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Science Foundations of *E. coli*-Based Water Quality Requirements

North Central Region FSMA Center
Listening Session 15 Aug 2019

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Produce Safety Rule water compliance dates (other than sprouts) now start in 2022

- The reason given for this extension is "*to address questions about the practical implementation of compliance with certain provisions and to consider how we might further reduce the regulatory burden or increase flexibility while continuing to achieve our regulatory objectives*
- Extension applies to ALL of subpart E, other than sprouts

FEB. 27-28, 2018
WATER SUMMIT

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Compliance Date Schedule Detail

Farms over $500,000 [a]

Production Water Sampling Schedule			
MWQP [b] for untreated water type:		Surface	Ground
Latest growing season to:	Begin Sampling	2022	2022
	Complete Sampling	2025	2022
	Use MWQP to Make Decisions	2026	2023

[a] 3-year average annual sales of all produce
[b] MWQP, Microbial Water Quality Profile
[c] 2019 adjusted 3-yr average annual sales of produce

Note: Requirements may change prior to dates

Other farm sizes

- **Farms up to $27,500** [c] annual produce sales, no compliance date
  - Excluded from the Produce Safety Rule
- **Farms up to $250,000** [a] annual produce sales, Table plus 2 years
- **Farms up to $500,000** [a] annual produce sales, Table plus 1 year

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The basic requirements for water quality

- Farms must inspect the agricultural water system
  - Identify hazards to water quality
  - Ensure the system is in good repair
- Farms must know the quality of agricultural water
  - Routine testing during the growing season
  - **Quantitative** analysis for generic *E. coli*
- Farms must maintain the quality of the water
  - Remember, adulterated produce may not be sold as food

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21 US Code § 342

A food shall be deemed to be adulterated:

(1) If it bears or contains any poisonous or deleterious substance which may render it injurious to health …

(2) If it bears or contains any added poisonous or added deleterious substance … that is unsafe …

(3) If it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food

(4) If it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health …

There are seven conditions in the full definition

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E. coli – based requirements for agricultural water used in production

- Geometric mean 126 CFU/100 mL or less

AND

- Statistical threshold value 410 CFU/100 mL or less

WHY? HOW?

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The history is with bathing beaches and goes back to 1922

- American Public Health Association Committee on Bathing Beaches report of 1922
  - Not enough science to support bathing water standards
  - Follow-up reports concluded that lacking epidemiological evidence, any standards created would be arbitrary
- By 1963, 38 States had taken the initiative
  - Limits ranging from 50 to 2400 coliforms/100 mL
  - About half used 1000 coliforms/100 mL

DUFOUR, A. P. AND S. SCHAUB. THE EVOLUTION OF WATER QUALITY IN THE UNITED STATES - 1922-2003. Chapter 1, L. J. Wymer (ed.), Statistical Framework for Recreational Water Quality Criteria and Monitoring. John Wiley and Sons, 1-12, (2007)

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In 1963, the **total coliform** test was one of the only routine tests for wastewater contamination available

Common methods

Broth culture: LTB, BGBL broth

Filtration: mENDO

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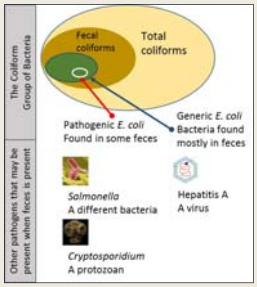
Remember this image from the PSA Grower Training course →

- Generic *E. coli* is a type of fecal coliform
- Fecal coliform is a type of total coliform



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Where did 1000 coliforms per 100 mL come from?

- Survey of marine coastline in Connecticut
  - 1000 coliforms/100 mL was 90% achievable in 1932
- Ohio River Valley Sanitation Commission calculations
  - Pathogen:total coliforms (pathogen was *S. typhosa*)
  - Risk of illness from ingesting one cell of pathogen
  - Concluded 1000 coliforms/100 mL would not increase illness rates over baseline in 1951
- California Bureau of Sanitary Engineering 1943
  - 1000 coliforms/100 mL was 500x the contemporary drinking water standard, acceptable by common sense

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Governing Concepts Emerged

Needs to be achievable

Should not appreciably increase observed illness rates

Consistent with other standards (by common sense)

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Where did 1000 coliform per 100 mL end up?

