

Report on  
**Agricultural Water Testing Methods**  
Colloquium

Colloquium sponsored by



Facilitated by



and



Report prepared by



Final Release 8.2.17

**April 6 & 7, 2017**  
at  
Western Growers  
15525 Sand Canyon Irvine, CA 92618

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*With special thanks to the Dr. Martha Roberts, Dr. Michelle Danyluk and Dr. Trevor Suslow for preparatory, background and technical work to support the Colloquium.*

**A**gricultural (ag) water applied during growing, harvesting, and postharvest handling activities associated with fresh produce may serve as a vehicle to contaminate fresh produce, if that agricultural water is contaminated with human pathogens.

Water is recognized as a potential conduit for microbiological hazards. Determining the means to accurately and effectively assess agricultural water microbial quality and the risk associated with its use has been the topic of much discussion among produce safety experts within industry, regulatory, and public health communities. Since the FDA's publication of the final FSMA Produce Safety Rule in November 2015, these discussions have continued in earnest as growers work to implement the Rule in advance of pending compliance dates. Divergent views have emerged in relation to the standards, mandated practices, and recognized technical methods FDA included in the Rule. The Produce Safety Rule's agricultural water provisions require that produce farms characterize the microbial quality of agricultural water (as defined in the Produce Safety Rule) to minimize the risks related to its use. The FDA has recently announced that it will be "revisiting" the current ag water requirements and will extend the compliance dates for the Produce Safety Rule's ag water provisions (FDA, 2017). The FDA has not yet outlined the agency's scope, course of action, process or timeline regarding this reassessment.

The current FDA agricultural water testing requirements and the methods associated with this testing are of significant concern for produce growers due to costs and lack of available laboratories to perform the required method. Industry has significant concerns regarding FDA's selection of the analyte, method, and sampling requirements for ag water in the Produce Safety Rule. The most pressing issue that challenges implementation of and compliance with the current Produce Safety Rule's water testing provisions is the requirement to use the U.S. Environmental Protection Agency's (U.S. EPA) Method 1603 (M1603) or an equivalent method, to analyze ag water for generic *Escherichia coli* (*E. coli*), the most commonly used indicator of fecal contamination.

The fact that FDA has not acknowledged other methods currently accepted by US EPA as equivalent methods for the enumeration of generic *E. coli* and with no clarification of standard for assessing "equivalent methods," growers are struggling to complete ag water testing, as required by the Produce Safety Rule and produce buyers. Although extensive testing of water sources and distribution systems for

# Introduction

generic *E. coli* has been routinely conducted by the fresh produce industry, in many cases using one of the methods recognized by US EPA as equivalent, laboratories serving the fresh produce industry have not traditionally used M1603 to enumerate generic *E. coli* in ag water. This has caused a dilemma in produce growing regions about how to test ag water so as to ensure compliance with the Produce Safety Rule requirements. Changing analytical methods not only devalues a company's historical water quality data produced using US EPA-approved methods other than M1603 and disconnects it from future data to be collected using different methodologies, but also creates a challenge for testing laboratories, most of which are not equipped to perform method M1603.

Experts in academia, extension, industry, and the national network of Produce Safety Alliance instructors and lead instructors are receiving input from constituents about the difficulties in moving forward. Changing water-testing methods from readily available, relatively inexpensive tests to M1603 would result in increased training and analytical costs, impractical (or even impossible) logistics, and limited availability of lab services in many regions. In the absence of other more practical test methods officially recognized as meeting equivalency criteria, the current standards will be especially burdensome for many mid- to small-size producers because of the reasons listed above. Implementation and compliance are also a significant concern among foreign produce suppliers and import industries.

In an effort to address the questions surrounding ag water testing methodologies, a panel composed

of experts from academia (in particular cooperative extension), government, and industry was convened by the Center for Produce Safety (CPS)<sup>1</sup>. Key FDA produce safety subject matter experts also attended to provide technical assistance. Sponsored by CPS and facilitated by Western Growers and the UC Davis Postharvest Technology Center, the panel met for a day and a half at Western Growers' headquarters in Irvine, CA, to review water monitoring data, develop a shared understanding of implementation issues, and evaluate the applicability of available water testing methods to the Produce Safety Rule requirements for public health protection. Panel members reviewed peer-reviewed research and agreed that using the current list of US EPA's approved method(s) for analyzing microbiological hazards in recreational water provided an immediate path forward for agricultural water quality testing. In parallel to this industry-led effort, the FDA continues their review of the ag water standards for guidance development and/or revision. The FDA recognized the urgent need for industry to move forward considering both impending compliance deadlines and demands from buyers and encouraged the ag water experts to address industry's questions and develop recommendations pertaining to ag water testing methods.

