


Temperature Control Sampling Tips

- You make three temperature readings from two sensors
 - pH & conductivity probes share a temperature sensor
 - dissolved oxygen probes has its own sensor
 - All three readings should agree within 0.5 C
 - **Troubleshooting tips**
- Drift
 - Last two measurements should be no more than 0.1 C different
 - **Troubleshooting tips**
- Gas exchange between your sample and the atmosphere
 - Prevent this by acclimating your sensors first
 - Collect sample water last
 - Transfer in your sensors ASAP and then read!
 - **Troubleshooting tips**





Updated *E. coli* measurement technique for Volunteer Water Quality Monitoring

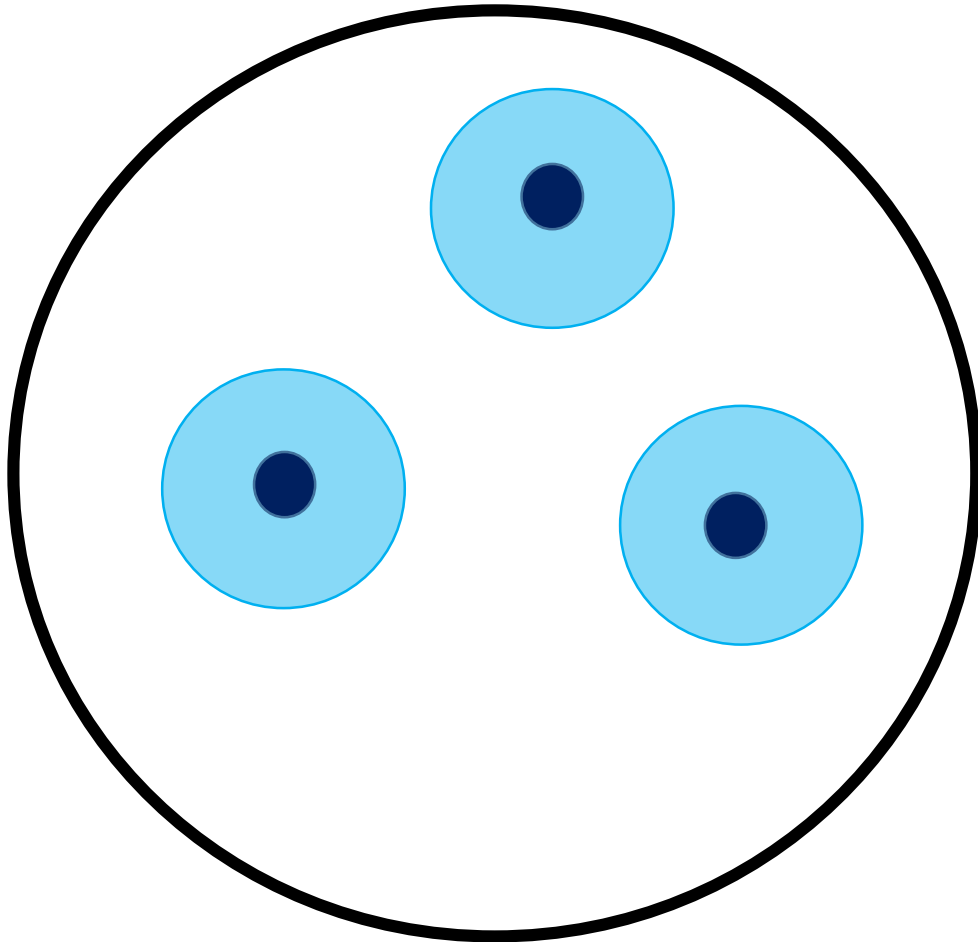
Kelly Hall

Albert Taylor

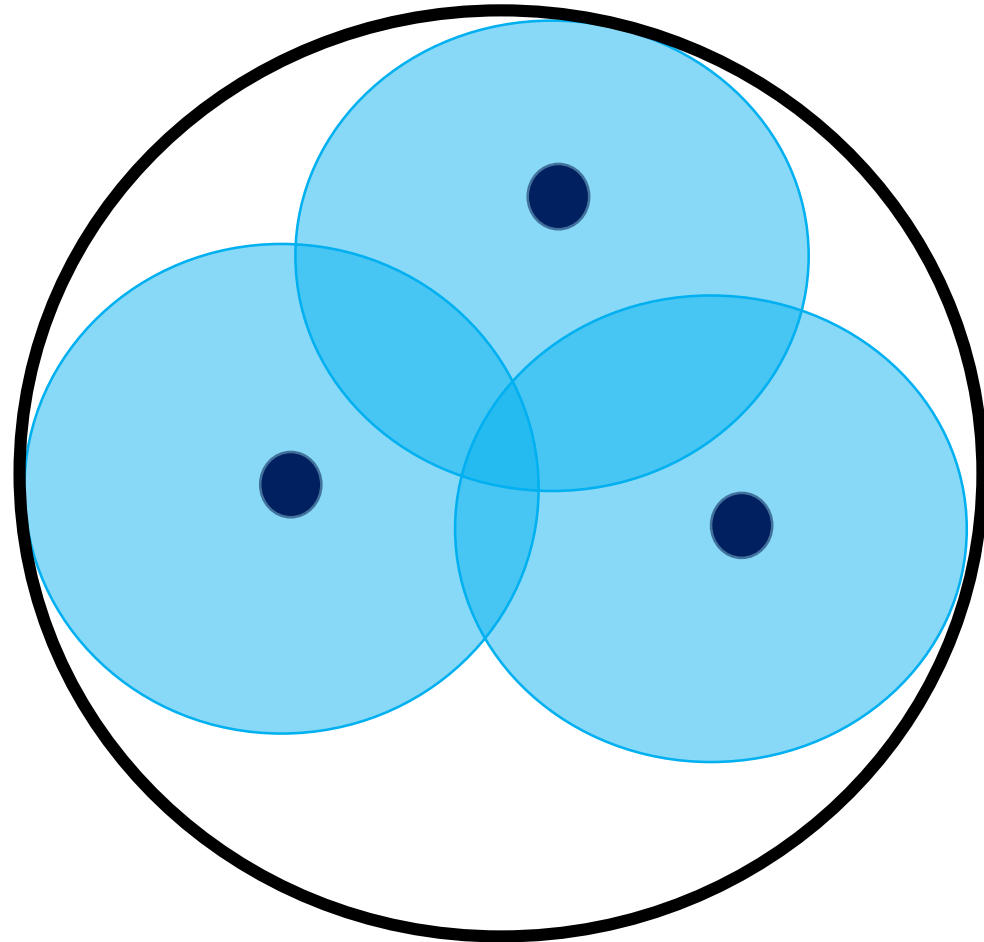
Cara Schildtknecht

Incubation Time

- Why 18 to 20 hr?
- How to accommodate 18 to 20 hr?



