Wednesday, April 13
1:20 p.m.–2:50 p.m.

Session 3: Recreation Water Monitoring and Implementation Challenges/Successes

Monitoring Beaches Statewide in Michigan for *E. coli* with qPCR (USEPA Draft Method C)

Shannon Briggs, PhD
Michigan Department of Environmental Quality

Abstract

In 2015, Michigan initiated a statewide, rapid beach testing program by providing 10 laboratories with $500,000 worth of qPCR-related equipment. In collaboration with Michigan State University (MSU) and the U.S. Environmental Protection Agency (EPA), laboratory personnel are being trained to use the EPA's Draft Method C: Escherichia coli in Water by TaqMan Quantitative Polymerase Chain Reaction (qPCR). The training effort includes developing manuals containing standard operating procedures that can be easily followed by laboratory staff. Michigan's qPCR network of 16 labs is connected with the MiqPCR listserv hosted by MSU. Beaches will be posted sooner and reopened faster because test results will be available the same day. Monitoring results are posted on Michigan's BeachGuard website at http://www.deq.state.mi.us/beach/. During the transition to qPCR methods, beaches will be monitored using both the culture and qPCR methods so that correlations between the two methods can be determined, allowing for future derivation of water quality standards for the new method.

Biosketch

Dr. Shannon Briggs is a toxicologist for the Water Resources Division of the Michigan Department of Environmental Quality (DEQ). She received her bachelor of science degree in animal science and her doctorate in pharmacology and toxicology from Michigan State University. She is a member of a planning team that will host the 2016 Great Lakes Beach Conference in Marquette, Michigan, October 5–7, 2016. Dr. Briggs assists local health departments with state and federal grants for monitoring beaches across the State of Michigan. She is leading a water quality initiative of the DEQ to provide rapid testing equipment and training for 10 new laboratories that will test beaches using the U.S. Environmental Protection Agency's draft Method C (i.e., qPCR method for *E. coli*). Dr. Briggs is an active member, past president, and cofounder of the Great Lakes Beach Association.

Monitoring Michigan Beaches Statewide for *E. coli* with QPCR (USEPA Draft Method C, June 2015)

Shannon Briggs
briggss4@michigan.gov
Michigan Department of Environmental Quality

Water Resources

11,000 inland lakes
77,000 river miles
1,200 public beaches
4 Great Lakes
3,288 miles of coast
5.5 million acres of wetlands

Michigan Public Health Code and Public Beaches

- Monitoring is Voluntary for all 45 Health Departments, however
- County Ordinance can require testing
- Health Officer has authority to test and close
- Requires signs at all public beaches
- Requires reporting if beach is tested

Beach Monitoring "typical stats"

- 200 inland lake beaches monitored
- 200 Great Lakes beaches monitored
- 3.6% samples exceed WQS, n =186 samples
- 80% of beaches are open all season, n= 332
- 20% of beaches report action, n= 84 beaches
- Most actions are 1 to 2 days

Path to qPCR for Beach Testing

QPCR Methods	Year	Beaches
Kary B. Mullis invents PCR	1985	
	1986	
	2000	BEACH Act

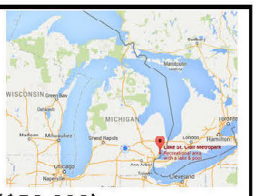
Path to qPCR for Beach Testing

QPCR Methods	Year	Beaches
Dr. Joan Rose at Michigan State University	2003	Monitor Beaches with local, state & federal funds
Water Fellows Lectures & Discussion	2005	Identify Impaired Beaches
Microbial Source Tracking (MST)	2007	Beach Sanitary Survey Tool

Path to qPCR for Beach Testing

Year	QPCR & Beaches
2010	MST at Impaired Beaches
2011	Training Manual & Video for Beach Testing with QPCR
2012	Public Meeting for MST Results U.S. EPA Rec Water Quality Criteria Includes Enterococci QCPR values

QPCR Lab at Lake St. Clair Metropark Beach



$100,000 for equipment

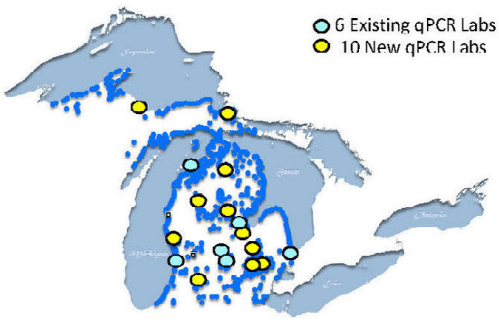
Park renovated office to lab ($50,000)

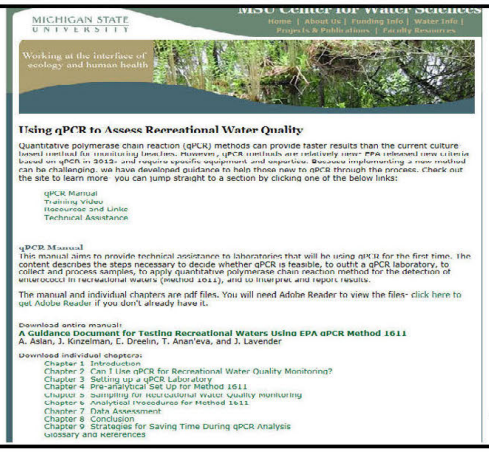
$500,000 for 10 New Labs

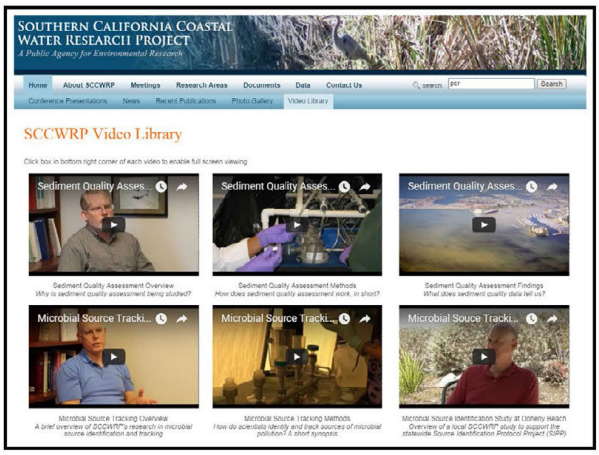
- State of Michigan provided $500,000 to DEQ for rapid beach testing equipment
- Only health departments have authority to test beaches
- DEQ sent letters of invitation to 45 health departments responsible for 83 counties
- 13 responses and description of lab capacity

Questions and Details

- Commitment & Expectations in Memorandum of Understanding between DEQ and HDs
- 10 Health Departments signed MOUs
- MOU included equipment list with 50+ items for each HD
- $30K for Training and Support from MSU
  just added $28,000 more





