



COOPERATIVE EXTENSION

UNIVERSITY OF HAWAII AT MĀNOA
COLLEGE OF TROPICAL AGRICULTURE AND HUMAN RESOURCES

Evaluating Water Remediation Options for Potential Agricultural Water Use

Update for Jimmy Nakatani

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University of Hawaii at Manoa

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FSMA Water Testing: Method 1603

- Prior to September 2017, Method 1603 was the only method that FDA has formally approved for use (in the text of the Rule)
- FDA was not willing to state whether Colilert or Colilert-18, used with Quantitray/2000 MPN format, would be approved methods for generic E. coli in 40 CFR 136.3, alongside method 1603
- Method 1603 has a 6-hour hold time from collection to delivery to the lab (8 hours to analysis)
- There is one testing lab in Hawaii that can offer this test (Manoa)
- It is possible the Recycled Water Branch also does this membrane testing method. UH CTAHR is currently evaluating this method (2016).



Difference Between Method 1603 & Colilert

- Colilert water test is a reagent based test that detects total coliform and *E. coli*
 - Used for drinking water
 - Presence vs. Absence type of test
 - Results read at 24 hours
- Method 1603
 - Recreational & waste water quality test
 - Membrane filtration method
 - Direct count of *E. Coli* in water based on the development of colonies (CFU/100 ml)
 - Within 6 hours of hold time