18 – 20 hrs

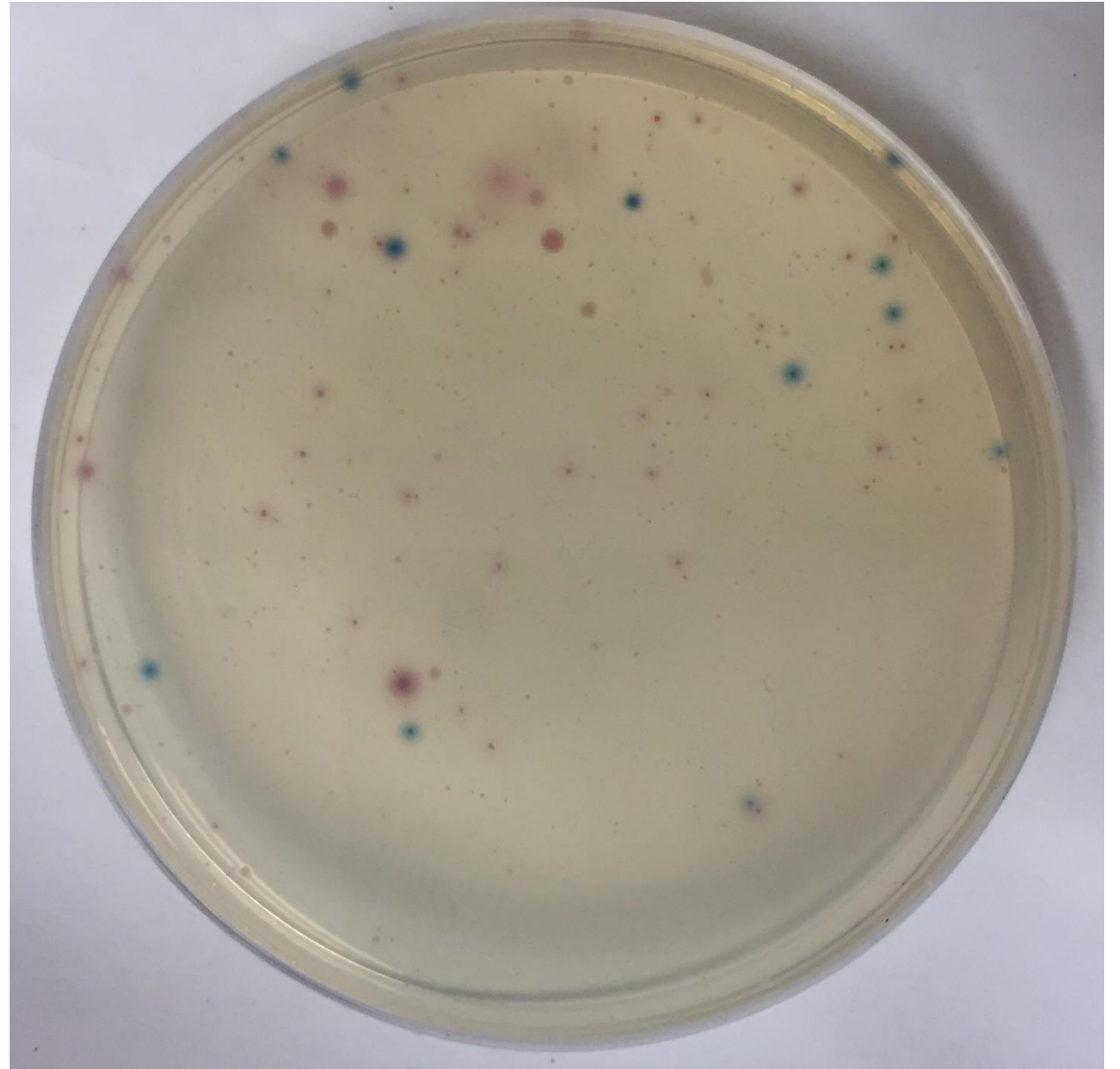


24 hrs

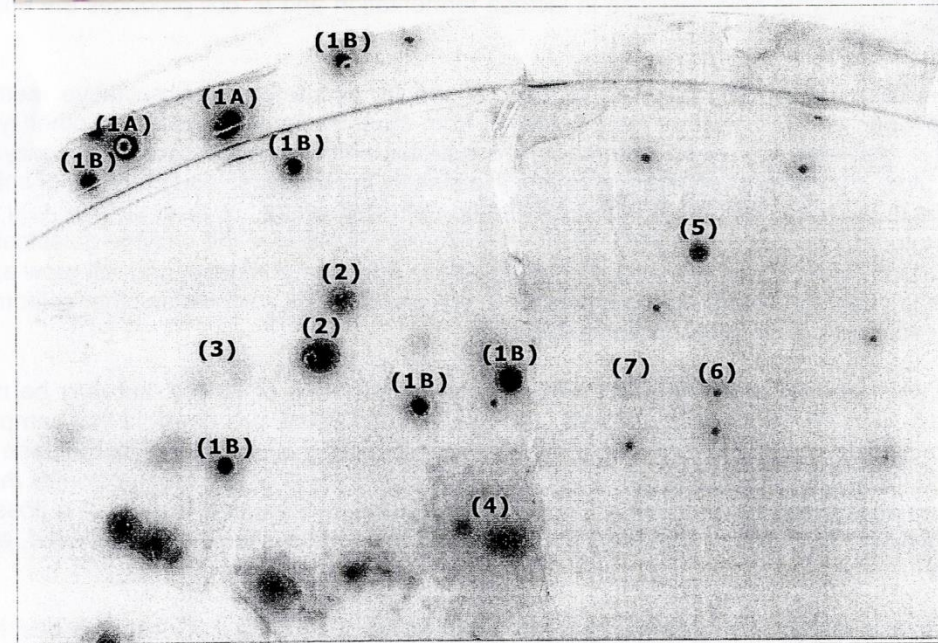
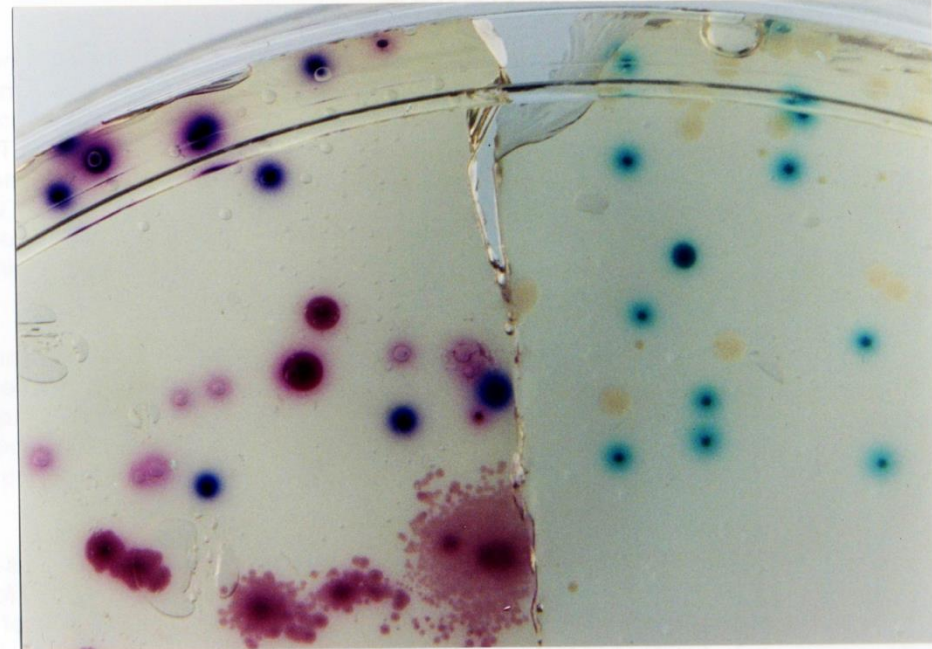
Visible Light

Count only pink with distinct pinpoint center.

Count blue colonies identified by Microbiology as *E. coli* colonies.



- This was the only guidance we got on how to determine what was an “acceptable” blue
- So we adopted a confirmation step using fluorescence

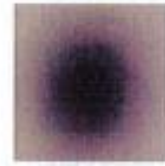


Identification Guide

This would have
been helpful ...

but Micrology just
sent this to us last
month! It was
compiled by the VA
volunteer monitors

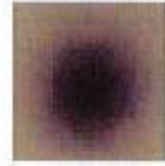
E. coli



Purple, with purple halo



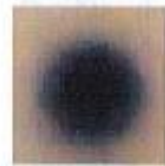
Purple, no halo



Purple with pink halo



Blue with purple or pink halo



Blue or dark blue, no halo



Dark blue with teal halo

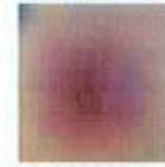


Dark blue with blue halo

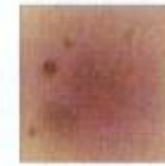
Not *E. coli*



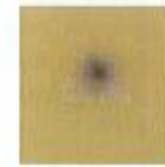
White



Pink, no halo



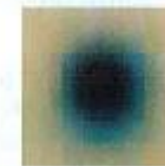
Pink with pink halo



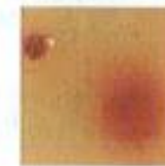
Pinpoints*
(If after incubation period)