- 1950s confirmation of 'best professional judgement' by epidemiological studies
- US Public Health Service studies at 3 geographic areas (Chicago, Cincinnati, Long Island Sound)
  - Concluded that 1000 per 100 mL was conservative; health effects observed only above about 2300 per 100 mL
  - Recommended using a new and improved indicator, fecal coliforms, geometric mean value 200 per 100 mL and 90th percentile 400 per 100 mL (equivalent to 2300 coliforms)

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The fecal coliform test is similar to the total coliform test but more selective

- Bile salts (similar to what is in the gut)
- Higher temperature (above body temp)

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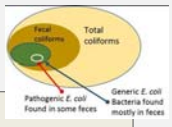

The fecal coliform standard is consistent with the prior total coliform standard

- Upper limit (400 fecal coliforms) based on observed health effects, associated with 2300 total coliforms
- Median value (200) comparable to the old standard of 1000 total coliforms

Common methods

Broth culture: same as coliform, 44.5 °C

Filtration: mFC agar

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How did we get generic *E. coli*?

- USEPA epidemiological studies in the 1970s
  - Marine and freshwater beaches
  - All contemporary indicator organisms: total coliforms, fecal coliforms, *E. coli*, and enterococci
- Recommendations published in 1986
  - Geometric mean 126 generic *E. coli* in 100 mL
  - Single-sample maximum values (among 5 samples; 80th percentile) differ by degree of contact
  - The calculations included the fecal coliform levels
    - The new standards were directly related to the fecal-coliform illness risk levels

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In 1972, when these studies were planned, the EPA was a new agency

In the late 1970s, when these studies were done, tests for generic *E. coli* were confirmation tests

Confirmation: Which colonies or tubes of total coliforms/ fecal coliforms in standard tests were *E. coli*

Usually involves transfer from the original broth or plate to a secondary culture medium

mENDO
Total coliforms have metallic sheen

NA-MUG
Generic *E. coli* glow under UV light

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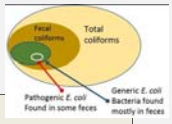

Common methods

In the 1980s a new agar called mTEC was developed for easier detection (Method 1103)

In the 2000s, an easier-to-use version called modified mTEC (Method 1603) was released

Another 2000s medium, MI, was developed primarily for drinking water tests

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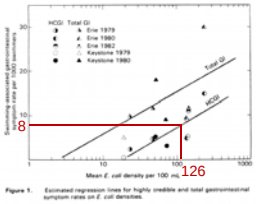
The science behind the numbers

- Evolution of standard as technology improved
  - Total coliforms, to fecal coliforms, to generic *E. coli*
- Several years of data collection at beaches across the United States



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2012 updated criteria

- Substantial work confirming the epidemiology
- Change in definition led to change in illness risk
  - 1986

CRITERIA ELEMENTS	Fresh Water Estimated Illness Rate 8/1000		Marine Water Estimated Illness Rate 19/1000	
Indicator	GM (CFU/100 mL)	Upper 90% (CFU/100 mL)	GM (CFU/100 mL)	Upper 90% (CFU/100 mL)
Enterococci	33	107	35	276
E. coli	126	409	--- Not Applicable ---	

  - 2012

CRITERIA ELEMENTS	Recommendation 1 Estimated Illness Rate 36/1000		Recommendation 2 Estimated Illness Rate 32/1000	
Indicator	GM (CFU/100 mL)	STV (CFU/100 mL)	GM (CFU/100 mL)	STV (CFU/100 mL)
Enterococci (marine &fresh)	35	130	30	110
E. coli	126	410	100	320

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  - 2012

CRITERIA ELEMENTS	Recommendation 1 Estimated Illness Rate 36/1000		Recommendation 2 Estimated Illness Rate 32/1000	
(marine &fresh)				
E. coli	126	410	100	320

These numbers ought to look familiar

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How did all that science help EPA with recreational water criteria?

