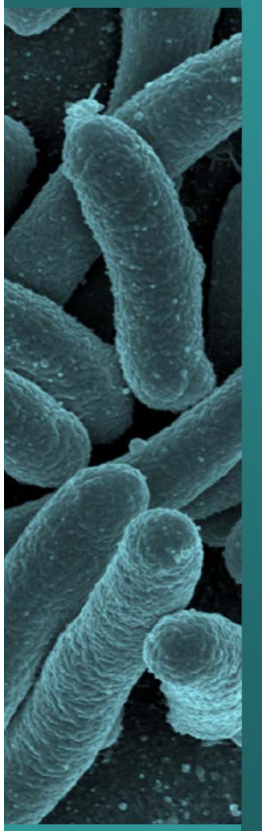
Multi-Laboratory Validation of Methods 1642 and 1643 for Male-specific and Somatic Coliphage in Fresh and Marine Recreational Waters and Wastewater Samples

Presented by
Yildiz T. Chambers-Velarde, MSPH
General Dynamics Information Technology

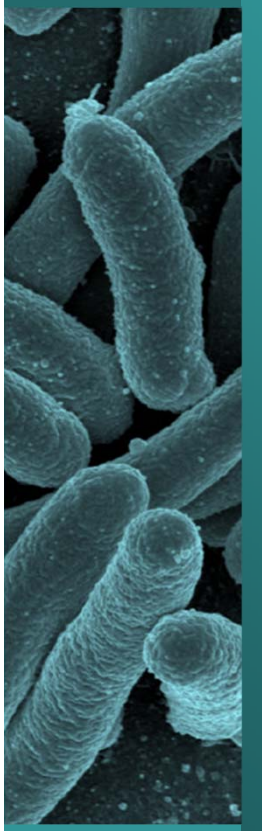
August 6, 2018



Co-authors

Rashmi Ghei, Environmental Scientist
General Dynamics Information Technology

Ken Miller, Statistician
General Dynamics Information Technology



Disclaimer

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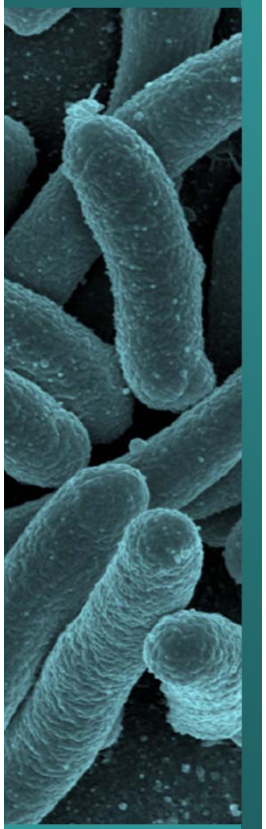
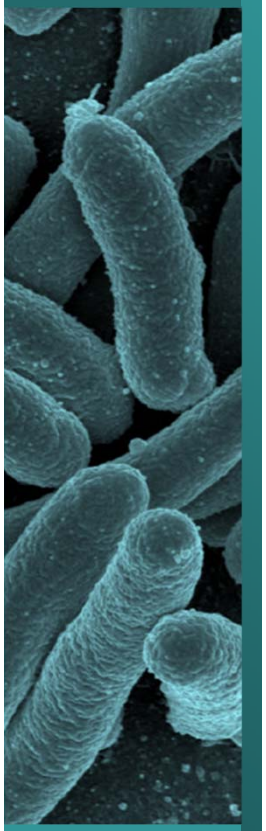
Background

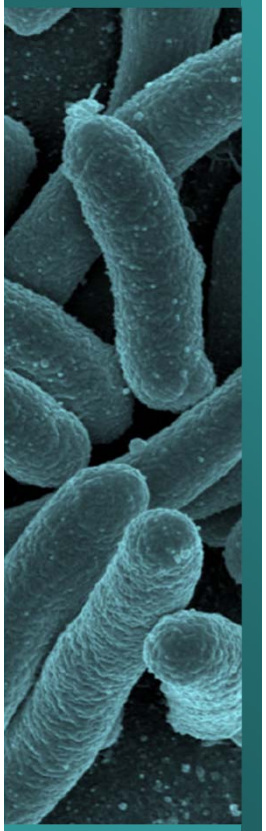


- EPA was evaluating the suitability of the use of coliphage as indicators of viral fecal contamination in ambient waters
- To address the need for larger sample volumes for ambient waters a UF procedure was evaluated in conjunction with the analytical procedure
- EPA developed and validated two coliphage methods for use in fresh and marine waters and/or wastewater effluents

Method 1642



- Validated for male-specific and somatic coliphage in marine and fresh recreational waters, and advanced treatment wastewater
- Dead end hollow fiber UF procedure coupled with EPA Method 1602
- After UF is used to concentrate large volume of water, samples are assayed using the Single Agar Layer (SAL) procedure
- Results are reported as plaque forming units (PFU/1 L) for male-specific and somatic coliphage