Teal green, no halo



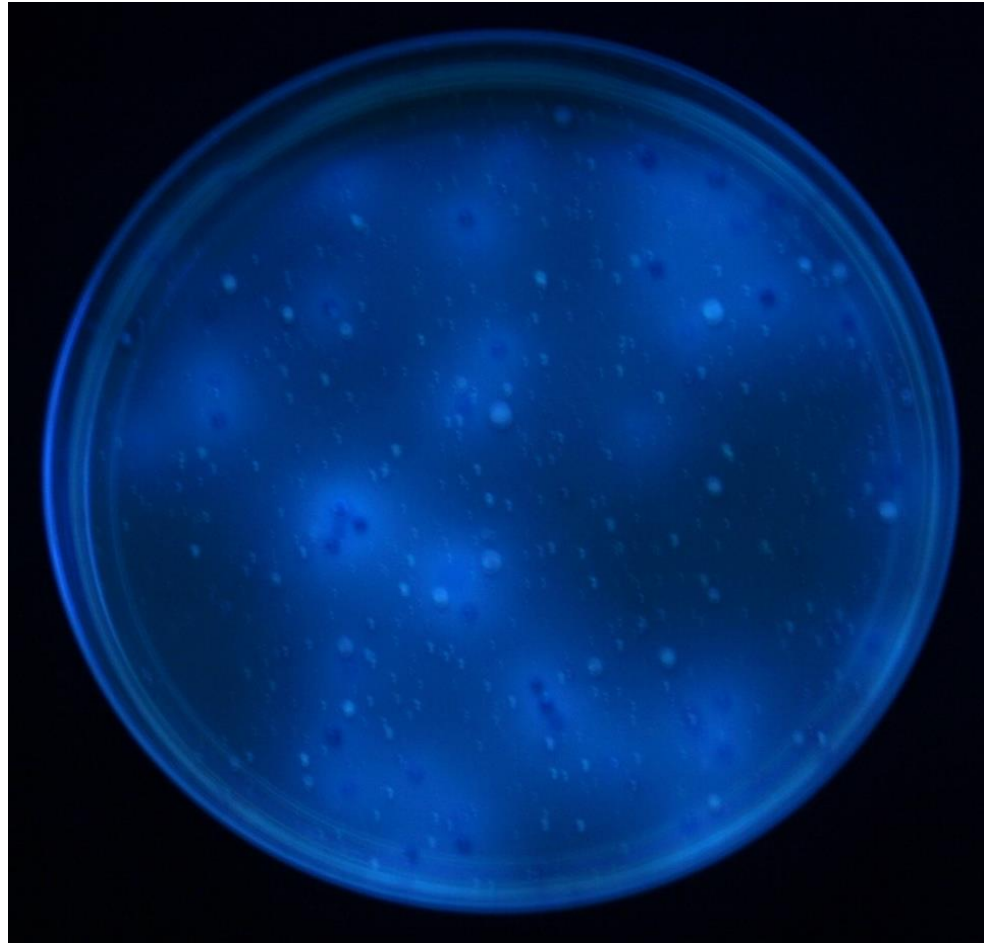
Teal with teal halo



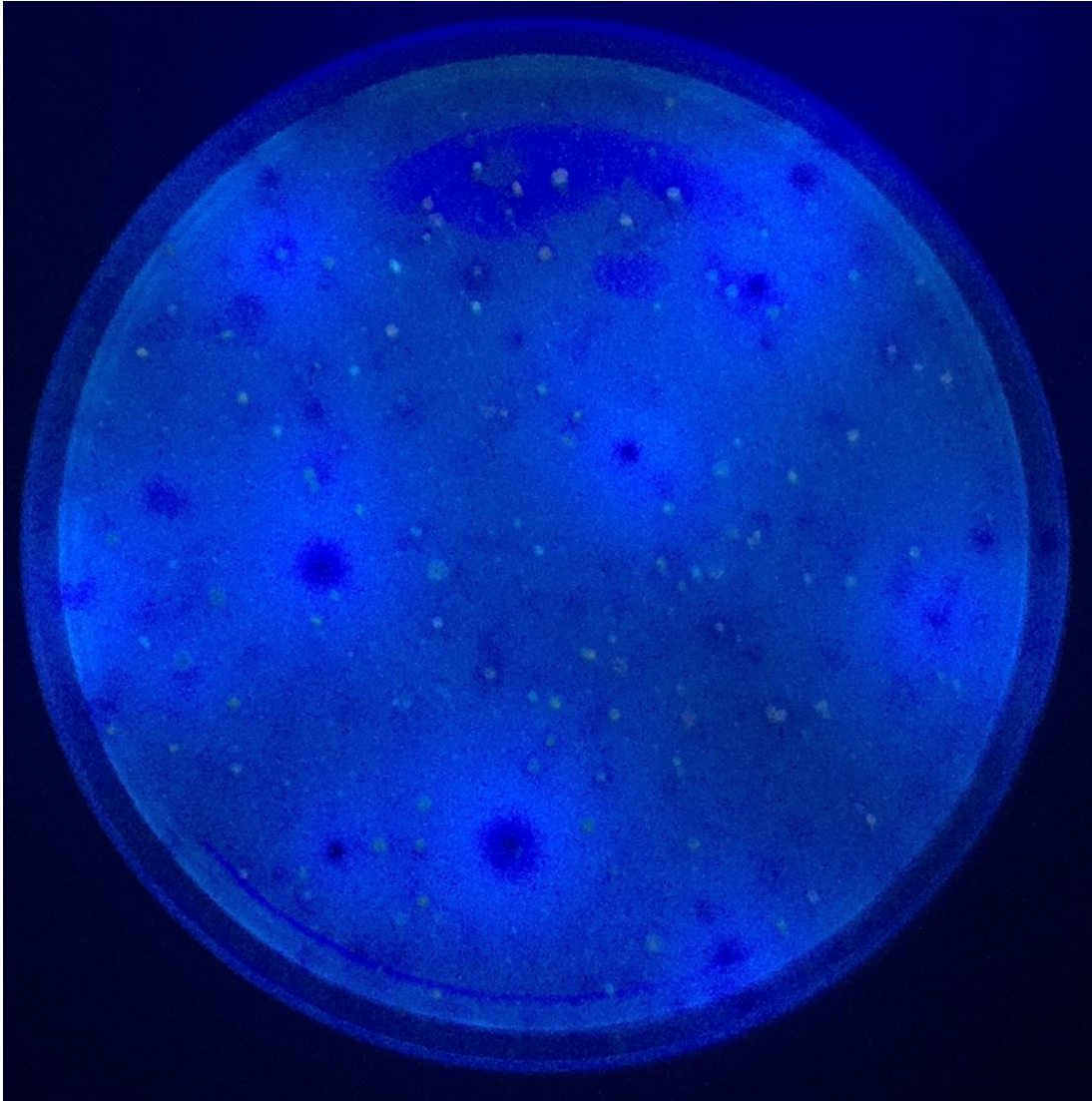
Red

Dual confirmation

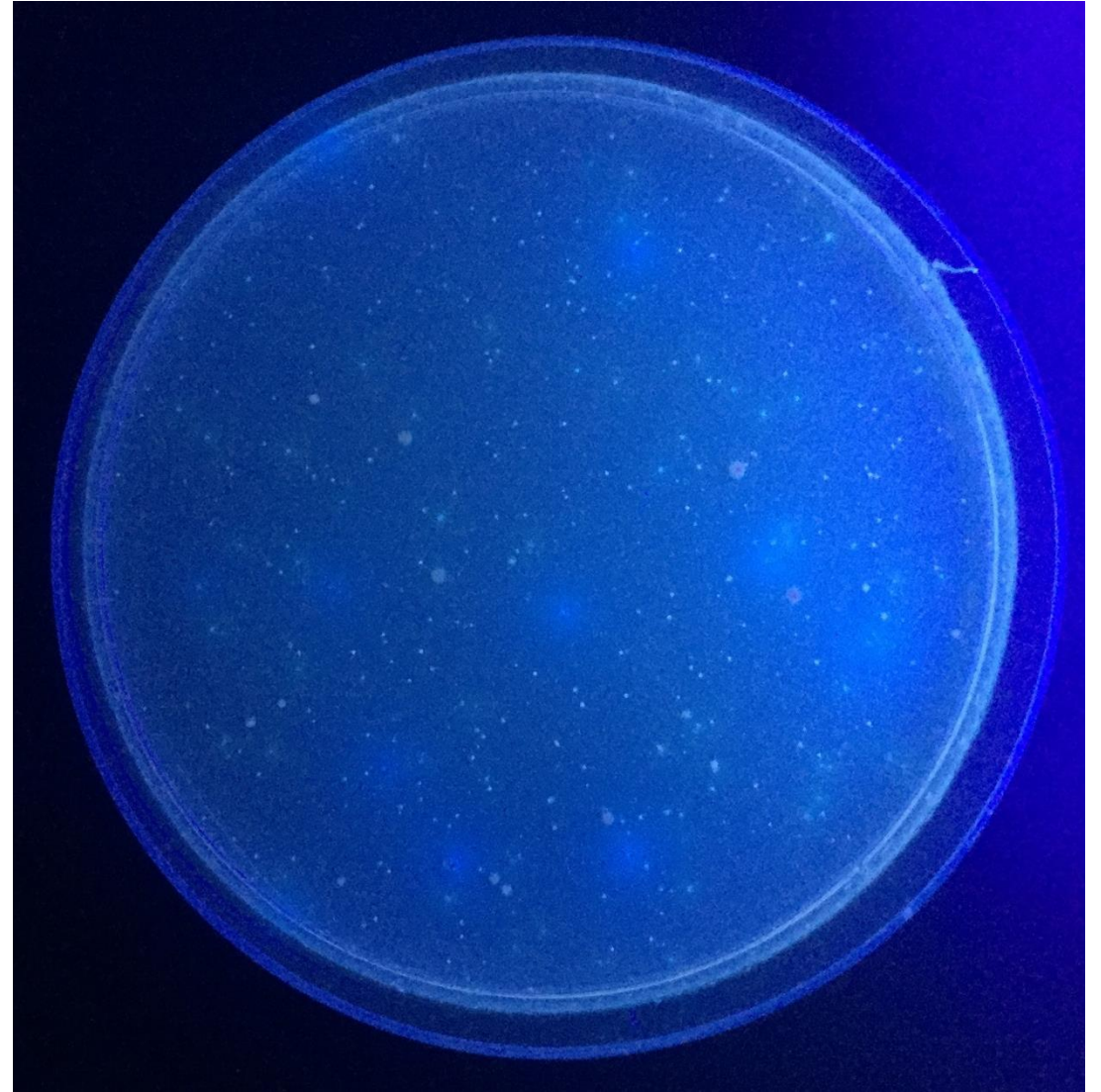
Count only colonies
with blue fluorescing
halos



See the Difference?

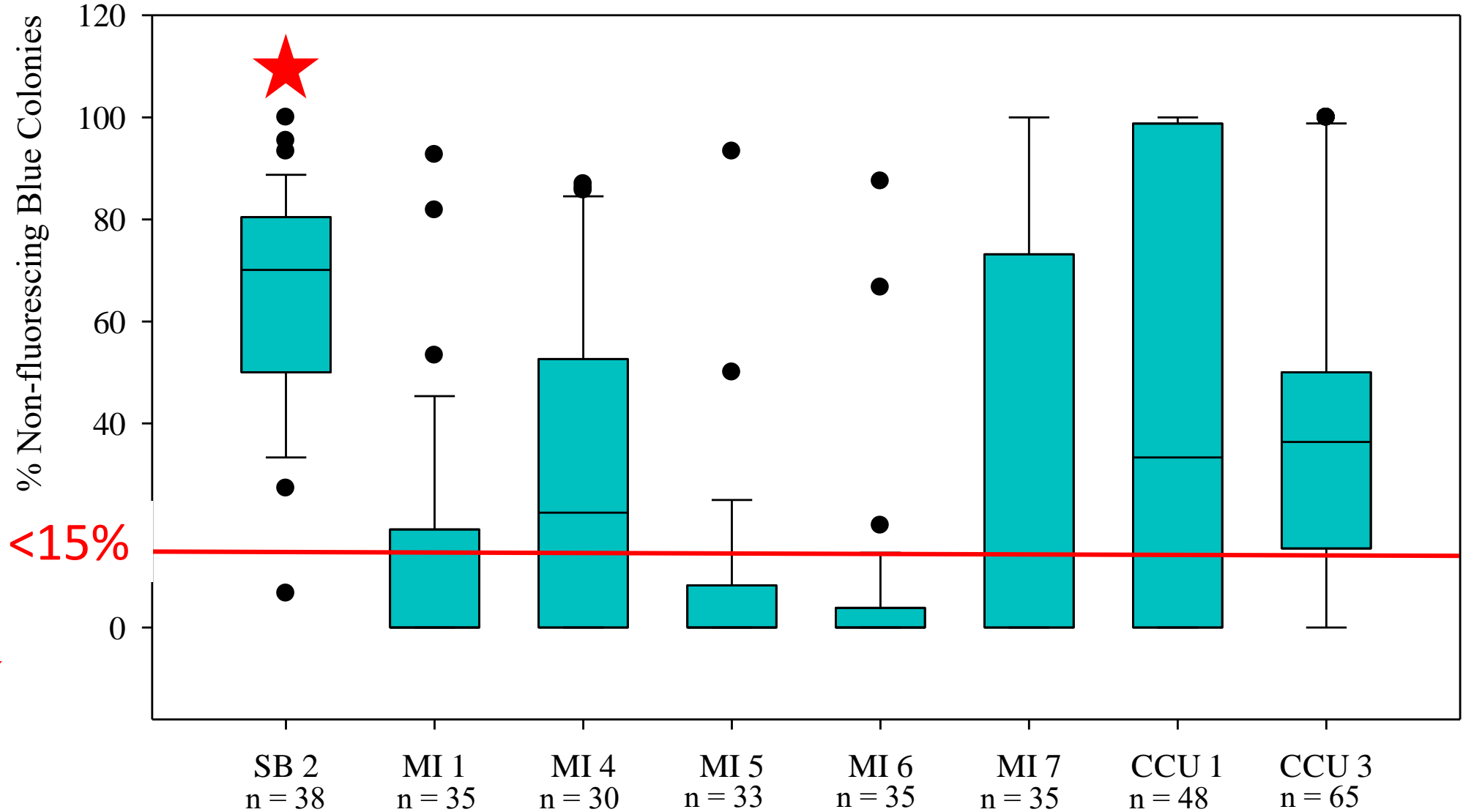


Spectrocorp
EQL Light

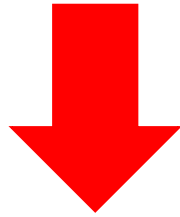


Spectroline
VM light

% Non-fluorescing Blue Colonies for Volunteer Water Quality Monitoring
Sites with *E. coli* concentrations consistently >100 CFU/100mL
(January 2015 – December 2016)



