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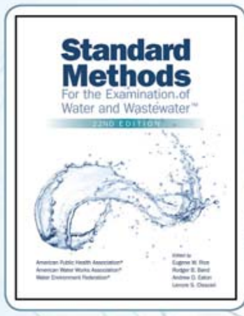

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Coliphages: What You Need To Know And How Will Laboratories, The Regulatory Community And The Public Be Impacted?

August 3rd, 2016
1:00 PM – 3:00 PM ET

Today's webcast is the result of collaboration between the WEF Laboratory Practices Committee, the American Public Health Laboratories and the WEF Disinfection & Public Health Committee

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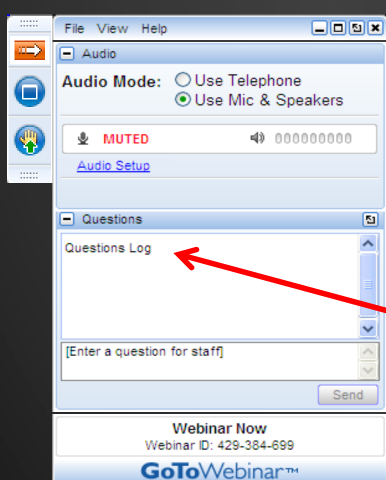
APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

How to Participate Today



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- **Submit your questions using the Questions pane.**
- **A recording will be available for replay shortly after this webcast.**

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Today's Moderator

Akin Babatola
Laboratory & Environmental Compliance Manager
ABabatola@cityofsantacruz.com
110 California Street Santa Cruz CA
WEF LPC
APHL

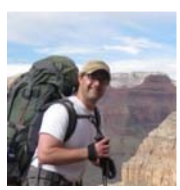
CITY OF SANTA CRUZ

Speakers

Sharon Nappier
US EPA

Naoko Munakata
Sanitation Districts of Los Angeles County
WEF D&PHC

Raul Gonzalez
Hampton Roads Sanitation District
WEF LPC



Jeremy Olstadt
Wisconsin State Laboratory of Hygiene

Sanjib Bhattacharyya
Deputy Laboratory Director
City of Milwaukee Health Department
APHL

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Recreational Water Quality Criteria for Coliphage: Updates and Experts Workshop Overview

Sharon P Nappier, MSPH, PhD
Office of Water, Office of Science and Technology
US Environmental Protection Agency
August 3, 2016

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Outline

- Recreational Water Quality Criteria
- Experts Workshop
- Next Steps

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Clean Water Act (CWA)

- Goal: Restore and maintain oceans, watersheds, and their aquatic ecosystems to protect human health, support economic and recreational activities, and provide healthy habitat for fish, plants and wildlife.
  - Establishes basic structure for state water quality standards, including regulation of pollutant discharge into the waters of the United States.

- Recreational Water Quality Criteria (RWQC), CWA 304(a)
  - Intended to be used by states adopting water quality standards to protect the designated use of primary contact recreation.
  - BEACH ACT requires EPA to review coastal RWQC every five years (next review: 2017)

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Recreational Water Quality Criteria (RWQC)

RWQC recommendations:

- **Prevent illness**

  By preventing fecal contamination and/or pathogens from entering surface waters

  – Point source permits (NPDES permits)
- **Identify impaired waters**

  – 303(d) Listing, Total Maximum Daily Loads (TMDLs)
- **Identify potentially hazardous conditions**

  – Beach notifications

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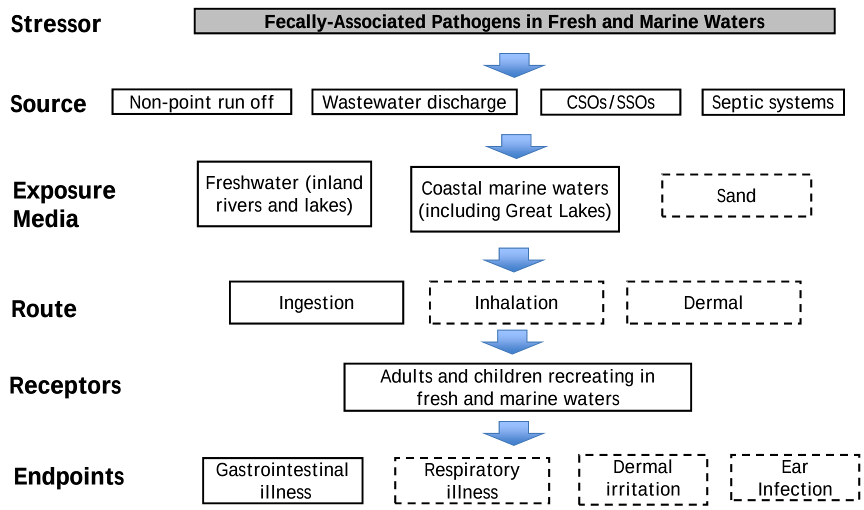
Conceptual Model



2012 Recreational Water Quality Criteria

The 2012 RWQC for primary contact recreation are associated with bacterial indicators of fecal contamination.

Highlights:

- Indicators:
  - Enterococci (marine/freshwater) and *E. coli* (freshwater)
- Specified magnitude, duration (30 day), and frequency
  - Two sets of recommended criteria, each corresponds to a different illness rate
- Includes supplemental tools
  - qPCR method for same-day notification
  - Beach action values for precautionary notification

Current Status

To prevent illness

- **Bacterial** pathogens targeted through bacterial indicators
  - Historically bacteria were thought to cause majority of illnesses
  - Wastewater treatment improvements and permits based on bacterial indicators effectively control bacterial pathogens
- QMRA, epidemiological, and microbial water quality studies indicate **viruses** cause majority of swimming-associated illnesses in human-impacted waters
  - Current WWTP, indicators, & permits do not specifically target viruses
  - Thus, viruses enter surface waters from treated & untreated human sources

To identify impaired waters or potentially hazardous conditions

- Culturable **bacterial** indicators used
  - Effective at predicting bacterial impairments of water quality
  - Epi studies indicate they may not always be predictive of **viral** illnesses

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Coliphage – A Viral Indicator

In use since the 1970's:

- EPA: Ground Water Rule recommended coliphage to detect and/or quantify viral indicators in ground water
- ISSC/FDA: Recommended the use of male-specific coliphage for shellfish bed closure decisions
- NWRI: Framework for Direct Potable Reuse recommends coliphage be used as a surrogate for evaluating virus removal in reuse configurations

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Recreational Water Quality Criteria - Coliphage

Coliphage advantages:

- Of fecal origin/highly concentrated in sewage
- Physically similar to enteric viruses of concern
- Similar persistence patterns to enteric viruses of concern
  - To treatment and to environmental insults
- No appreciable re-growth in ambient waters
- Non-pathogenic

Indicators rather than pathogenic viruses:

- Currently not feasible to assess all pathogenic viruses due to methodological and time constraints

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Recreational Water Quality Criteria - Coliphage

- Prevent viral illness √
  - Coliphage-based discharge permits can prevent viruses entering source waters, thus preventing viral illnesses
- Identify impaired waters or potentially hazardous conditions √
  - Epidemiological studies indicate coliphage may provide a tool to better protect from viruses

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Coliphage Experts Workshop: March 1-2, 2016

Coliphage Experts Workshop

Purpose: Have internationally recognized experts engage on the topic of how best to protect public health from viral contamination of water given currently available information.

Specific Goals:

- Obtain input on science questions from experts in fields of environmental microbiology, microbial risk assessment, and environmental epidemiology.
- Gather scientific insight to determine the best coliphage type (male-specific and/or somatic) for use in CWA 304(a) criteria.
  - Identify situations where these coliphage types may be most useful for preventing illnesses and identifying impaired waters
- Identify research needs that can be addressed by 2017.