Ultrafiltration Set-up

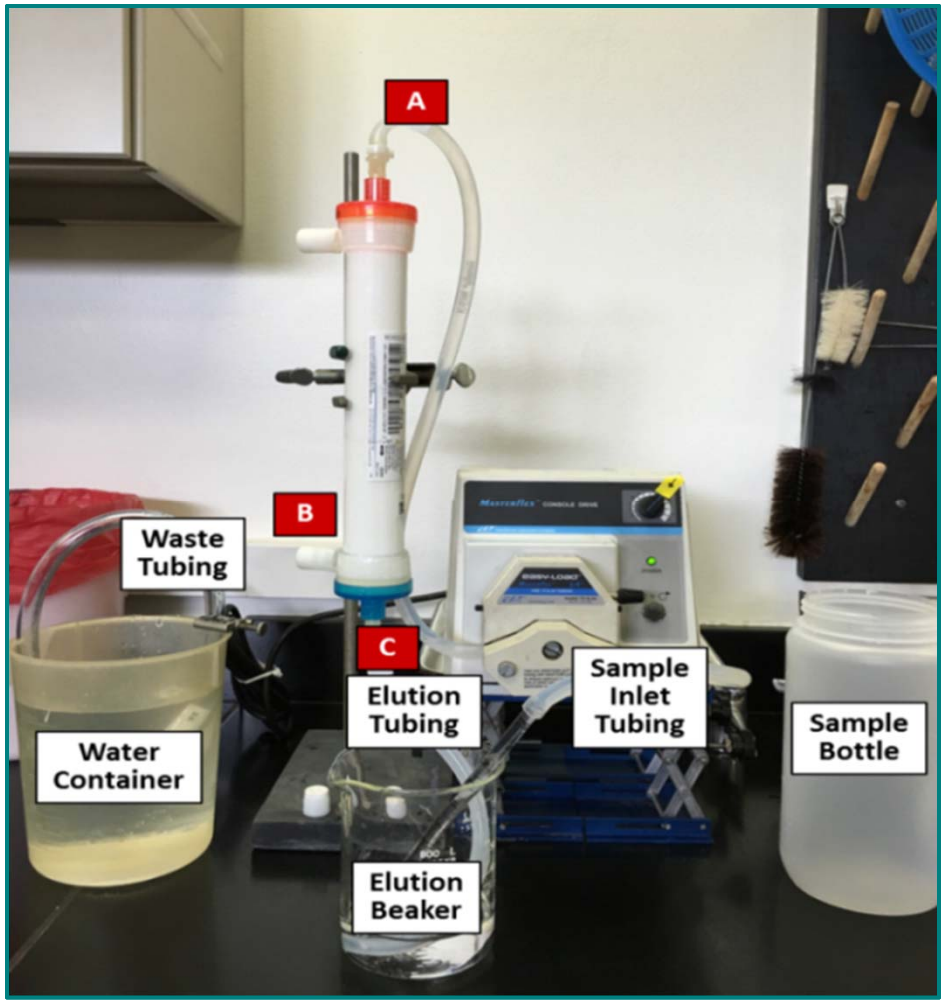
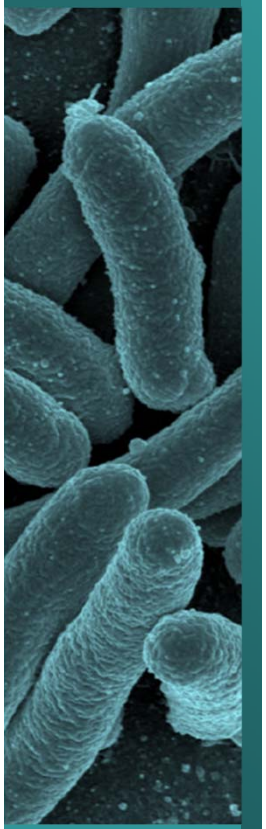


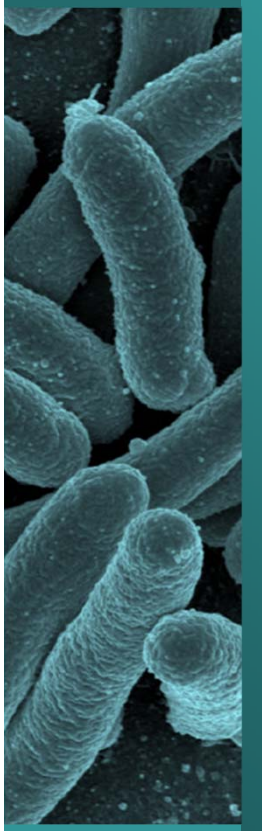
Photo credit: Jennipher Quach-Cu
(San Jose Creek Water Quality Laboratory - County Sanitation Districts of L.A. County)

Method 1643



- Validated for male-specific and somatic coliphage in secondary (no disinfection) wastewater samples
- 100 mL samples are assayed using the SAL procedure
- Results are reported as plaque forming units (PFU/100 mL) for male-specific and somatic coliphage

