Analytical and Sampling Methods

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FAC Questions Addressed

- What are the data quality concerns related to methods and sampling?
  - Of the positives, how many are false?
  - Of the negatives, how many are false?
- Method performance - what are the false positive and false negative rates for approved TC, FC, and EC methods?
- Method prevalence - what methods are most commonly used?
- Sampling – do sampling procedures have any effect on whether total coliform or *E. coli* is detected?

Sources of Error for Samples

Measured Result = True Result ± Error

Analytical Error	+	Laboratory Error	+	Sample Contamination
↓		↓		↓
Associated with method, sample matrix, etc.		Associated with laboratory personnel and procedures (not addressed in this presentation)		Associated with faucet, transport, bottle preparation, etc.

Background

	True State	
Indicated Result (Measured)	Present	Absent
Present	True Positive a	False Positive b
Absent	False Negative c	True Negative d

$$\text{Sensitivity} = \frac{a}{a + c} \qquad \text{Specificity} = \frac{d}{b + d}$$

Definitions of False Positive & False Negative

- **Analytical False Positive —** A non-target organism incorrectly identified as the target organism using the method of interest
  - Example: *Aeromonas* is not part of the coliform group, is ubiquitous in the environment, not related to fecal contamination, and interferes with many coliform methods
- **Analytical False Negative —** A target organism incorrectly identified as a non-target organism or not identified at all using the method of interest

EPA Methods Approval Process

- Proposed methods are divided into two categories
  - Alternate Test Procedures (ATPs) - Procedures which use the same analyte but a different technique than an approved method
  - New Methods - Procedures which introduce new analytes and determinative techniques or concepts, such as a change from colorimetry to ion selective electrode methods, or a change in WET test endpoints
- Method performance is measured against existing standard tests
- The method approval program also reviews requests for variances to sample collection, preservation, and incubation times requirements specified in approved methods and the *Code of Federal Regulations* (CFR)

Method Performance Data

- Analytical false positive and false negative rates are defined differently in different studies
  - Results depend on the sample matrix, protocol, and confirmation procedure used
  - Need to understand how false positive and false negative rates were determined before they can be compared across studies
- High number of samples needed for statistical power of false+ and false– rates
- Method performance rates from these studies should not be used to interpret false+ / false– rates in the field since
  - Study conditions not always representative of field conditions

Method Performance Data (2)

- Different methods have varying specificities in organisms recovered
  - Total coliform is a functional definition consisting of many different bacterial genera/species; not a taxonomic group
    - Organisms recovered may differ based on method technology
      - Enzyme substrate methods typically recover a larger group of organisms
  - *E. coli* isolation easier: only one species to isolate
    - Several strains of *E. coli* exist
    - FP/FN rates generally less for this organism
- Alternative Test Protocol (ATP) compares new methods to existing standard tests
  - There are no regulatory criteria for TC/FC/EC method performance
  - Performance goals are not linked to the expected occurrence rate of coliforms in drinking water

Method Performance Data

- Summarized data from ATP protocol (EPA Data) and two other studies that evaluated false positive and false negative rates for approved methods
  - EPA data for each method published in FR notices at time of approval.
  - Fricker, C., P. Warden, D. Silvaggio, E. Gleeseman, R. Tamanaha, J. Rust, B. Eldred. 2003. Comparison of Five Commercially Available Methods for Detection of Coliforms and *E. coli.* In *Proceedings 2003 Water Quality Technology Conference.* American Water Works Association, Denver, CO. CD ROM.
  - Olstadt, J., J.J. Schauer, J. Standridge, S. Kluender. 2007. A comparison of ten USEPA approved total coliform/*E. coli* tests. *J Water and Health*. 05(2), 267-282.
  - Fricker, C., P. Warden, M. DeSarno, B. Eldred. 2007. Performance Characteristics of methods approved for the detection of coliforms in drinking water. In *Proceedings 2007 Water Quality Technology Conference.* American Water Works Association, Denver, CO. CD ROM.

