



Medford Wastewater  
TREATMENT FACILITY

# **E. COLI TESTING IN WASTEWATER- TIPS FROM A WASTEWATER OPERATOR**

BROOKE KLINGBEIL

LABORATORY DIRECTOR & WASTEWATER  
OPERATOR

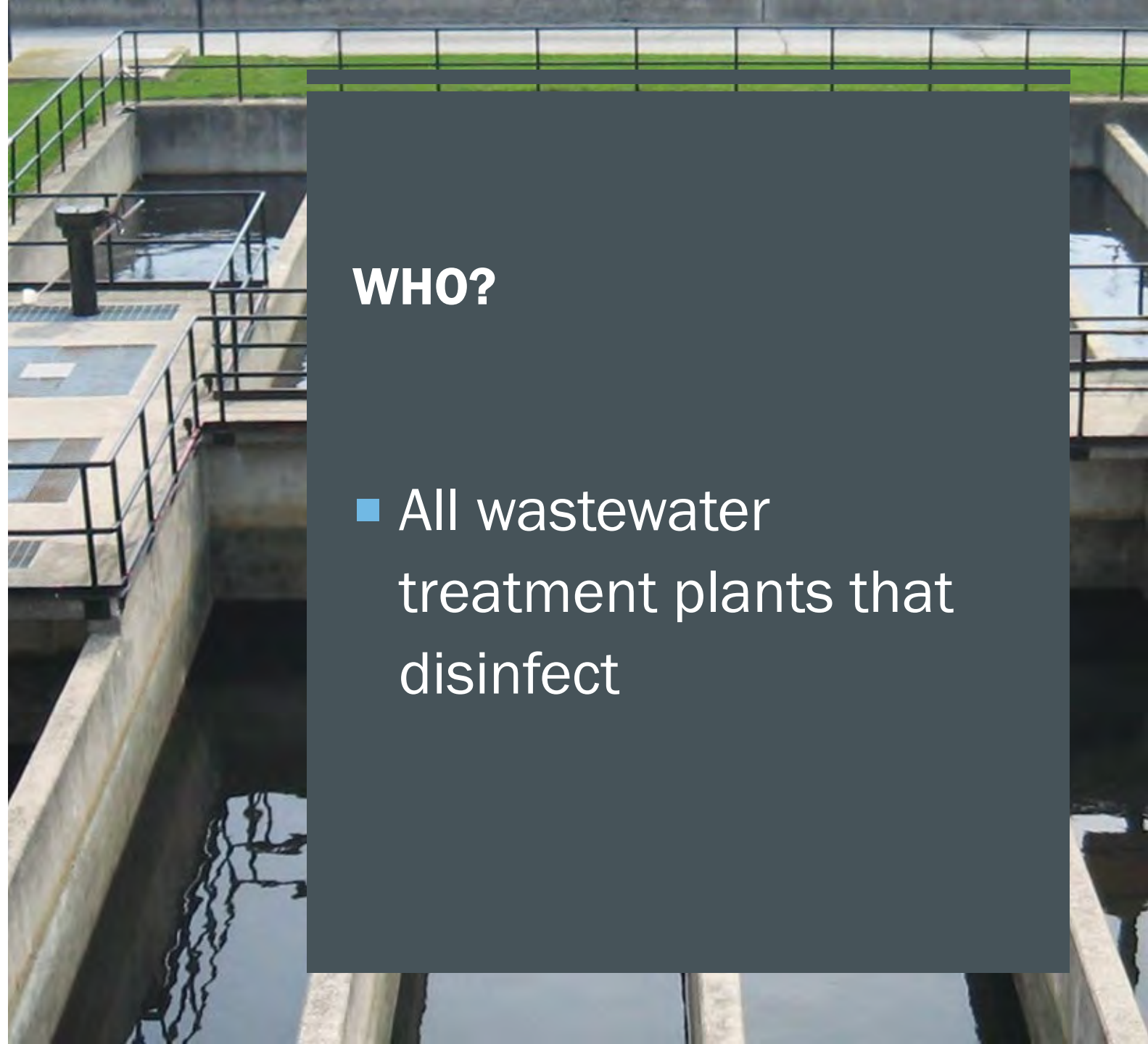


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# OVERVIEW

- Who?
- What?
- When?
- Why?

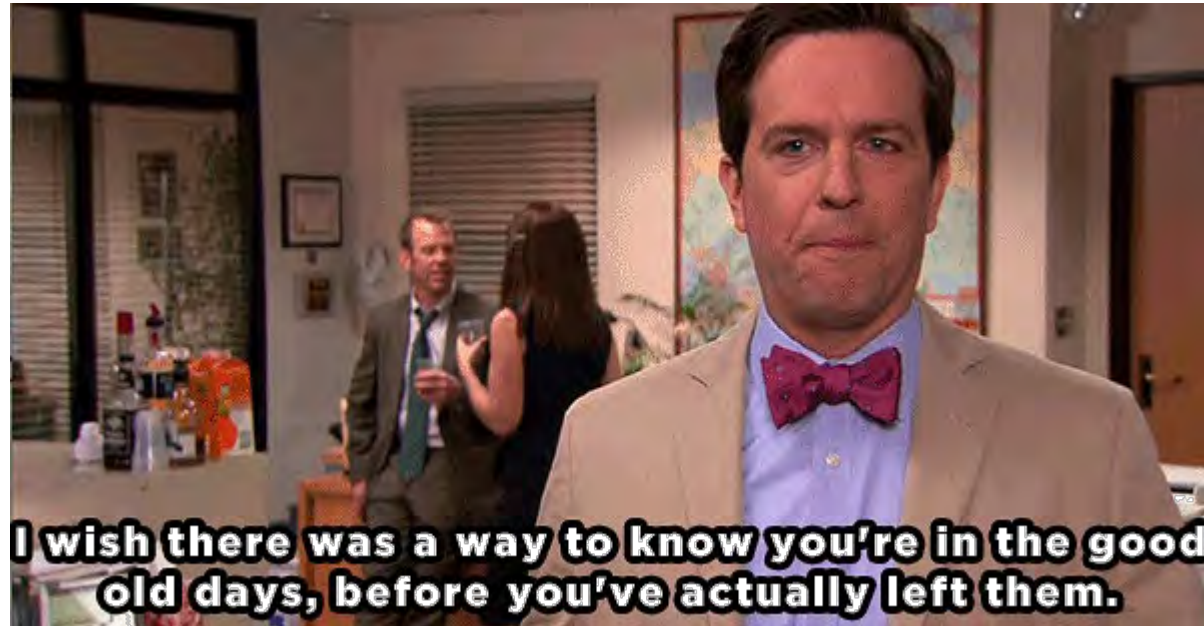


## WHO?

- All wastewater treatment plants that disinfect

## WHAT?

- Replacing fecal coliform bacteria standards with *E. coli*



**From:** Strickland, Wade K - DNR <[Wade.Strickland@wisconsin.gov](mailto:Wade.Strickland@wisconsin.gov)>  
**Sent:** Monday, June 1, 2020 2:28 PM  
**To:** Strickland, Wade K - DNR <[Wade.Strickland@wisconsin.gov](mailto:Wade.Strickland@wisconsin.gov)>  
**Subject:** Revised Bacteria Water Quality Standards