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<sup>1</sup> CPS has funded a significant amount of research on agricultural water summarized in a June 2014 CPS [five-year research review](#) specifically detailing the agricultural water research findings. This CPS report provides a review of the scientific body of knowledge and places research results into context for industry. Highlighting how CPS-funded research has added significantly to the ag water body of knowledge, the report also details research needs and provides excellent technical background information on this issue.

## MEETING PURPOSE

The overarching goal of the meeting was to provide a forum where relevant data could be presented to improve understanding of ag water-related risk and risk mitigation and to recommend ag water testing methods that would be comparable in public health protection to M1603. The intended meeting outcome was to provide recommendations of equivalent test methods for generic *E. coli* enumeration. It was agreed that any recommended method would also support the required microbial water quality profile (MWQP) and permissible die-off interval between application and harvest/storage calculations as mitigation for noncompliant test results. An additional purpose was to inform key FDA staff as they develop industry guidance and consider changes to the water provisions in the Produce Safety Rule.

The meeting also served the purpose of:

1. Ensuring FDA's technical staff and leadership are aware of, and have opportunity to discuss, current



data that will inform any recommendations for re-assessment of recognized test methods.

2. Providing an opportunity to review current US EPA approved methods for the analysis for generic *E. coli* in water
3. Developing an industry consensus for addressing test method variance.
4. Identifying priority research areas in ag water test methods.

In addition, the panel sought to provide state departments of agriculture, through the National Association of State Departments of Agriculture, with subject matter experts' input on current ag water data sets and practical issues of implementation and compliance with current recognized test method restrictions.

## Variability in Ag Water Quality

Many produce growing regions have been conducting extensive water quality monitoring projects to better understand and define factors that influence the microbial quality of their ag water sources. During the first day's proceedings, panel members gave short presentations of their research findings related to ag water monitoring projects in growing regions in Arizona, California, Florida, Georgia, and Washington. The consistent message coming from these research findings as well as published research in all growing regions is the high variability in microbial water quality data (Benjamin, 2013; McEgan, 2013; Sbodio, 2013; Topalcengiz, 2017). Microbial water quality consistently varies among water sources in each growing region and trends are not easily correlated with critical factors. Some study findings suggest higher false-positive rates for generic *E. coli* when temperatures are cooler (i.e., during late fall and winter) (McLain, 2008 & 2011). Researchers also consistently reported high temporal variability in water samples – for example in samples taken in the morning compared to samples taken in the afternoon from the same location (relevant [CPS research reports](#) are available [here](#) and [here](#)). The variability of water

*...although method comparison results varied among investigators, neither data variability nor differences among methods have translated into significant differences in relation to meeting Produce Safety Rule water quality requirements or permissible mitigation provisions.*

monitoring data, the innumerable factors that affect microbial levels, and an incomplete understanding of how they affect microbial levels have made model development likely impossible (Partyka, 2017).

Several water monitoring studies also compared test methods where samples were applied to generic *E. coli*-specific growth media on plates and colonies are counted after an incubation period (CFU results) compared to more commonly used methods where generic *E. coli* are detected via fluorogenic development (e.g., Colilert®) and estimated using [most probable number](#) (MPN) calculations. Many of these studies comparing commonly used MPN methods to M1603 (or similar methods) suggest that MPN methods are more conservative, resulting in greater estimated populations in replicated paired samples (Brassill, 2013; Rock, unpublished; Suslow, unpublished). In addition, many of these studies indicated that fluorogenic based *E. coli* detection methods are better able to handle highly problematic or turbid irrigation water samples where the M1603 is unable due to filter clogging resulting in the need for increased sample numbers and dilutions.

Numerous peer-reviewed papers comparing CFU and MPN methods for surface water also demonstrate that the differences in paired samples are not typically significant although MPN methods often have a higher generic *E. coli* estimated viable count than CFU methods (Buckalew, 2006; Budnick, 2001; Cowburn, 1994; Fricker, 2010; Gronewold, 2008; Hargett, 2004). Therefore, although method comparison results varied among investigators, neither data variability nor differences among methods have translated into significant differences in relation to meeting Produce Safety Rule water quality requirements or permissible mitigation provisions.