SAL Procedure



Add $MgCl_2$ to 100 mL samples
and temper at 36°C for 5 minutes

↓

Add log-phage host to samples
and transfer to 46°C water bath

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Add samples with host
to 100 mL 2X TSB with agar

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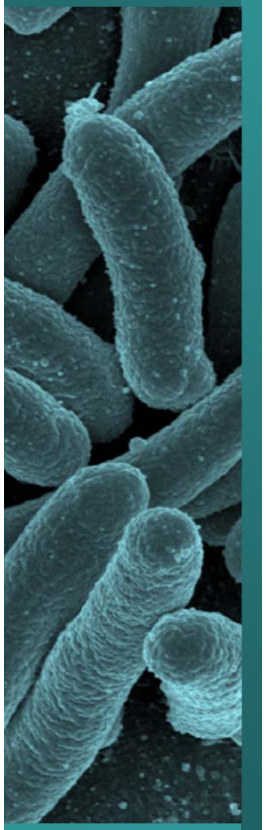
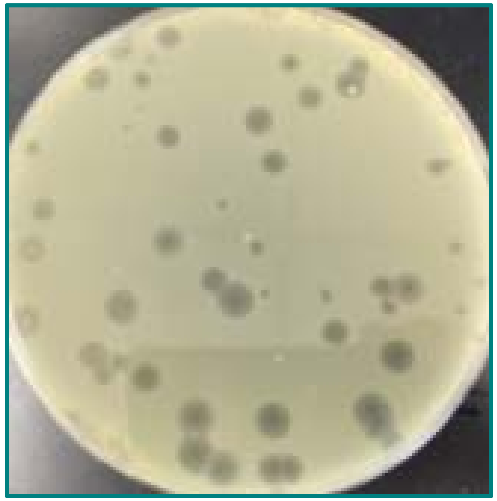
Pour plates and incubate
at 36°C for 16-24 hours

↓

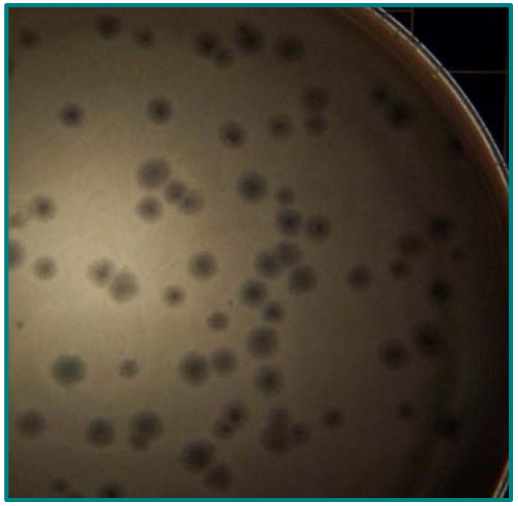
Count plaque forming units

Coliphage Plaques





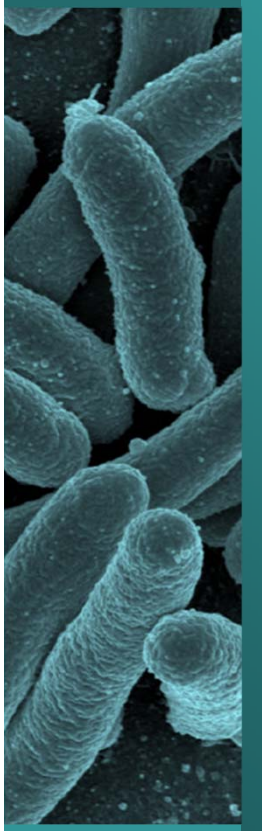
Somatic Coliphage Plaques (CN-13)



Male-specific Coliphage Plaques (F_{amp})

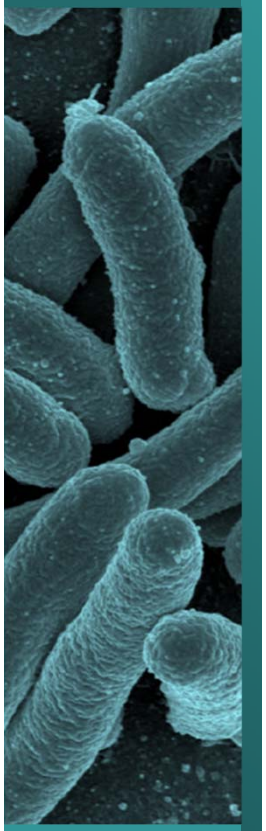
Photo credits: Jeremy Olstadt (Wisconsin State Laboratory of Hygiene) and Richard Danielson (IEH Biovir)

Study Design



Objectives

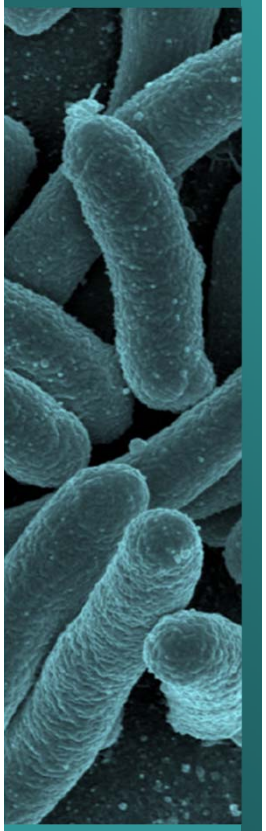
- Develop quantitative quality control (QC) acceptance criteria
- Assess method performance of Method 1642 across multiple laboratories and matrices
- Assess method performance of Method 1643 across multiple laboratories and secondary wastewater (no disinfection) matrices



