Set MCL for E. Coli.
- Eliminated Fecal Coliforms.
- Changed monitoring and public notification requirements.



# RTCR INNOVATION

- **Eliminated Fecal Coliform Pos verification in favor of E.Coli verification.**
- **Made switch from EC media to EC with MUG.**



# **HAB**

**HARMFUL ALGAL BLOOM**



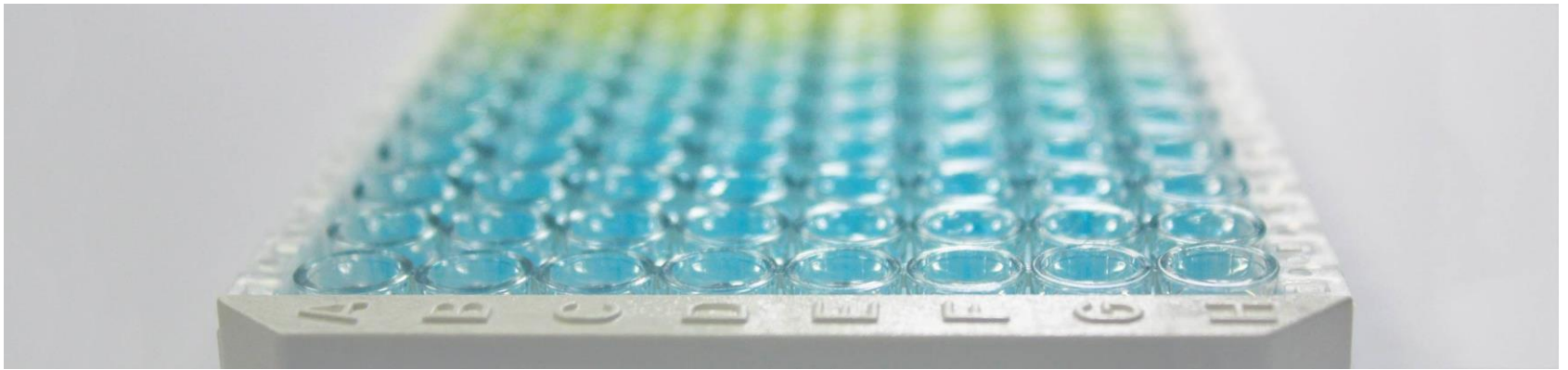
# HAB REGULATION

- **Harmful algal blooms are large growths of cyanobacteria.**
- **Cyanotoxins can cause illness and even death.<sup>4</sup>**
- **The USEPA has implemented health advisory levels for the cyanotoxins- microcystins and cylindrospermopsin.<sup>4</sup>**
- **Monitoring is required only for public water systems sourcing surface water or groundwater under the influence of surface water.**



# HAB INNOVATION

- **Harmful Algal Blooms are tested via the ELISA method, innovated by ABRAXIS.**
- **ELISA is enzyme-linked immunosorbent assay.**
- **ELISA is an immunoassay detection method for microcystins in water.**<sup>12</sup>
- **Specific antibodies compete with proteins on the plate for binding sites.**<sup>12</sup>
- **When combined with a color substrate, a blue color is given off and the intensity is read at 450 nm.**<sup>12</sup>



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## 3. SimPlate HPC

- <https://www.idexx.com/water/products/simpla.html>

## 4. HAB Rules Factsheet

- <http://epa.ohio.gov/Portals/28/documents/habs/HAB%20rules%20factsheet%20March%202016.pdf>

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## 8. Revised Total Coliform Rule and Total Coliform Rule

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## 10. LT2 Sample Collection Pocket Guide

- <http://nepis.epa.gov/Exe/ZyPDF.cgi?Dockkey=2000ZZBU.txt>

## 11. MPA for Filtration Plant Optimization

- EPA 910-R-96-001 (1996)

## 12. Ohio EPA Total Microcystins- ADDA by ELISA

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# Questions



**THANK YOU**