### **Ag Water Regulations**

The FDA's ag water microbial standards are based on the US EPA's recreational water quality standards used to protect the health of swimmers and those engaged in other recreational activities that bring them in direct contact with water. Because the FDA has adopted the US EPA's criteria for measuring generic *E. coli* levels in recreational water for measuring generic *E. coli* in agricultural water, discussions included how these two water types compare.

Recreational water quality is monitored to protect individuals who are utilizing those waters for activities that involve full body contact, such as swimming. For large water bodies such as rivers, reservoirs and lakes, public health risk due to recreation arises from exposure to waterborne pathogens via direct human contact (e.g., dermal or ingestion) with the water. Agricultural water is different because it comes from both surface and ground water sources and may flow through various canals and channels

before it arrives at the point of use. Since ag water is commonly located in mixed agricultural production areas, it may contain runoff from ag production surfaces and contain different types and concentrations of nutrients that create potentially unique chemistries. How these chemistries affect *E. coli* survival, growth, and detection is known to some degree, but is also highly variable in different regions and climates. One known impact from ag-runoff is an increase in false positives during *E. coli* enumeration compared to many other water sources. Research has shown that, depending on the test method, false positive rates as high as 45% may be common in some growing regions (Rock, 2015; McLain, 2008; McLain, 2011). The risk to public health from ag water is quite different from recreational water in that consumers do not have direct contact with the water, but are exposed via ingestion of produce crops that have been contacted by ag water. In the Produce Safety Rule, the FDA appropriately recognized ag water distinction and developed corrective measures to allow for environmental and post-harvest die-off of bacteria.

The Produce Safety Rule defines *agricultural water* as:<sup>1</sup>

*“water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces, including*

*water used in growing activities (including irrigation water applied using direct water application methods, water used for preparing crop sprays, and water used for growing sprouts) and in harvesting, packing, and holding activities (including water used for washing or cooling harvested produce and water used for preventing dehydration of covered produce).”*

The Rule regulates the microbial quality of water that is:<sup>2</sup>

- Intended to, or is likely to, contact the plants’ harvestable portions or food contact surfaces during growing, harvesting, packing, and holding activities
- Used to wash hands during and after harvest activities

### Current Ag Water Testing Methods

Produce growers recognize that agricultural water used in the production environment may present a food safety risk and many have been testing their ag water for years. Water testing is commonly required by Good Agricultural Practices (GAP) audits and is generally a buyer requirement. Laboratories in major growing regions serving those communities are using test methods such as the Quanti-Tray Systems® where results are reported as MPN (Kinzelman, 2005; Sutton, 2010). These methods have been widely used by growers for many years, are recognized as acceptable

by US EPA, and, therefore, the panel contends that the experience and data amassed generate high confidence and wide comparability across broad regions using those methods.

### US EPA Method 1603

As currently written, the Produce Safety Rule requires ag water quality to be tested using the US EPA’s [Method 1603](#) or alternatively -

1 § 112.3 What definitions apply to this part?



2 § 112.44 What specific microbial quality criteria apply to agricultural water used for certain intended uses?

*Water testing is commonly required by Good Agricultural Practices (GAP) audits and is generally a buyer requirement.*

*“a scientifically valid method that is at least equivalent...in accuracy, precision, and sensitivity”.*<sup>3</sup> M1603 was originally designed as a test for drinking water, but is applied to ambient environmental water and treated wastewater. The procedure provides a direct count of generic *E. coli* in water based on the development of colonies growing on the surface of a membrane filter overlaying a semi-selective and differential agar media. After the membrane is incubated at two specific temperatures for specified time periods, those colonies appearing red or magenta in color are counted as presumptive generic *E. coli* and reported as [colony-forming units](#) (CFU) (US EPA, 2009). M1603 requires specified multiple colony verification protocols using standard determinative taxonomic testing. Additionally, M1603 requires highly trained technical staff and maintains a considerable amount of subjectivity in evaluation to address false positives.