Coliphage Experts Workshop – Experts

Name	Affiliation
Nicholas Ashbolt	University of Alberta
William Burkhardt	U.S. Food and Drug Administration
Kevin Calci	U.S. Food and Drug Administration
Jack Colford	University of California, Berkeley
John Griffith	Southern California Coastal Water Research Project
Vincent Hill	Centers for Disease Control and Prevention
Juan Jofre	University of Barcelona, Spain
Naoko Munakata	Sanitation Districts of Los Angeles County
Rachel Noble	University of North Carolina, Chapel Hill
Joan Rose	Michigan State University
Mark Sobsey	University of North Carolina, Chapel Hill
Timothy Wade	U.S. Environmental Protection Agency

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Coliphage Experts Workshop - Scope

- Focused on recreational risks associated with fecal contamination
  - Other risks not considered: sunburns, shark attacks, etc.
- Focused on science aspects of criteria development
  - Minimized policy and implementation discussions

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Coliphage Experts Workshop – Topic Areas

1. Need for a Viral Indicator
2. Coliphage as a Predictor of Gastrointestinal Illness
3. Coliphage as an Indicator of WWTP Performance
4. Male-specific vs Somatic Coliphage
5. Systematic Literature Review of Viral Densities

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Coliphage Experts Workshop – Meeting Format

- Experts assigned a topic with associated charge questions
  - Experts provided written responses to charge questions to EPA prior to Workshop
  - Responses compiled and provided to all experts prior to Workshop
- Each expert gave 10-15 min presentation, based on their answers to charge questions
- Group collectively discussed charge questions
- Group captured main points in discussion summary

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Coliphage Experts Workshop – Highlights (1)

Topic 1: Need for a Viral Indicator

- Individual experts agreed that viruses are a source of illness in recreational water exposures.
- Viruses can enter surface waters via WWTP effluent.
  - Especially during wet weather and when WWTPs exceed design flows.
- Coliphages are more similar to human pathogenic viruses compared to the traditional fecal indicator bacteria (FIB).
  - Mimic human pathogenic viruses.
- Coliphages have demonstrated value added for managing risks and are used full-scale to address WWTP water quality and related applications.
  - Ex: NC reclaimed water, Ground Water Rule, and by FDA for reopening shellfish harvesting areas after catastrophic spills.
- Coliphage methods are available, inexpensive, and could be developed into easy-to-use commercial kits.

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Coliphage Experts Workshop – Highlights (2)

Topic 2: Predictor of GI Illness

- Future epidemiological studies should specifically include coliphages as measured indicators.

Topic 3: Indicator of WWTP performance

- Coliphages are consistently present in municipal sewage, and provide a baseline for looking at different WWTP processes under varied conditions.
  - Some experts indicated the literature suggests coliphage and human viruses have more similar log-reductions during wastewater treatment, compared to traditional FIB.

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Coliphage Experts Workshop – Highlights (3)

Topic 4: Male-specific vs Somatic Coliphages

- Opinions ranged on whether somatic, male-specific coliphage, or both would be better for various applications.
  - Evidence for both showing relationship to GI illness.
  - Male-specific coliphage behave more similarly to RNA viruses under some conditions and are currently used successfully by FDA/ISSC.
  - Somatic may persist longer than male-specific coliphage and may be present in greater concentrations in raw sewage.
  - Hosts are available that can detect both.

Topic 5: Review of Viral Densities

- Individual experts supported how the systematic analysis was structured and conducted.

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Status and Timeline

date	milestone
04/17/2015	*Review of Coliphages as Possible Viral Indicators of Fecal Contamination for Ambient Water Quality*
10/15/2015	Stakeholder Webinar
03/01/2016	Coliphage Expert Workshop fact sheet (July 2016) and proceedings (winter 2017)
2016	Listening Sessions/Webinars • Conferences (New Orleans and Chapel Hill) • States • Other stakeholders (industry/environmental groups)
early 2017	Analytical method multi-lab validation
late 2017	Drafting of the Criteria

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Questions?

Contact:

Sharon Nappier

Nappier.Sharon@epa.gov

(202)566-0740

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Naoko Munakata, PhD, PE

- Chair, WEF Disinfection and Public Health Committee
- Supervising Engineer, Sanitation Districts of Los Angeles County

SANITATION DISTRICTS OF LOS ANGELES COUNTY

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Implications of Coliphage Criteria for Water Resource Recovery Facilities

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How would proposed phage criteria affect WRRFs?

- Laboratory/monitoring requirements
- Disinfection/treatment requirements

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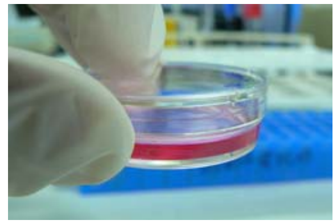
Laboratory requirements

- Need to learn/certify/ implement new method (based on existing EPA virus methods)
- Additional costs, particularly if the criteria are in addition to (rather than instead of) current bacterial criteria



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Changes to treatment processes will depend on many factors

- Phage (male specific/F+, somatic)
- Level/concentration limit
- Level of upstream treatment (primary, secondary, tertiary)
- Disinfectant

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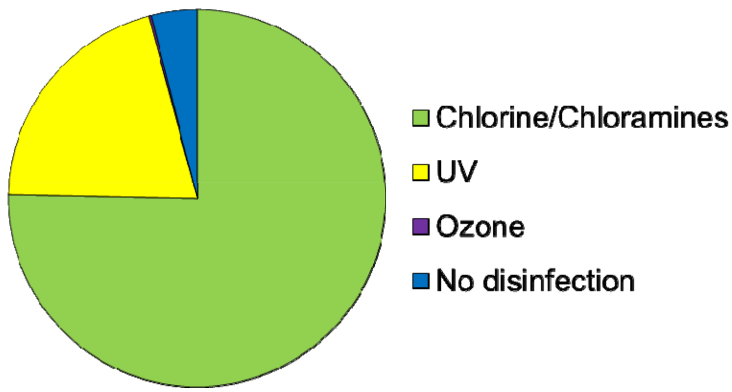
Disinfectants used at WRRFs*



*At publicly owned treatment works (POTWs) with flow > 0.95 MGD.
From WERF 04-HHE-4, 2008.

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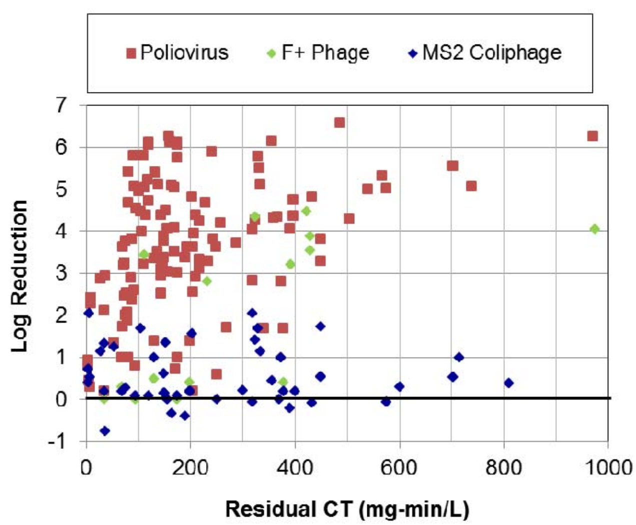
Chloramines



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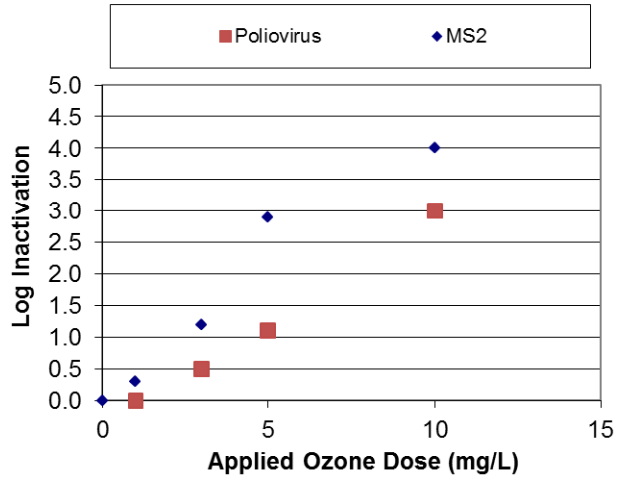
Ozone



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Summary

- Disinfection is inherently variable
- Finding a "good" indicator is difficult
  - Indicators that are conservative for one disinfectant may not be for another
  - "F+ phage" were disinfected by chloramines but MS2 coliphage (a type of F+ phage) was not
- Disinfection depends on water quality and upstream processes
- Impacts on WRRFs will depend on the indicator chosen, the limit, upstream plant processes, and the disinfectant used

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The ultimate goal:

- To protect human health and beneficial uses
- To identify POTWs that do not provide adequate disinfection
- To exempt POTWs with adequate levels of disinfection from equipment upgrades that are unnecessary and expensive

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More data are needed!