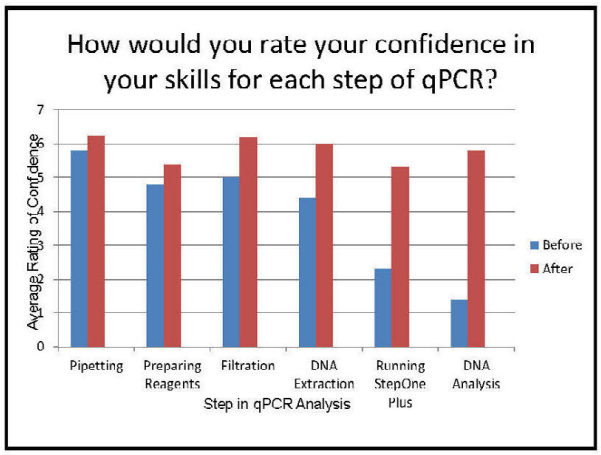


Path to qPCR for Beach Testing

Year	QPCR & Beaches
2014	10 New QPCR Labs (15 total) U.S. EPA draft Method C (QPCR - *E. coli*)
2015	Equipment Ordered & Delivered Samples filtered & frozen Train the Trainer for QPCR

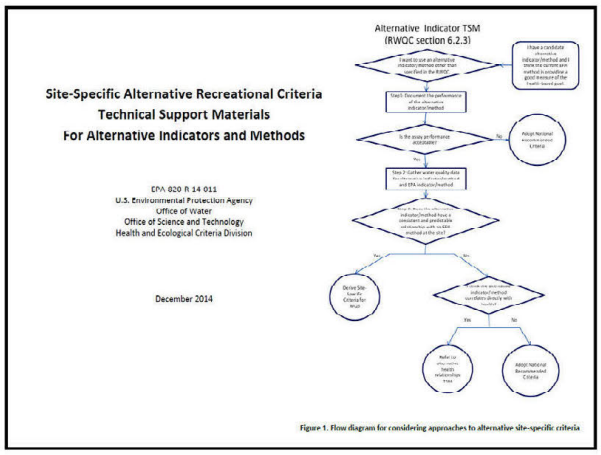
Path to qPCR for Beach Testing

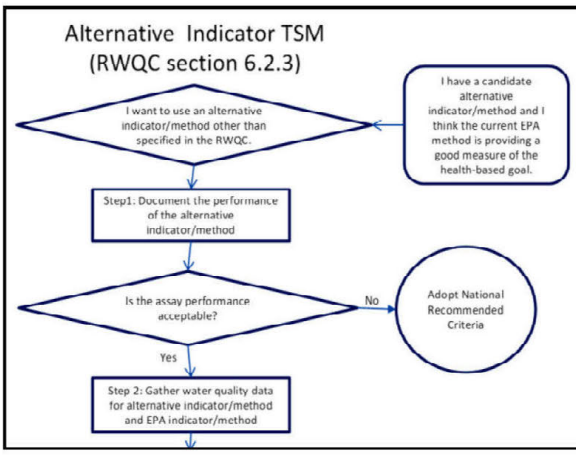
Year	QPCR & Beaches
2016	4-day Training on Draft Method C Stockholm Water Prize awarded to Dr. Joan Rose

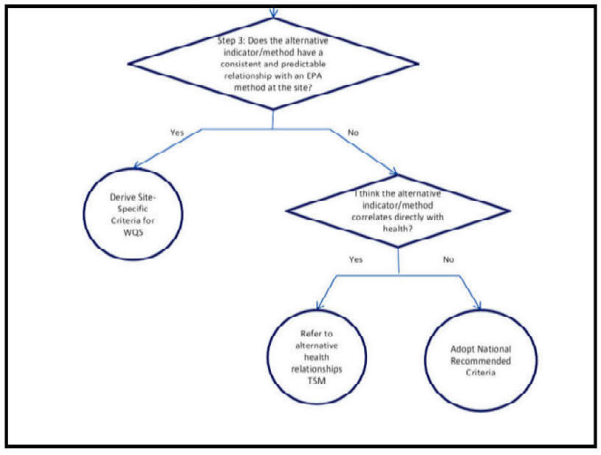


Path to qPCR for Beach Testing

Year	QPCR & Beaches
2016	Multi-lab Validation Study 2015 & 2016 Samples tested Review Colilert & QPCR Results



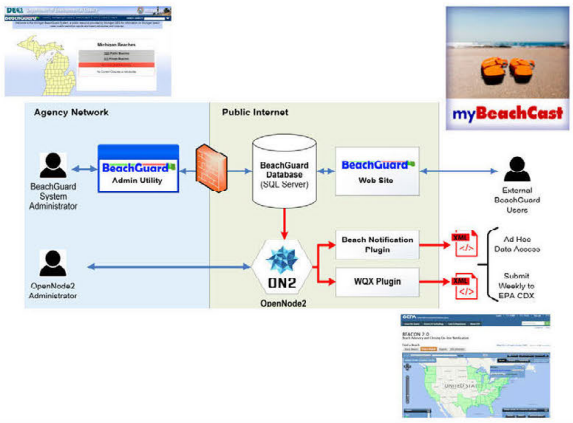




Path to qPCR for Beach Testing

Year	QPCR & Beaches
2017	Samples tested and reviewed with previous 2 years
2018	Continue sampling Present equivalent QPCR results to USEPA and Local Health Officers
2019	Beach status determined by QPCR methods Molecular Source Track Training?

Report Data

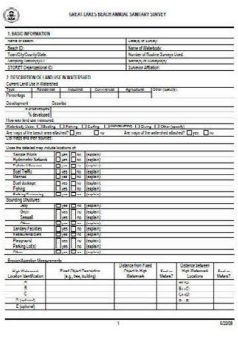


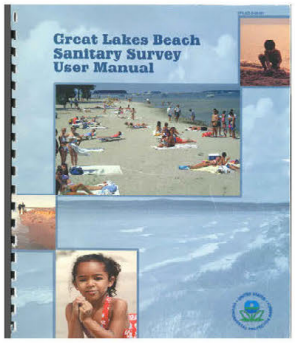
Sleeping Bear Dunes Photo by Steve Keighly, Winner of the Instagram Beach Photo Challenge for favorite beach to take a long walk.