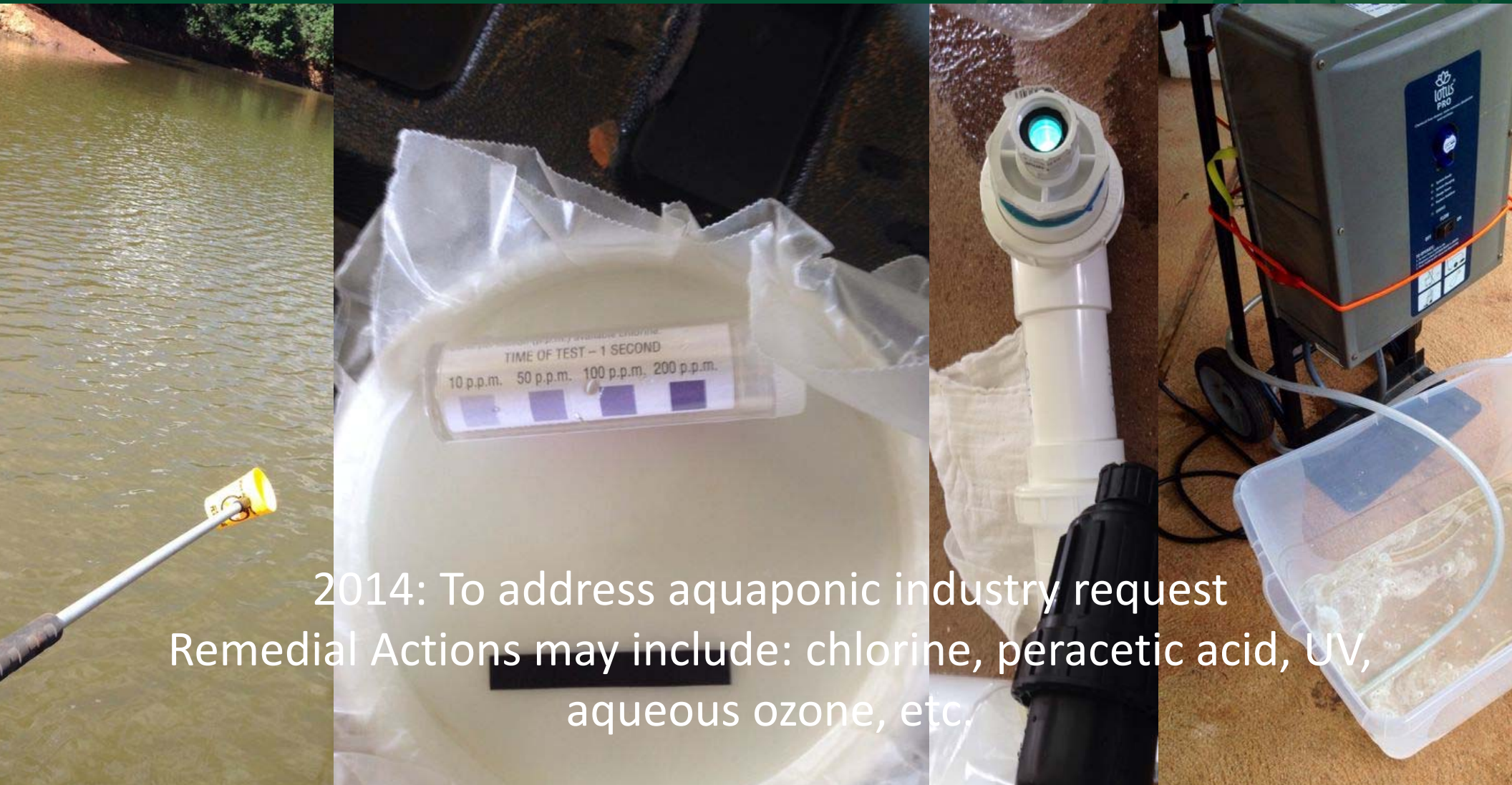
Agriculture Water Thresholds

- FSMA: EPA Recreational Water Standards
 - Geometric mean (GM) is **126 CFU or less** of generic *E. coli*/ 100 ml of water AND
 - Statistical Threshold Value (STV) is **410 CFU or less** generic *E.coli* in 100 ml/ water
- GAP Previous Audit Standards
 - Geometric mean (GM) is **126 CFU or less** of generic *E. coli*/ 100 ml of water
 - No more than **235 CFU** generic *E. coli* per 100 ml for any single water sample



Water Remediation Options

- If water does not meet FSMA criteria or exceeds thresholds, corrective action is required:
 - **Passive treatments:**
 - Longer harvest time to allow for microbes to die off in the field (die off rate (log))
 - Longer harvest time to allow for microbes to die off between harvest and end of storage
 - **Active Treatment:**
 - Treating the water



2014: To address aquaponic industry request
Remedial Actions may include: chlorine, peracetic acid, UV,
aqueous ozone, etc.



Evaluation of Remedial Water Treatments- Colilert

Treatment	Beginning E. coli	Ending E. coli	ORP
Chlorine: 200 ppm	130	1	600
Chlorine: 200 ppm	130	1	661
Chlorine: 400 ppm	130	1	620
UV	130	<1	495
Aqueous ozone: 25% 1 ppm	<200	83	523
Aqueous ozone: 50% 1 ppm	<200	6	462
Aqueous ozone: 75% 1 ppm	<200	<1	359
Aqueous ozone/ UV	130	<1	495
Peracetic acid: 3 ppm	200	<1	332

J. Sugano, J. Uyeda, S. Fukuda, and J. Odani, August 2014

2017: Can we assist growers in remediating surface water for use in food crop systems and meet FSMA's definition of agricultural water?







Evaluation of Remedial Water Treatments- Method 1603

Treatment	Beginning E. coli	Ending E. coli	ORP
Chlorine: 200 ppm (MultiChlor)	3200	0	331
Chlorine: 500 ppm (MultiChlor)	3200	0	558
Peracetic acid: 4.4 ppm (Bioside HS 15%)	3200	0	315
Peracetic acid: 19.7 ppm (Bioside HS 15%)	3200	0	398

Zero E.coli does not mean no other pathogens exist such as Cryptosporidium, Leptospirosis, Hepatitis A, Giardia intestinalis, etc. We submitted a duplicate sample set to Food Quality Lab to test for Salmonella kill rates. No Salmonella detected on the starting sample.