- Experts' Workshop: coliphage are promising indicators, but more data are needed
- Examples
  - Epidemiology (risk of illness in rec. waters)
  - Performance with different disinfectants and comparison with pathogens
  - Typical concentrations in POTW effluents (with different treatment processes) and receiving waters

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A POTW's Perspective

- Hampton Roads Sanitation District
- WEF Lab Practices Committee Member

HRSD
Cleaning wastewater every day for a better Bay.

Raul Gonzalez, Ph.D

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Diel variation of fecal indicators and pathogens in wastewater

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Coliphage Criteria?

EPA
interest in new viral criteria

ISSC
2015 National Shellfish
Sanitation Program Guide

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Preparation for Coliphage Criteria

- Understanding of fate and transport
- Hampton Roads specific data

HRSD Projects:
Dilution study, Baseline study, Wet weather transport, Seasonal Variation Study, Treatability Study

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Coliphage Pilot

Goal: Examine the diel variability of indicators and pathogens in 4 POTWs with differing biological treatment

Objectives:

1. Determine if hydrologic retention time should be incorporated into sampling
2. Characterize the indicator-pathogen relationship within the POTWs

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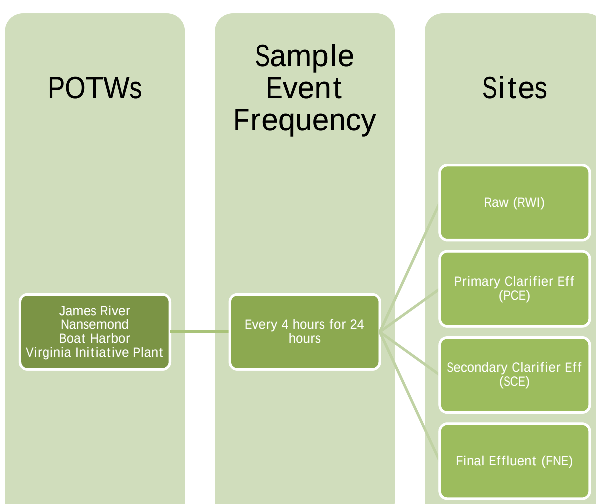
Sample Scheme



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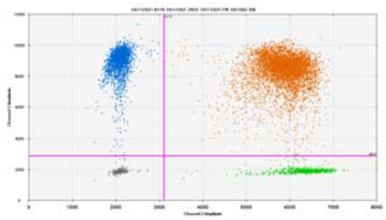
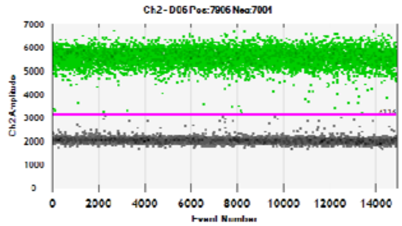
Measured Parameters





Indicators
Enterococci: IDEXX
E. coli: IDEXX
Male-Specific Coliphage: EPA method 1602

Enteric Pathogens
Adenovirus: ddPCR
Norovirus GI: ddPCR
Enterovirus: ddPCR

Environmental
Ammonia, salinity, turbidity, temperature, dissolved oxygen, free chlorine, combined chlorine

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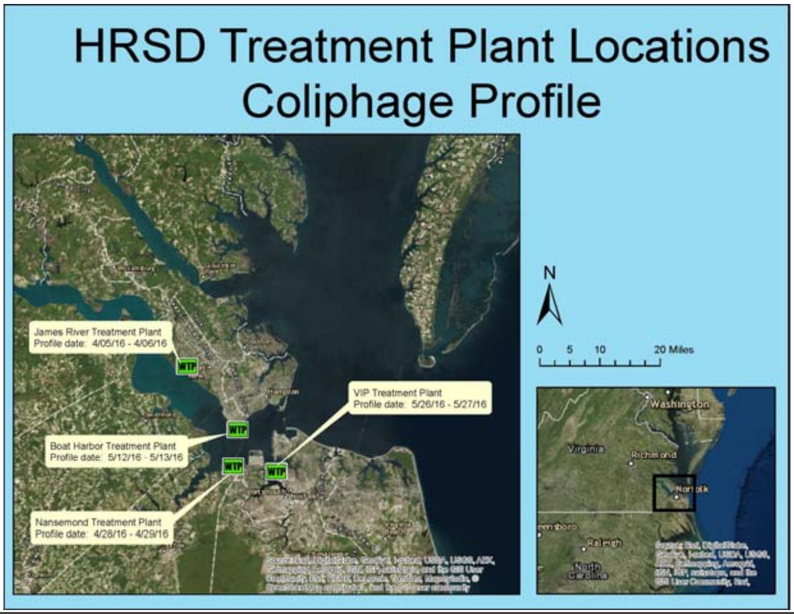

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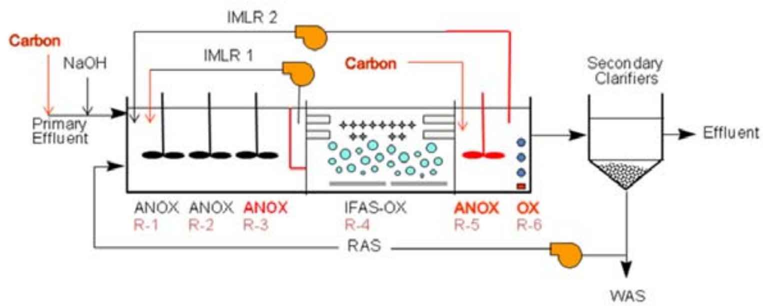
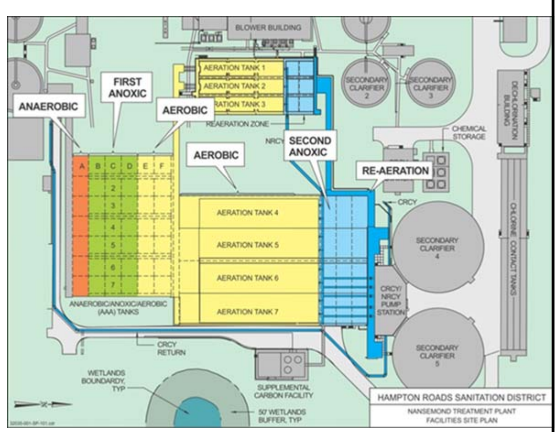
James River	
Average daily design flow	20 MGD
Average flow during sampling	13.8 MGD
Primary treatment	Circular, peripheral feed, center weir
Secondary treatment	4 Stage MLE with 2 anoxic and 2 aeration regions. NRCY recycle to primary anoxic zone. IFAS media is used for fixed growth BNR
Sludge SRT	3.1 days
MLSS	2740 mg/L
Disinfection type	Sodium Hypochlorite
Avg. design disinfection contact time	37.15 minutes