Beach Sanitary Surveys

http://www2.epa.gov/beach-tech/beach-sanitary-surveys

Beach Sanitary Surveys





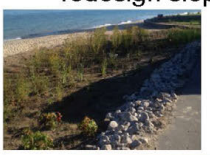
Canine Scent Tracking

Environmental Canine Services

Remediation

landscaping

redesign slope of beach, groom beach



Beach listservs

beachnet@great-lakes.net

MIQPCR@LIST.MSU.EDU

beachinfo@lists.epa.gov

Rapid Analyses of Water Quality at Five Chicago Beaches, 2015

Abhilasha Shrestha
University of Illinois, School of Public Health

Abstract

In the summer of 2015, the Chicago Park District (CPD) enhanced its beach monitoring and notification through a pilot program of rapid molecular testing of beach water. Water samples were provided at approximately 8:30 a.m. 4 days per week to the University of Illinois at Chicago School of Public Health (UIC SPH) Water Research Laboratory. The results of the rapid testing method, qPCR, were reported on the same day by 1:00 p.m. The CPD used the qPCR results to notify the public about measured bacterial concentrations. Previously, the CPD posted notifications based on the most probable number (MPN) of *E. coli* obtained from overnight cultures.

Water samples from five Chicago beaches were tested using the Enterococci qPCR. Similar samples were set up for *E. coli* culture analysis by a commercial laboratory on the same days that UIC performed the qPCR test. The CPD used the U.S. Environmental Protection Agency's (EPA's) Beach Action Values (BAV) for both the qPCR test results and the culture test.

Of the 270 qPCR tests, 23 exceeded EPA's BAV, and of the 270 culture tests, 67 exceeded the BAV. The results of *E. coli* culture testing that became available on a given day (e.g., results that became available on a Thursday from tests of beach water samples collected on Wednesday) were frequently inconsistent with the current qPCR results (from water samples collected on Thursday). Our data suggest that beach water notifications based on qPCR testing presented a more accurate picture of same-day water quality than the prior-day's culture test results.

Biosketch

Ms. Abhilasha Shrestha is a doctoral student in the Environmental and Occupational Health Sciences Department at the University of Illinois at Chicago School of Public Health (UIC SPH). She earned her bachelor of science degree in biology from the University of Minnesota-Duluth and then worked as an aquatic toxicologist in a private laboratory in Minnesota for more than 2 years. She completed her master's degree from UIC SPH in 2013, focusing on environmental and occupational health sciences with a concentration in water quality and health. Ms. Shrestha's research interests include studying the use of different indicator targets/genes for water quality assessment. In her dissertation research, she is focusing on molecular methods for rapidly evaluating infectious agents in surface water.

Rapid Analyses of Water Quality at Five Chicago Beaches, 2015

Abhilasha Shrestha, PhD Student
Ira Heimler, Cathy Breitenbach, Samuel Dorevitch
U.S. EPA's Recreational Waters Conference
April 13, 2016
University of Illinois at Chicago
School of Public Health
Chicago, IL

Overview

- Introduction
- Methods
  - Beach Action Value (BAV)
- Results
  - Data quality
  - *E. coli* culture results
  - *Enterococci* qPCR results
  - One day delay in *E. coli* results, and associations with qPCR results
  - BAV exceedance after 0.5 inch of rain
- Conclusion
- Future projects
- Acknowledgment

Introduction

- Chicago: 26 miles of public beaches
- ~20 million visitors annually
- Chicago Park District: 27 beaches
- Point source discharges are rare
- Monitoring: Culture-based methods such as Colilert®
- Prior-day culture → poor predictor of current conditions
- 2015: Pilot program with UIC
  - 5 Chicago beaches

Methods

- qPCR at UIC lab, Tuesday-Friday, May 26 - August 30, 2015
- Culture tests: Commercial laboratory, Colilert® method
- 1L samples, 2 transects each at 5 Chicago beaches (N=270)
- Delivered at approximately 8:30 AM
- Quantified for *Enterococci* DNA using the USEPA Method 1611 with one modification
- Results reported to the CPD on the same day by 1:00 PM

Beach Action Value (BAV)

Indicator	Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators BAV (Units per 100 mL)		Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators BAV (Units per 100 mL)
Enterococci – culturable (fresh and marine)[a]	70 cfu		60 cfu
E. coli – culturable (fresh)[b]	235 cfu	OR	190 cfu
Enterococcus spp. – qPCR (fresh and marine)[c]	1,000 cce		640 cce

[a] Enterococci measured using EPA Method 1600 (U.S. EPA, 2002a), or another equivalent method that measures culturable enterococci.
[b] *E. coli* measured using EPA Method 1603 (U.S. EPA, 2002b), or any other equivalent method that measures culturable *E. coli*.
[c] EPA *Enterococcus* spp. Method 1611 for qPCR (U.S. EPA, 2012b). See section 5.2.

Source: USEPARecreational Water Quality Criteria, 2012

Data quality

- **qPCR Accuracy**: Standard curves
  - Nine standard curve runs, each in triplicate, initially and every two weeks
  - R^2= 0.9957 (high accuracy)

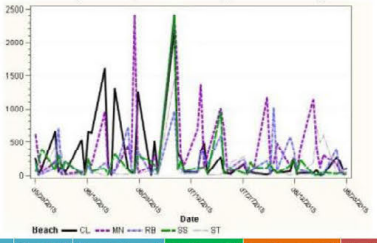
Parameter	Mean	Standard deviation	95% lower bound	95% upper bound
Slope	-3.4945	0.0202	-3.5345	-3.4545
Intercept	38.2329	0.061	38.1122	38.3535

- **qPCR Precision**: Calibrators & sample processing controls (SPC)
  - 55 calibrators

Variable	CT mean	CT standard deviation	Coefficient of variation
Sample processing control	23.00	0.26	1.11%
Enterococci cells	26.09	0.53	2.05%

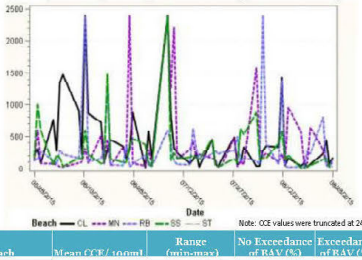
- **qPCR Inhibition**:
  - Of the 540 total beach samples, only two (0.37 %) exceeded the 3 CT unit offset; other two (0.37%) had offsets in the 2-3 cycle range

E. coli culture (MPN/100mL geomean)



Beach	Mean MPN	Range (min-max)	E. coli<235 (%)	E. coli 235-999 (%)	E. coli ≥1,000 (%)
Montrose	383.1	(14.9 - >2420.0)	33(61.1)	14(25.9)	7(13.0)
63rd Street	171.6	(1.4 - >2420.0)	46(83.3)	7(13.0)	2(3.7)
South Shore	167.8	(5.5 - >2420.0)	46(85.2)	7(13.0)	1(1.9)
Rainbow	186.9	(2.8 - 1020.7)	42(77.8)	11(20.4)	1(1.9)
Calumet	257.8	(6.0 - 2192.3)	37(68.5)	13(24.1)	4(7.4)
All beaches	233.4	(1.4 - >2420.0)	203(75.2)	52(19.3)	15(5.6)