False +/False - data and study conclusions in Appendix A

Observations from Method Performance Studies

- Data from the ATP shows that all coliform methods have varying levels of false positive and false negative results.
- Olstadt (2007) and Fricker (2003 and 2007) demonstrate that the performance of coliform methods vary in the field.
  - Olstadt suggests that method performance can be influenced by water matrix effects.
- Some studies have suggested that false positive results could be due to variations in the manufacture of the media.
  - Suppression of *Aeromonas* may be an issue under some circumstances.

Prevalence of Analytical Methods

- Based on data from 5 States (6 year review data), enzyme substrate methods are used nearly exclusively for *E. coli assays*
  - *Standard Methods* 9223B: Colilert, Colilert-18, and Colisure
  - Approx 10-20% samples assayed for FC in dataset
- As mentioned at September meeting, Colilert is thought to be the most widely used method based on anecdotal information

Coliform Sampling

- Several sampling factors can affect the results of TC/*E. coli* monitoring results
  - Sample tap design
  - Sampling location
  - Sample collection procedure
  - Samplers aseptic handling/collection of sample
  - Maintenance of the tap
  - Storage time and temperature between sampling and assay
- Positive coliform samples due to sample collection should not be confused with analytical false positives
  - **Common Misperception —** A positive routine sample followed by repeat samples that are negative is sometimes referred to as a “false positive”

Overall Conclusions about Error due to Analytical Methods and Sampling

- Analytical methods for TC/EC introduce some error around the actual result
  - Progression of knowledge in methods since 1989 presents an opportunity to set performance criteria for coliform methods
- Other sources of error can come from sample collection, sampling taps, interferences, etc.

- Based on these data (and other method studies and sampling case studies), TCR indicator results require some additional follow up (repeat sampling, site evaluation, etc.) before a compliance decision should be made

Appendix

Summary of method performance studies

ATP Protocol & Changes

- Method performance data is generated by EPA during the method approval process
- Coliform methods have been approved under different versions of the ATP protocol
  - 3 different versions of ATP protocol in existence
    - pre-1995, 1995, and 2004 versions
- False positive and false negative values generated using different versions of the protocol are not directly comparable

False +/False - Rates for Total Coliform from ATP Protocol

Type of Method	Method	Approved	False+%	False-%
Enzyme substrate	Colilert	FR 6-10-92	20.2 [1]	10.9 [1]
	Colilert-18	Letter 9-10-96	Not given[2]	Not given[2]
	Colisure	FR 12-5-94	3.5	0
	Readycult	FR 3-7-02	7	5.1
	E*Colite	FR 12-1-99	16.0	3.7
	Colitag	FR 2-13-04	5[3]	0[3]
Membrane filter	MI medium	FR 12-1-99	4.9	8.8
	m-ColiBlue24	FR 12-1-99	26.8	1.6
	Coliscan	Letter 8-10-00	Not given	0.7

1 Federal Register, Vol. 56, No. 188, Friday September 27, 1991, page 49153, Proposed Rules
2 Oct. 1994 Method Description; Colilert-18 Performance Data Summary by David E. Townsend and Andy J. Croteau Oct. 3, 1994.
3 From 8/15/2001 FAX coversheet from Chris Woodruff of CPI to Khouane Ditthavong of EPA referencing a September 15, 1998 letter from Robert Bordner of EPA to James B. Knight of CPI which gives the false positive and negative rates for total coliforms and *E. coli* using data that were acceptable to EPA.