Should be <15%

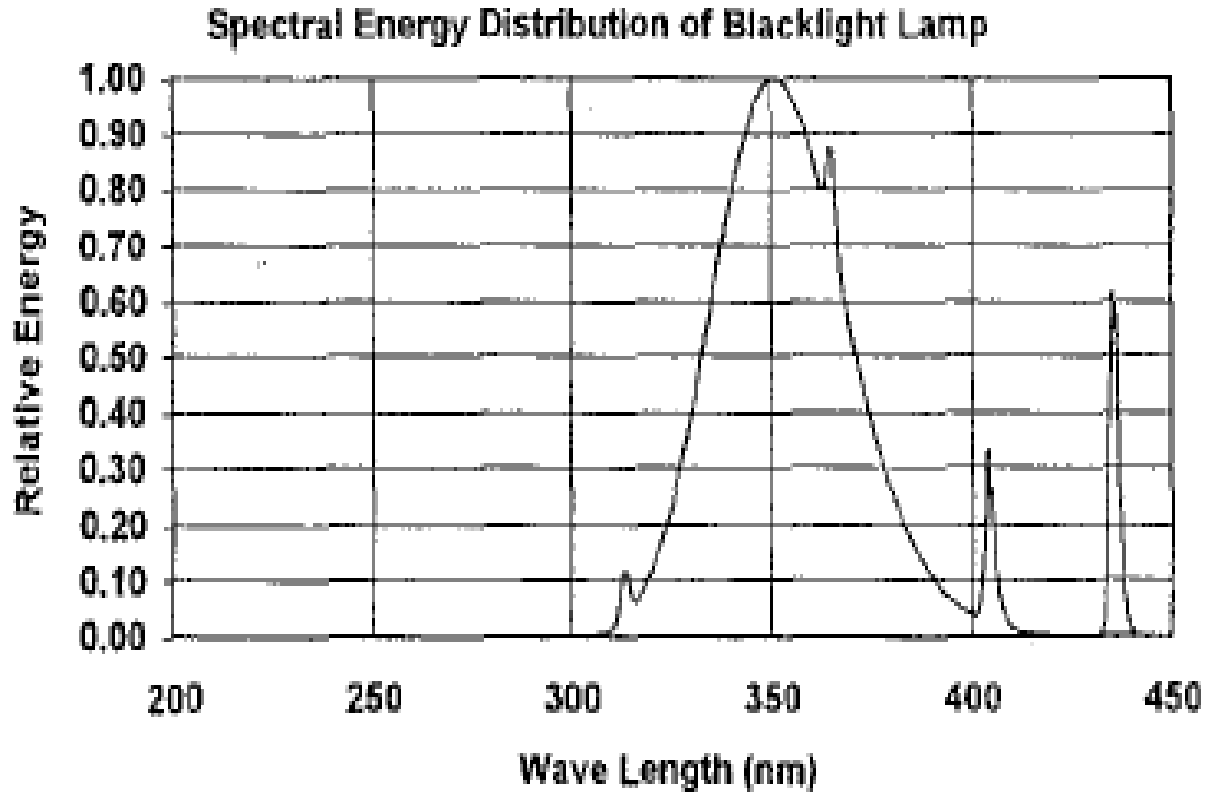


Shining some light on the problem

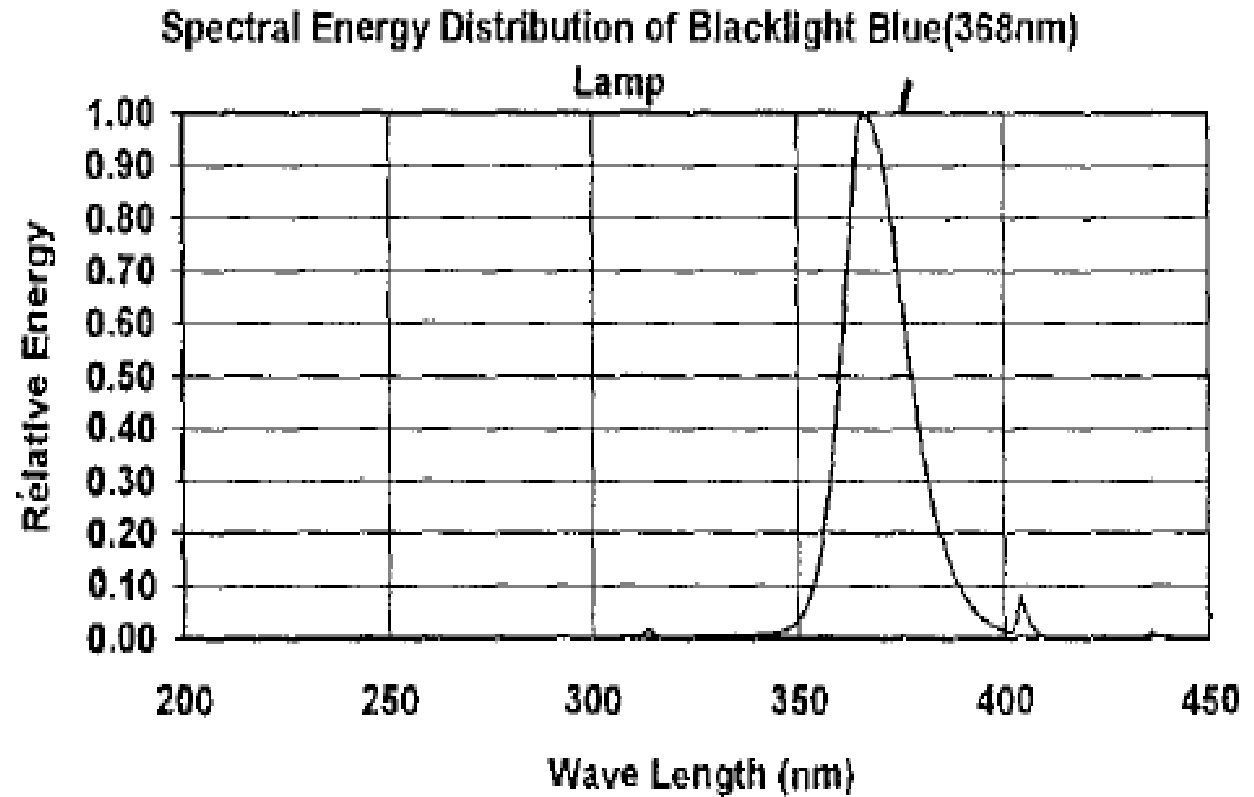
- The black light we have in the lab is 15+ years old
- The bulbs you all have are much newer
- We hypothesize, the composition of the black light bulb changed some time after 2005
- For months, the company denied any difference in the product
- They even have it listed as the same catalogue number on both the new and the old bulbs!



Spectral Curves Provided by the Spectroline



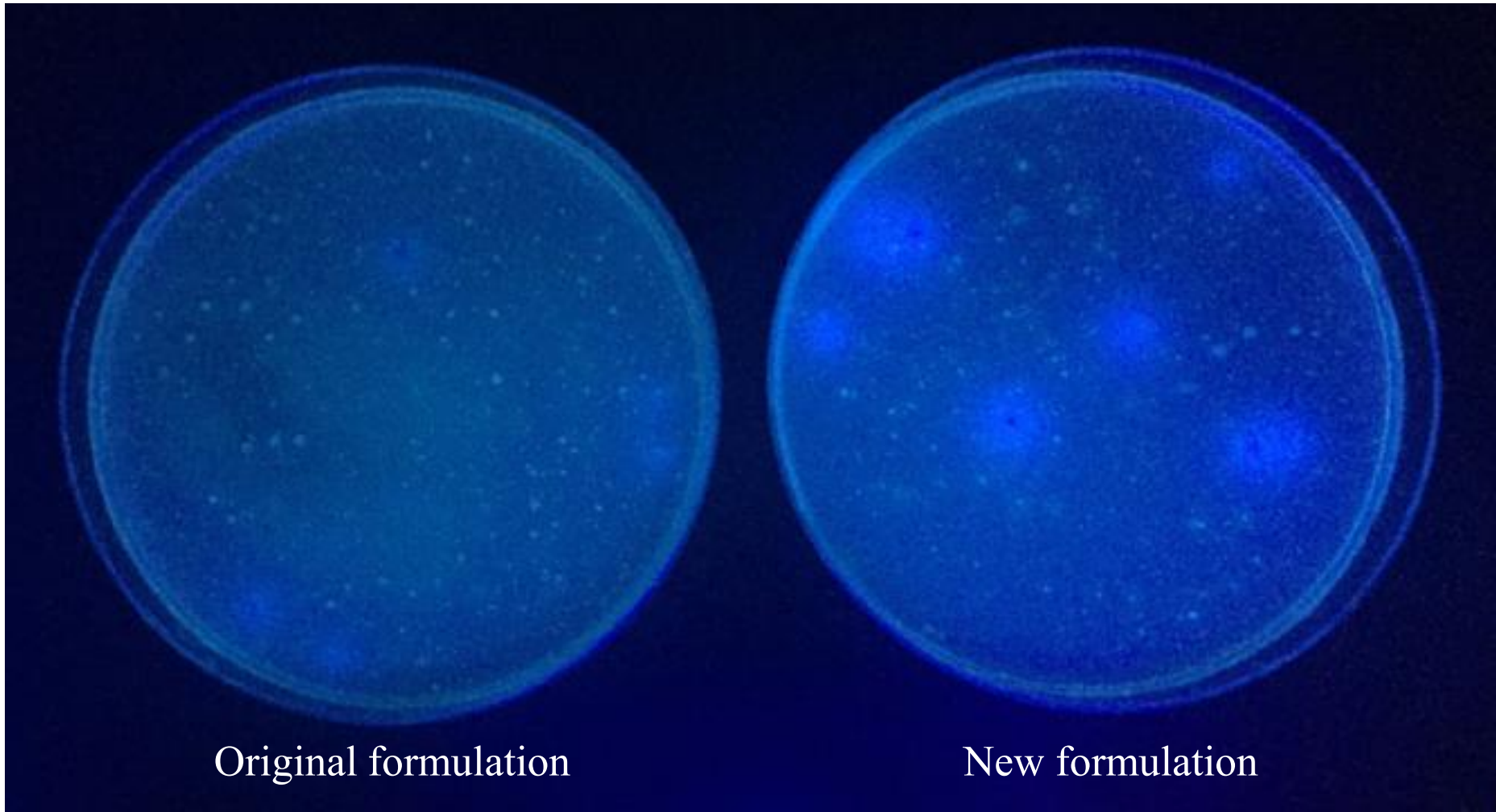
Black Lights prior to 2005
wavelength peaks around 350nm



Black lights made since 2005
wavelength peaks at 368 nm

Resolution

Because we cannot find any more “good” black lights, Micrology Labs has developed a new formulation that generates more fluorescence



Original formulation

New formulation

Black Light and Coliscan Easygel Plus Media comparisons

Conclusion: new media generates significantly higher fluorescence confirmations than the old media

Black Lights			Myrtle Lake			MI 5: HS			MI 6: BHR			Crabtree	
OEM	Wavelength	Power	Sampling date: 4/11/17			Sampling date: 4/11/17			Sampling date: 4/11/17			Analysis Date: 4/6/2017	
	nm	watt	New Media	Old Media	VM	New Media	Old Media	VM	New Media	Old Media	VM	New Media	Old Media
t-Test: Paired Two Sample for Means			p = 0.28			p = 0.00			p = 0.04			p = 0.00	
Blue Colonies present			1	2	TBD	5	4	TBD	9	11	TBD	35	42
Eiko	365	6	1	0	TBD	5	2	TBD	9	8	TBD	35	16
Bulbrite	365	6	1	0	TBD	5	2	TBD	9	7	TBD	35	29
Phillips	365	6	1	0	TBD	5	2	TBD	9	8	TBD	35	23
Black light	350	6	1	0	TBD	5	2	TBD	9	7	TBD	35	25
Spectrocorp	352	6	1	2	TBD	5	4	TBD	9	11	TBD	35	39
Spectroline	365	6	1	0	TBD	5	2	TBD	9	7	TBD	35	12
Intsun	365	4	1	0	TBD	5	2	TBD	9	5	TBD	35	13
Assassin	365	4	1	2	TBD	5	2	TBD	9	7	TBD	34	14
Li-Ion	365	4	1	2	TBD	5	2	TBD	9	7	TBD	33	8

We're getting acceptable fluorescence with the VM's Spectroline bulbs using the new media

	Myrtle Lake		MI 5: HS		MI 6: BHR		Crabtree	
	Sampling date:4/11/17		Sampling date: 4/11/17		Sampling date:4/11/17		Analysis Date: 4/6/2017	
	New Media	Old Media	New Media	Old Media	New Media	Old Media	New Media	Old Media
Visible Light	1	2	5	4	9	11	35	42
Spectrocorp	1	2	5	4	9	11	35	39
Spectroline	1	0	5	2	9	7	35	12