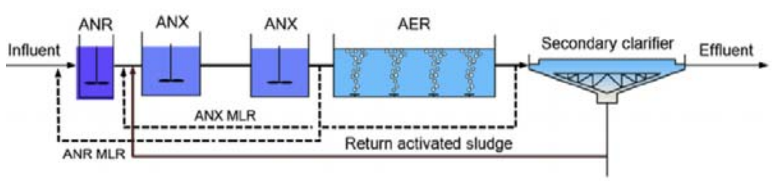


Nansemond	
Average daily design flow	30
Average flow during sampling	17.63
Primary treatment	Circular, center feed with peripheralweir & rectangular clarifiers
Secondary treatment	5 Stage Bardenpho for BNR and Phosphorus removal
Sludge SRT	15.1 days
MLSS	3080 mg/L
Disinfection type	Sodium Hypochlorite
Avg. design disinfection CT	38 minutes



Boat Harbor	
Average daily design flow	25
Average flow during sampling	14.7
Primary treatment	Rectangular clarifiers
Secondary treatment	Aeration tanks for carbonaceous BOD removal, BNR not a primary function
Sludge SRT	43.8 days
MLSS	2720 mg/L
Disinfection type	Sodium Hypochlorite
Avg. design disinfection contact time	59 minutes

VIP	
Average daily design flow	40
Average flow during sampling	29.33
Primary treatment	Circular, center feed with peripherial weir & rectangular
Secondary treatment	Virginia initiative process for BNR and phosphorus removal
Sludge SRT	9.4 days
MLSS	2280 mg/L
Disinfection type	Sodium Hypochlorite
Avg. design disinfection contact time	32.3

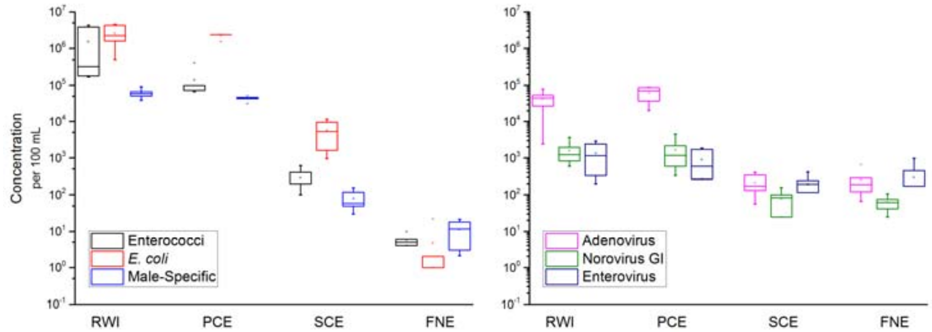


Results

Environmental Parameters

Parameter During Sampling	POTW James River	Nansemond	Boat Harbor	Virginia Initiative Plant
Combined Chlorine (mg/L)	0.0 - 0.01	0.0 - 0.03	0.01 - 0.18	0.0 - 0.42
Free Chlorine (mg/L)	0.0 - 0.02	0.0 - 0.02	0.0 - 0.07	0.0 - 0.05
Ammonia (mg/L)				
influent	25.9 - 33.0	34 - 40.4	19.7 - 28.7	20.7 - 25.4
effluent	0.24 - 1.36	0.78 - 1.55	20.9 - 23.2	0.092 - 0.124
DO (mg/L)				
influent	0.92 - 1.63	0.73 - 1.38	0.91 - 1.66	0.61 - 2.39
effluent	8.14 - 8.75	7.61 - 8.55	7.98 - 9.13	7.06 - 8.19
pH				
influent	6.7 - 6.79	6.96 - 7.47	6.82 - 6.98	6.02 - 6.88
effluent	6.9 - 7.18	6.93 - 7.15	7.26 - 7.39	6.34 - 6.78
Temp				
influent	14.09 - 17.06	18.19 - 19.29	19.77 - 20.68	20.95 - 26.80
effluent	16.25 - 17.66	19.72 - 20.62	21.37 - 22.818	22.07 - 26.81
Salinity				
influent	0.31 - 0.45	0.52 - 0.77	0.49 - 0.68	0.01 - 1.01
effluent	0.13 - 0.29	0.46 - 0.51	0.56 - 0.61	0.31 - 0.69
Turbidity				
influent	102 - 155	112 - 377	78.6 - 171	60.5 - 114
effluent	1.67 - 7.09	2.54 - 4.64	5.6 - 12.3	4.22 - 5.77

James River



N=6 for each individual box and whisker plot

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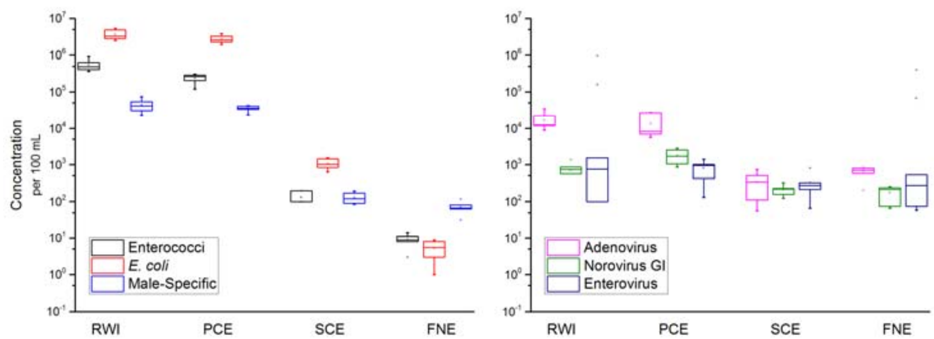
Nansemond



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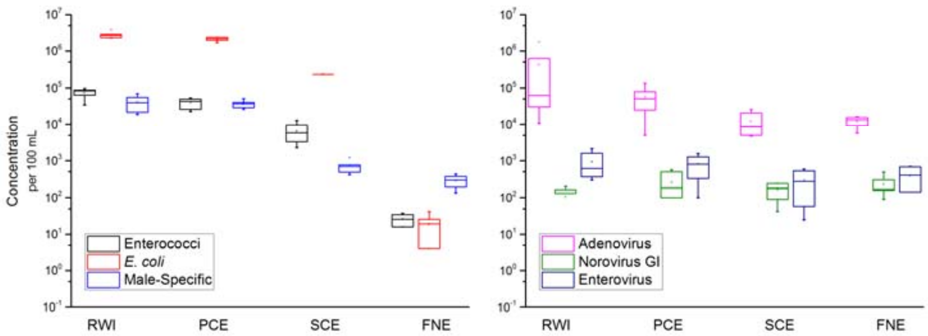
Boat Harbor



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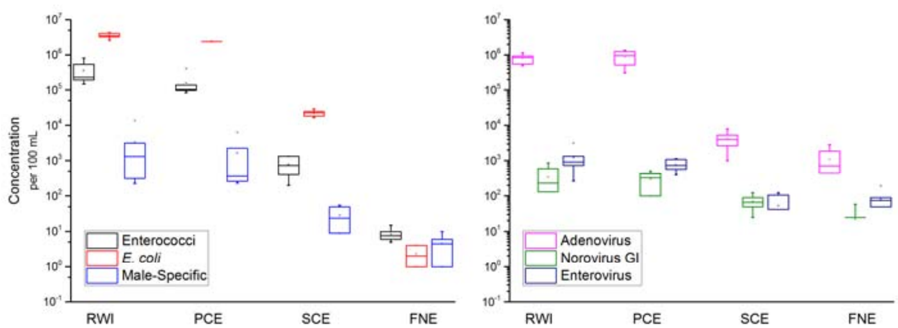
Virginia Initiative Plant