False +/False - Rates for *E. coli* from ATP Protocol

Type of Method	Method	Approved	False+ %	False- %
Enzyme substrate	Colilert	FR 6-10-92	0.8 [1]	9.2 [1]
	Colilert-18	Letter 9-10-96	Not given[2]	Not given[2]
	Colisure	12-5-94	4.3	2.4
	Readycult	FR 3-7-02	5	6.86
	E*Colite	FR12-1-99	7.2	9.2
	Colitag	FR2-13-04	17.8[3]	0[3]
Membrane filter	MI medium	FR 12-1-99	4.3	4.3
	m-ColiBlue24	FR 12-1-99	2.5	0
	Coliscan	Letter 8-10-00	3.8	<1

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Fricker et al. (2003) Comparison of Five Methods for Detection of Coliforms and *E. coli*

- Study performed in two parts
  - Examined the specificity of methods, particularly with regard to *Aeromonas* spp.
    - Surface water samples from sources known to have relatively low coliform counts
  - Investigated method sensitivity for *E. coli*
    - Surface water known to usually contain <10 *E. coli*/100mL
- Confirmation of positives using MacConkey agar and enzyme tests
  - Coliforms = colonies with typical coliform morphology that were oxidase negative
  - *E. coli* = organisms that produced indole from tryptophan

Results from Fricker et al. 2003

		Phase 1, Coliform results				Phase 2, *E. coli* results			
Medium	Incubation (hr)	# Samples	Pres. TC	Confirm TC	Confirm Rate	# Samples	Pres. TC	Confirm TC	Confirm Rate
Colilert-18	18	186	106	105	99.0	400	208	208	100.0
	22	186	107	106	99.0	400	214	214	100.0
Colilert	24	186	109	103	94.5	400	195	195	100.0
	28	186	111	105	94.6	400	207	207	100.0
Colisure	24	186	110	101	91.8	400	197	197	100.0
	48	186	116	105	90.5	400	204	204	100.0
ReadyCult	18	--	--	--	--	400	158	158	100.0
	24	186	116	103	90.8	400	186	186	100.0
	29	186	123	104	87.6	400	218	215	98.6
Colitag	22	186	149	78	52.3	400	--	--	--
	26	186	159	81	50.9	400	--	--	--

Not tested denoted with "—"

Conclusions from Fricker et al. 2003

- Authors concluded that:
  - Three defined substrate methods are all similar with respect to recovery of TC and EC
  - Readycult (29hr) is sensitive but loses specificity
    - Up to 12% positive reactions due to non-coliform organisms
  - Colitag appears to have higher false positive and false negative rates than other approved methods

Various methods have range of false positive and false negative rates

Olstadt et al. (2007)
A comparison of ten USEPA approved total coliform / *E. coli* tests

- Used three geographically and chemically diverse ground waters in Wisconsin
- Tested performance of approved methods for their ability to recover the target organisms (total coliforms and *E. coli*) and suppress *Aeromonas*
- Study is well defined with respect to test organisms (*E. coli*, various strains of *Klebsiella*, *Enterobacter, Citrobacter, Serratia* and *Aeromonas*), concentrations used, spiking methods, water quality characteristics of the samples, and sample processing.
- Study is limited by the low number of sample types tested