Matrices Evaluated

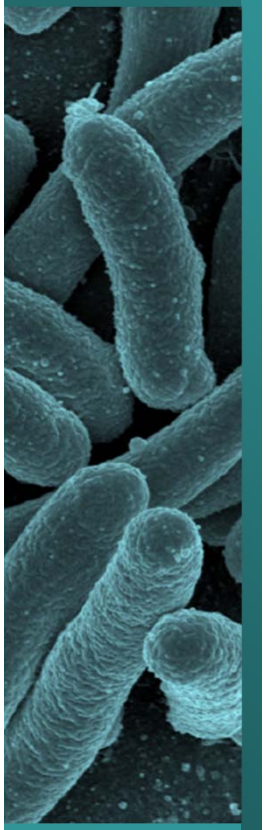
- **Reference Matrix**
  - Phosphate buffered saline (PBS)
- **Fresh water**
- **Marine water**
- **Wastewater**
  - Advanced treatment
  - Secondary (no disinfection)

Study Considerations



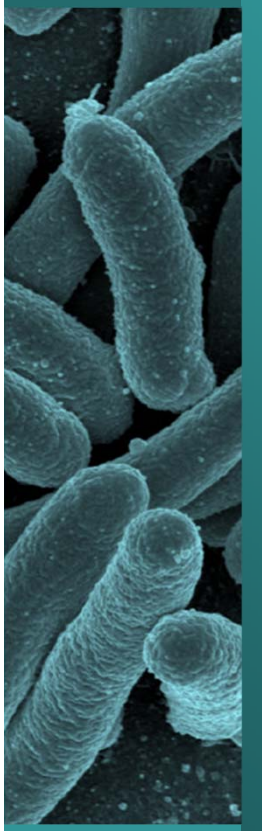
- **Laboratories may have limited experience with:**
  - Ultrafiltration
  - Method 1602
- **Laboratories may not have all of the appropriate equipment**
- **Supplies would need to be customized for the different laboratories**

Study Schedule

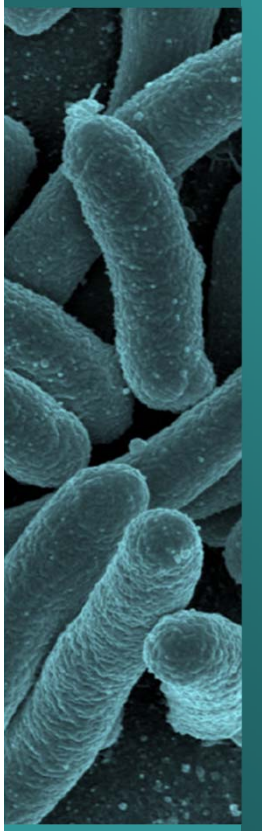


ANALYSIS	START DATE
2 L Samples	
Practice – Method 1600 coupled with UF	March 7, 2016
Practice – Method 1602 (no UF)	April 11, 2016
Preliminary	April 25, 2016
PBS and matrix samples (QC criteria)	May 16, 2016
Wastewater samples (QC criteria) – Additional analyses	November 6, 2017
100 mL samples	
PBS analyses	April 11, 2016
Range-finding	July 25, 2016
Wastewater samples (QC criteria)	August 15, 2016
Wastewater samples (QC criteria) – Additional analyses	October 28, 2016

Spiking Approach



- **Referee Laboratory**
  - Prepared and shipped spiking suspensions to the participant laboratories.
    - MS2 coliphage [ATCC® 15597-B1™]
    - phi-X174 coliphage [ATCC® 13706-B1™]
  - Enumerated suspensions prior to shipping and each day of sample spiking
- **Participant Laboratories**
  - Enumerated spiking suspensions using the Double Agar Layer (DAL) procedure



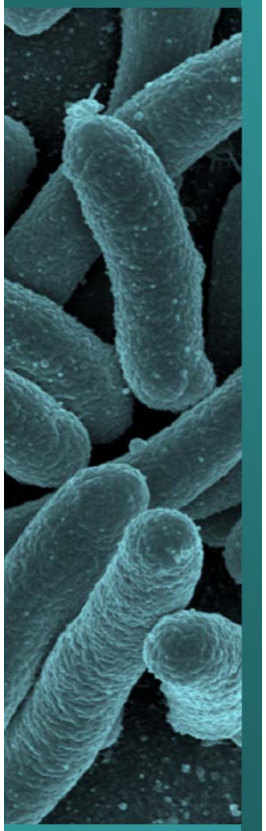
Sample Analyses
Assessment of Method Performance