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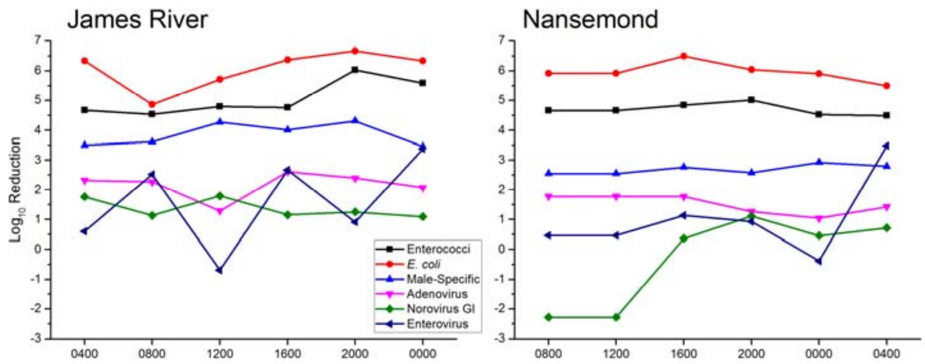
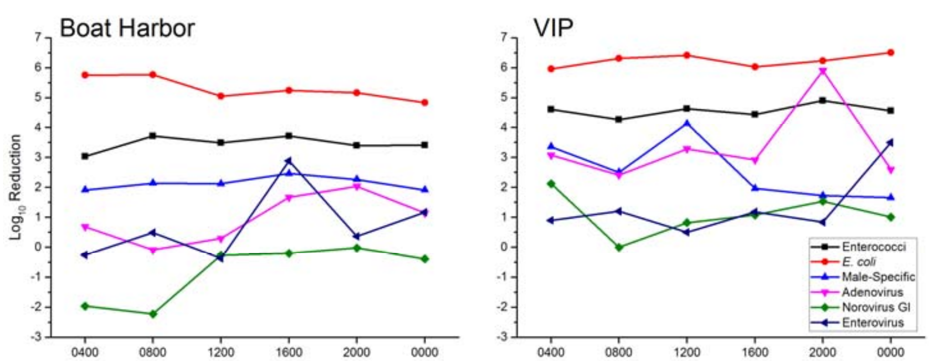
Log_{10} Reduction Variability



Log Reduction = $\log_{10}$(influent) – $\log_{10}$(effluent)

Log_{10} Reduction Variability



Log Reduction = $\log_{10}$(influent) – $\log_{10}$(effluent)

Indicators and Pathogens	Mean log_{10} reduction ± sd: Whole Process	Secondary Treatment	Chlorination
James River			
Enterococci	5.06 ± 0.79	2.67 ± 0.22	1.65 ± 0.24
E. coli	6.05 ± 1.25	2.74 ± 0.38	3.34 ± 0.87
Male-Specific Phage	3.86 ± 0.39	2.81 ± 0.23	0.91 ± 0.35
Adenovirus	2.16 ± 0.56	2.50 ± 0.17	-0.05 ± 0.3
Norovirus GI	1.37 ± 1.07	1.26 ± 0.32	0.05 ± 0.45
Enterovirus	1.57 ± 1.48	0.88 ± 0.84	0.58 ± 1.51
Nansemond			
Enterococci	4.70 ± 0.20	3.26 ± 0.18	1.19 ± 0.34
E. coli	5.96 ± 0.32	3.39 ± 0.17	2.38 ± 0.27
Male-Specific Phage	2.70 ± 0.16	2.46 ± 0.23	0.27 ± 0.15
Adenovirus	1.51 ± 0.31	1.69 ± 0.55	-0.37 ± 0.54
Norovirus GI	-0.32 ± 1.55	0.90 ± 0.13	0.12 ± 0.34
Enterovirus	1.02 ± 1.32	0.37 ± 0.36	-0.40 ± 1.44
Boat Harbor			
Enterococci	2.53 ± 0.25	0.82 ± 0.30	1.98 ± 0.15
E. coli	3.95 ± 0.38	0.95 ± 0.06	3.42 ± 0.43
Male-Specific Phage	1.60 ± 0.21	1.73 ± 0.12	0.66 ± 0.15
Adenovirus	0.61 ± 0.82	0.59 ± 0.37	0.17 ± 0.41
Norovirus GI	-0.50 ± 0.98	-0.15 ± 1.03	-0.07 ± 0.37
Enterovirus	0.52 ± 1.20	0.52 ± 0.22	0.33 ± 0.88
Virginia Initiative Plant			
Enterococci	4.57 ± 0.21	2.30 ± 0.25	1.92 ± 0.31
E. coli	6.25 ± 0.21	2.04 ± 0.09	4.04 ± 0.29
Male-Specific Phage	2.56 ± 1.00	1.49 ± 0.68	0.83 ± 0.59
Adenovirus	3.37 ± 1.29	2.36 ± 0.45	1.02 ± 1.45
Norovirus GI	1.10 ± 0.71	0.60 ± 0.32	0.81 ± 0.63
Enterovirus	1.36 ± 1.08	1.63 ± 1.09	-0.39 ± 0.77

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Correlations

	Enterococci	*E. coli*	Male-Specific	Adenovirus	Norovirus GI	Enterovirus
Enterococci	-	0.93	0.79	0.71	0.61	0.53
E. coli	0.93	-	0.77	0.76	0.51	0.55
Male-Specific	0.79	0.77	-	0.68	0.66	0.55
Adenovirus	0.71	0.76	0.68	-	0.44	0.55
Norovirus GI	0.61	0.51	0.66	0.44	-	0.42
Enterovirus	0.53	0.55	0.55	0.55	0.42	-

N=96

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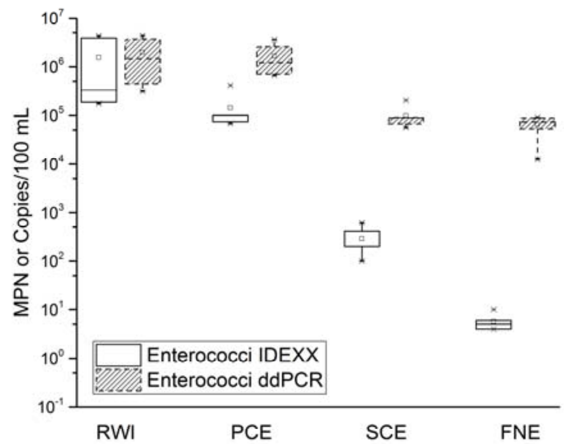
Molecular vs. Culture



James River Treatment Plant

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Take Home Messages

- Diel variability was minimal
  - HRT will not be incorporated into sampling scheme
- Indicator log reductions were greater than enteric pathogens, and consistent with literature values
- POTWs that performed BNR in 2º treatment had enhanced log removal

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COLIPHAGES

Dr. Sharon Long and Jeremy Olstadt
Wisconsin State Laboratory of Hygiene

Viruses

- Considered the smallest and most basic life form (prions are not considered "alive")
- Much smaller than bacteria (nm vs μm)
- Consists of nucleic acid (genetic material) and a capsid (protein shell)
- Genetic material can be dsDNA, ssDNA, or RNA
- Strict requirement for a host to replicate
- More than 140 enteric viruses

Coliphages

- Coliphages – both somatic and F-specific coliphages may be simultaneously detected using *E. coli* C3000 host. Their presence can be detected in as little as 16 hours. (See EPA Methods 1601 and 1602)

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Somatic Coliphages

- Somatic coliphages – good general indicators of fecal contamination
- Four viral families *Myoviridae, Siphoviridae, Podoviridae* and *Microviridae*; the first 3 families consist of icosahedral heads with tails of varying length and contain dsDNA, the last group do not possess tails and contains ssDNA
- Use *E. coli* CN13 or *E. coli* C to detect via a plaque assay

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Male-specific, F-specific or F+coliphages

- F-specific coliphages – typically do not replicate in the environment, and subtyping can be useful in discriminating between human and non-human microbial inputs
- Two viral families *Levivirodae* and *Inoviridae*, the *Levivirodae* contain RNA and are small icosyhedral viruses without tails, the *Inoviridae* contain ssDNA and are filamentous
- Use *E. coli* Famp or *S. typhimirum* WG49 (a genetically constructed host) to detect via a plaque assay

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More on Coliphages

- Additionally, serotyping of F+RNA coliphages can discriminate between contamination of human and non-human origin
- Disadvantages of this indicator system include significant temperature affects on organism die-off, no consensus in the scientific community as to which host to utilize, and variable presence of these phages in animal populations (i.e. non-uniform inoculation).