% Fluorescing

Spectrocorp	100%	100%	100%	100%	100%	100%	100%	93%
Spectroline	100%	0%	100%	50%	100%	64%	100%	29%

New media does not generate significantly different results under visible light (blue colonies) than the old media

t-Test: Paired Two Sample for Means

p = 0.139

	New Media	Old Media
Myrtle Lake	1	2
HS	5	4
BHR	9	11
Crabtree	35	42

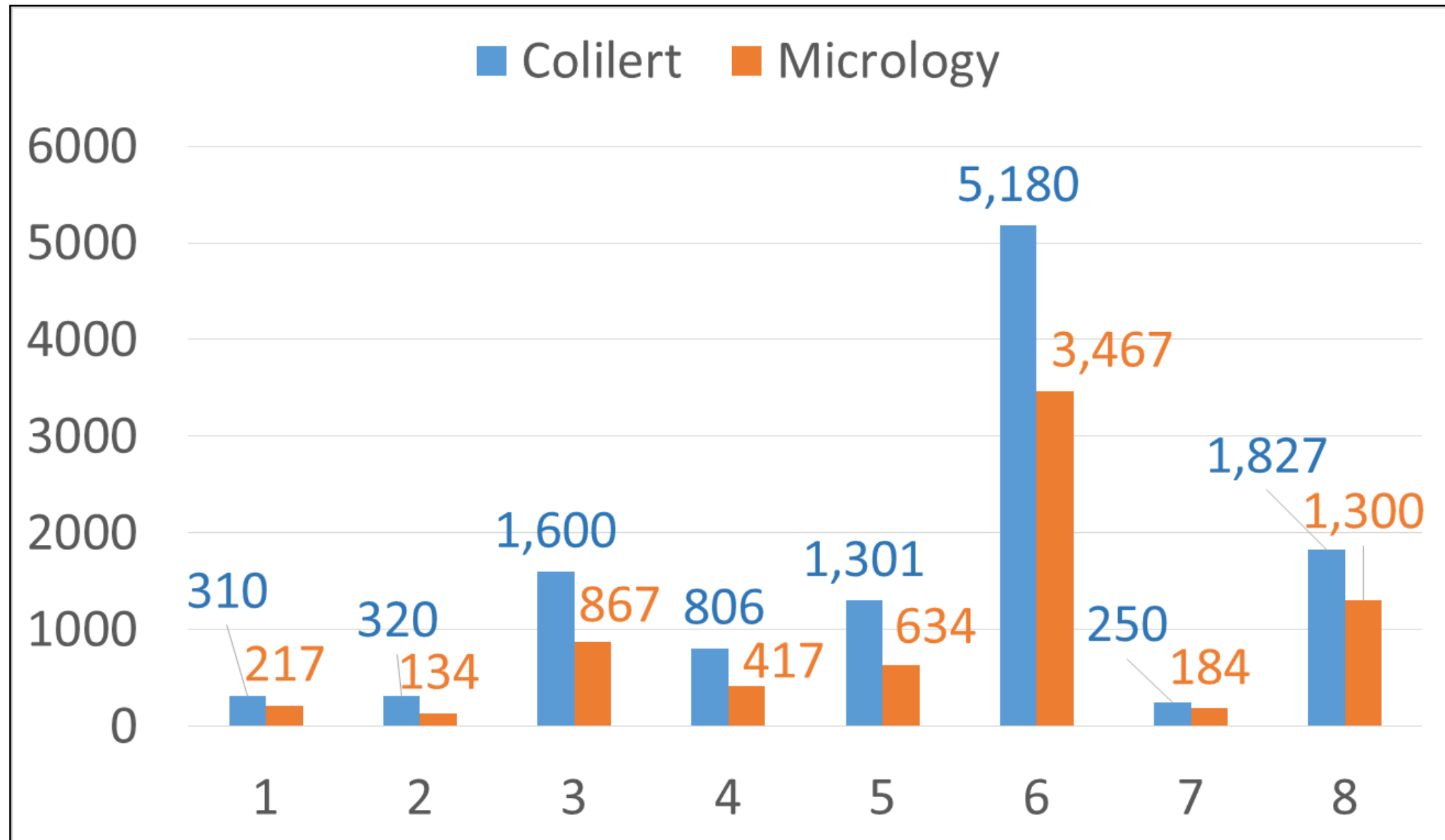
What To Do Now

- With this new media, we want to get a year's worth of data before amending any previous data
 - To do this we will compute the average percent fluorescence for each site and apply this to all previous samples
- We have added a Quality Control technique which involves us looking at the percent fluorescence

$$QC Formula = \frac{\# Fluorescing Blue Colonies}{\# Total Blue Colonies} \times 100$$

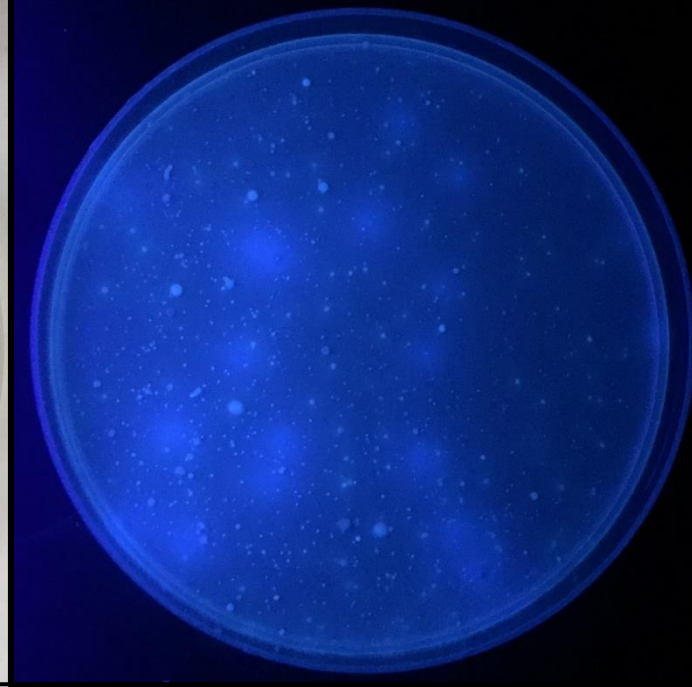
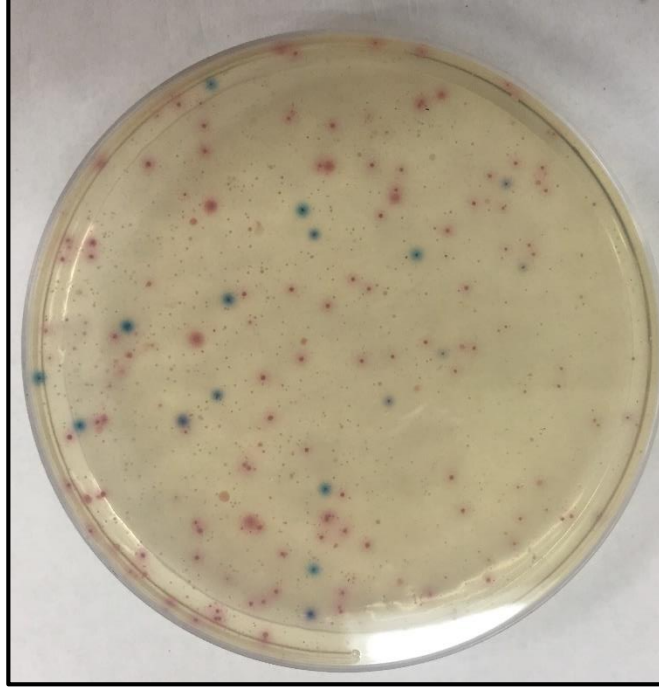
- This percentage should be greater than or equal to 85%
- Micrology is switching over to the new media formulation permanently!

Colilert results are higher than the Easygel results. This is statistically significant.