2 L Samples (Method 1642)

- 1, 2 L unspiked sample
- 4, 2 L spiked samples per matrix

100 mL Samples (Method 1643)

- 2, 100 mL unspiked sample
- 8, 100 mL spiked samples



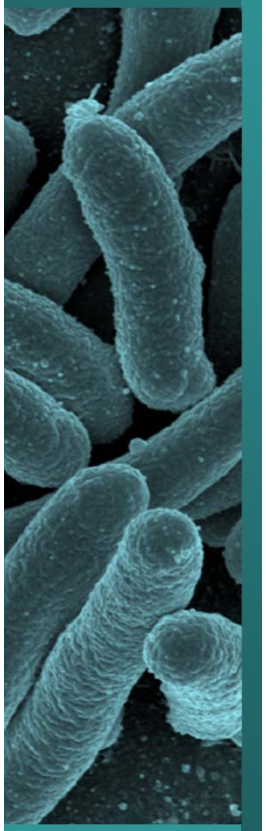
Sample Analyses
Development of QC Criteria

QC Criteria for Initial (IPR) and Ongoing (OPR) Method/Laboratory Performance Assessments

- 4, 2 L PBS samples spiked MS2 and phi-X174
- 4, 100 mL PBS samples spiked with MS2
- 4, 100 mL PBS samples spiked with phi-X174

Matrix Spikes (MS) Method Performance Assessments

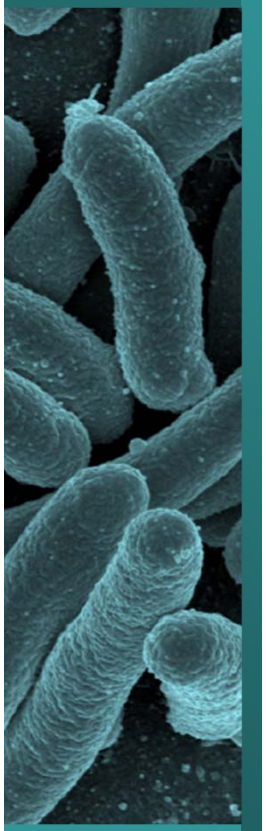
- 4, 2 L Matrix samples spiked with MS2 and phi-X174
- 4, 100 mL matrix samples spiked with MS2
- 4, 100 mL matrix samples spiked with phi-X174



Quality Control Analyses

- **Positive Controls**
  - Somatic (phi-X174) coliphage
  - Male-specific (MS2) coliphage
- **Sterility Checks**
  - Media sterility checks
  - Dilution blank sterility checks
  - Method blanks (sterile unspiked PBS)

Data Reporting and Validation



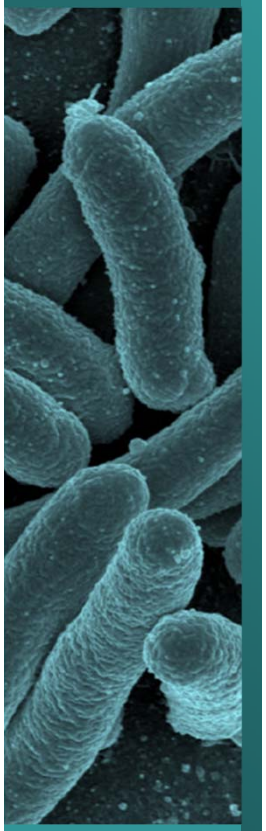
Standardized Data Checklists

Standardized data validation checklists were used to evaluate laboratory results against method and study-specific requirements including:

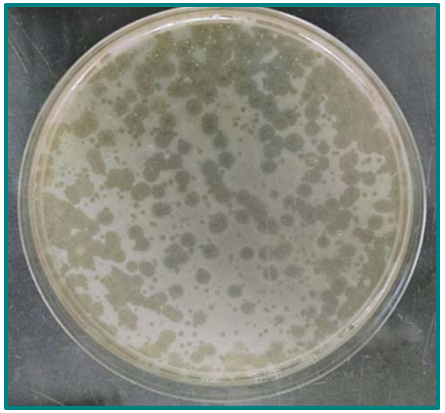
- Incubation times and temperatures
- Media and reagent preparation data
- Spiking volume
- QC results
- Confirm calculations
- Holding times