****NOTE:** Label does not allow for shock treatment of reservoir water. Wash water rates differ and may require a post treatment rinse with potable water. Experiment was conducted to determine if rates would kill E.coli. Label changes would need to be pursued to explore this further. **Inline treatments are currently acceptable if to be used as irrigation water for algae control, not for pathogens.**

J. Sugano, J. Uyeda, J. Silva, and L. Nakamura-Tengan- August 2017



New Equivalent Testing Methodologies Approved (9/2017)

- FDA has determined that the following methods are “scientifically valid” and “at least equivalent to the method of analysis (method 1603) in § 112.151(a) in accuracy, precision, and sensitivity[1]:
 - Method 1103.1
 - Method 1604
 - 9213 D
 - 9222 B
 - D 5392-93.
 - Hach Method 10029 for Coliforms – Total and *E. coli*
 - IDEXX Colilert® Test Kit
 - IDEXX Colilert-18® Test Kit

FDA FACT SHEET

EQUIVALENT TESTING METHODOLOGIES FOR AGRICULTURAL WATER

FDA has determined that the following methods are “scientifically valid” and “at least equivalent to the method of analysis in § 112.151(a) in accuracy, precision, and sensitivity”¹:

1. [Method 1103.1](#) - *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC) (March 2010). U.S. Environmental Protection Agency. EPA-821-R-10-002.
2. [Method 1604](#) – Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002). U.S. Environmental Protection Agency. EPA-821-R-02-024.
3. 9213 D – Natural Bathing Beaches (2007). *In: Standard Methods for the Examination of Water and Wastewater*, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).
4. 9222 B – Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. *In: Standard Methods for the Examination of Water and Wastewater*, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington, DC: American Public Health Association. (2005).

¹ 21 CFR § 112.151(b)(1).

5. D 5392-93 – Standard Test Method for Isolation and Enumeration of *Escherichia coli* in Water by the Two-Step Membrane Filter Procedure. *In: Annual Book of ASTM Standards*, Volume 11.02. ASTM International. (1996, 1999, 2000).
6. [Hach Method 10029 for Coliforms – Total and *E. coli*](#), using m-ColiBlue24® Broth PourRite Ampules.
7. [IDEXX Colilert® Test Kit](#), but only if using IDEXX Quanti-Tray/2000 for quantification.
8. [IDEXX Colilert-18® Test Kit](#), but only if using IDEXX Quanti-Tray/2000 for quantification.

For more information:

- [FSMA Final Rule on Produce Safety](#)

Have you seen our Blog? [FDA Voice](#)



The FDA, an agency within the U.S. Department of Health and Human Services, protects the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, and products that give off electronic radiation, and for regulating tobacco products.



Wash Water Sanitizer Options for Growers

	Brand Name	Active Ingredients	Company Contact	Where to Buy	Label Instructions	Desired ppm Active Ingredient ¹	Amount per 100 gal. Tank	Standard Operating Procedure ²
Peroxyacetic acid	Tsunami 100 EPA# 1677-64	11.2% Hydrogen peroxide, 15.2% Peroxyacetic acid	Ecolab Inc. Institutional Division 370 N. Wabasha St. St. Paul, MN 55102 1-800-352-5326	contact - Ecolab 4 gal ~ \$110	36-575 ppm total product (5-80 ppm PAA)	80 ppm PAA (FDA regulation)	6.7 oz \$1.45/ use	1. Remove soil 2. Prepare 80 ppm solution 3. Submerge produce 4. Monitor PAA level with strips, adjust as needed 5. Contact time ~ 90 s 6. Drain and dry
	SaniDate 5.0 EPA# 70299-19	23% Hydrogen peroxide, 5.3% Peroxyacetic acid	BioSafe Systems, LLC 22 Meadow St. East Hartford, CT 06108 1-888-272-3089	Johnnys.com 2.5 gal ~ \$159 Helena Chemical Co. 5 gal ~ \$280 Crop Prod. Serv. 5 gal ~ \$218	462-1636 ppm total product (24-85 ppm PAA)	80 ppm PAA (FDA regulation)	19.3 oz \$9.59/ use	
	VigorOx 15 F&V EPA# 65402-3	15% Peroxyacetic acid, 10% Hydrogen peroxide	FMC Corporation - Peroxygens Division 1735 Market Street Philadelphia Pennsylvania 19103		30-45 ppm PAA	80 ppm PAA (FDA regulation)	6.83 oz \$1.48/ use	
Chlorine	Clorox Regular Bleach EPA# 5813-50	6% Sodium Hypochlorite	The Clorox Company P.O. Box 493 Pleasanton, CA 94566	online - amazon.com -Walmart ~ \$3.50	25 ppm available chlorine	25 ppm free chlorine (label)	5.33 oz \$0.55/ use	1. Remove soil 2. Prepare 25 ppm 3. Submerge produce 4. Monitor Cl levels 5. Monitor pH 6. Monitor turbidity 7. Rinse required
	Clorox Germicidal Bleach EPA# 5813-100	8.25% Sodium Hypochlorite	The Clorox Company P.O. Box 493 Pleasanton, CA 94566	online - amazon.com 4.5 gal ~ \$27.95 -Walmart ~ \$32	25 ppm available chlorine	25 ppm free chlorine (label)	3.88 oz \$1.02/ use	