Dear WPDES Permittee and/or Wastewater Operator-

The Department's Water Quality Program recently revised administrative code to promulgate new bacteria water quality standards for *E. coli* in chapter NR 102, Wis. Admin. Code, to comply with federal requirements. A central objective of the new standard(s) is to better protect recreational users (e.g., swimmers) from human health impacts related to the exposure to bacteria. The Department also concurrently codified *E. coli* WPDES permit limitations for all sewage treatment works in chapter NR 210, Wis. Admin. Code, specifically s. NR 210.06, Wis. Admin. Code (please view here: [http://docs.legis.wisconsin.gov/code/admin\\_code/nr/200/210/06](http://docs.legis.wisconsin.gov/code/admin_code/nr/200/210/06)). The *E. coli* standards became effective May 1, 2020. As part of the rulemaking process, representatives for all sewage treatment works that disinfect were notified of the proposed rulemaking during the public notice period for the economic impact analysis associated with the proposed rule and the public notice period for the proposed rule language. During the public notice period, the Municipal Environmental Group reviewed and provided comments to the Department for consideration.

As part of future WPDES permit reissuances, including those permits being publicly noticed now or in the next week or two, permits will include the new *E. coli* standards. Permits on public notice may be viewed here: <https://dnr.wi.gov/topic/wastewater/publicnotices.html>. In most instances, *E. coli* will replace fecal coliform bacteria standards and associated permit limitations. Analytical methods for determining representative *E. coli* counts are discussed in the attached for reference.

The Department will require *E. coli* data with your next permit application. Permittees are encouraged to collect *E. coli* effluent monitoring data ahead of this requirement in order to evaluate whether effluent quality will meet new effluent limitations. If your facility already has collected *E. coli* data, please send applicable data to your DNR compliance engineer.

Should you have any questions regarding this approach, please reach out to your DNR compliance engineer or permit drafter.

Thank you.

Wade

**We are committed to service excellence.**  
Visit our survey at <http://dnr.wi.gov/customersurvey> to evaluate how I did.

Wade Strickland  
Chief, Water Permits Section  
Water Quality Bureau  
Wisconsin Department of Natural Resources  
101 S. Webster Street, Madison, WI 53707  
Office Phone: (608) 266-7420

# WHEN?

■ May 1<sup>st</sup>, 2020



**WHY?**

Because we are  
now required to do  
so...



# WHAT DO WE KNOW?

- <https://dnr.wisconsin.gov/search/google?keys=e.%20coli%20testing%20methods#gsc.tab=0&gsc.q=e.%20coli%20testing%20methods&gsc.sort=>

## Test Methods for Measuring *E. coli* in Wastewater



### Introduction

The EPA has approved three approaches in 40 CFR 136 for quantifying *E. coli* in wastewater: membrane filtration, multiple tube/multiple well, and multiple tube fermentation. These three approaches are also approved by the Wisconsin DNR for *E. coli* monitoring in wastewater and are listed in Ch. NR 219, Wisc. Admin. Code.

A description of each of these approaches is provided below and a summary of the advantages and disadvantages of each is included in the table on page 3.

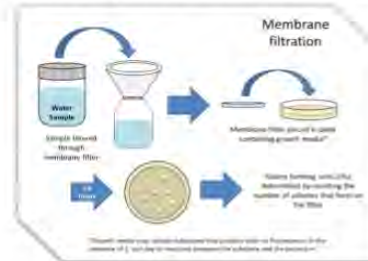
#### Recreation Water Quality Criteria for Bacteria

Recreation water quality criteria for bacteria protect people from exposure to bacteria that are present in water contaminated by human fecal matter.

Because pathogens can be difficult to measure directly, a pathogen indicator is used to signal the potential for illness caused by fecal contamination. The U.S. EPA recommends that *E. coli* or enterococci be used as the pathogen indicator.

### Membrane Filtration

In the membrane filtration approach, a water sample is filtered through a membrane. The membrane is then placed on culture media that is selective for *E. coli*. Because the bacteria are retained on the surface of the filter, they grow on the media and develop into a visible colony.



The number of colonies that are formed are counted and reported as the colony forming units (CFUs).

mColiBlue-24<sup>®</sup> by Hach Company is a commercially available culture media that can be used to quantify *E. coli* via the membrane filtration approach.

EGAD #: 3200-3400-2020-02

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EPA and Wisconsin DNR Approved Analytical Approaches for Quantifying <i>E. coli</i>				
Analytical Approach	Standardized Test Method	Commercial Technology	Advantages	Disadvantages
<b>Membrane filtration:</b>				
Single-step or Two-step	EPA 1603 SM 9222B-2015 SM 9222I-2015	mColiBlue-24 <sup>®</sup> N/A	<ul style="list-style-type: none"> <li>Readily available</li> <li>Used to establish EPA's <i>E. coli</i> criteria<sup>3</sup></li> <li>Results can be compared directly to fecal coliform results</li> <li>Media less costly</li> </ul>	<ul style="list-style-type: none"> <li>Labor and material intensive</li> <li>Require high degree of technical skill to evaluate results</li> <li>Additional analysis may be needed for samples with high turbidity, high levels of noncoliform bacteria, or organisms stressed by chlorine</li> </ul>
<b>Multiple tube/multiple well</b>	SM 9223-B-2016 AOAC 991.15	Colliert <sup>®</sup> 1 Colliert-18 <sup>®</sup> 1	<ul style="list-style-type: none"> <li>Commercially available</li> <li>Standardized media and procedure</li> <li>Less labor, material, and time intensive</li> <li>Requires minimal technical skill to evaluate results</li> </ul>	<ul style="list-style-type: none"> <li>May yield higher values than membrane filtration methods<sup>2</sup></li> <li>Reagent more costly</li> <li>Requires specialized equipment</li> </ul>
<b>Multiple tube fermentation</b>	SM 9221B.3-2014 SM 9221F-2014	N/A	<ul style="list-style-type: none"> <li>One of the first approved methods for quantifying <i>E. coli</i></li> </ul>	<ul style="list-style-type: none"> <li>Not commonly used</li> <li>Labor and time intensive</li> <li>May underestimate bacterial density</li> </ul>

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### Multiple Tube/Multiple Well

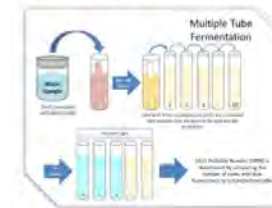
In the multiple tube/multiple well approach, a water sample is mixed with a commercial reagent containing methylumbelliferyl-β-glucuronide (MUG). *E. coli* enzymatically cleaves MUG forming a fluorescent product. Samples are distributed into a multi-well plate. After incubating for 24 hours, the MPN is estimated from the number of wells that are positive for the presence of bacteria growth using a standardized table. The MPN is a statistical estimate of the mean bacteria density.