Method Performance

Study Issues

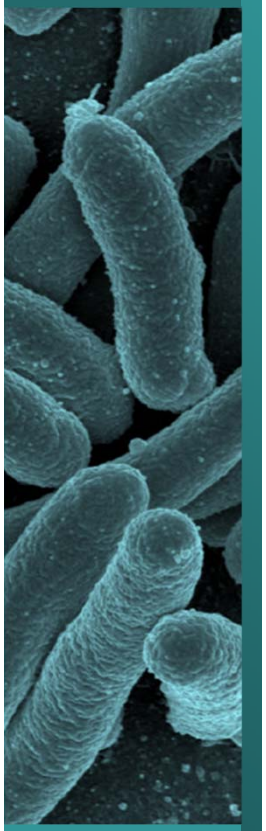


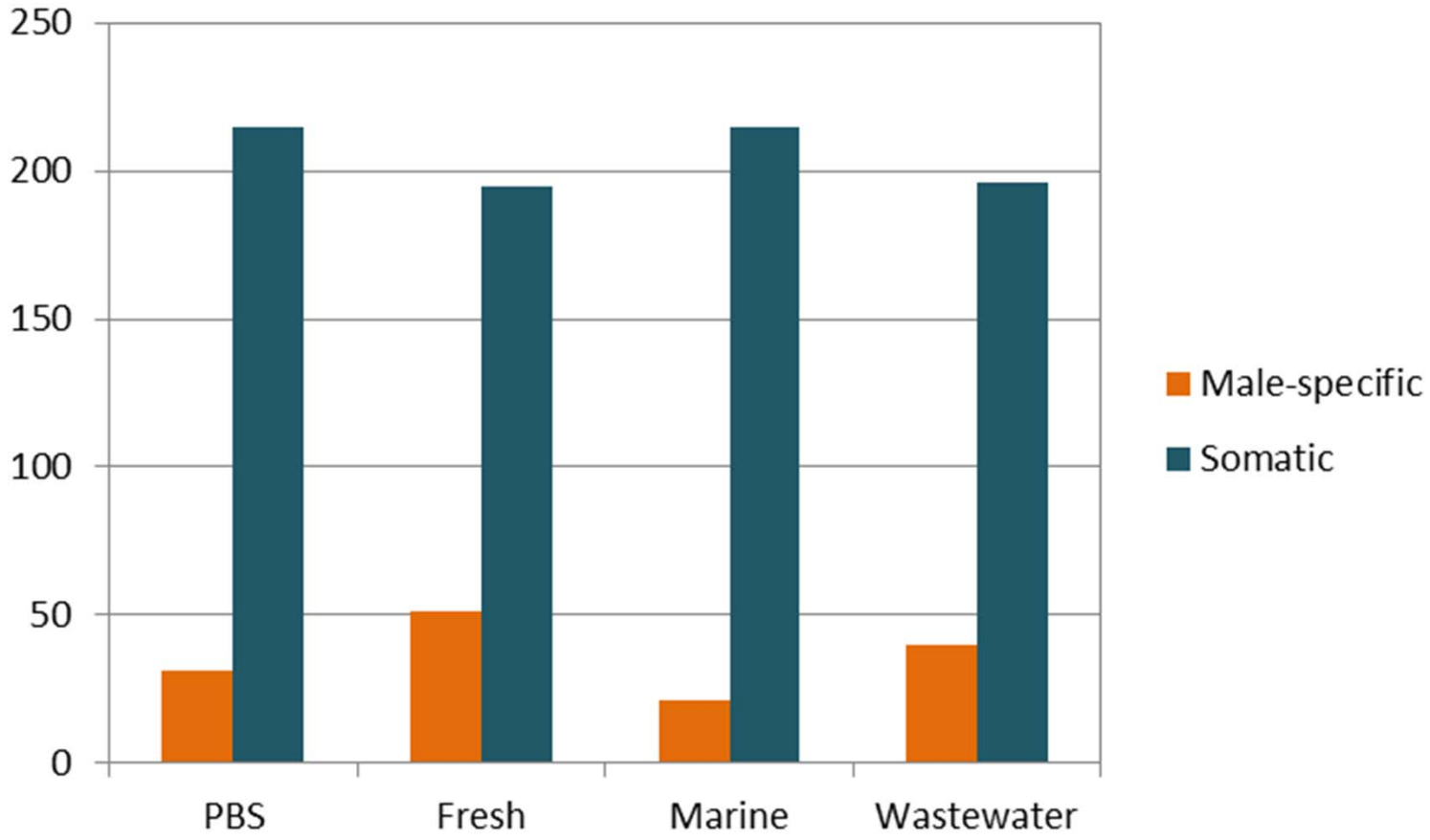
- **Ultrafiltration**
  - Leaking at connection points
  - Lack of constant flow
- **SAL**
  - Flasks tipping over in water bath
  - Plates with streaks of colonies, clumping, particulates, or TNTC
- **General**
  - Higher background levels of coliphage than expected