¹Suggestion based on recommendations for chlorine, see publication by Trevor Suslow for various commodities

<http://ucfoodsafety.ucdavis.edu/files/26414.pdf>

²Sample standard operating procedure

-Authors: Marie Lawton and Amanda Kinchla



Ran Multi-dose at UH Microbiology Lab (9/14/17)

Ran under controlled environment at UH Microbiology Lab (Dr. Yong Li)

Starting E. Coli Colony		3137CFU
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(251 CFU / 10 ml)

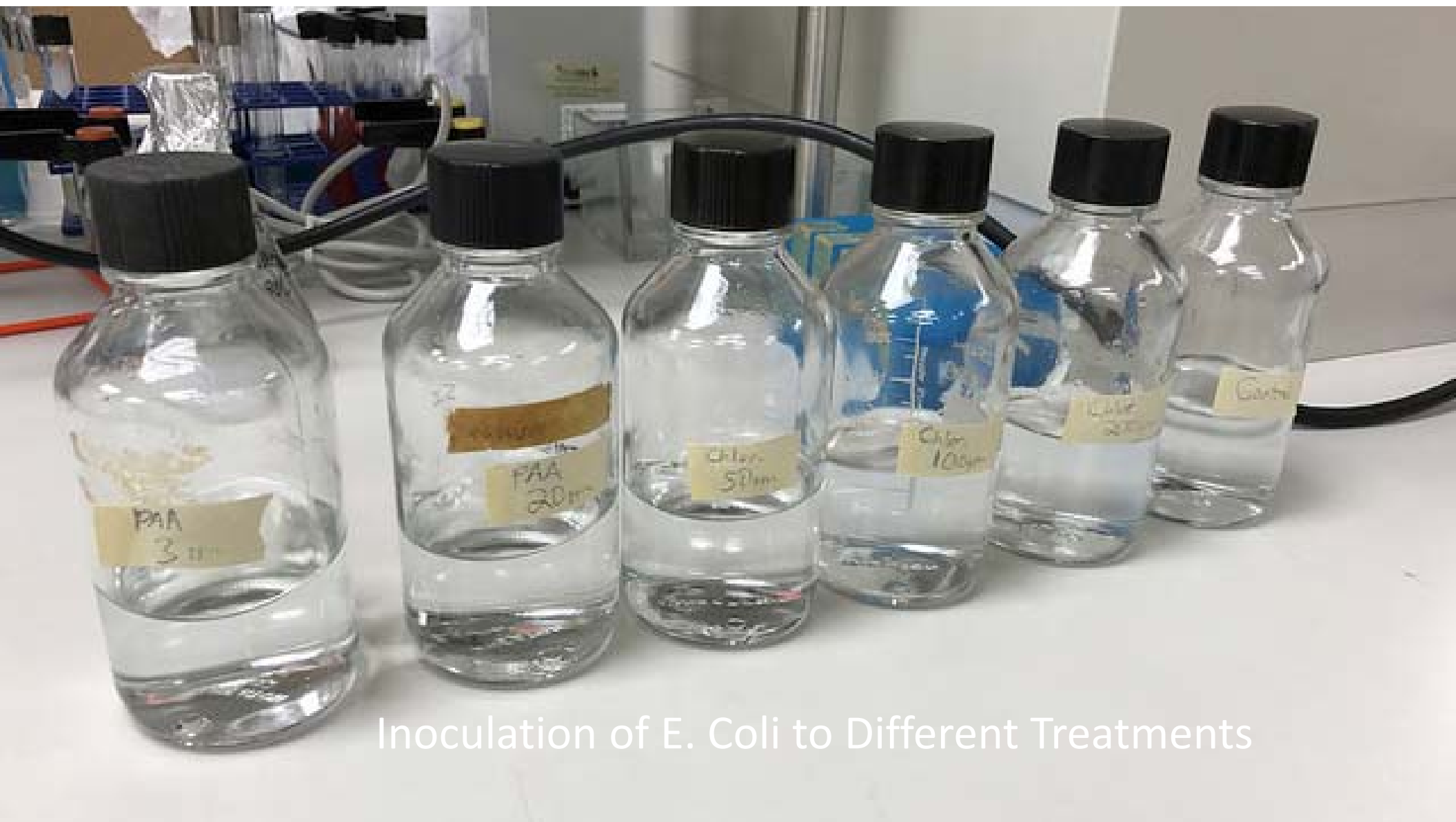
251 + 12.5

Ending E. Coli after 15 minutes

Method 1603

1) Treated with 50 ppm Chlorine		0
2) Treated with 100 ppm Chlorine		0
3) Treated with 200 ppm Chlorine		0
4) Treated with 3 ppm PAA		0
5) Treated with 20 ppm PAA		0
6) Control		3137 CFU

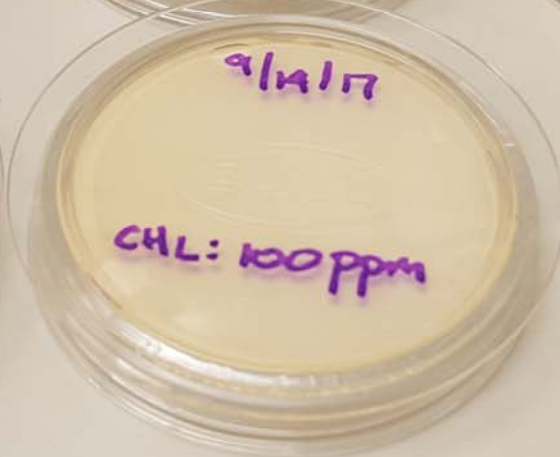
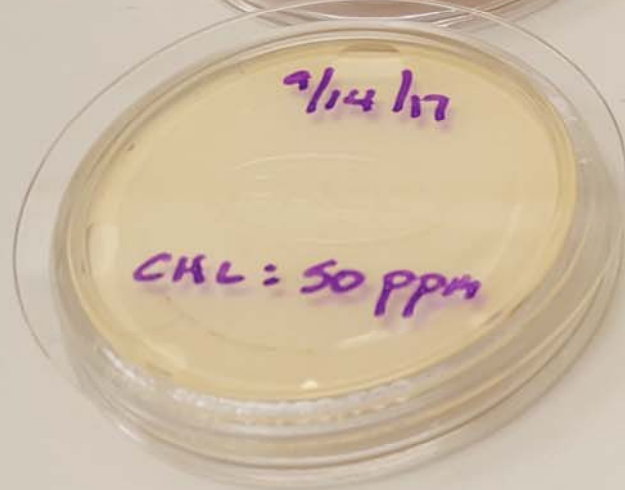
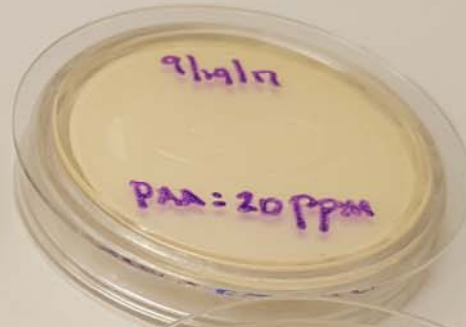
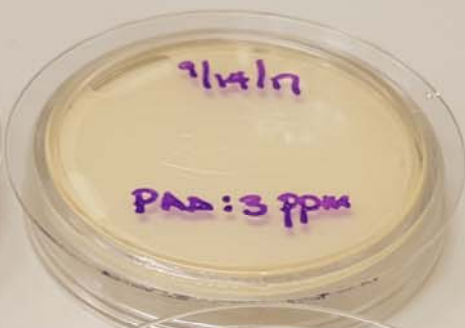