**Precision** is the quantitative reproducibility and measurement consistency of a test method. **Accuracy** is the trueness of a result to a known or standardized value. The technical panel members discussed multiple datasets which highlight the variability of replicated samples from single surface water sources. The panel concluded that **this high data variability largely negates the technical requirement for equivalent precision and accuracy across already recognized and characterized test methods.**

Practical and logistical problems with Method 1603 include:

- The current shortage of laboratories that perform this method or are able to perform it according to US EPA’s instructions. It is estimated that few private laboratories currently can run this test.
- Requirement for a maximum 8 hour pre-test interval for validity. Samples must be kept chilled and delivered to the laboratory within 6 hours of collection so analysis can begin within 8 hours of collection. This is highly impractical and unachievable in many parts of the U.S. due to the limited commercial availability of the method and the remote locations of many farming communities worldwide.
- A complex protocol requiring increased sample handling i.e., dilutions, filter rinsing, two incubation periods, etc.
- Greater need for duplicates/replicates to adhere to validity requirements.
- Requires access to equipment (e.g., water bath; multiple incubators) and skills not commonly used by laboratories analyzing ag water.

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<sup>3</sup> § 112.151 What methods must I use to test the quality of water to satisfy the requirements of § 112.46? In Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption

- Subjective analysis is required in determining what is a colony; introduces greater chance for technician error.
- Increased cost as it is significantly (projected as 2-3 times) more expensive for laboratories to perform. The increased costs include an increased level of operator training and proficiency and associated base-salary scale. Increased costs are also associated with colony-verification requirements, which can be time-consuming (enzyme and gas production testing), or can involve high-tech molecular laboratory equipment.
- Clogged filters if water sample is highly turbid. Sediments common in ag water may require multiple filters for one sample and are known to increase difficulty in diagnostic colony color development.

The Produce Safety Rule language is intended to provide flexibility in allowable methods for testing

ag water for generic *E. coli*, but the current realities of individual laboratory capability in effect negates this stated flexibility on test method options. For reasons listed in the above bullets, flexibility is particularly questionable for cultural methods with reportable test units of CFU per 100 ml sample. Standard colony enumeration is itself, at best, an estimate of viable populations on a selective and differential media. These visually counted colonies may have originated from single cells to small aggregates of cells, thus making a statistical argument for stringent numerical equivalency in comparative tests impractical. By extension from this knowledge, implementation of corrective measures necessitated under the Produce Safety Rule by differences in tens or twenties of estimated generic *E. coli* in a single test method is not supported by available science. Growers are also concerned that the Rule states the FDA will not pre-approve the use of a different method, leaving



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the proof of equivalency burden on the grower. To date, the FDA has not provided specific criteria or guidance for establishing method equivalency, with the effect that growers are left in regulatory jeopardy if they use an alternative method and the FDA rejects their rationale regarding equivalency during an inspection. Moreover, growers will be unable to utilize the post irrigation die-off equation and the ag water calculators without the recognition of quantitative method equivalency.

Within the current state-of-the-science no one method is perfect. Although the FDA has not defined equivalency, **the US EPA has an established process in place for determining equivalency and has evaluated and deemed several other methods to be equivalent in measuring *E. coli* in 100 mL water samples** (Parshionikar, 2009). These methods, published in the Code of Federal Regulations, are as follows (adapted from [40 CFR 136.3 Table IA](#)) (See Table 1 on the following page)

### Conclusions

Key panel findings such as “the high variability of *E. coli* populations in ag water largely negates the technical requirement for equivalent precision and accuracy across already recognized and characterized test methods” and “the EPA has an established process in place for determining equivalency and has evaluated and deemed several other methods to be equivalent in measuring *E. coli* in 100 mL water samples” coupled with the FDA’s stated intent to extend compliance dates for the Produce Safety Rule’s ag water provisions, develop and publish regulatory guidance, and reexamine the water provisions the panel issues the following statements and recommendations for close consideration and use by growers, researchers and FDA:

### Panel Statements

- An evaluation of the microbial quality of ag water used on produce during growing and harvesting operations is an important part of a systems based approach at reducing the risk of foodborne illness related to produce consumption.
- An abundance of regional water test data, published comparative studies, and extensive equivalency assessments evaluated by US EPA, provide support for FDA to recognize and/or adopt the US EPA’s approved methods and definitions of equivalency.
- Water monitoring research has produced a preponderance of evidence indicating that MPN test methods are often more conservative at estimating *E. coli* populations than membrane filtration methods.