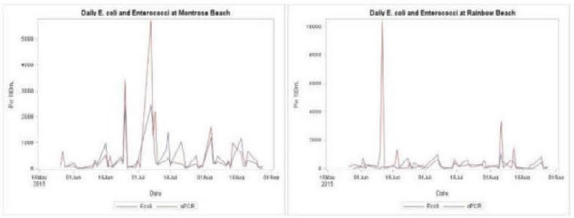
Enterococci qPCR (CCE/100mL geomean)



Note: CCE values were truncated at 2400

Beach	Mean CCE/100ml	Range (min-max)	No Exceedance of BAV (%)	Exceedance of BAV (%)
Montrose	447.9	(27.1-5999.0)	49 (90.7)	5 (9.3)
63rd Street	240.9	(14.2-1977.0)	49 (90.7)	5 (9.3)
South Shore	305.0	(10.6-3435.8)	51 (94.4)	3 (5.6)
Rainbow	501.9	(23.3-10385.8)	49 (90.7)	5 (9.3)
Calumet	450.1	(20.6-3894.3)	49 (90.7)	5 (9.3)
All beaches	389.3	(10.5-10385.8)	247 (91.5)	23 (8.5)

Time series graphs of daily measures of culture and qPCR



Beach	Pearson's R	p-value
Montrose	0.78	<.0001
63rd Street	0.78	<.0001
South Shore	0.68	<.0001
Rainbow	0.29	0.035
Calumet	0.69	<.0001

One day delay in *E. coli* results, and associations with qPCR results

- Beach management decisions based on today's qPCR results and the *E. coli* results from yesterday's water sample were not associated, with the exception of 63rd Street beach.
- Prior day culture results frequently lead to the erroneous decisions when compared to the same day qPCR results as the gold standard.

63RD STREET	Prior day Culture		
qPCR	Advisory	No Advisory	Total
Advisory	3	2	5
No Advisory	9	37	46
Total	12	39	51
	OR 6.17 (0.89, 42.56)		

MONTROSE	Prior day Culture		
qPCR	Advisory	No Advisory	Total
Advisory	3	4	7
No Advisory	20	26	46
Total	23	30	53
	OR 0.98 (0.20, 4.86)		

SOUTH SHORE	Prior day Culture		
qPCR	Advisory	No Advisory	Total
Advisory	1	3	4
No Advisory	9	39	48
Total	10	42	52
	OR 1.44 (0.13, 15.55)		

CALUMET	Prior day Culture		
qPCR	Advisory	No Advisory	Total
Advisory	2	5	7
No Advisory	16	29	45
Total	18	34	52
	OR 0.73 (0.13, 4.17)		

BAV exceedance after 0.5 inch of rain

- Odds of exceeding either the *E. coli* culture MPN BAV or the *Enterococci* qPCR CCE BAV were increased
- *Enterococci* qPCR: Odds ratio 4.26 (1.59 – 11.43)
- *E. coli* Culture: Odds ratio 1.90 (0.85 – 4.24)

	CCE <1,000	CCE≥ 1,000	Total
<0.5 inches past 24 hours	224 (93.3%)	16 (6.7%)	240 (100%)
≥0.5 inches past 24 hours	23 (76.7%)	7 (23.3%)	30 (100%)
Total	247	23	270

	MPN<235	MPN≥ 235	Total
<0.5 inches past 24 hours	184 (76.7)	56 (23.3%)	240 (100%)
≥0.5 inches past 24 hours	19 (63.3%)	11 (36.7%)	30 (100%)
Total	203	67	270

Conclusions

- Accurate, precise qPCR results can be available by 1:00 PM.
- Daily qPCR CCE values resulted in BAV exceedance less frequently than the *E. coli* culture results (8.5% vs 24.8% of samples).
- Inhibition of the qPCR reaction was rare (<1% of samples).
- Results of *E. coli* testing (from prior day water samples) were not consistently related to qPCR results.
- Beach management decisions should be based on same-day rather than prior-day information.
- Heavy precipitation tends to increase *Enterococci* qPCR CCE results significantly, and to a lesser degree, *E. coli* MPN.

Future Projects

- **Archived filters**
  - Evaluate the concentration of a human-specific molecular target like HF 183.
- **Summer 2016**
  - qPCR testing expanded to additional beaches, particularly those that tend to have relatively frequent BAV exceedance based on *E. coli* culture results.
  - 9 beaches, 5 days a week, Wednesday- Sunday.
  - **Goal**: Earlier sample collection and results by noon.

Acknowledgement

Funding for this project was provided by the Chicago Park District.

ENVIRONMENTAL AND OCCUPATIONAL HEALTH SCIENCES SCHOOL OF PUBLIC HEALTH UIC

CHICAGO · PARK · DISTRICT · SEAL · HORTUS IN URBE · ORGANIZED · 1934 ·

THANK YOU

Application of Rapid qPCR-Based Tests for *Enterococci* (Method 1611) in Hawaiian Coastal Waters

Marek Kirs, PhD
University of Hawaii

Abstract

To evaluate the applicability of the U.S. Environmental Protection Agency's (EPS's) enterococci qPCR method 1611 for beach waters of Hawaii, a total of 127 water samples were collected from 12 beaches on Oahu over a 10-month period. The samples were analyzed using EPA methods for Enterolert®, 1600, and 1611. *Clostridium perfringens*, human-associated Bacteroides, and human polyomaviruses also were enumerated. Concentrations of enterococci and C. *perfringens* varied from < 10 to 389 colony-forming units (CFU) 100ml-1 (Enterolert®), from < 1 to > 151 CFU 100ml-1 (1600), and from < 1 to 96 CFU 100ml-1 (mCP). Four samples (3.1%) analyzed using Enterolert, and two samples (1.6%) using method 1600 exceeded the EPA-recommended statistical threshold value (STV) of 130 CFU 100ml-1, while *C. perfringens* concentrations exceeded 50 CFU 100ml-1 in a single sample (0.8%), indicating generally good water quality at the beaches studied. In the samples exceeding the STV, human-associated Bacteroides was detected in a single sample, while human polyomaviruses were not detected. Importantly, 88 samples (69.3%) tested using method 1611 could not be quantified because of the PCR inference. After those samples were diluted in molecular grade water (1:10), the majority of the samples (85 samples, 66.9%) remained compromised by the PCR inference. In contrast, for an additional set of monthly samples (n=39) collected at three sites from the brackish Ala Wai Canal, only a single sample was compromised (2.5%). Although good agreement existed between the methods for enterococci when samples were not compromised, our data indicate serious shortcomings for the recommended qPCR method 1611 for enterococci enumeration for Hawaiian beaches. New technology that alleviates inhibition issues for qPCR is being evaluated.