- The 1986 recommendations, based on 1970s data, were published but it was up to the States to use them.
  - In 1988, 46 states used fecal coliforms and 1 state used generic *E. coli* … 3 states also used total coliforms
  - By 1992, 7 states were using the EPA-recommended indicators (either *E. coli* or enterococci)
  - By 2003, 11 states were using the EPA-recommended indicators but 40 states also still used fecal coliforms
  - Today (2019) most states use the EPA-recommendations

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Change is slow

Science and data alone did not accomplish change to regulatory systems

Ultimately, though, the science was convincing and resulted in more consistent application

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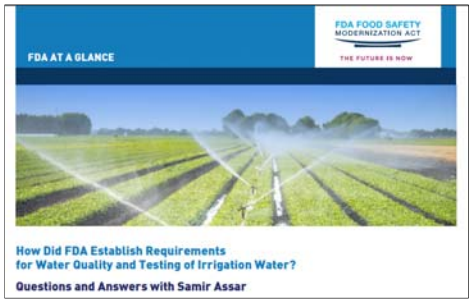
Produce Safety Rule Subpart E



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FDA thinking (as of 2017)

- Some challenge the scientific basis of the microbial water quality criteria … (and) they question the use of Environmental Protection Agency (EPA) criteria for recreational water … in the final rule. What scientific support was used to craft the final standards?

After reviewing scientific literature, we determined that generic E. coli, bacteria found in the intestinal tract of both people and animals, are consistent indicators of the presence of feces.
Identifying fecal contamination is important in assessing the safety of agricultural water. As such contamination increases, so does the likelihood that disease-causing microorganisms are also present.
The science behind EPA's recreational water criteria is based on recent epidemiological studies, and the scientific evidence showed that people have gotten sick by swallowing recreational water that is contaminated with feces. …

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Where you have *E. coli*, you have poop

Where you have poop, you might have fecal-oral pathogens

Many foodborne illness outbreaks are caused by fecal-oral pathogens

Many recreational water-related illnesses are caused by fecal-oral pathogens

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FDA future directions

- The final rule on compliance date extension of Subpart E (other than sprouts) provides insights

These compliance dates have been extended while the FDA considers how best to protect public health while addressing widespread concerns about the complexity of the agricultural water requirements and the practicality of implementing them across a wide variety of farms, water sources and uses. The FDA intends to use this time to work with stakeholders to address these concerns.

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Where are we now?

- ***Module 5: Agricultural Water*** is important information
  - Updated in v1.2 with revised compliance dates and methods guidance
  - The requirements in 2022 may be very similar to existing language
  - Helps to avoid adulterating produce per FD&C
- While FDA is reconsidering the standards, farms are encouraged to test water
  - Follow Good Agricultural Practices (GAPs) to protect and maintain water quality
  - Develop water management strategies, such as water system surveys, to identify and reduce risks

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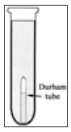
A little more on methods

- Before 1950, only broth culture was used
  - Dilution series to obtain no growth
  - Growth in the broth was indicated by cloudiness, or gas production
  - Most-probable-numbers (MPN) enumeration



- Membrane filtration was an innovation of the 1950s
  - Filtration of increasingly large volumes
  - Colony-forming units (CFU) enumeration

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CFU or MPN?

- As early as 1968, when membrane filtration was relatively new, professional organizations recommended either to be used interchangeably
  - EPA only recommended membrane filtration in the 1986 recreational water criteria publications
- EPA maintains a list of acceptable methods in 40 CFR 136.3 that includes both CFU and MPN methods
- FDA published a different list of equivalent methods that includes both CFU and MPN methods

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What is a CFU?

- A count of the colonies on the surface of a gel (agar) plate
  - For water quality tests, the water is first filtered then the filter (with bacteria) is transferred to the plate
  - A colony will form anywhere there was **one or more** cells
- The agar is formulated to grow only target cells
  - A colony may not form from damaged or stressed cells
  - A colony may form from a clump of cells

From US Geological Survey Field Manual, chapter 7.1

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What is a MPN?

- A statistical estimate of original number, based on multiple Presence/Absence tests.
- As an example:
  - There are three bacteria in a sample
  - The sample is divided into three equal test volumes
  - Each bacterial cell MIGHT be in any of those volumes

Original sample volume — Test sample volumes

- The test might be positive in 1, 2, or all 3 test volumes
  - 1
  - 2
  - 3

Most Probable Result
Many possible combinations that result in 2 positives

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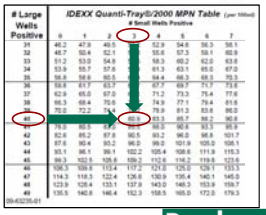
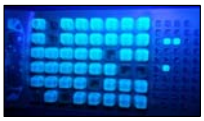
Where the MPN comes from