**Table 1. List of US EPA Approved Methods for Enumeration of *E. coli* in Water Samples**  
(adapted from [40 CFR 136.3 Table IA](#))

Water	Method	US EPA	Standard methods	AOAC ASTM	Other
Wastewater, Ambient	MPN multiple tube,		<a href="#">9221B.1 followed by 9221E</a> for presumptive positives		
Wastewater, Ambient	Multiple tube/multiple well		<a href="#">9223B</a> – Enzyme substrate test	<a href="#">991.15</a>	Colilert® Colilert-18®
Wastewater, Ambient	Membrane filtration, single step	1603			mColiBlue-24®
Ambient	Membrane filtration, single step	1604			mColiBlue-24®
Ambient	Membrane filtration, two step	1103.1	<a href="#">9222B followed by 9222G, 9213D</a>	<a href="#">D5392</a>	



## Panel Recommendations

- We recommend that FDA issue clarifying language publicly, as soon as possible, to convey the FDA acceptance of the US EPA's list of approved methods (as noted in [40 CFR 136.3 Table IA](#)) to be equivalent to M1603 in meeting scientifically valid criteria for adequately protecting public health. This will provide the flexibility intended in the Final Produce Safety Rule.
- We urge the incorporation and use of MPN calculations within the available Microbial Water

Quality Profile auto-calculator spreadsheets for untreated surface and groundwater (<http://wafs.ucdavis.edu/>; <https://cals.arizona.edu/fps/node/57/>; <http://agwater.arizona.edu/onlinecalc/>; <http://agwater.arizona.edu>).

- We recommend that language in the Produce Safety Rule describing recognized alternative test methods be revised to remove the expectation of equivalency and substitute 'comparable and adequate for the purpose of public health protection' or that FDA issue draft guidance indicating this interpretation of equivalency.

Center for Produce Safety. 2014. Agricultural water - Five year research review. <http://www.centerforproducesafety.org/amass/documents/document/247/CPS%20Ag%20Water%20Research%20Report%202014%20with%20corrections%201.1.pdf>

Produce Safety Alliance: The Water Analysis Method Requirement in the FSMA Produce Safety Rule <https://producesafetyalliance.cornell.edu/sites/producesafetyalliance.cornell.edu/files/shared/documents/Water-Analysis-2017.pdf>

Produce Safety Alliance: FSMA Produce Safety Rule water requirements: Insights to get you organized! <https://producesafetyalliance.cornell.edu/resources/educational-materials/fsma-produce-safety-rule-water-requirements-insights-get-you-organized>

Western Growers – FSMA Portal: Produce Safety Rule <https://www.wga.com/resources/produce-safety-rule>

### Ag Water Calculators

Ag water application and online calculator available at the University of Arizona's Fresh Produce Safety: Information from Farm to Fork: <http://cals.arizona.edu/fps/node/57/>

Microsoft Excel calculator tool available at the Western Center for Food Safety: <http://wcfs.ucdavis.edu/>

### US EPA Documents

U.S. EPA. 2009. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* Agar (Modified mTEC) [https://www.epa.gov/sites/production/files/2015-08/documents/method\\_1603\\_2009.pdf](https://www.epa.gov/sites/production/files/2015-08/documents/method_1603_2009.pdf)

U.S. EPA. 2002. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI medium) <https://nepis.epa.gov/Exe/ZyPDF.cgi/P1002D57.PDF?Dockkey=P1002D57.PDF>

U.S. EPA. 2010. Method 1103.1: *Escherichia coli* (*E. coli*) in water by membrane filtration using membrane-Thermotolerant *Escherichia coli* agar (mTEC). [https://www.epa.gov/sites/production/files/2015-08/documents/method\\_1103-1\\_2010.pdf](https://www.epa.gov/sites/production/files/2015-08/documents/method_1103-1_2010.pdf)

U.S. EPA. 2012. Recreational water quality criteria: <https://www.epa.gov/wqc/2012-recreational-water-quality-criteria-documents>

# Resources

Code of Federal Regulations, Title 40, Part 136 – Guidelines establishing test procedures for the analysis of pollutants <https://www.ecfr.gov/cgi-bin/text-idx?SID=8b77d4b2377934941b41c3dfa10dbf02&mc=true&nde=pt40.25.136&rgn=div5>