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Viruses in Water: Monitoring

Sources of Enteric Pathogens

Landfills

Biosolids - application on land

wastewater

- application on land
- ocean disposal

septic systems

leaky sewers

stormwater

CSOs and SSOs

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Challenges for Monitoring

- Viruses have a lower infectious dose than bacterial waterborne pathogens (10s vs 100s to 1000s)
- Viruses are present at lower concentrations in environment: Need to concentrate large volumes of sample
- Viruses cannot be "cultured" as easily as bacterial waterborne pathogens

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Sample Concentration

Physical basis

- Adsorption – 1MDS filter
- Physical entrapment – Ultrafilter
- Centrifugation
  - Polyethylene glycol precipitation
  - Ultracentrifugation

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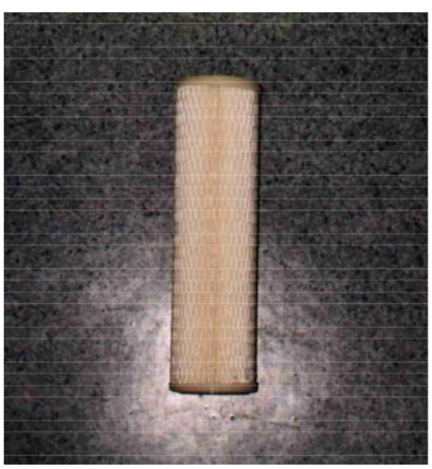
EPA Sample Concentration

- Filter 100L+ of sample
- Desorb viruses from filter using 1 liter beef extract solution
- 1-liter sample concentrate:
  - Add acid to decrease pH to 3.5
  - Organic material (with viruses attached) precipitates
  - Centrifuge
  - Viruses pellet out
  - Resuspend pellet in buffer

Source: Marylynn Yates, Prof. UC Riverside

1MDS Collection Apparatus



Ultrafiltration

- 30K Da molecular weight cut off
- Block filters with calf serum (proteins) to prevent adsorption
- Add polyphosphates to create "charged cloud" around microorganisms to prevent adsorption
- Collect concentrate (retentate)
- Wash ultrafilter with polyphosphate and surfactant (Tween 80), combine with concentrate

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Flow-through Ultrafiltration

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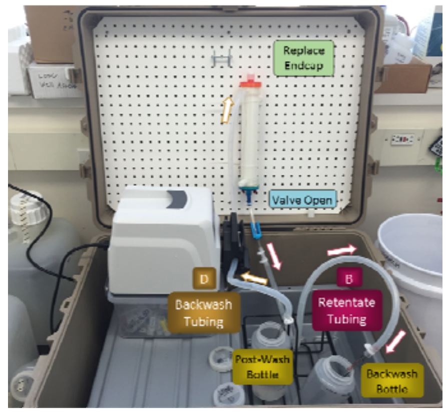
Dead-end Ultrafiltration



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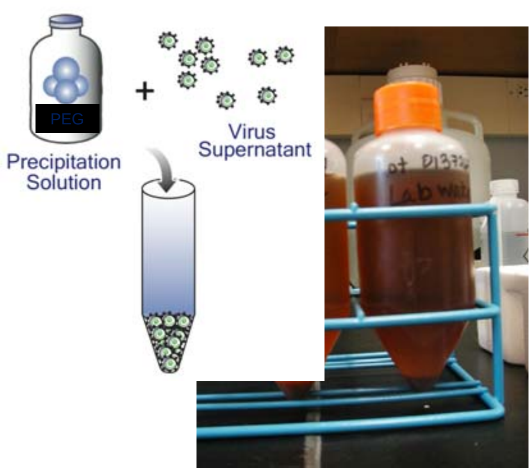

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Ultracentrifugation

1 hour
10,000 to 77,000xg

Source: http://www.igb.fraunhofer.de

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COLIPHAGE METHODS

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Method 1601: Enrichment

- Collect Sample, Measure 1L
- Incubate 16-24 hours at 37ºC
- Spot 10uL of each enrichment onto spot plate
- Incubate 16-24 hours at 37ºC
- Lysis zone formation indicates a positive sample

Add $MgCl_2$, host, 10x tryptic soy broth, 100x antibiotic

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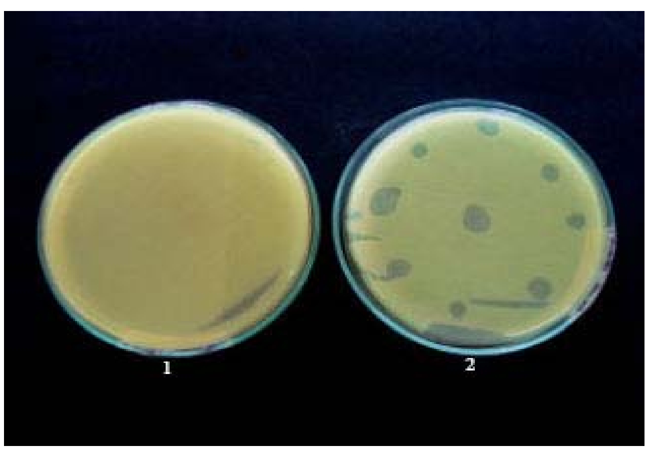
Coliphage Spot Plate



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Method 1602: Single Agar Layer

- Collect sample, measure 100 mL
- Add Host
- Mix with 2x TSB-1.5% agar and antibiotics
- Pour into five 150mm petri dishes
- Count plaques

Add $MgCl_2$,
Heat to 36ºC

Heat to 43ºC

Incubate 16-24 hours
At 36ºC

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Coliphage Plate

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Coliphages in Groundwater

- Monitoring in Wisconsin yielded low occurrence from groundwater
- Less than10% of animals(including humans) shed coliphages
- Analysis in groundwater for recent project has ceased due to lack of detection
- Money may be better spent on other indicator

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Coliphages in Recreational Water

- Recreational water analysis locally has shown detection almost inevitable
- Analysis for coliphages in recreational water **not** routine.
- Presence of a large amount of waterfowl often the source
- Coliphage useful as source tracking tool if detection is inevitable

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Sanjib Bhattacharyya, PhD

Deputy Laboratory Director,
City of Milwaukee Health Department Laboratory

Adjunct Faculty, Joseph J. Zilber School of Public Health
Clinical Associate Professor, College of Health Sciences
University of Wisconsin-Milwaukee

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Benefits of Multi-agency Partnership as a Model Practice to Bring in Novel Testing in Public Health Laboratories

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Outline

- MHD Laboratory Programs and Partnerships
- Community Engagements and System Partnerships
- Partnership Challenges and Keys to Success

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City of Milwaukee Health Department
- Today's Public Health Laboratory

- Established in 1872
- 16,000 sq. ft.
- Totally rebuilt 1957 → 2000
- Dedicated one pass air- HEPA-in
- Dedicated exhaust- HEPA-out
- Clinical & Environmental Chemistry
- Clinical & Environmental Microbiology
  - BSL-3 Suite
  - Renovated 2003- added BSCs/room
  - STD Clinic laboratory (offsite)
- Virology & Molecular Science