Colliert<sup>®</sup> and Colliert-18<sup>®</sup> by IDEXX Technologies are commercially available kits that can be used to quantify *E. coli* via the multiple tube/multiple well approach.



### Multiple Tube Fermentation

The multiple tube fermentation approach is a two-step process. First, a water sample is added to test tubes containing bacteria growth media and incubated for 24-48 hrs. Tubes that are positive for the production of acid and/or gas are then added into a series of tubes with media containing MUG. After 24 hours, the tubes are examined for fluorescence.

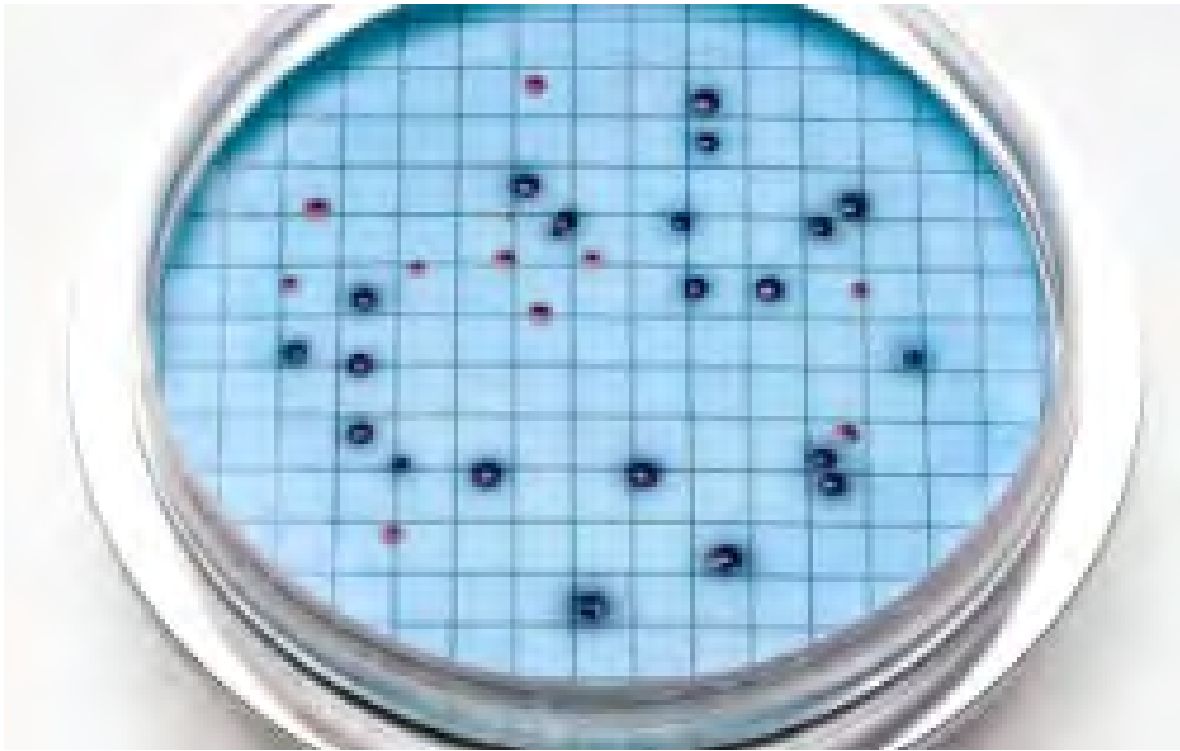


The bacteria level is reported as the most probable number (MPN). The MPN is estimated from the number of tubes that are positive for the presence of bacteria growth using a standardized table.

This approach is not used frequently as the precision is low unless a large number of samples are collected and it is more labor and time intensive than the other approaches.

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# MEMBRANE FILTRATION VS MULTIPLE TUBE/MULTIPLE WELL

LET'S TALK ABOUT IT!