## Citations

- Benjamin L, Atwill ER, Jay-Russell M, Cooley M, Carychao D, Gorski L, Mandrell RE. 2013. Occurrence of generic *Escherichia coli*, *E. coli* O157 and *Salmonella* spp. in water and sediment from leafy green produce farms and streams on the Central California coast. *International Journal of Food Microbiology*, 165(1):65-76. <https://www.ncbi.nlm.nih.gov/pubmed/23697918>
- Brassill N. 2013. The assessment of *Escherichia coli* as an indicator of microbial quality of irrigation waters used for produce. Master's thesis, University of Arizona. Available at: <http://arizona.openrepository.com/arizona/handle/10150/293596>
- Buckalew D, Hartman L, Grimsley G, Martin A, Register K. 2006. A long-term study comparing membrane filtration with Colilert defined substrates in detecting fecal coliforms and *Escherichia coli* in natural waters. *Journal of Environmental Management*, 80:191-197. <http://www.sciencedirect.com/science/article/pii/S0301479705002859>
- Budnick GE, Howard RT, Mayo D. 2001. Comparison of Colilert-18 to the mTEC agar method for the enumeration of *Escherichia coli* in recreational waters. Proceedings from the American Society of Microbiologists Annual Convention May 2001. <https://www.idexx.com/resource-library/water/water-reg-article6l.pdf>
- Cowburn JK, Goodall T, Fricker EJ, Watler KS, Fricker CR. 1994. A preliminary study of the use of Colilert for water quality monitoring. *Applied Microbiology*, 19(1):50-52. <http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.1994.tb00902.x/abstract>
- FDA. 2017. FDA considering simplifying agricultural water standards. <https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm546089.htm>
- FDA. 2017. FDA Intends to Extend Compliance Dates for Agricultural Water Standards. <https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm561844.htm>
- Fricker CR, Warden PS, DeSarno M, Eldred BJ. 2010. Significance of methods and sample volumes for *E. coli* and total coliform measurements. Water Research Foundation. <http://www.waterrf.org/publicreportlibrary/4024.pdf>
- Gronewold AD, Wolpert RL. 2008. Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration. *Water Research*, 42(13):3327-3334. <https://www.ncbi.nlm.nih.gov/pubmed/18490046>
- Hargett E, Goyn L. 2004. Modified mTEC agar, Colilert®, and M-FC agar – field trial comparison of bacteria enumeration methods in surface waters of eastern Wyoming. [http://deq.wyoming.gov/media/attachments/Water%20Quality/Surface%20Water%20Monitoring/Publications/wqd-wpp-monitoring\\_comp\\_study\\_e\\_coli\\_final2.pdf](http://deq.wyoming.gov/media/attachments/Water%20Quality/Surface%20Water%20Monitoring/Publications/wqd-wpp-monitoring_comp_study_e_coli_final2.pdf)