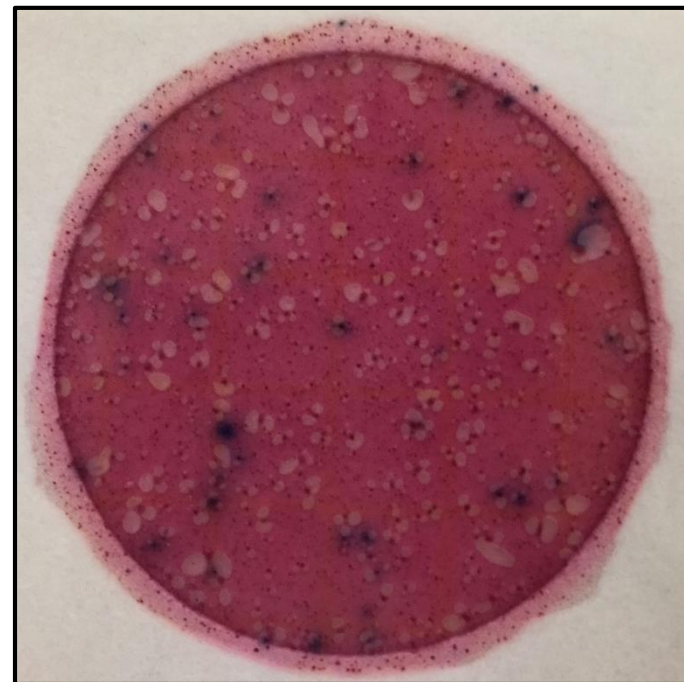




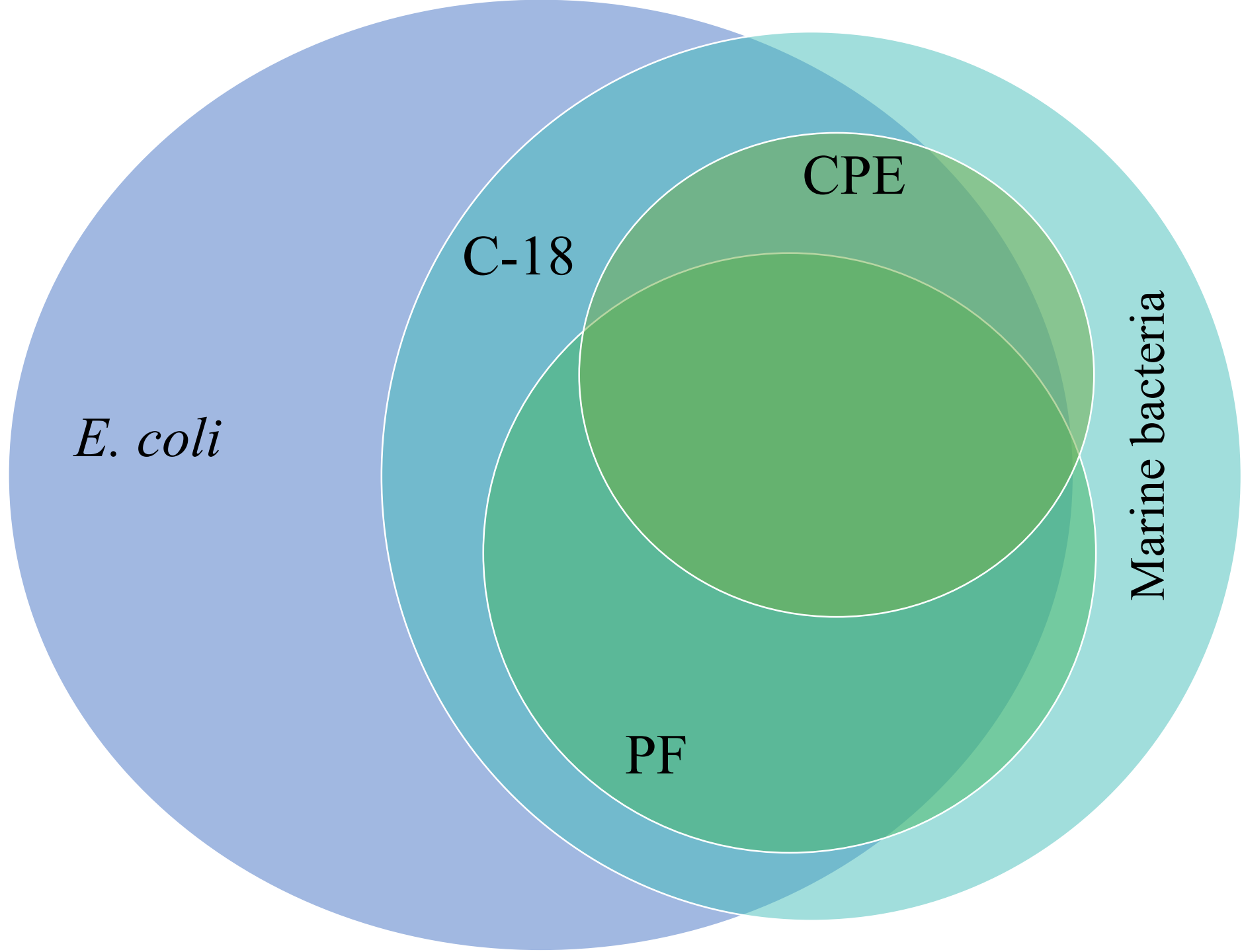
Colilert®-18
(C-18)



Coliscan® Plus
Easygel®
(CPE)



3M® Petrifilm™
(PF)



E. coli

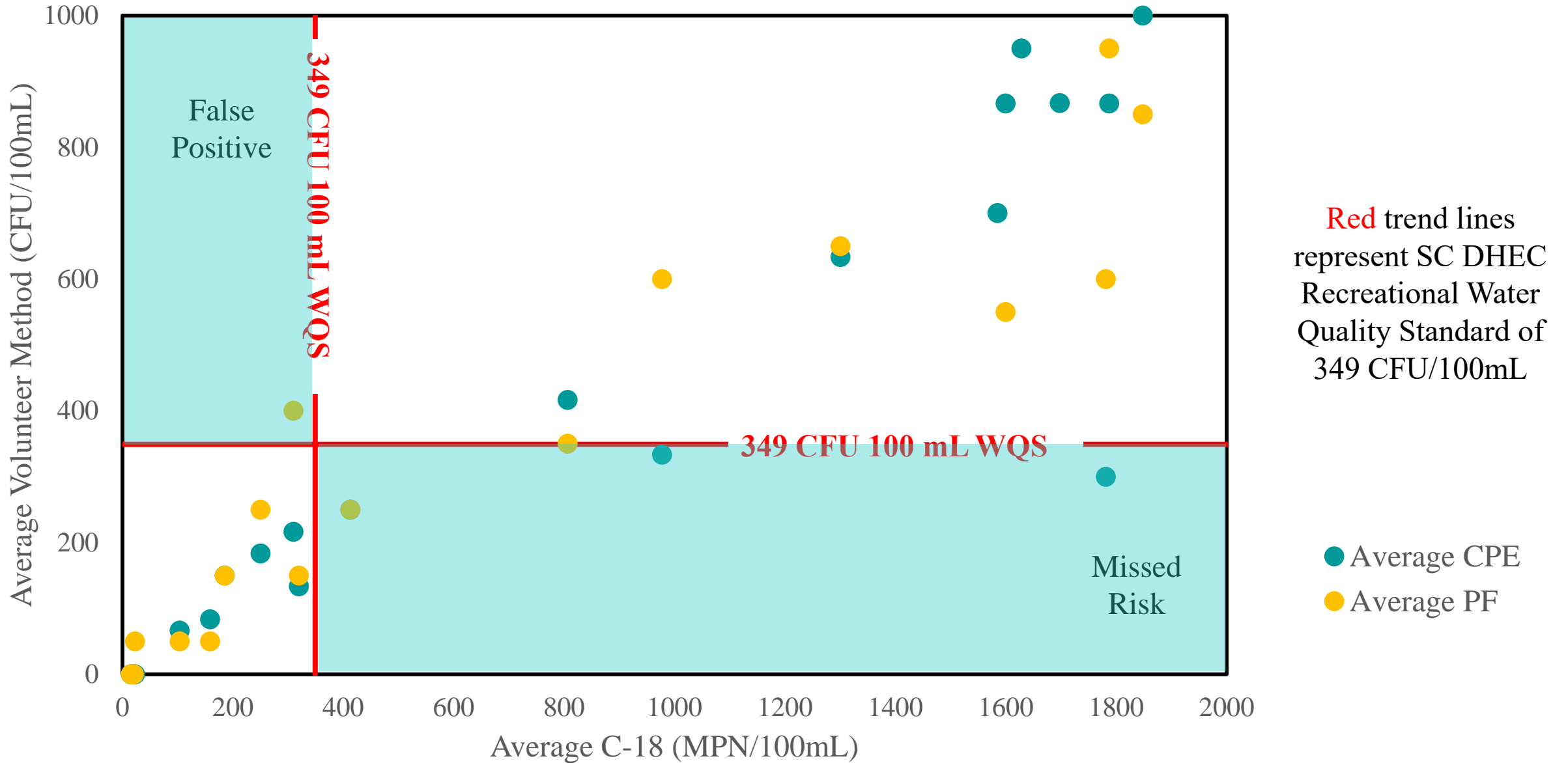
C-18

CPE

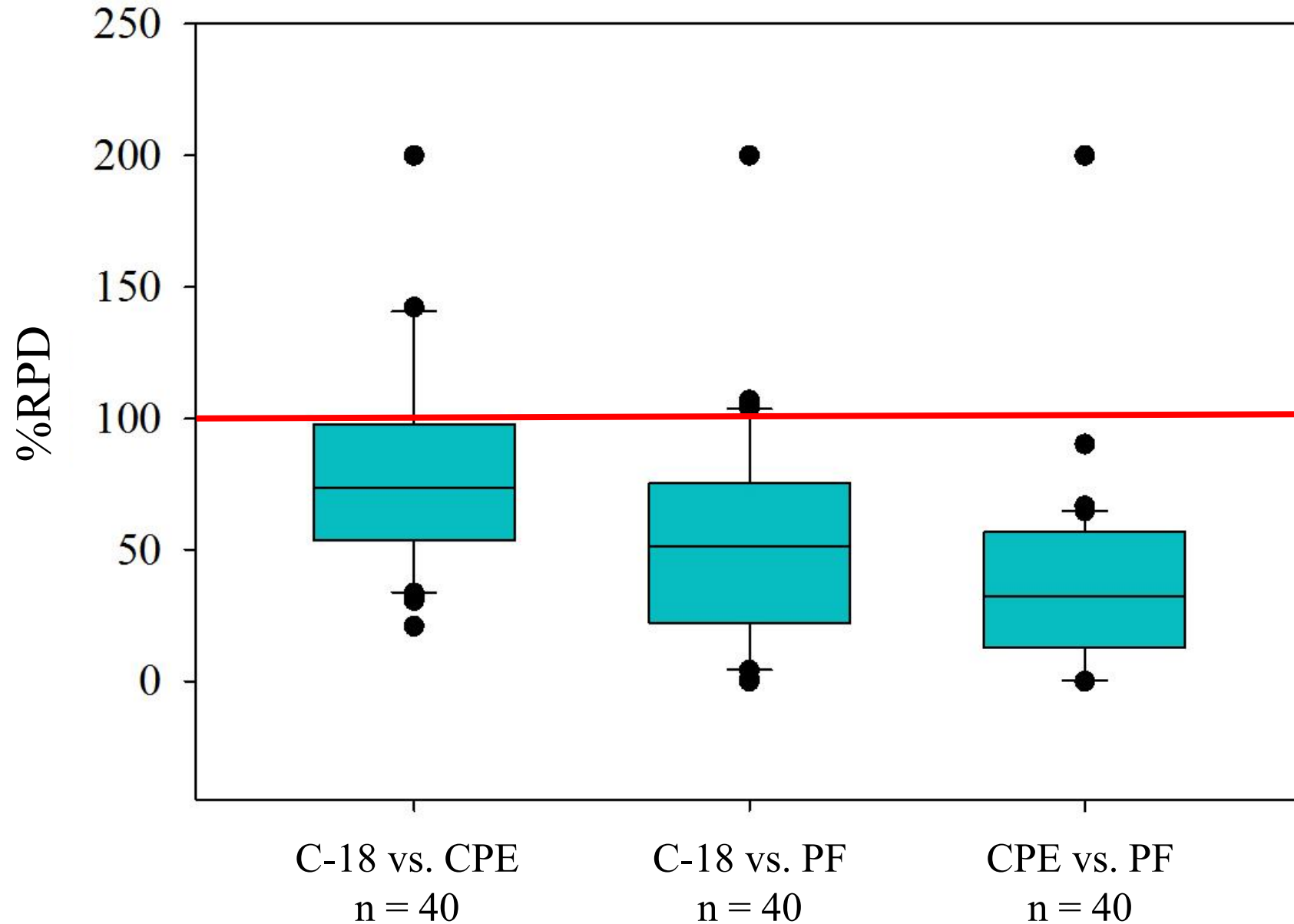
PF

Marine bacteria

Agreement over SC DHEC Water Quality Standard (349 CFU/100mL) between Volunteer Methods and C-18



Distribution of %RPD between Enumeration Methods



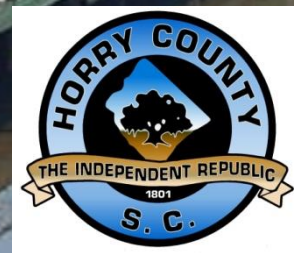
The **red line** represents the EQL's regulatory acceptance threshold for replicate measurements of *E. coli* $\leq 100\%$ when ≥ 150 CFU/100mL

Conclusions

- There is a statistically significant difference between Colilert and Easygel
- The difference is within the acceptance range for sample duplicates
- The difference rarely causes a difference in the regulatory interpretation of the data results, i.e. a missed risk from the Easygel.
- According to the peer-reviewed literature, the higher Colilert results are possibly due to false positives. In the case of Myrtle Lake, BHR and HS which are tidally influenced sites, this could be from marine bacteria.