Biosketch

Dr. Marek Kirs is an assistant researcher at the Water Resources Research Center of the University of Hawaii. He received his bachelor of science degree from Tartu University in Estonia, his master of science degree from the University of Edinburgh in the UK, and his doctorate from the University of Rhode Island. He also has completed postdoctoral training at the University of North Carolina at Chapel Hill. More recently, Dr. Kirs worked at the Cawthron Institute in New Zealand, where he was involved in establishing microbial source tracking services and lead microbial water quality research and consultancy projects. His research focuses on a wide range of microbial water quality and related public health issues.

Application of rapid qPCR-based tests for enterococci (Method 1611) in Hawaiian coastal waters

Marek Kirs, Denene Blackwood, Rachel Noble, Philip Moravcik

April 13, 2016

U.S. EPA's 2016 Recreational Water Conference, New Orleans

Hawaii and rapid methods

HI extremely well suited:

- ~8 million tourists per year, many high use beaches (Waikiki beaches, Ala Moana)
- Beaches are easy to reach (easy to sample and post)

Rapid accurate methods would make difference (ruining on not ruining a person vacation)

So far two samples have been analyzed from Hawaii(?)

Water Resources Research Center at the University of Hawaii

Mission

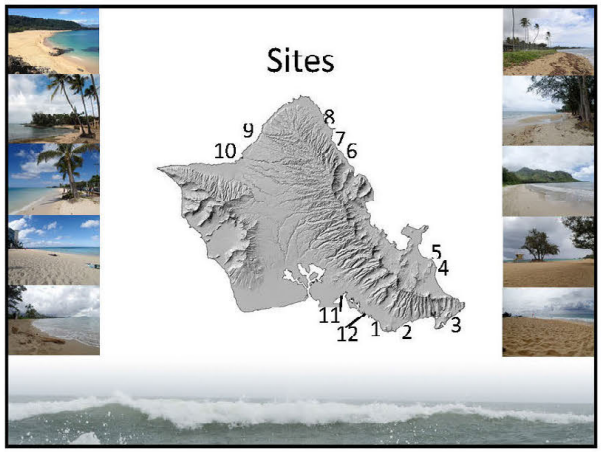
The Center's mission is to identify water and environmental problems and provide solutions by:

- Conducting research that identifies, characterizes and develops solutions for water and environmental problems in Hawaii;
- Providing opportunities for graduate and undergraduate students to prepare them to be leaders in water and environmental research;
- Assisting communities in Hawaii and the Pacific to address water and environmental problems;
- Providing science-based information to help inform decision-making activities in Hawaii and Pacific Islands.

The study

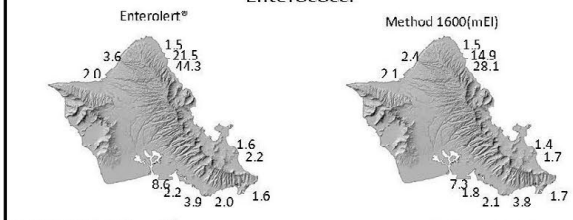
- June, 2013 – April 2014
- 12 beaches (HI DOH)
- 11 samples per beach (except Waimea Beach) ,
- Total 127 samples
- Measurements: enterococci by membrane filtration (mEI), Enterolert® , and by qPCR (1611) as well as analyzed for MST markers (human associated *Bacteroides*, and human polyomaviruses)
- Another parallel study June 2013-2014 in Manoa Stream - Ala Wai Canal: 9 sites, 12 samples per site

Sites



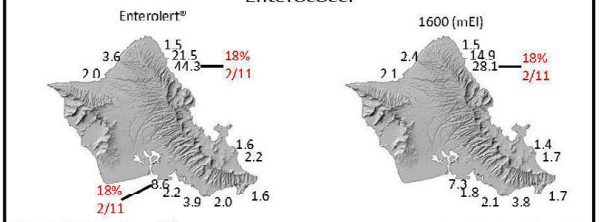
Water quality

Enterococci



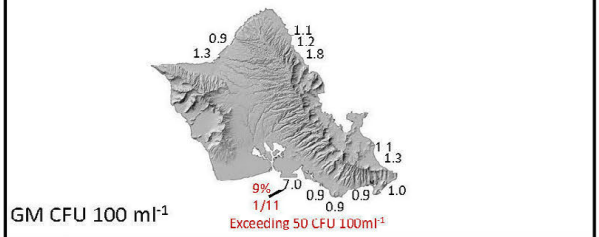
Water quality

Enterococci



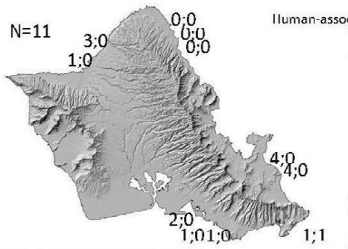
Water quality

Clostridium perfringens



Water quality

Human-associated *Bacteroides* ; human polyomaviruses



Human-associated *Bacteroides* sources in III:

Source	Concentration (gc g^{-1})
Humans	$2.5x10^8$
Cats	$<1x10^2$
Chickens	$3.1x10^2$
Dogs	$1.3x10^3$
Mongoose	$1.6x10^3$
Pigs	$<1x10^2$

- Other than human sources exist

Water quality

Summary

- Enterococci and *C. perfringens* indicated good water quality on the beaches studied
- 3.1% of the samples exceeded STV for enterococci by Enterolert®
- 1.6% of the samples exceeded STV for enterococci by method 1600
- Only a single sample exceeded both , the STV for enterococci and threshold level for *C. perfringens*
- Human sewage was not conclusively identified as the contamination source in any of the coastal samples based on the markers

Rapid Method Application (1611)

PCR Inference (inhibition)

PCR inference can be caused by:

- Mechanical blocking of the enzyme, template
- Physical and chem. modification of the enzyme, template
- Binding and chelating of other chemicals necessary in PCR
- Other....(see Schrader et al., 2012, J. Appl. Microbiology 113: 1014-1026)

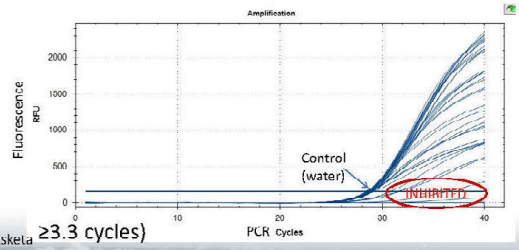
PCR inference results in:

- Severe underestimate of bacterial concentrations
- False negatives

Rapid Method (1611)

PCR Inference (inhibition)

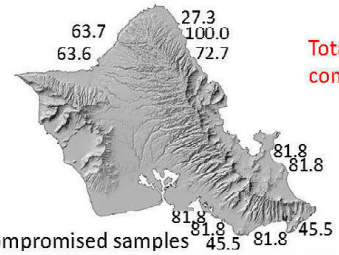
PCR inference was measured:



(ΔC_{tsketa} ≥3.3 cycles)

Rapid Method (1611)

PCR Inference



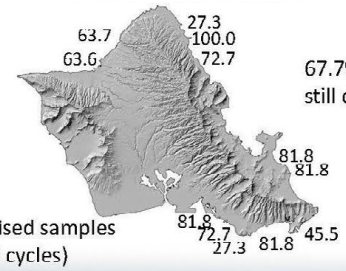
Total of 69.3% samples compromised

0% samples extracted using commercial kit compromised (ΔC_{tsketa} ≤ 0.61) (mixed cellulose filters)

% compromised samples (ΔC_{tsketa} ≥3.3 cycles)

Rapid Method (1611)

PCR Inference 1:10 diluted



67.7% samples still compromised

% compromised samples (ΔC_{tsketa} ≥3.3 cycles)

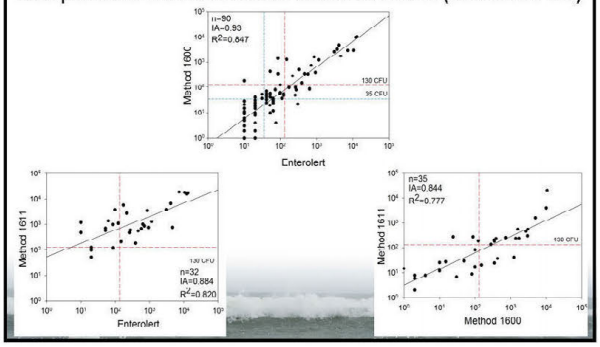
Rapid Method (1611)

Ala Wai

Site	Description	n	Salinity (ppt)	Compromised (%)
A	Coastal	13	34.9	**76.9**
B	Canal	13	27.6	**7.7**
C	Canal	13	23.4	**0**
D	Stream	13	6.8	**0**

Rapid Method (1611*)

Comparisons with cultivation based methods (combined set)



Rapid Method (1611*)

Beach management decisions (combined set)

%		Method 1600 Close	Method 1600 Open
Enterolert®	Close	68	5
	Open	7	20

%		Method 1611 Close	Method 1611 Open
Enterolert®	Close	11	33
	Open	0	56

%		Method 1611 Close	Method 1611 Open
Method 1600	Close	40	9
	Open	11	40

Rapid Method (1611)

Summary

Good water quality of the beaches sampled

PCR inhibitors can compromise application of rapid qPCR based methods in Hawaiian coastal waters

There was good agreement between enterococci concentration estimates as well as beach management decisions based on all three methods

Rapid accurate methods are highly desired in HI (number of beach goers, distances, impact)

Rapid Method (1611)

Future plans

A study funded by the Sea Grant College Program/NOAA:
1) identify cause,
2)troubleshoot, and
3)secondary assay needed

Coral sand?

Acknowledgements

Contributors:

Dr. Roger Fujioka
Dr. Valerie Jody Harwood
Dr. Mayee Wong
Ms. Martina Frycova
Clean Water Branch (HI DOH)

Funding:

National Institute of Water Resources (USGS) and start up

Multi-Laboratory Survey of U.S. EPA *Enterococci* qPCR Methods Acceptability for Analyses of U.S. Coastal and Inland Waters

Richard Haugland, PhD
U.S. Environmental Protection Agency, Office of Research and Development

Abstract

The U.S. Environmental Protection Agency (EPA) offers two similar quantitative polymerase chain reaction (qPCR) methods, method 1611 and method 1609, for the rapid estimation of enterococci fecal indicator bacteria densities in recreational surface waters. Water quality monitoring results from either of these methods can be compared with 2012 EPA Recreational Water Quality Criteria (RWQC) values for site-specific notification programs if the methods are demonstrated to meet performance acceptability guidelines at the site. Current site acceptability guidelines that are available from EPA recommend a maximum frequency of 10% of samples that can exhibit excessive sample matrix interference to the EPA methods as assessed by results and acceptance criteria of the sample processing and/or amplification control assays prescribed in the methods. Here we report the results of a multi-laboratory survey of 22 different marine, Great Lakes, inland lake, and river or stream sites from across the U.S. for their potential acceptability in implementing methods 1611 and 1609 based on these guidelines. Combined laboratory results from 20 and 16 of these sites were found to meet the guidelines using methods 1609 and 1611, respectively. The benefits of augmenting the control assay results with qPCR analysis estimates of recoveries of target sequences from enterococci that are spiked into the test samples also are presented. Results from the analyses in this study indicated that the recommended protocol in method 1609 provided the greatest assurance (>98%) of preventing excessively underestimated enterococci densities (< 50% recovery) caused by matrix interference in samples meeting control assay results acceptance criteria.

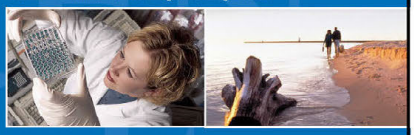
Biosketch

Dr. Richard Haugland is a microbiologist in the Environmental Methods & Measurements Division of the National Exposure Research Laboratory. He received his bachelor of science degree in biology from Muskingum College, Ohio, and his doctorate in developmental biology from the Ohio State University. His past research has addressed diverse problems including biodegradation of hazardous chemicals in the environment, assessment of the microbiological quality of indoor environments, detection of biothreat agents for homeland defense, and most recently, monitoring ambient water quality using bacterial indicators of fecal pollution. Since joining the U.S. Environmental Protection Agency (EPA) in 1991, Dr. Haugland has authored or coauthored more than 60 publications and has received a number of awards for his work, including the EPA bronze and gold medals.

EPA United States Environmental Protection Agency

Multi-laboratory survey of U.S. EPA enterococci qPCR methods acceptability for analyses of U.S. coastal and inland waters

Richard Haugland, Shawn Siefring, Manju Varma, Kevin H. Oshima, Mano Sivaganesan, Yiping Cao, Meredith Reith, John Griffith, Stephen B. Weisberg, Rachel T. Noble, A. Denene Blackwood, Julie Kinzelman, Tamara Anan'eva, Rebecca N. Bushon, Erin A. Stelzer, Valarie J. Harwood, Katrina V. Gordon, Christopher Sinigalliano



Office of Research and Development, National Exposure Research Laboratory

Notice: Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official agency policy.

May 12, 2016

EPA United States Environmental Protection Agency

Study background

- QPCR methods can provide rapid (same day) estimates of fecal indicator bacteria (FIB) densities in recreational waters.
- Enterococci FIB densities determined by qPCR have been found in a series of epidemiological studies. (U.S.EPA NEEAR studies and others) to correlate with bather gastrointestinal illness rates.
- Based on these observations, qPCR density values for enterococci are provided in the U.S.EPA (EPA), 2012 Recreational Water Quality Criteria (RWQC).