- Kinzelman JL, Singh A, NgC, Pond KR, Bagley RC, Gradus S. 2005. Use of IDEXX Colilert-18® and Quanti-Tray/2000 as a rapid and simple enumeration method for the implementation of recreational water monitoring and notification programs. *Lake and Reservoir Management*, 21(1):73-77. <http://www.tandfonline.com/doi/pdf/10.1080/07438140509354414>
- McEgan R, Mootian G, Goodridge LD, Schaffner DW, Danyluk MD. 2013. Predicting *Salmonella* populations from biological, chemical, and physical indicators in Florida surface waters. *Applied and Environmental Microbiology*, 79(13):4094-105. <http://aem.asm.org/content/79/13/4094.full>
- McLain JE, Rock CM, Lohse K, Walworth J. 2011. False-positive identification of *Escherichia coli* in treated municipal wastewater and wastewater-irrigated soils. *Canadian Journal of Microbiology*, 57(10):775-84. <https://www.ncbi.nlm.nih.gov/pubmed/21936679>
- McLain JE, Williams CF. 2008. Seasonal variation in accurate identification of *Escherichia coli* within a constructed wetland receiving tertiary-treated municipal effluent. *Water Research*, 42(15):4041-8. <https://www.ncbi.nlm.nih.gov/pubmed/18674793>
- Partyka ML, Bond RF, Chase JA, Atwill ER. 2017. Monitoring bacterial indicators of water quality in a tidally influenced delta: A Sisyphean pursuit. *The Science of the Total Environment*, 578:346-356. <http://www.sciencedirect.com/science/article/pii/S0048969716323671>
- Parshionikar S, Haugland RA, Shanks O, Hunt ME, Cottrill M, Grimm A, Genthner F, Harris S, Oshiro RK, Lincoff A, Pfaller S. 2009. Method validation of US Environmental Protection Agency microbiological methods of analysis. In *The EPA Forum on Environmental Measurements* (pp. 2009-01). Available from: [http://www.iremsaglik.com/UM/EPA\\_Guide.pdf](http://www.iremsaglik.com/UM/EPA_Guide.pdf)
- Rock C. 2015. Evaluation of risk-based water quality sampling strategies for the fresh produce industry. Center for Produce Safety, Final Report. [http://www.centerforproducesafety.org/amass/documents/researchproject/365/CPS%20Final%20Report\\_Rock\\_January%202016.pdf](http://www.centerforproducesafety.org/amass/documents/researchproject/365/CPS%20Final%20Report_Rock_January%202016.pdf)
- Sbodio A, Maeda S, López-Velasco G, Suslow TV. 2013. Modified Moore swab optimization and validation in capturing *E. coli* O157:H7 and *Salmonella enterica* in large volumes of field samples of irrigation water. *Food Research International*, 51(2): 654–662. <http://www.sciencedirect.com/science/article/pii/S0963996913000306>
- Sutton S. 2010. The most probable number method and its use in QC microbiology. *Journal of GXP Compliance*, 14(4):28-33. <http://www.ivtnetwork.com/sites/default/files/QCMicrobiology.pdf>
- Topalcengiz Z, Strawn LK, Danyluk MD. 2017. Microbial quality of agricultural water in Central Florida. *PloS one*, 12(4), p.e0174889. <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0174889>
- Wohlsen T, Bayliss J, Bates J, Gray B, Johnson S, Schneider P. 2008. An evaluation of membrane faecal coliform agar and Colilert-18® for the enumeration of *E. coli* bacteria in surface water samples. *Journal of Water Supply: Research and Technology-AQUA*, 57(8):569-576. <http://aqua.iwaponline.com/content/57/8/569>

## Additional Critical Issues Pertaining to Ag Water Provisions in the Produce Safety Rule That Were Beyond the Scope of This Meeting

Numerous policy issues relating to agricultural water provisions are in need of deliberation and clarification although beyond the scope of the CPS Colloquium on Agricultural Water Testing Methods. The Colloquium Panel did set aside time to identify other issues for continued discussion and consideration by FDA, academia and industry as we work together to perfect both food safety policy and programs. Many of the issues relate to the absence of comprehensive research studies to formulate agricultural water use policy, regulation and programs.

The following is a list of issues which should be considered when one is developing procedures, policies and practices to assess the safety of ag water for the growing, harvesting and postharvest handling of fresh produce.

### Analyte(s)

- Are there other analytes that would be better for measuring public health risk or levels of fecal indicators?
- *E. coli* vs. pathogen prediction in water
- In the rule it is fecal indicator bacteria and not pathogens
- *E. coli* (fecal indicator bacteria) vs. pathogen prediction on crop
- Using *E. coli* as a fecal indicator in harvest and post-harvest water
- Total coliforms are used as the measure for wastewater management and drinking water evaluations.
- What is the best analyte to test for to make informed decisions?
- Use of a microarray results in good data but is it reasonable, affordable?
- There will always be limitations
- Variability of analyte in different water sources and regions... includes globally

### Method

- *Equivalency* – how was this defined?
- Equivalency could be different based on what samples were evaluated (laboratory standards vs environmental samples); e.g. environmental samples vs. bioballs
- Important for methods to be available and affordable

- Accurate, ease of use, sensitive, fit for purpose
- Facilitates operator reproducibility
- Variability of methods (laboratory tests vs environmental samples)
- Historic data cannot be compared with changing methods

## Sampling

- Sample frequency (how often and how many and when)
- Since ag water is defined as that which contacts the harvestable portion of the crop – there is a need to better define “harvestable portion”; for example – when does a tree fruit or nut become “harvestable”?
- Use of water for freeze protection differs regionally and by crop. Recommendations for sampling need to be for months associated with highest risk of freeze events. Needs to be better defined by major crop groups.
- Prior to harvest of what (especially important in mixed crop farms)?
- What testing required for yearly sampling of the water distribution system?
- How is sampling per water source defined in practice (e.g., aquifer or individual wells)?
- Where in the delivery system should one test?
- Pooling contiguous water source data needs to be allowed. Use of historic samples from sources other than the individual farmer should be permitted.
- Rotational water sources and leased land:
  - Can you use data collected by other growers, academic institutions, industry associations, etc?
  - How should you sample when you know you cannot collect enough samples to generate a full (20 sample) microbial water quality profile?