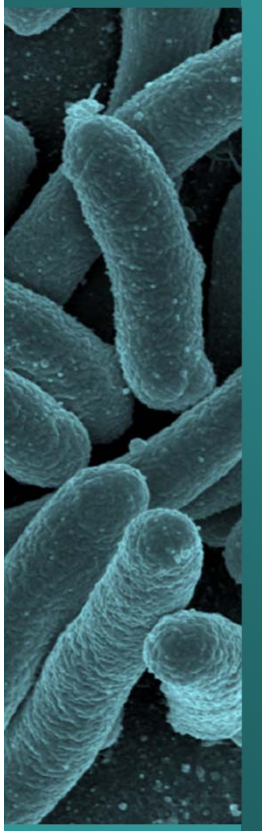
Results

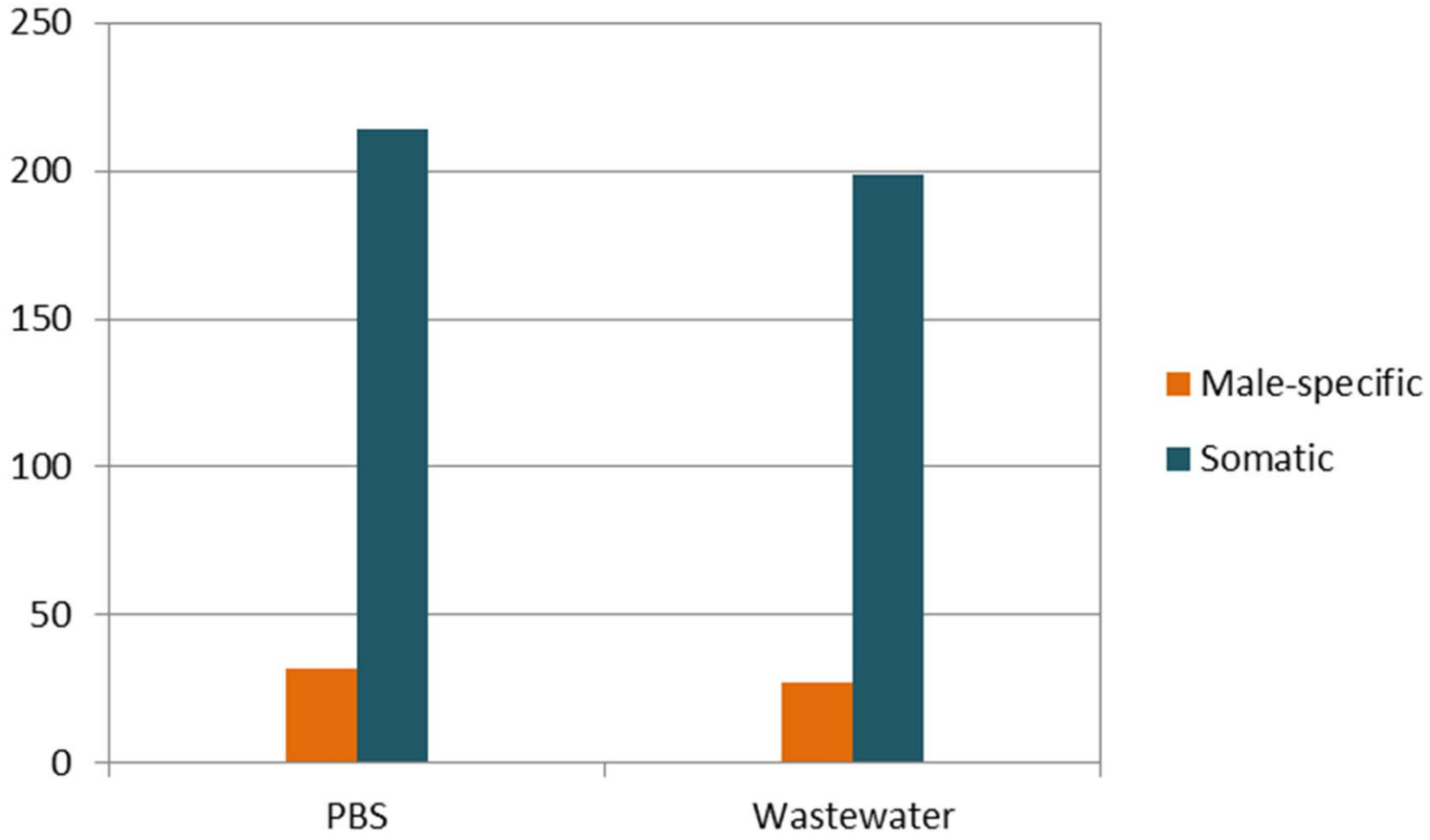
Method 1642 – Overall Percent Recoveries