Inoculation of E. Coli to Different Treatments

Method 1603: Membrane Filtration Method







Counting Coliform Fecal Units (CFU) Indicates Presence of Fecal Material



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Proposal: Drop surface water E.coli levels to zero

Hypothesis #1: 200 ppm is not suffice to eliminate E.coli in surface water to 0

Hypothesis #2: 3 ppm of peracetic acid is not suffice to reduce E. Coli to 0

Collection: 8/9/17, Method 1603

Start Time: 9:05 am, end time: 10:48 am

**Note: current label does not allow for shock treatment of reservoir water. Wash water rates differ.

Experiment was conducted to determine if rates would kill E.coli. Label changes would need to be pursued to explore this further

In line treatments are acceptable for algae not pathogen control

In line treatments are acceptable for algae not pathogen control		Method 1603	FQL	FQL		
Extracted water into a trash can and spiked with 2 bird droppings		PH	ORP	E.coli	Total Colif	E.coli
Lake Wilson Water	1) Lake Wilson Water (spiked)	7.5	331	3200	>2,419.6	>2,419.6
	Collect 30 gallons (Lake Wilson Water)			SPIKED		
	Test starting E.coli levels	Temp: 30.9 °C				
	125 ml	NATURAL Lake Water		10	>2,419.6	10.2
		*re-did tested at UH				
200 PPM (CL)	2) Treated with 200 ppm Multichlor	Chlorine Strip				
	24 hours later chlorine reading	9.8	588	0	<1	200+
	8/30/17: Chlorine reading					200+
Rate: 1 ounce into 5 gallon water: Temp: 28.7 °C						100
500 ppm	3) Treat with 500 ppm Multichlor (take 2 samples)	Chlorine Strip				
	24 hours later chlorine reading	10.2	584	0	<1	200+
	8/30/17: Chlorine reading					200+
Rate 2.5 oz into 5 gallons: Temp: 28.4 °C						200+
4.4 ppm (PAA)	4) Treat with 4.4 ppm Peracetic acid	PAA Meter				
	Temp: 28.5 °C	7.1	315	0	435.2	4.4
20 ppm (PAA)	5) Treat Peracetic acid at 20 PPM					
	Temp: 28.5 °C	4.6	398	0	<1	19.7

*** Zero E.coli does not mean no other pathogens exist such as Cryptosporidium, Leptospirosis, Hepatitis A, Giardia intestinalis, etc. We submitted same sample set to Food Quality Lab to test for Samonella kill rates. No samonella was present at the start from Lake Wilson Water

We conclude 200 PPM (or less) of Multiclor or 20 PPM of PAA (or less) maybe ample to reduce E. coli levels in surface water to zero, depending on H2O turbidity. Under FSMA, ag water only has to meet the criteria below. We wanted to evaluate the potential of water sanitation treatment options for growers Geometric mean (GM) is **126 CFU or less** of generic *E. coli* / 100 ml of water AND Statistical Threshold Value (STV) is **410 CFU or less** generic *E.coli* in 100 ml/ water We conducted a multi dose trial in a controlled lab environment to test this theory further. Additional funding is needed to replicate this work and evaluate new label changes

9/14/2017
Ran under controlled environment at UH Microbiology Lab (Dr. Yong Li)

Starting E. Coli Colony 3137 CFU
(251 CFU / 10 ml)
251 + 12.5

Ending E. Coli after 15 minutes

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- 1) Treated with 50 ppm Chlorine
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- 3) Treated with 200 ppm Chlorine
- 4) Treated with 3 ppm PAA
- 5) Treated with 20 ppm Chlorine
- 6) Control

0
0
0
0
0
3137 CFU