- How does sampling variability influence the consistency of test results
- Can the number of samples be reduced if previous data over multiple years shows consistent compliance?
- How does one collect and utilize composite samples?
- How should sampling vary for moving water sources (canals, rivers, streams) vs still water sources (ponds, lakes, reservoirs, groundwater)

## Industry Tools

There is a need for development and broader dissemination of grower-based publications that outline best practices in sampling methodology. These publications should emphasize the issues that may impact the integrity and interpretation of data as well as foster the use of historical data and data collected by associations, academia, grower cohorts using established industry methods in the development of robust microbial water quality profiles for water systems.

- Can we establish guidelines for representative water sampling on properties with several wells drawing from the same aquifer?
- Can we establish guidelines for representative water sampling in canal systems drawing water from the same source? Under control of a single management agency?
- Can we develop a decision tree that provides growers a green, yellow and red light system to react to water quality results?

## Research Priorities to Address Ag Water Usage Data Gaps

The participants in the Colloquium felt that continued investment in further research on the use of water in agricultural production is vital. During the course of the colloquium several key search needs were identified and have been listed below for consideration by CPS leadership.

### Water Treatment

- What are the viable alternatives for treatment of agricultural water that exceeds the water criteria? Environmental issues of treatment? Design of existing equipment modification would make it more economically feasible?
  - Cost effective, environmentally-compatible, soil-friendly, crop-friendly, treatment options.
  - Available treatment options; how to adequately manage the treatment option (scientifically valid, verification).
  - U.S. water treatment chemicals are often not EPA labeled for reducing *E. coli*.
  - If you use it in a given environment do you know the impact of its use?
- Better information/data that would inform better methods/ approaches to sampling around water source?

### Analyte(s)

- Alternative indicators? CPS has supported research in this area but discussion suggested this should really be moved to USDA as it is a long-term research issue.

### Method

- A regionally coordinated and standardized Rapid Response comparative test method analysis. Is this needed if FDA broadens recognized test methods acceptance?
- How do ground water, surface water, ionic strength, turbidity affect filtration and methods.

### Sampling

- What is appropriate number of samples based on usage and historic data?
- What is “representative of use”?
- What types of sampling schemes would be useful?

- Minimize burden around testing
  - Pool samples, share data across shared water sources (how close is close enough?). e.g., irrigation districts.
  - What is a water source? Wells that are influenced by surface water (e.g., faulty sub-surface distribution system, field, aquifer, spring box).
  - Where and when is sample compositing a valid approach?
  - Building interfaces or centralized repositories for already available data so that it is easily accessible.
  - When could reduced sampling based on compliance history be considered?

### Assessment of Risk

- Hazard analysis, risk ranking, preventive controls
  - For water: 1. Monitoring; 2. Treatment
- Die-off differences by commodities, region, season?
- Data interpretation, risk communication
- Recommend rapid response research (CPS) when outbreaks or other incidents (crop contamination captured in pre-harvest testing) occur that might inform the assessment of risk.

- Encourage FDA to also allocate resources to follow up on environmental assessments when outbreaks occur: Do we have the appropriate protocols, sample collection, methods, etc.

### Ag Water Comprehensive Research Study

- Since agricultural water provisions are currently based on EPA recreational water standards, examine available research and formulate a comprehensive agricultural water study to craft recommendations for regulatory requirements tied to science.
- Mechanism for funding
- Mechanisms to collaborate on funding identified research priorities should be found.
- **FDA** – FDA should place priority on funding identified research priorities
- **USDA** – USDA should place priority on funding identified research priorities
- **CPS**
  - Mechanism or provision for establishing longer-term research projects (outside the current 2-year maximum) for continuity where projects would benefit.
  - Rapid response funding mechanism exists.
  - Short term regional baseline trials



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