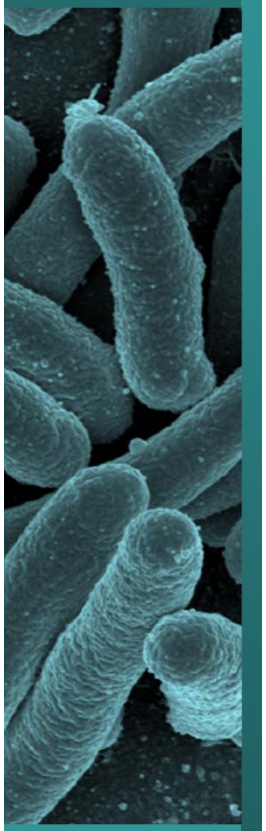


Method 1643 – Overall Percent Recoveries



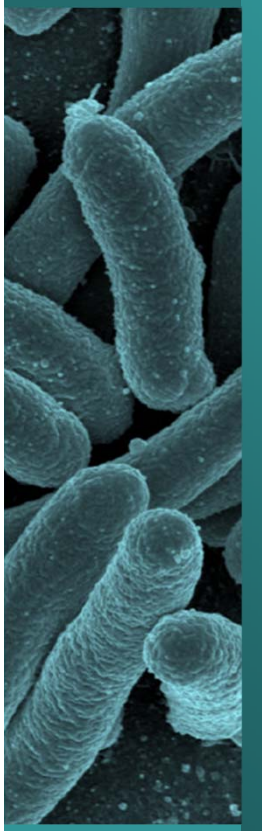


Quantitative QC Acceptance Criteria



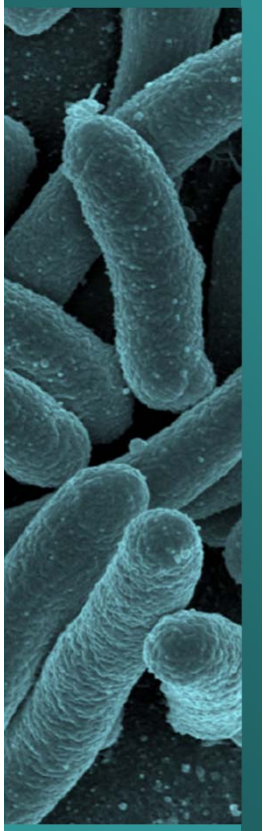
QC Acceptance Criteria: IPR and OPR

Method	Phage Type	IPR Mean Recovery	IPR RSD	OPR Recovery
UF and Method 1602 (2 L)	Male-specific	Detect – 100%	31	Detect – 100%
	Somatic	68 – 397%	28	59 – 406%
Method 1602 (100 mL)	Male-specific	9 – 100%	17	9 – 100%
	Somatic	139 – 278%	16	134 – 283%



QC Acceptance Criteria: MS and MSD

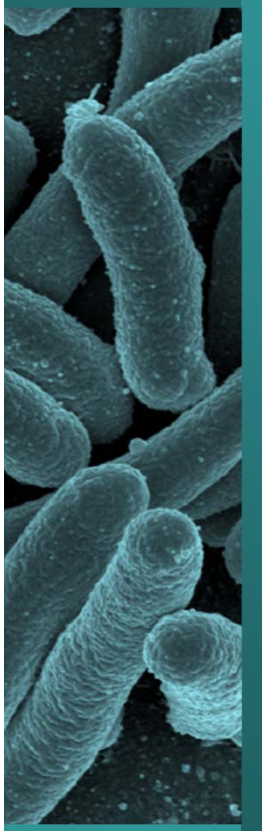
Method	Matrix	Phage Type	MS/MSD Recovery	MS/MSD RPD
UF and Method 1602 (2 L)	Fresh Water	Male-specific	Detect – 152%	130
		Somatic	Detect – 450%	59
	Marine Water	Male-specific	9 – 100%	53
		Somatic	66 – 303%	55
	Advanced Treatment Wastewater Effluent	Male-specific	10 – 100%	87
		Somatic	Detect – 388%	84
Method 1602 (100 mL)	Secondary Wastewater (no disinfection)	Male-specific	Detect – 100%	79
		Somatic	7 – 385%	51



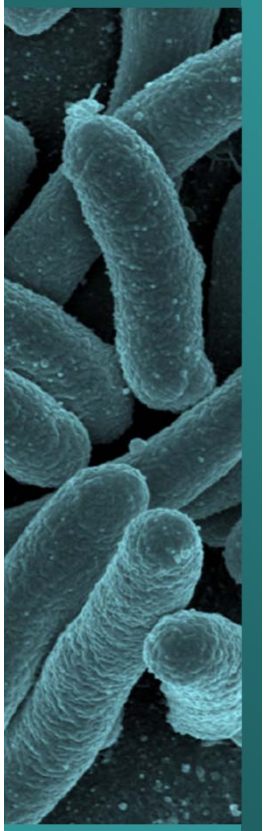
Key Points

- Ambient levels of coliphage were variable in the matrices evaluated
- Range-finding analyses can be extremely beneficial
- Prior to implementation laboratories should become proficient with UF, SAL, and DAL procedures
- Water bath space will dictate the number of samples that can be analyzed

Conclusions



Results of the study indicate that Methods 1642 and 1643 are appropriate for the analyses of male-specific and somatic coliphage in the matrices analyzed during the study



Volunteer Laboratories

Participant

- American Interplex
- Alabama Department of Public Health
- County Sanitation Districts of L.A. County – JWPCP
- Hampton Roads Sanitation District
- Hoosier Microbiological Laboratory (HML)
- IEH BioVir
- Orange County Public Health Laboratory
- Orange County Sanitation District
- San Francisco Public Water Utilities
- San Jose Creek Water Quality Laboratory – County Sanitation Districts of L.A. County
- SVL Analytical, Inc.
- Southwest Research Institute
- Texas A&M University – College Station
- University of Georgia Marine Extension Service
- University of Hawaii – Water Resources Research Center
- U.S. Environmental Protection Agency
- Wisconsin State Laboratory of Hygiene

Referee

- New York State Department of Health