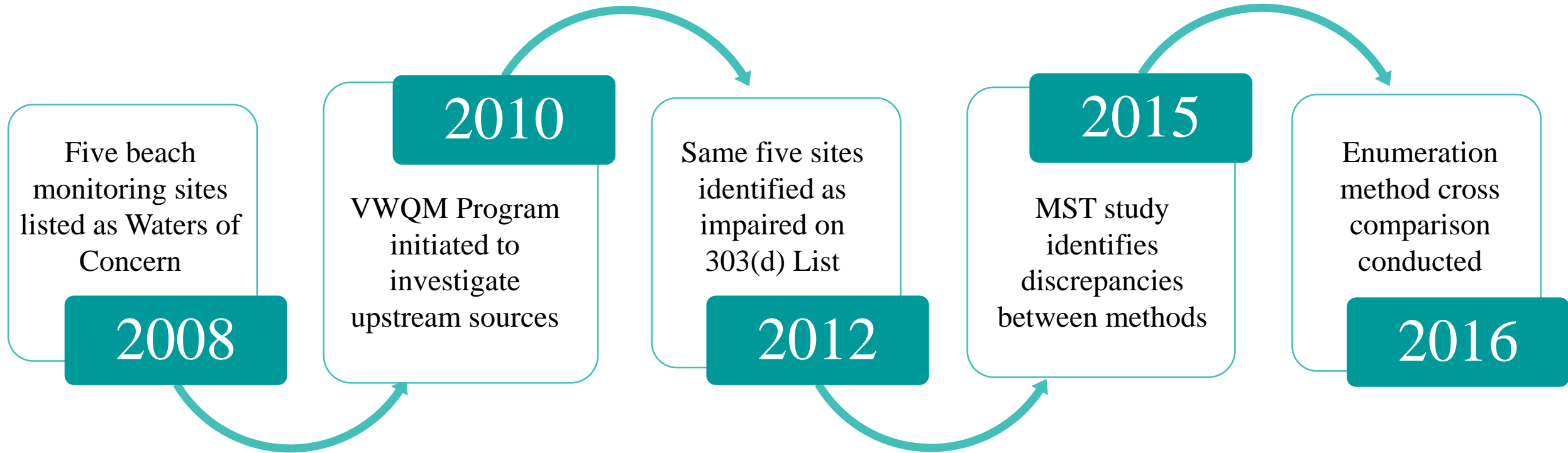


Volunteer Water Quality Monitoring Easygel Plus Training



Enumeration Method Cross Comparison

Case Study: Myrtle Lake, Surfside Beach, SC



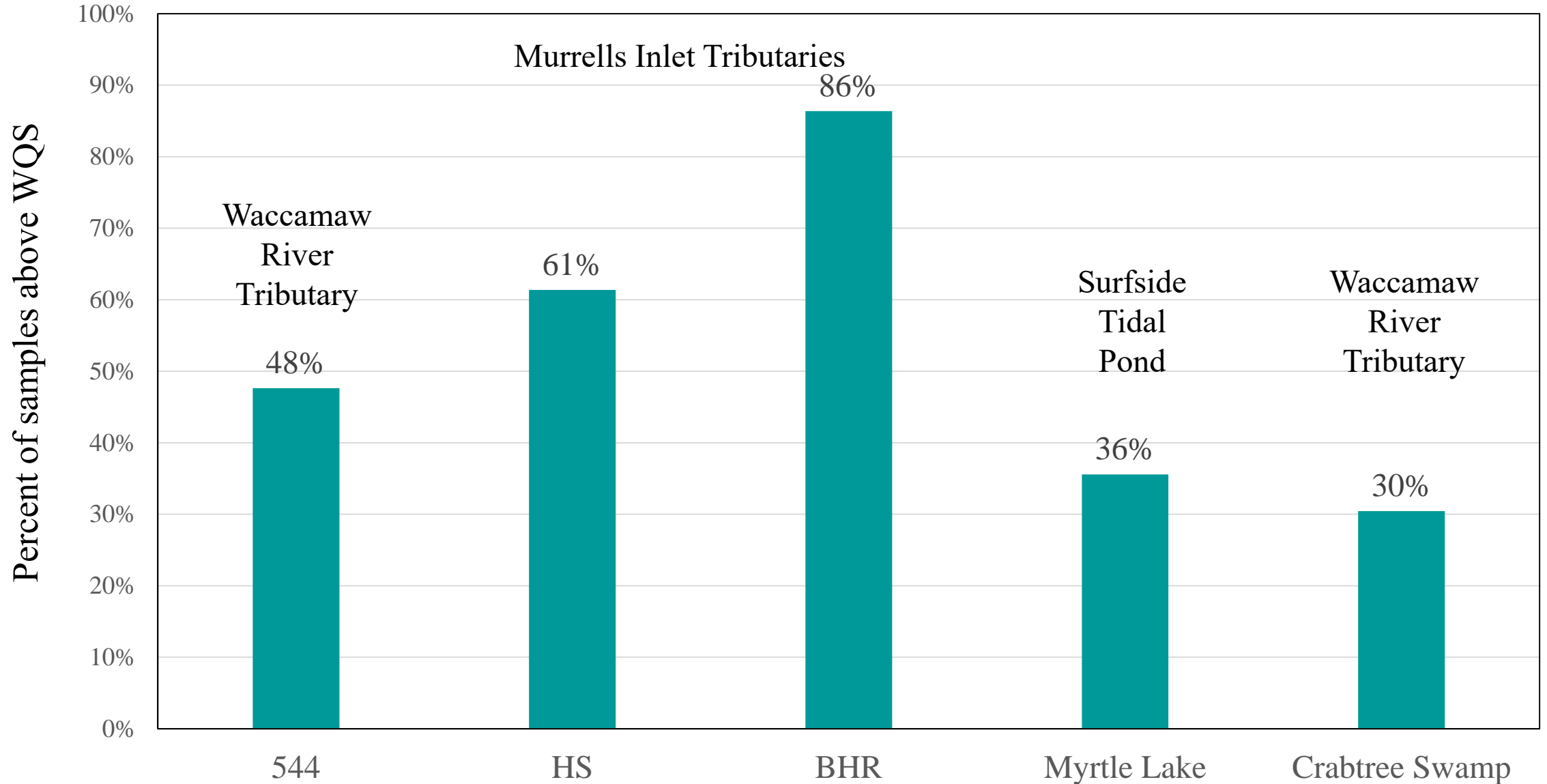
First Evidence of Method Discrepancy

Date of sampling	Coliscan[®] Plus Easygel[®] (CFU/100mL)
09/09/2015	400
09/22/2015	67
11/03/2015	1000
11/17/2015	116
12/08/2015	482
01/12/2016	367
	533

E. coli concentration values in red exceed the SC DHEC recreational water quality standard daily maximum for *E. coli* of 349 CFU/100 mL

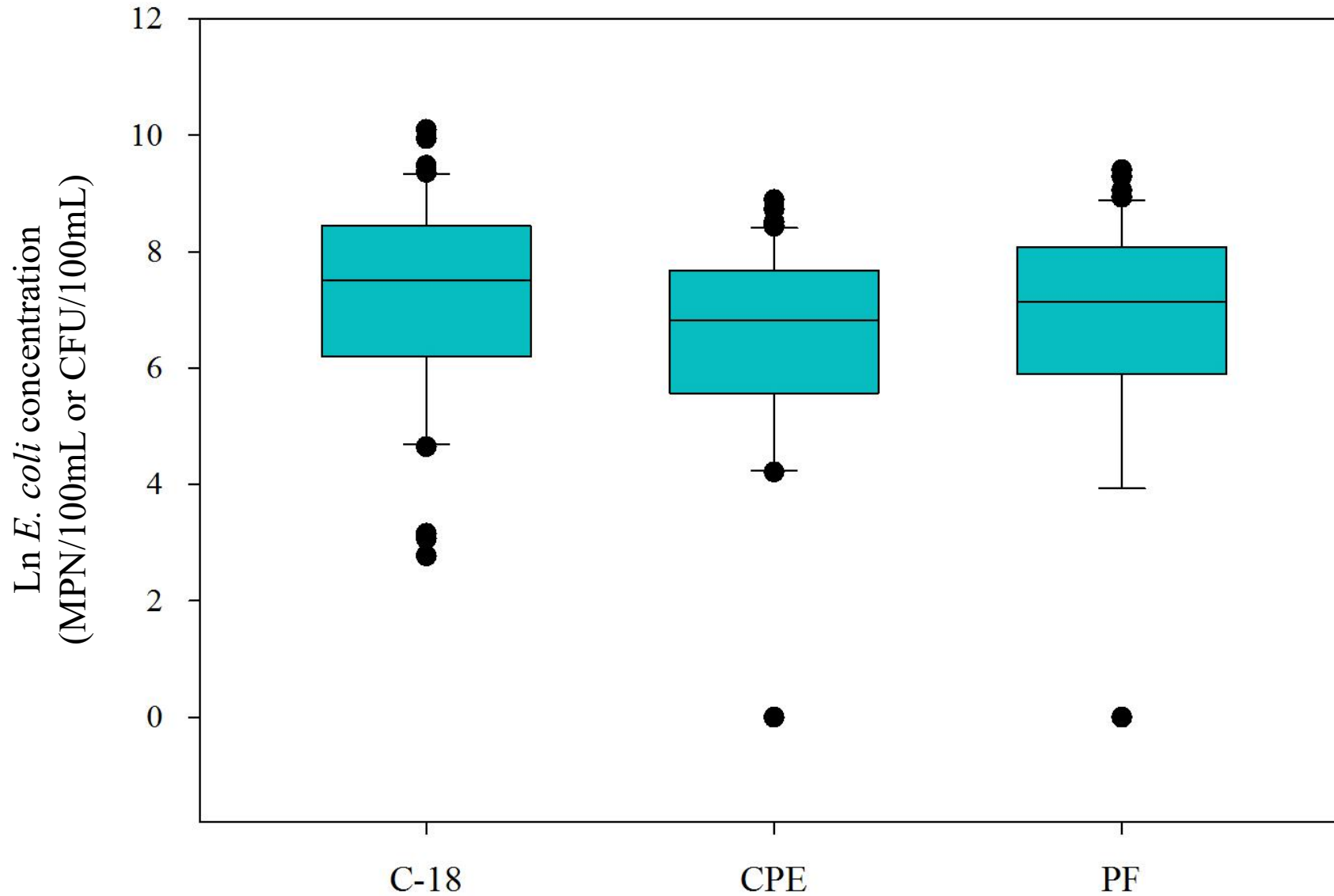
Site Selection

Percent Exceedance of *E. coli* above
SC DHEC Recreational Water Quality Standard (349 CFU/100mL)
from June 2014 through April 2016 (n ≈ 45)