~ 100,000 tests/year

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MHD Laboratory Programs

Sexually Transmitted Disease
- Resistance surveillance: NAAT ID, GS-AST- CDC

Foodborne Diseases

Emergency Preparedness

Molecular Diagnostics
- Real-time PCR (Bacterial and viral pathogens)
- Luminex (Respiratory virus surveillance; enteric pathogens)
- Molecular sequencing- Sanger & Pyroseq (ref. bacteria and fungus ID, TB, anti-viral resistance)- Next Generation Sequencing *(outsourced)*

Communicable Diseases
- Microbiology: Clinical, Env. & TB
- Virology: culture, NAAT, serology
- Surveillance programs: Wisc. CDC. WHO

Waterborne Pathogens

Water Quality – Recreational and Potable- Colilert, qPCR- *E. coli*, waterborne viruses, Crypto/Giardia

Chemistry- Analytical and Clinical
- Env. & Blood lead, Heavy metals, Asbestos, Household allergens- ELISA, MARIA
- AA's, GC/LC-MS- VOC/SVOC/Env. tox/Soil- heavy metals, nutrients

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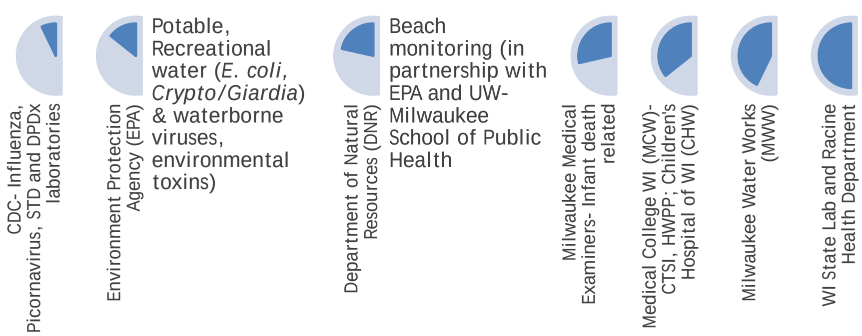
Research and Academic Partners
- A Snapshot Schematic



……..and many other partners

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Outline

- MHD Laboratory Programs and Partnerships
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Partnership & Communication

One of the **11 Core Functions of Public Health Laboratories (PHLs)**, as defined by the Association of Public Health Laboratories (APHL)

Partnership and Communication

Support their respective state public health laboratory systems

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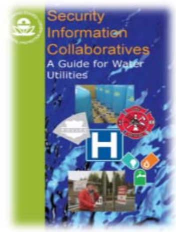
Hands Across the Hallway:
Partnerships Protect Public Health

- April 2013 marked the 20-year anniversary of Milwaukee's *Cryptosporidium* outbreak (1993)- the largest waterborne disease outbreak in US history
- A leading national example of partnerships between health departments, drinking water utilities and public health stakeholders- the **Milwaukee Inter-Agency Clean Water Advisory Council (IACWAC)**
- Endorsed by Milwaukee Common Council legislation in 1994; charged with overall coordination of water quality issues in community
- **IACWAC** was highlighted in the U.S. EPA's guide
- Multi-agency team approach to water and public health highly relevant to managing security concerns involving both water and public health; possible contamination of a public water system
- Key partners: MWW, MHD, MMSD, DNR, WI- DPH, DPW
- Cryptosporidium/Giardia- EPA Method 1623.1 (DNR & EPA LT2)



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Definition of Local PHL System or Network
(within State PHL System)

"A public health laboratory system is an alliance of organizations and individuals that operate in an interconnected and interdependent way to facilitate the exchange of information, optimize laboratory services, and help control and prevent disease and public health threats."

Developed by MHDL when embarked upon Laboratory System Improvement Program (L-SIP) assessment in November 2010- became the first *local* PHL to do so

Gradus MS, Bhattacharyya S, Murphy A, Becker JN, Baker BK. 2013. Milwaukee Laboratory System Improvement Program (L-SIP). Public Health Rep. Suppl. 2:40

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Benefits of PHL Partnerships

An Innovative System's Approach to Assess and Define Roles and Responsibilities

- **Diverse group of partners**
  - Diversity in applied research areas & innovations
  - Expanded research capabilities, themes & collaborations
- **Identify laboratory systems need & priorities**
  - Define roles & responsibilities
  - Regulatory vs. Research
- **Create a PHL system resource inventory**
  - Current research
    - Research methods (e.g., chemical, biological, microbial, engineering), biological systems, modeling & PH surveillance
  - Research interests
    - Linking to other disciplines (outreach & interdisciplinary)- environmental microbiology, chemistry, toxicology (bio-monitoring); genomic, molecular source tracking, novel biological indicators
  - Resources
    - Models/centers of excellence, databases, sample repositories, technologies (AMD), instrumentations (analytical platforms), students/interns, training and support staff

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Technology Advancement and PHL Interventions

- **Evaluation of new platforms/technology**
  - PH labs routinely use and evaluate new instruments and provide input on the next generation of products
- **Training on new technology**
  - Develop joint training courses- new tech, bio-safety & security
  - Provide opportunities for corporate members to learn about PH preparedness and response capabilities

Work together with partners to understand testing priorities and system needs

- Clinicians, Environmental, Agricultural, Food n Feed
- Epidemiologists, Law Enforcement, FBI, Bio-Watch, PH emergency
- Academic and Corporate partners

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Partnerships to Bring in Novel Environmental Testing

Environmental Health and Protection

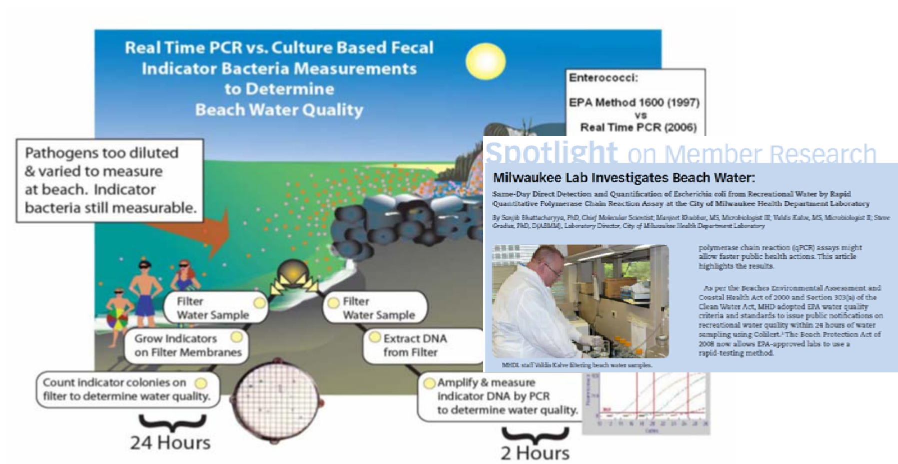
Collaborate with partners to coordinate and ensure scientific analysis of environmental and human samples to identify, quantify and monitor potential threats to health

Recreational Water Testing Partnership

- **2006-'07: MHDL, CDC, EPA, other local PHLs**
  - Enterococcus, *E. coli*- validate qPCR, compare with plating, Enterolert/Colilert
- **2010-'11: Racine HD, UW-Oshkosh, Dane County HD and MHDL**
  - *E. coli* qPCR- Site compare, interpretation criteria
- **2012- '15: DNR- Predictive modeling** (ongoing)
  - Colilert, USGS and qPCR data
- **MHD Disease Control Environmental Health** (internal partners)
  - Regulatory decision making
- **UW-Milwaukee Zilber School of Public Heath** (ongoing)
  - Academic/research- multiple beach models, auto-sampling- multi-time points sampling throughout the day; algal toxins