Results from Olstadt et al. 2007

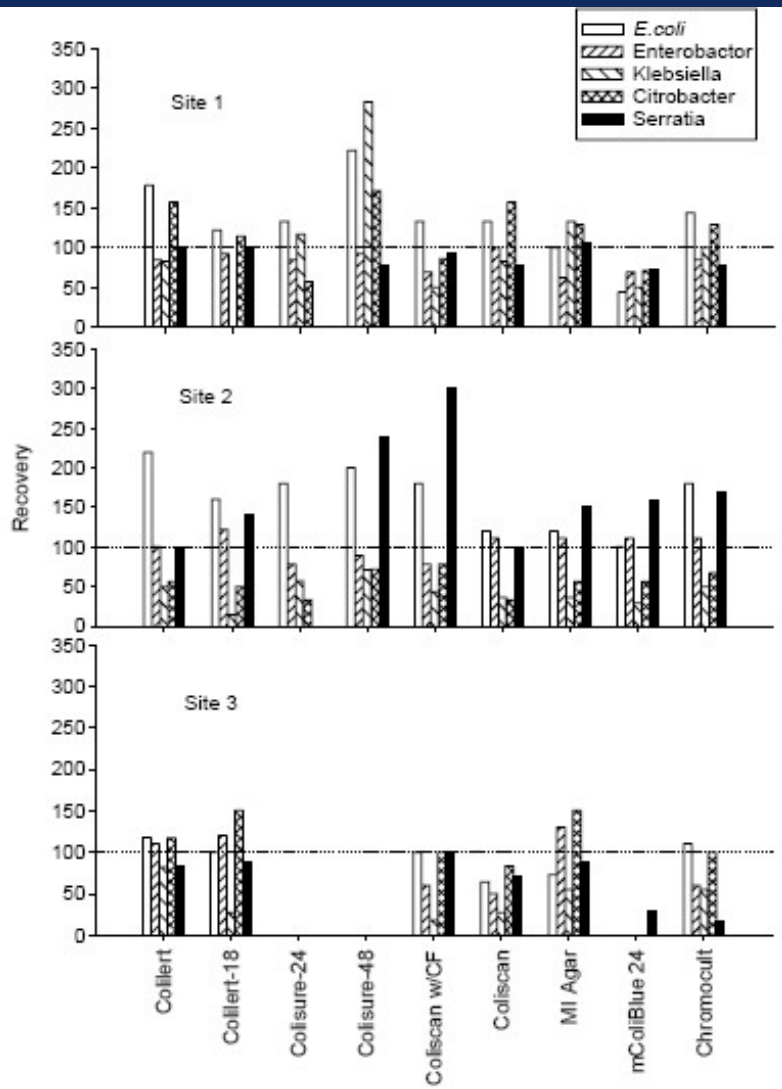

Figure 1 | Percent recovery data for water samples from each sampling site spiked with 1–10 bacteria of each total coliform genus.

Conclusions from Olstadt et al. (2007)

- Differences observed between approved methods both in ability to detect TC and EC and in their ability to suppress false positive results from non-coliform organisms
- Performance differences attributable to sample matrix
  - Study tested three geographically, geologically and chemically diverse ground waters
  - Some methods unable to detect certain coliform species from all matrices examined
    - pH and alkalinity – buffer capacity
    - Background bacteria
- Differences between products and their ability to suppress *Aeromonas* spp.
  - Between sites and between strains.

Fricker et al. 2007 – Performance characteristics of methods approved for the detection of coliforms in drinking water

- Preliminary results for AwwaRF #4024 reported at WQTC in November 2007
- Initial investigations focused on the sensitivity and specificity of various methods
  - Used both “seeded” (with Bioballs) and naturally contaminated samples

Preliminary Results from Fricker et al. 2007

Method	Number colonies/wells examined	Number colonies/wells confirmed	Number which did not confirm	Confirmation rate (per cent)
1 coliforms	183	154	29	84.2
2 E.coli	87	80	7	92.0
3 E.coli	124	95	29	76.6
4 coliforms	63	58	5	92.1
5l coliforms	61	59	2	96.7
5 E.coli	98	95	3	96.9
6 coliforms	49	41	8	83.7
6 E.coli	1	1	0	100
7 coliforms	30	30	0	100
7 E.coli	49	37	12	75.5
8 coliforms	1	1	0	100
8 E.coli	2	1	1	50
9 coliforms	98	91	7	92.6
9 E.coli	63	55	8	87.3
10 coliforms	79	79	0	100
11 coliforms	77	67	10	87.0
11 E.coli	39	36	3	92.3

From 2007 WQTC Proceedings

Preliminary Conclusions from Fricker et al. 2007

- Authors' conclusions:
  - Considerable differences seen between the various approved methods
    - Some approved methods are significantly less sensitive and specific than others
- Experiments are ongoing