1

EPA United States Environmental Protection Agency

Study background

- 2012 RWQC further indicates that: *"overall testing of the qPCR method with different types of ambient waters, and by different laboratories, remains limited and (EPA) anticipates that there may be situations at some locations where the performance of the qPCR method may be inconsistent".*
- For this reason, the RWQC suggests that: *"states evaluate the qPCR method with respect to laboratory performance and sample interference in their prospective waters prior to developing new or revised standards relying on this method".*

2

EPA United States Environmental Protection Agency

Study background

- EPA has provided guidelines for determining acceptability of qPCR method performance at prospective sites based on the percentage of samples passing the control assay acceptance criteria specified in the EPA methods: (http://www2.epa.gov/owa methods/other clean water act test-methods-microbiological).
- EPA offers two methods (Method 1611 and Method 1609) that can be evaluated at prospective sites for their ability to meet these performance acceptability guidelines.

3

EPA United States Environmental Protection Agency

Study objectives

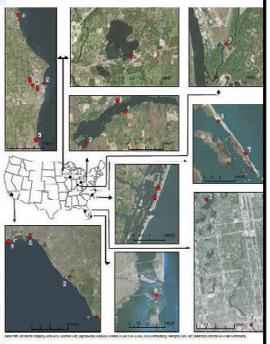
- Use the two EPA methods and different EPA-recommended or alternative method permutations to:

1. Determine the percentage of samples passing EPA Method specified and alternative control analysis acceptance criteria from a variety of different water body types based on analyses of shared samples by multiple labs.
2. Evaluate the reliability of the controls in identifying accurate sample analyses based on estimated recoveries of target gene sequences from spiked enterococci in these water sample matrices.

4

Study sites

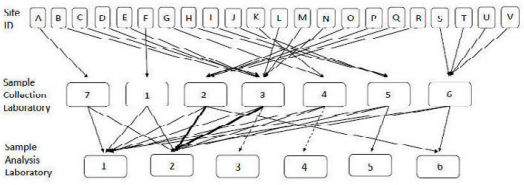
Site name	Site ID	Water body type (abbreviation)	Location
Virginia Key Wetlands	A	Brackish Stream (BS)	Miami, Florida
Horlick Dam, Root River	B	River or Stream (RS)	Racine, Wisconsin
Liberty Street, Root River	C	River or Stream (RS)	Racine, Wisconsin
Oak Creek	D	River or Stream (RS)	South Milwaukee, Wisconsin
Pike River	E	River or Stream (RS)	Kenosha, Wisconsin
Little Miami River	F	River or Stream (RS)	Near mouth, Cincinnati, Ohio
Hillsborough River	G	River or Stream (RS)	Riverfront Park, Tampa, Florida
Bronks Beach	H	Inland Lake (IL)	Buckeye Lake, Ohio
Crystal Beach	I	Inland Lake (IL)	Buckeye Lake, Ohio
Fairfield Beach	J	Inland Lake (IL)	Buckeye Lake, Ohio
White Sands Beach	K	Inland Lake (IL)	Lake Carroll, Florida
Fischer Park Beach	L	Inland Lake (IL)	Browns Lake, Wisconsin
Chesney Lake Park Beach	M	Inland Lake (IL)	Racine, Wisconsin
North beach	N	Great Lakes (GL)	Racine, Wisconsin
Cabrillo Beach	O	Pacific Ocean (PO)	San Pedro, California
Doheny Beach	P	Pacific Ocean (PO)	Dana Point, California
Long Beach	Q	Pacific Ocean (PO)	Long Beach, California
Newport Dunes Beach	R	Pacific Ocean (PO)	Newport, California
Jockey's Ridge Beach	S	Atlantic Ocean (AO)	Outer Banks, North Carolina
South Nags Head Beach	T	Atlantic Ocean (AO)	Outer Banks, North Carolina
Hula, Wrightsville beach	U	Atlantic Ocean (AO)	Wilmington North Carolina
Snyder, Wrightsville Beach	V	Atlantic Ocean (AO)	Wilmington North Carolina



5

Study design

Laboratory 1: U.S. EPA, National Exposure Research Laboratory, Cincinnati, OH
Laboratory 2: Southern California Coastal Water Research Project, Costa Mesa, CA
Laboratory 3: City of Racine Health Department, Racine, WI
Laboratory 4: Department of Biology, University of South Florida, Tampa, FL
Laboratory 5: U.S. Geological Survey, Columbus, OH
Laboratory 6: Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC
Laboratory 7: National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratories, Ocean Chemistry Division, Miami, FL



From "Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters/Haugland," by R. A. Haugland, S. Siefring, M. Varma, K.H. Oshima, M. Sivaganesan, Y. Cao, M. Rath, J. Griffith, S.B. Weisberg, R.T. Noble, A.D. Blackwood, J. Kinzelman, T. Anan'eva, R.N. Bushon, V.J. Harwood, K.V. Gordon, and C. Sinigalliano, 2016, *J. Microbiol. Methods*, 123, pp. 114–126.

6

Sample analysis methods and permutations

Method	PCR Master Mix Reagent	Extract Dilution Analyses	Calculation Models
EPA Method 1611	Universal Master Mix	5x-diluted extracts (recommended in Method), undiluted extract data collected but not recommended in Method	Delta-Delta Ct (recommended in Method) & Delta Ct
EPA Method 1609	Environmental Master Mix	Undiluted extracts (recommended in Method) & 5x-diluted extracts (optional in Method)	Delta-Delta Ct (recommended in Method) & Delta Ct

7

Matrix interference control analyses & acceptance criteria

Control Analysis	Acceptance Criterion	Reference
Salmon DNA sample processing control (SPC) assay	test sample Ct within 3 units of positive control samples	EPA Methods 1611 & 1609
Competitive Internal Amplification Control (IAC) assay	test sample Ct within 1.5 units of negative control samples	EPA Method 1609 & updated Method 1611
Enterococcus assay Ct shift across undiluted - 5x sample extract dilutions	test sample Ct shift within 2.32 ± 1 units	Cao et al., 2012*

*J. Appl. Microbiol. 113, 66-75

8

Spike recovery estimations

- Spiked test matrix (STM) samples: ~10^4 E. faecalis (Ent) cells added to filters containing water sample retentates.
- Spiked control matrix (SCM) samples: same number of Ent cells added to clean filters.
- Ratios of total Ent target sequences recovered from STM/SCM samples calculated by Delta & Delta-Delta Ct formulas:
  - ΔCt ratio = AF^(-(a - c))
  - ΔΔCt ratio = AF^(-((a - b) - (c - d)))
  - where AF = amplification factor (amp efficiency + 1), a = mean STM sample *Ent* Ct, b = mean STM sample SPC Ct, c = mean SCM sample *Ent* Ct, d = mean SCM sample SPC Ct
- Ratios converted to STM/SCM recovery percentages
- The same analyses and calculations were performed for corresponding unspiked samples and recoveries subtracted from the spiked sample recoveries to determine net spike recoveries.
- Net recoveries within 50-200% were considered as acceptable