## MEMBRANE FILTRATION SIMILARITIES AND DIFFERENCES

### Fecal Coliform

\*MF-C/Rosalic Acid Broth  
PourRite® Ampoules



\*Incubation at  $44.5 \pm 0.2$  °C

\*47mm petri dishes with  
absorbent pads

\*0.45µm membrane filters

\*Sterile disposable or  
magnetic filter funnels

### E. Coli

\*m-Colibblue24® Broth  
PourRite Ampoules



\*Incubation at  $35 \pm 0.5$  °C

\*47mm petri dishes with  
absorbent pads

\*0.45µm membrane filters

\*Sterile disposable or  
magnetic filter funnels

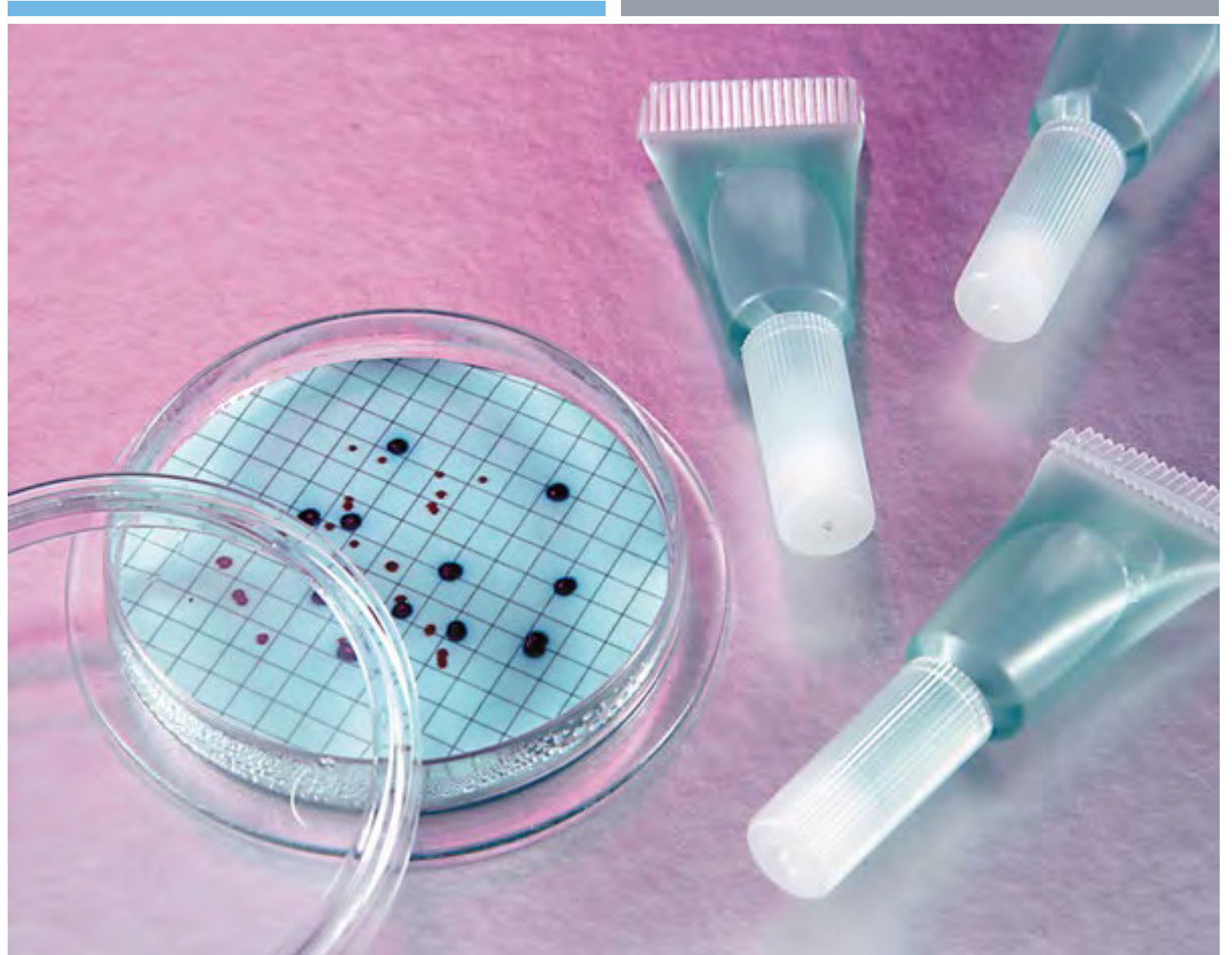
# MEMBRANE FILTRATION: EPA 1603

<https://www.hach.com/m-coliblu24-broth-plastic-ampules-pk-50/product-downloads?id=7640249626&callback=qs>

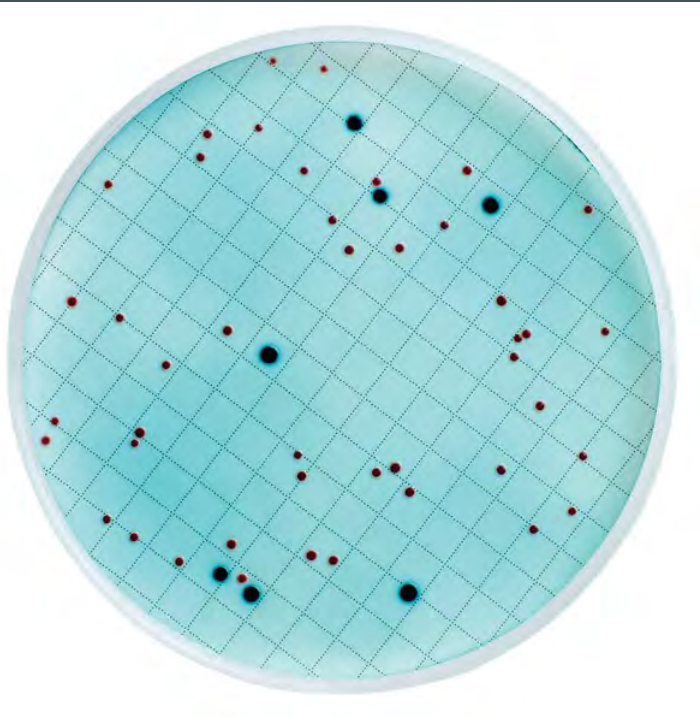


North Central Laboratories

800-648-7836



$$(7/100) \times 100 = 7 \text{ cfu}/100$$



1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated coliform colonies.
2. Determine the coliform density as follows:

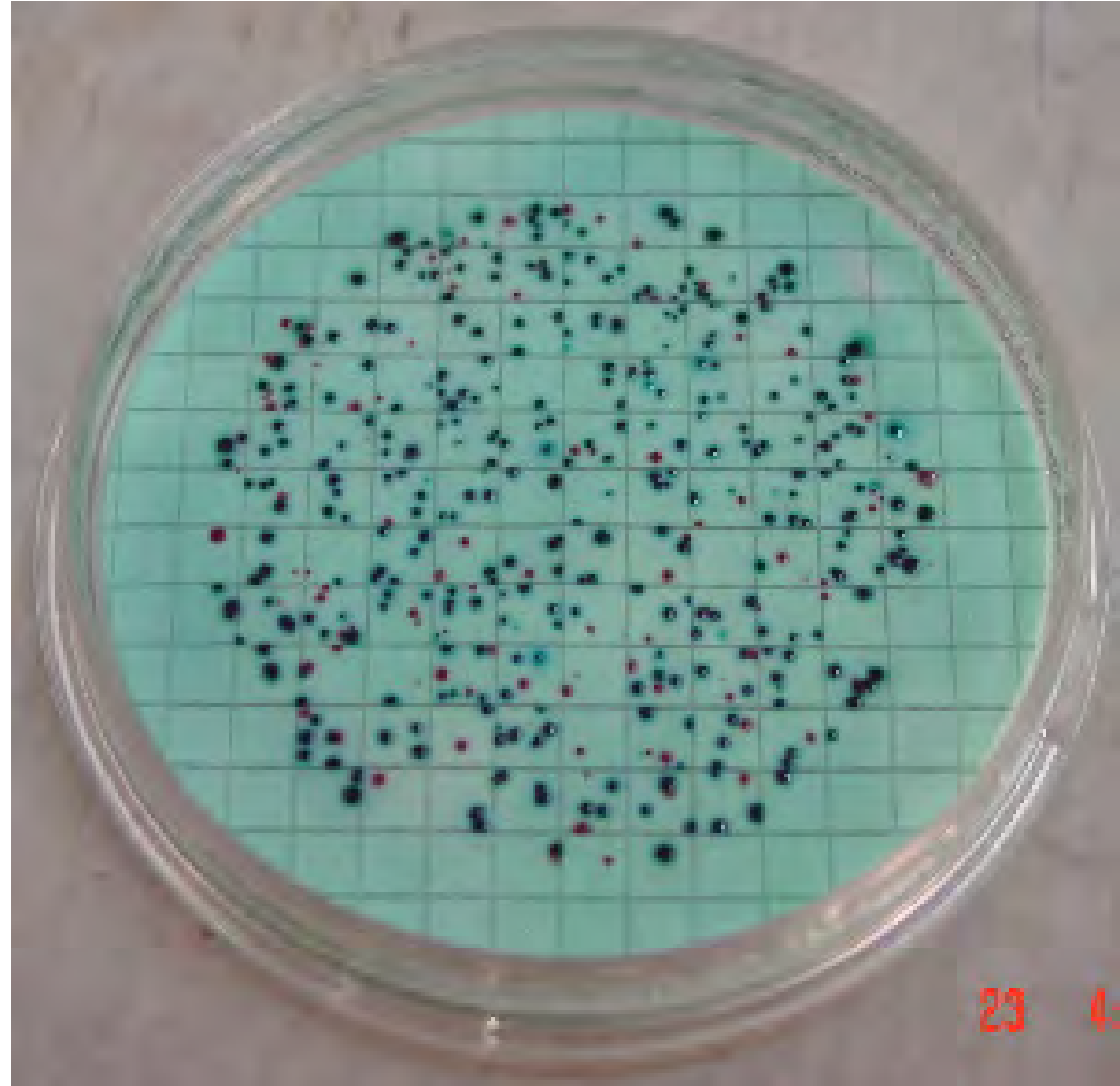
Membrane filter(s)	Coliform density determination
<b>One membrane filter</b>	Coliform colonies in 100 mL = Coliform colonies counted $\div$ mL sample $\times$ 100  <i>Example: 50 coliform colonies were counted. The sample volume was 20 mL. The coliform density is <math>50 \div 20 \text{ mL} \times 100 = 250</math> coliforms in 100 mL of sample.</i>
<b>Multiple filters, dilutions or duplicates for each sample</b>	Average coliform colonies in 100 mL = Sum of coliform colonies in all samples $\div$ sum of mL sample $\times$ 100  <i>Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The coliform density is <math>(5 + 9) \div (50 + 50) \times 100 = 14</math> coliforms in 100 mL of sample.</i>