- This image shows 40 positive "large" wells and 3 positive "small" wells
  - Result is 81 MPN/100 mL

The Table gives a MPN result





The exact calculation gives the MPN and a 95% confidence interval

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Comparing CFU and MPN

- Both CFU and MPN are estimates, neither is the actual number of generic *E. coli* cells in the sample
  - There is no current test that gives actual number
- There are pro and con factors for each method type
  - Commercial MPN tests tend to be easier and cheaper, and can handle turbid (cloudy) samples better
  - Membrane filtration (CFU) is generally seen as a more direct measure, can handle a wide range of concentrations

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Best practice is to use the same method for your entire sample set

The results *should* be the same but there can be differences between methods, affects apparent trends

It's especially helpful to have the same units (CFU or MPN) for all results

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Equivalent Water Testing Methodologies

- The FDA fact sheet on equivalent methods was updated in 2018 to include presence/absence methods
- Different methods can apply to water used different ways
  - Production water criteria; use quantitative methods
  - Postharvest water criteria; use either quantitative or presence/absence methods
- PSA updated fact sheets about water testing to reflect changes
- APHL webinar with PSA, SSA to inform laboratories

Note: Requirements may change prior to compliance dates

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Where Do I Go For Testing?

- Be certain the lab can provide the test you need
  - Analysis using a method accepted by FDA
  - Upper limit high enough to get a number to calculate GM and STV, when needed
- Be sure the lab provides sampling instructions
  - Labs should provide instructions for acceptable sampling containers, hold times, storing, and transport expectations

- Lab certification is recommended, but is not a requirement in the FSMA Produce Safety Rule

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Manual slide 31

Equivalent Water Testing Methodologies

Equivalent quantitative methods to EPA Method 1603 (membrane filtration with modified mTEC). These methods can be used for both production AND harvest/postharvest agricultural water.

- Membrane filtration methods (colony forming units, CFU/100 mL)
  - mTEC agar (EPA 2010, APHA 2012, ASTM 2000)
  - mColiBlue PourRite Ampules (Hach method 10029)
  - mEndo followed by NA-MUG agar (APHA 1997)
  - MI agar (EPA 2012)
- Most Probable Number (MPN/100 mL) methods
  - Colilert (using Quantitray 2000 tray)
  - Colilert 18 (using Quantitray 2000 tray)

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Many labs call their MPN method Standard Method 9223B

There are many industry-accepted variations of SM9223B

Encourage your farms to confirm that the lab uses the FDA-accepted variations: Colilert or Colilert 18 with Quantitray 2000

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Other variations of SM 9223B that may be acceptable in other settings

None of these is on the FDA list

Colisure using Quantitray 2000

Colilert 18 using Quantitray 200

Colilert using Quantitray 200

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Equivalent Water Testing Methodologies

Equivalent presence/absence methods to EPA Method 1603 (membrane filtration with modified mTEC).

- These methods can be used for agricultural water used during harvest and postharvest:
- Broth-based methods for detection in 100 mL water
  - Veolia TECTA™ EC/TC medium and the TECTA™ Instrument
  - CPI Modified Colitag™ Test method
  - IDEXX Colilert, Colilert 18, and Colisure
  - Charm Sciences E*Colite Bag or Vial Test
  - Millipore Readycult Coliforms 100

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References and Resources

- Dufour, A. P. and S. Schaub. *The Evolution of Water Quality in the United States-1922-2003*. Chapter 1, L. J. Wymer (ed.), Statistical Framework for Recreational Water Quality Criteria and Monitoring. John Wiley and Sons, LTD, , Uk, , 1-12, (2007)
- USEPA *Health Effects Criteria for Fresh Recreational Waters.* EPA 600/1-84-004 (1984)
- USEPA *Ambient Water Quality Criteria for Bacteria – 1986.* EPA 440/5-84-2 (1986)
- USEPA *Recreational Water Quality Criteria*. Office of Water 820-F-12-058 (2012)
- FDA *FSMA Final Rule for Produce Safety: How Did FDA Establish Requirements for Water Quality and Testing of Irrigation Water?* (2015)
- FDA Fact Sheet *Equivalent Testing Methodologies for Agricultural Water* (2018)

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Questions?

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