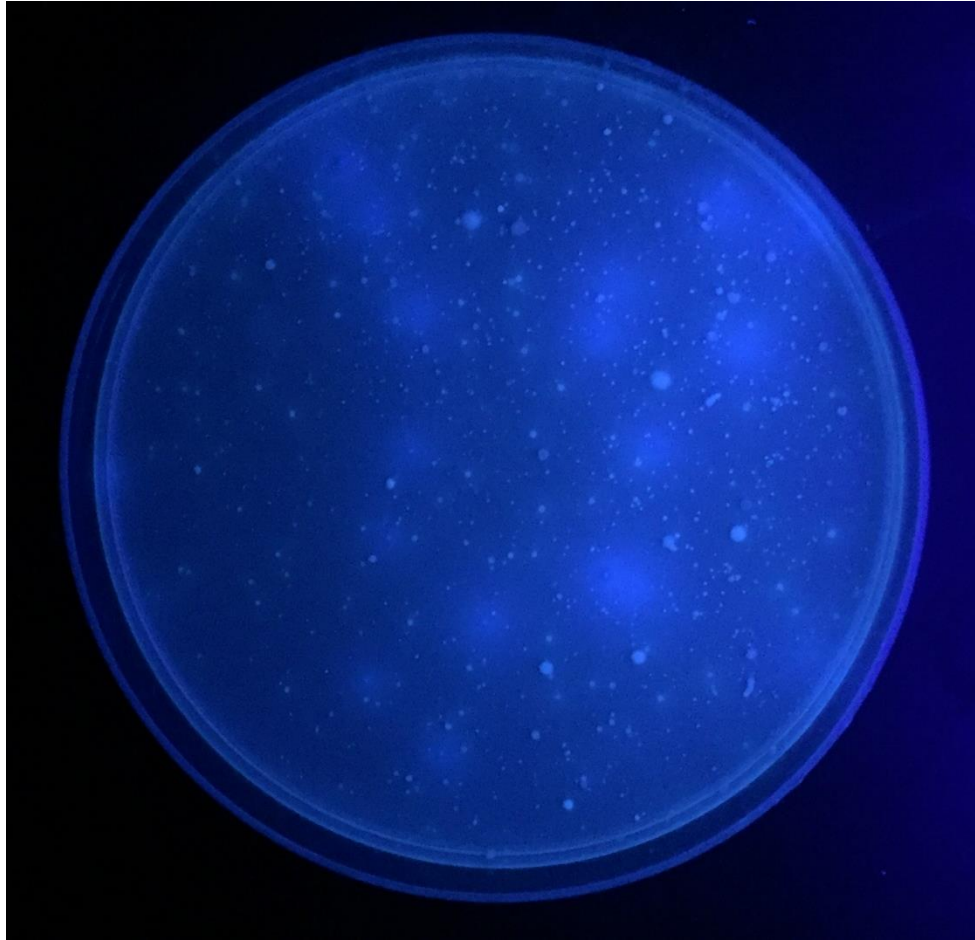
Distribution of Ln *E. coli* Concentrations (n = 40)

Results are significantly different between methods ($p < 0.01$)



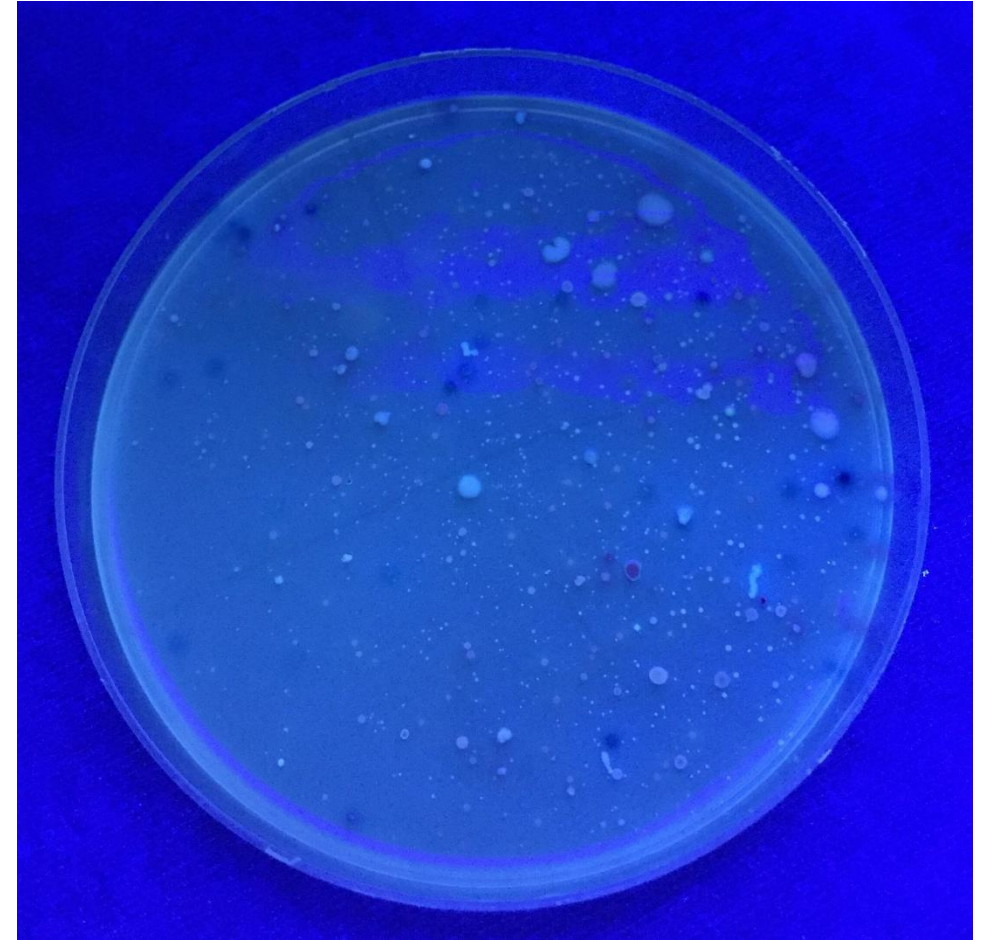
Same CPE plate from Myrtle Lake on 8/9/16 under two different black lights

Plate read with EQL's "old" black light



600 CFU/100mL *E. coli* concentration
5% Non-Fluorescing Colonies

Plate read with the VM "new" black light



67 CFU/100mL *E. coli* concentration
92% Non-Fluorescing Colonies

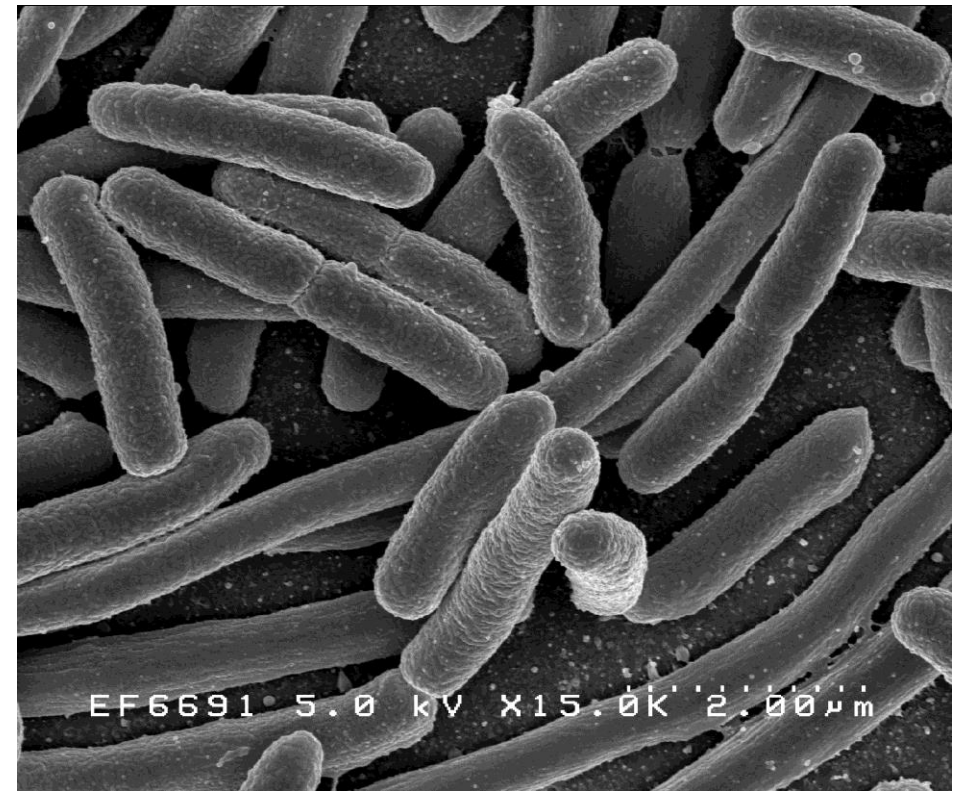
Black Light Issues



- During side by side comparisons done by Cara for her graduate research, she noticed her *E. coli* concentrations were different than what some of the volunteers were measuring
- The biggest issue occurred at Myrtle Lake in Surfside but why????
- After much debate, research, calls to Micrology and Sprectroline, we found an answer!

E. Coli

- Pathogen indicator
- Predictor of gastroenteritis risk
- Recommended by US EPA 1986
- Water Quality Standards
 - Single Sample
 - Designated Beach Area: 235 CFU/100 mL
 - Moderate Full Body Contact: 298 CFU/100 mL
 - Lightly Used Full Body Contact: 406 CFU/100 mL
 - Infrequently Used Full Body Contact Recreation: 576 CFU/100 mL
 - Steady State Geometric Mean Indicator: 126 CFU/100 mL



When Nature Calls, Don't Get Caught

- In Charleston, cats and dogs produce about 10,000 pounds of waste each day.
- Pet waste can wash into storm drains and go directly into waterways without being treated.
- Pet waste can also wash directly into marshes and waterways.

Other Sources?

- Broken sewer lines
- Dysfunctional septic tanks
- Livestock
- Wildlife



Help keep pet waste from polluting our water:

- Scoop the poop. Take a plastic bag with you on your walk.
- Wrap pet waste in a plastic bag and put it into the trash.
- Flush pet waste down the toilet (if you are on a public sewer). Do NOT flush kitty litter.
- Bury pet waste in your yard. Bury it 6 inches deep, away from waterways and gardens.
- Don't put pet waste in the compost pile.



PROMOTE PROTECT PROSPER

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and the environment.*

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Help keep our pets from polluting. Scoop the poop.

CPE Results for Myrtle Lake

Date	EQL R1 vs. EQL R2
6/14/16	15%
6/28/16	50%
7/12/16	8%
7/26/16	8%
8/9/16	11%
8/23/16	6%
9/13/16	18%
9/27/16	0%

Comparison by %RPD of results for Myrtle Lake samples. Values in red exceed EQL QC criteria for *E. coli* enumeration.

≤100% when ≥150 CFU/100mL