3. If colonies are not isolated or if there are more than 200 colonies of all types:
  - a. Report the results as "Confluent growth with or without coliforms" when the bacteria grows together across some or all of the membrane filter.
  - b. Do the test procedure again with half the sample volume. If the total number of colonies (coliforms plus non-coliforms) is more than 200 for each membrane or the colonies are not isolated, report the results as "Too numerous to count" (TNTC).
  - c. Do the test procedure again with a dilution that gives approximately 50 coliform colonies and not more than 200 colonies of all types.

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## WHY DO WE RUN MULTIPLE VOLUMES?

- A membrane having more than 200 colonies would be reported as TNTC (too numerous to count).
- Re-sample and run smaller volumes until you reach the desired range of 20-80 colonies per membrane.



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# HOW SHOULD WE BE REPORTING RESULTS?

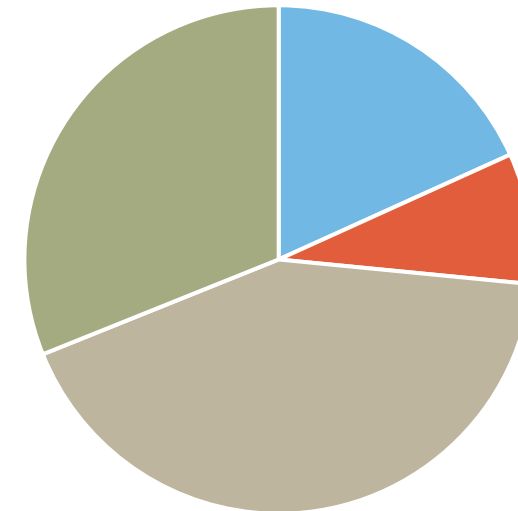
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- How should we be reporting *E. coli* results?
  - If using the mColiBlue24 it would it be CFU/100mL
  - If using the IDEXX Quanti-Tray it be MPN/100mL
  - The DMR will only have #/100 mL.

## EQUIPMENT/MEDIA/SUPPLIES

- Incubator or Water Bath capable of maintaining  $35 \pm 0.5^{\circ}\text{C}$
- m-ColiBlue24® ampules
- 47 mm petri dishes with absorbent pads
- 0.45 $\mu\text{m}$  membrane filters
- Sterile disposable or magnetic filter funnels
- Sterile Buffered dilution water

\$780

Start-Up Cost



■ m-ColiBlue24

■ Petri Dish with Pad

■ Milliopore 0.45um Filters

■ Sterile Disposable Filter Funnel



## MULTIPLE TUBE/MULTIPLE WELL: SM9223-B-2016

- <https://www.idexx.com/en/water/resources/water-resources/>
- Procedures and QC information found online!





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## COLILERT®

- 24 HOURS UP TO 28 HOURS
- *E. coli* 35°C±0.5
- Drinking Water & Wastewater

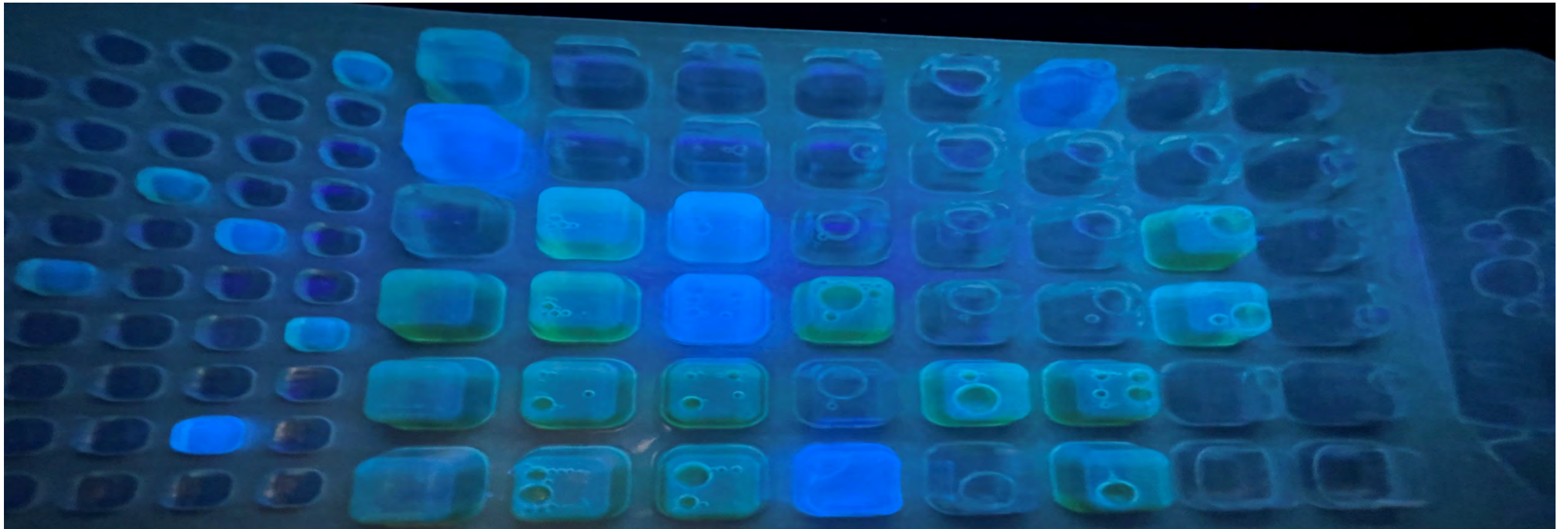




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## COLILERT-18®

- 18 hours up to 22 hours
- Fecal Coliform @  $44.5^{\circ}\text{C}\pm 0.2$
- *E. coli* @  $35.5^{\circ}\text{C}\pm 0.5$
- Drinking Water & Wastewater