Multi-laboratory: Multi-jurisdictions

EPA Validation Study of Rapid Method "qPCR"
Water Quality- Milwaukee Beaches



Manuscript under preparation-
Appl. Env. Micrbiol (ASM)

Best Poster Award (Local category- 2012 APHL AM)

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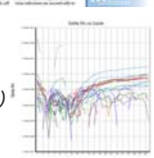
Detection of Waterborne Viruses

- Primary regulations on drinking water quality related to viral contaminants- **Surface Water Treatment Rule** (SWTR; 40 CFR Part 141) and the **Ground Water Rule** (GWR; Federal Register 71:65573-65660)
- **Information Collection Rule** (ICR)- All utilities serving population >100,000 requires to monitor source water for viruses monthly (since 1990s)
- In 2009, MHDL with 4 other federal and private labs participated in study to provide a side-by-side evaluation of the **NanoCeram** and **1MDS filters** for detection of Enteroviruses (EV) from ground water sources *(AEM 2009)*
- Recovery of poliovirus, coxsackievirus B5, and echovirus 7- culture (NanoCeram filtration followed by **virus culture on BGMK cell lines), real-time PCR and EV sequence typing** *(AEM 2009)*
- In 2014, MHDL began collaborating with EPA on **Method 1615** for 'Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR' (UCMR 3- Enterovirus and Norovirus)



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Community Involvement in PHL Research Practice

- **Community involvement**
  - Meeting community needs
  - PHL research partnership in practice- Citizen Scientist/Scientific Citizen concept
- **Community feedback**
  - Practice and priority of research topic
  - Dissemination of data from PHLs
- **Engage community partners**
  - Different stages of research
- **Celebrate community-PH research success**
  - Visibility by community members and leaders

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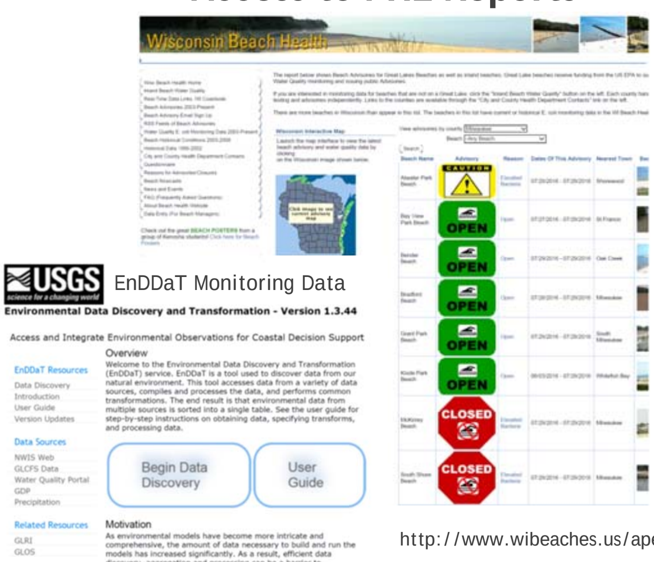
Community Interface and Access to PHL Reports



http://www.wibeaches.us/apex/f?p=BEACH:HOME

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HWPP Grant Collaboration 2014-2018

Growing Healthy Soil for Healthy Communities - Urban Gardening

- Minimize environmental lead exposure
- Innovative multi-agency approach
  - 16th Str. CHC
  - UW Madison
  - Walnut Way
  - MCW
  - MHDL
- Soil intervention
- Education
- Policy
- Build knowledge, skills; increased capacity to reduce soil lead- access to soil analysis
- Two Milwaukee neighborhoods
  - Lindsey Heights
  - KK neighborhood

- Create action at the individual, community and societal level
- Desired long-term effect
  - Increased safe gardening practices
  - Reduce soil lead concentrations
  - Groundwork for policy recommendations

- Goal of increasing demand for and improving access to soil testing for urban gardeners while informing best practices in safe urban gardening
- MHDL now poised to begin offering soil testing to the public

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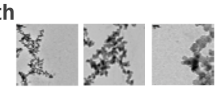
Potential PHLs Partnership & Research Areas

…….but not limited to

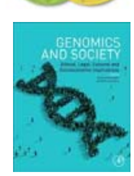
- **Novel Microbial Indictors and next generation source tracking**
- **Nanotechnology- Impact of Human and Environmental Health**
  - Analyze the nanoparticle in water and food sources
  - Public health impact and understanding



APHL white paper
Manuscript in progress

- **Microbiome approach- Complex matrix analysis for potential microbial impact on human, plant & animal health- Advanced Molecular Detection (e.g. next. gen. sequencing)**
  - Impact of pathogen/microbial load on health

- **Environmental Health Genomics-** New paradigm to address children's environmental health- NIEHS priority areas
- **Genomic and Society-** Community understanding of genomic applications- *Scientific Citizen and/or Citizen Scientists*



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PHL Partnership and Research
Potential Road blocks

- **Leadership buying**
  - Perception- routine vs. applied research/developmental
  - Legal issues- sharing materials, safety, PHI, maintaining confidentiality
- **Admin support**
  - Justifying the need
  - Research areas
- **Sustained funding**
  - Limited operations cost
  - Challenges in obtaining grant funding
- **Workforce**
  - Staff Vs. Researcher
  - Motivation & expertise
- **PH routine surveillance & emergencies**
  - How do you manage and sustain the demands of day-to-day service and surges while also continuing research/developmental projects?

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Quality Control and Critical Workforce for PHL Sustainability and Innovation

- System partnership-building and integrity
- Adhere to the Quality Control Practices
- Workforce development- students, interns and faculty development
- Partnership with community organizations, industry, academic- *explore non-traditional partners*
- Explore sustained funding
- Publications, seminars
- Client feedback

Quality Control in PHLs

Students & Workforce Development

Collaborations for Applied Research: Developing Public Health Tools

Partnerships within the Public Health Laboratory System….

…..allows **system-strengthening and improvements** along with **further development of system partnerships**

Acknowledgements

- Steve Gradus, PhD, D(ABMM)
- Julie Becker
- Staff at the MHD Laboratory
- APHL Environmental Laboratory Science Committee
- All our system partners

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Questions?

Think Health Act Now!
CITY OF MILWAUKEE
HEALTH DEPARTMENT
Tom Barrett, Mayor
Bevan K. Baker,
Commissioner of Health
www.milwaukee.gov/health

Sanjib Bhattacharyya, PhD
Deputy Laboratory Director

sbhatt@milwaukee.gov

(414) 286-5702

August 3, 2016

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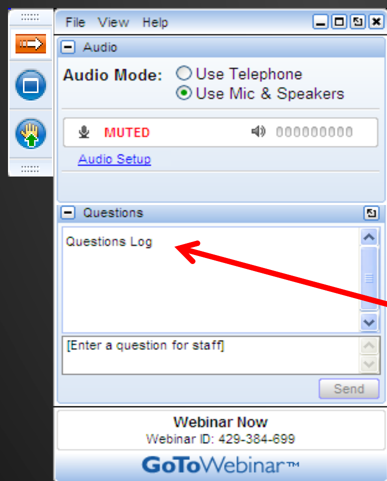
How to Participate Today



- **Audio Modes**
  - Listen using Mic & Speakers
  - Or, select "Use Telephone" and dial the conference (please remember long distance phone charges apply).
- **Submit your questions using the Questions pane.**
- **A recording will be available for replay shortly after this webcast.**

WEFTEC 2016 Workshop
Bacteriophage Analyses in Wastewater, Ambient Water, and for Biosolids Quality Compliance Measurements

Speakers:
Professor Mark Sobsey Ph.D
Professor Charles Gerba Ph.D
Professor Anicet Blanch Ph.D
Akin Babatola
Professor Juan Jofre Torroella Ph.D

Saturday, Sept 24, 2016 8:30 am – 5:00 pm
http://www.weftec.org/workshops/