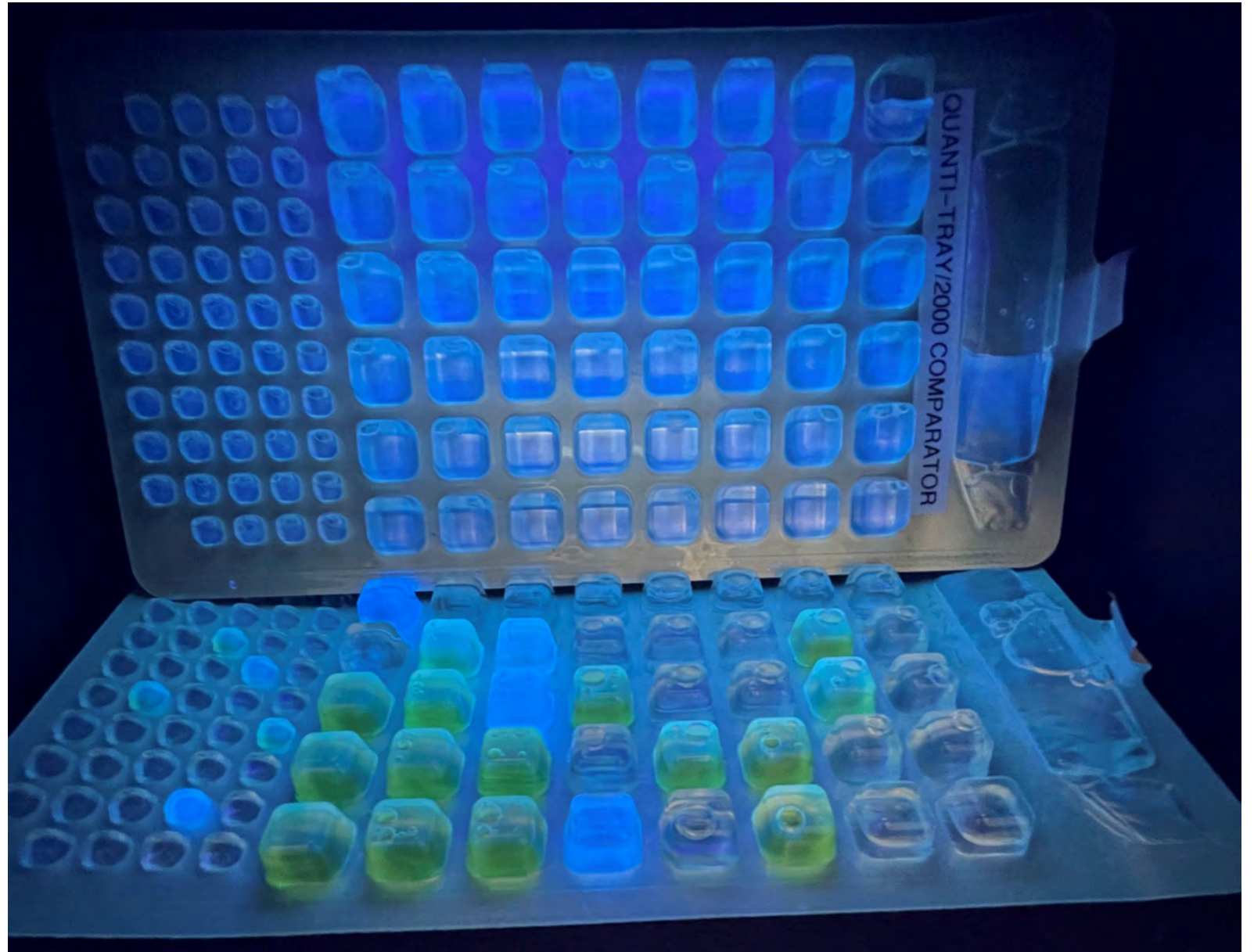


***E. COLI POSITIVE WELLS AFTER 18-22 HRS AT 35±0.5 °C***

# Large Wells Positive	IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)																								
	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.8	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.0	246.1	257.5	270.5	284.2	298.6	305.5	344.8	365.4	387.3	410.6	435.2

## ***E. COLI* POSITIVE COMPARISON**

- Only wells that fluoresce equal to or greater than the comparator are positive for *E. coli*

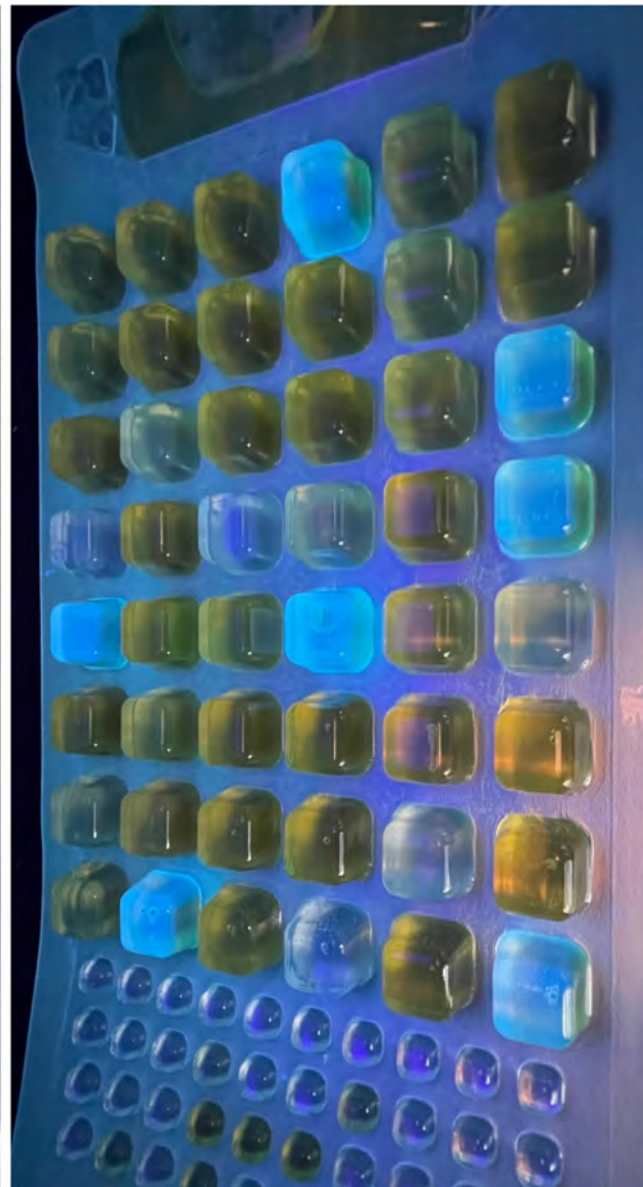




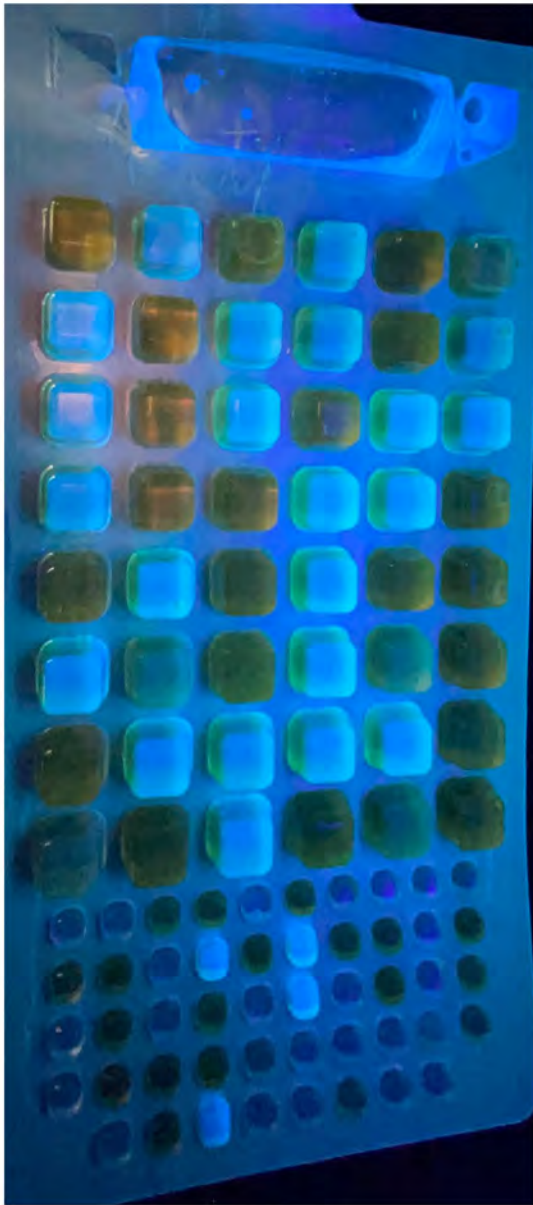
## MORE COMPARISONS...

- Remember...when incubated at  $35\pm 0.5^{\circ}\text{C}$  only the wells that FLUORESCCE are positive for *E. coli*.
- For Fecal Coliform, when incubated at  $44.5\pm 0.2^{\circ}\text{C}$ , only the YELLOW colored wells are positive for Fecal Coliform

- 7 LARGE 0 SMALL
- 7.5 MPN/100 ML



23 LARGE, 4 SMALL



35.5 MPN/100



## QUANTI-TRAY

- Quantification from 1 up to 200 MPN/100mL
- Fecal Coliform
- *E. coli*



## QUANTI-TRAY 2000

- Quantification from 1 up to 2,419.6
- Fecal Coliform
- *E. coli*



# MEDFORD EFFLUENT

Sample Type: Grab

Sample Date:

Sample Time:

Analyst in:

Test date/time in:

Incubator temp: °C

Analyst out:

Test date/time out:

Incubator temp: °C

## FECAL COLIFORMS

# of positive large wells:

# of positive small wells:

MPN from table:

## E. COLI

# of fluoresced large wells:

# of fluoresced small wells:

MPN from table:

# BENCH SHEET INFORMATION

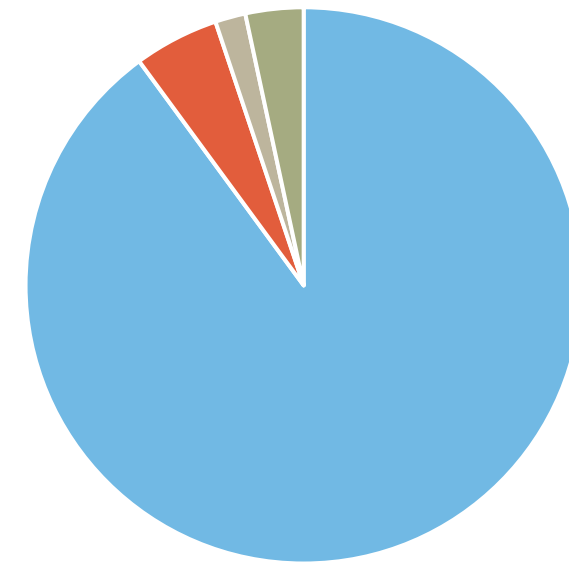
[HTTPS://WWW.IDEXX.COM/FILES/QT97MPNTABLE.PDF](https://www.idexx.com/FILES/QT97MPNTABLE.PDF)

# Large Wells Positive	IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)																								
	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.3	16.4	17.4	18.4	19.4	20.5	21.5	22.5	23.6	24.6	25.7	26.8
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.4	27.5	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.5	11.6	12.7	13.8	14.8	15.9	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.2	29.3	30.4	31.5	32.6	33.7
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.5	29.6	30.7	31.8	32.9	34.0	35.1
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.5	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.0	62.6	64.1	65.6	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.9	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.5	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.8	88.9	91.1	93.3	95.5	97.7	100.0	102.3	104.6	107.0
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
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38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.6	93.1	95.6	98.1	100.7	103.3	105.9	108.6	111.4	114.2	117.1	120.0	122.9	125.9	128.9	132.0	135.1	138.2
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5				

## EQUIPMENT/MEDIA/SUPPLIES

- Incubator or Water Bath capable of maintaining  $35.0^{\circ}\text{C} \pm 0.5$
- Quanti-Tray Sealer Model 2.0 or Quanti-Tray Sealer PLUS
- 365-nm long wave UV lamp
- Quanti-Tray® (counts up to 200) or Quanti-Tray/2000® (counts up to 2419)
- Colilert® or Colilert-18® Test Pack
- Sterile Vessels
- Presence/Absence Color Comparators

**\$5,000**  
Start-Up Cost



■ Sealer PLUS ■ Media + Trays ■ Sterile Bottles ■ Sealer Tray




## CERTIFICATIONS REQUIRED **NONE**

Will not be on an audit checklist

Will not need to run PT's

Will not need accreditation



During the annual/bi-annual inspection, basin engineer will be responsible for making sure it's being done appropriately

## QUALITY CONTROL RECOMMENDATIONS

- Standard Operating Procedures specific to your facility
- Initial Demonstration of Capability
- Bench sheets
- Incubator Temperature Log
- Standard/Reagent Logbook
- Comparators
- Quality Control Organisms
- Quality Control Certificates (vendor provided)
- SDS Sheets



# CITY OF MEDFORD WASTEWATER TREATMENT FACILITY



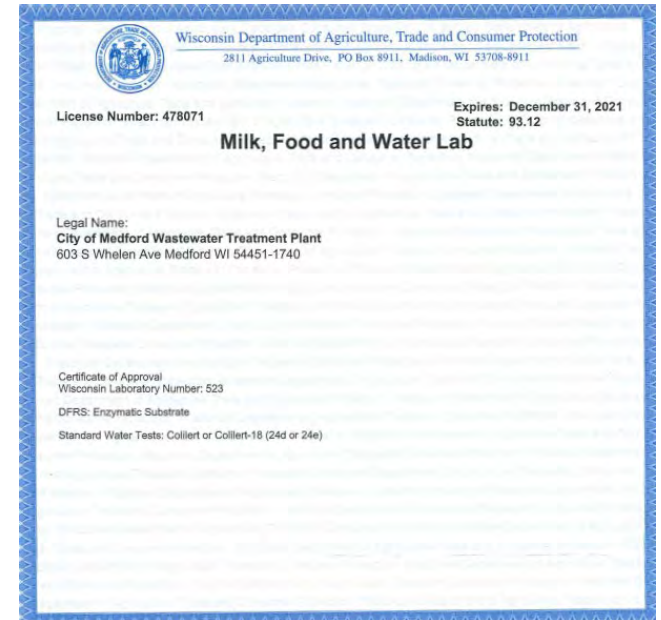
Ben Brooks-  
Superintendent

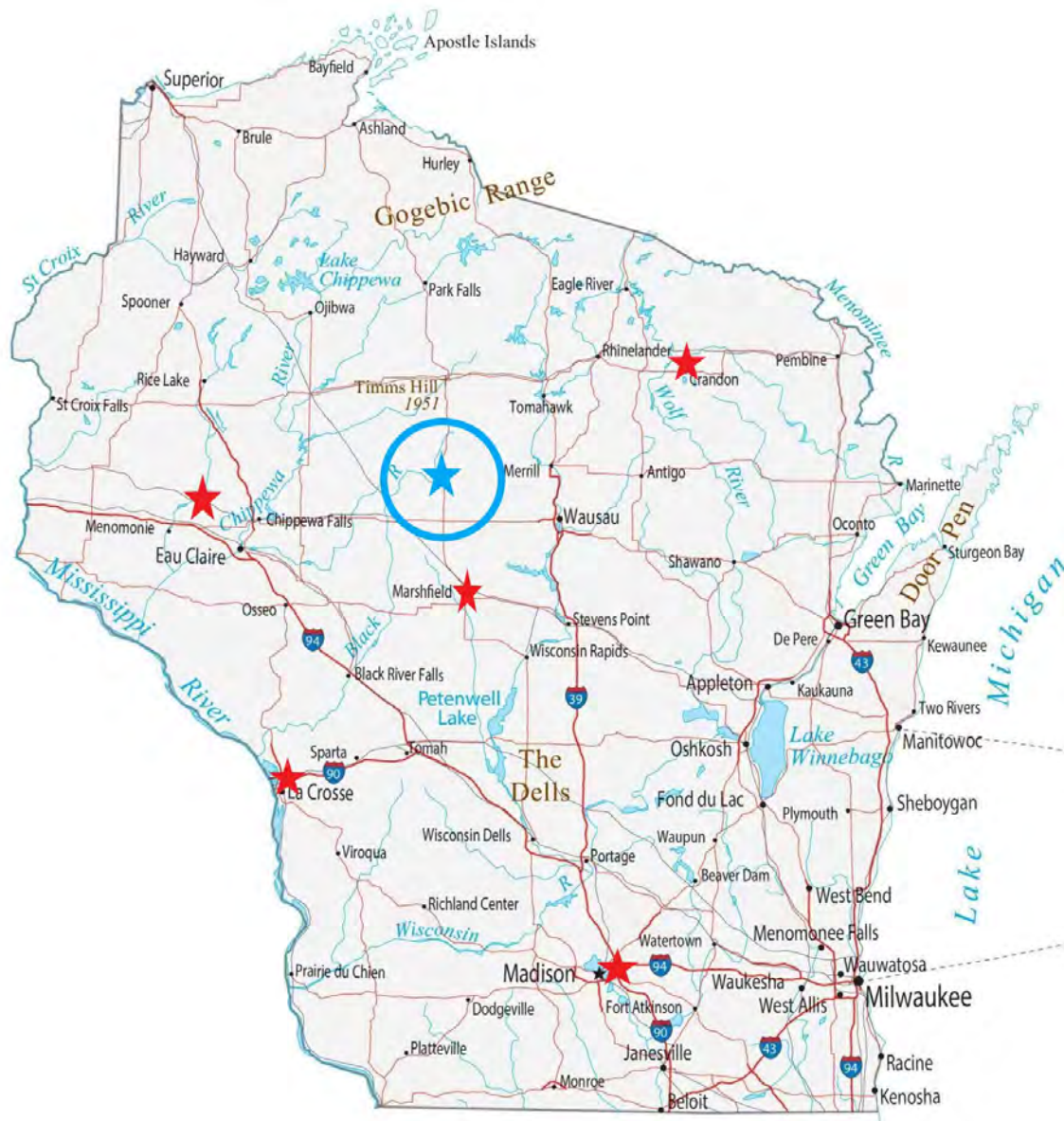


Brooke Klingbeil-  
Laboratory  
Director/Operator



Alex Zenner- Lead  
Operator





## REVENUE POTENTIAL- DRINKING WATER LAB

- 2019= \$3,488.00/81 PWS Samples
- 2020= \$7,286.00/203 PWS Samples
- 2021= \$5,330.00/69 (138) PWS Samples
- As of August 16, 2021...868 Drinking Water Samples received total.

**\$16,104**



## ASSOCIATED COSTS FOR START-UP

- WDATCP Licensing Fee Colilert® or Colilert-18®= \$350
- WSLH PT Samples= \$296
- WDNR Lab Certification Fee SDWA Nitrate Hach Method 10206= \$792
- WSLH PT Samples=\$138



# WDNR CONTACTS

- Tom Trainor  
Program Chemist – Certification Services  
Wisconsin Department of Natural Resources  
Phone: (920) 412-5970  
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## QUESTIONS?



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