9

Overall results

Method (sample extract dilution)	Total analyses (N)	Percent of analyses passing SPC & IAC control assay criteria	Percent of analyses passing Enterococcus assay Ct shift criterion (Cao et al)	Percent of ΔCt net recovery analyses within 50-200% recovery range STM/SCM	Percent of ΔΔCt net recovery analyses within 50-200% recovery range STM/SCM
1609 (1x)	732	69%	81%	71%	91%
1609 (5x)	775	97%	Not determined	85%	93%
1611 (1x)	732	~ 60%	Not determined	Not determined	Not determined
1611 (5x)	778	94%	Not determined	87%	84%*

* Percentage reduced by a group of sample analyses that would not meet current QC criteria

10

Summary of site acceptability analyses based on current control assay criteria

Method (extract dilution)	Sample analysis acceptability criterion	Sites passing EPA guidelines (≥ 90% sample analyses pass criteria)	Sites passing or approaching EPA Guidelines (≥ 80% sample analyses pass criteria)
Method 1609 (undiluted)	SPC and IAC assay controls	14/22 (64%)	18/22 (82%)
Method 1609 (undiluted)	Enterococcus assay Ct shift	13/22 (59%)	17/22 (77%)
Method 1609 (5x-diluted)	SPC and IAC assay controls	20/22 (91%)	22/22 (100%)
Method 1611 (5x-diluted)	SPC and IAC assay controls	16/22 (73%)	21/22 (95%)

11

Great Lakes, Lake Michigan site

North Beach, Racine, Wisconsin

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	59	3	93	97	90	95
1609 (5x)	44	3	93		89	98
1611 (5x)	44	2	92		86	98

Passes EPA site guidelines (≥90%)
Approaches EPA site guidelines (80-90%), further analyses warranted?
Fails EPA site guidelines (< 80%)

12

Pacific Ocean sites

Newport Dunes Beach, Newport, California

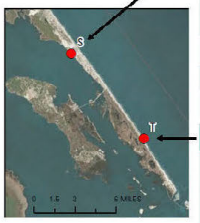
Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	32	2	78	69	75	59
1609 (5x)	40	3	94		88	97
1611 (5x)	50	3	86		90	84

Doheny Beach, Dana Point, California

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	31	2	100	100	90	100
1609 (5x)	36	3	100		97	97
1611 (5x)	36	3	100		97	42*

13

Atlantic Ocean sites



Jockey's Ridge Beach, Outer Banks, North Carolina

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	20	2	100	95	85	100
1609 (5x)	24	2	100		92	100
1611 (5x)	24	2	100		88	92

South Nags Head Beach, Outer Banks, North Carolina

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	21	2	81	86	67	67
1609 (5x)	24	2	100		92	100
1611 (5x)	24	2	100		92	100

14

Midwest inland lake sites

Fischer Park Beach, Browns Lake, Wisconsin

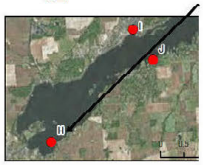
Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	33	2	91	85	70	94
1609 (5x)	21	2	100		81	62
1611 (5x)	21	2	100		95	81

Quarry Lake Park Beach, Racine, Wisconsin

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	53	3	77	74	60	96
1609 (5x)	37	3	86		57	97
1611 (5x)	37	3	84		73	95

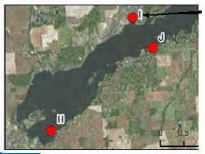
15

Midwest inland lake sites



Brooks Beach, Buckeye Lake, Central Ohio

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	35	3	94	29	3	91
1609 (5x)	36	3	100		89	97
1611 (5x)	36	3	100		100	97



Crystal Beach, Buckeye Lake, Central Ohio

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	33	3	76	12	0	97
1609 (5x)	34	3	97		38	79
1611 (5x)	34	3	97		62	85

16

Florida brackish stream site

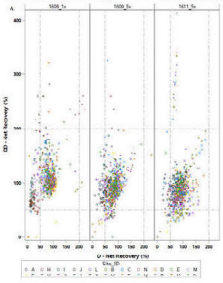
Virginia Key, Miami

Method (extract dilution)	Total Analyses	Labs doing analyses	% Analyses passing SPC & IAC control assay criteria	% Analyses passing Enterococcus assay Ct shift criterion	% ΔCT net recovery analyses within 50-200%	% ΔΔCT net recovery analyses within 50-200%
1609 (1x)	27	2	93	93	59	70
1609 (5x)	30	2	100		90	100
1611 (5x)	30	2	100		87	97

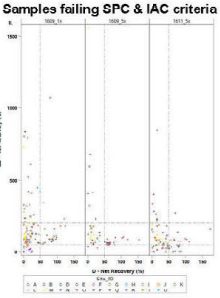
17

Spike Recovery Estimates

Samples passing SPC & IAC criteria



Samples failing SPC & IAC criteria



From "Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters" by R. A. Haugland, S. Siefring, M. Varma, K.H. Oshima, M. Sivaganesan, Y. Cao, M. Reith, J. Griffith, S.D. Weisberg, R.T. Noble, A.D. Blackwood, J. Kinzelman, T. Anan'ina, R.N. Dodson, V.J. Harwood, K.V. Gordon, and C. Sinigalliano, 2016, *J. Microbiol. Methods*, 123, pp. 114–125.

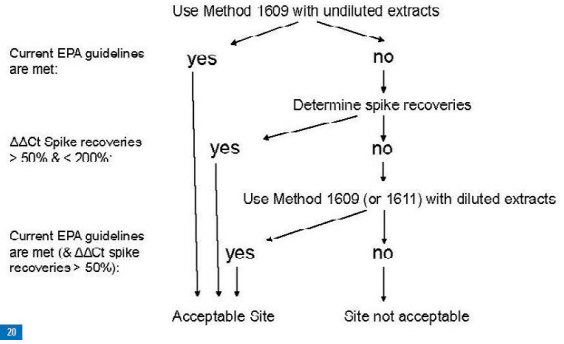
10

Summary

- Using data from all labs, 20 out of 22 of the sights passed the current EPA site acceptability guidelines based on Method 1609 analyses of either undiluted or diluted sample extracts. 16 sites passed based Method 1611 analyses of diluted sample extracts.
- Agreement on site acceptability by different labs was 76% (several factors may be involved in the differences)
- The current controls were generally, but not always, accurate in predicting acceptable (50-200%) spike recoveries.
- Enterococcus assay Ct shift and spike recovery results from delta Ct analyses suggested that some of the samples (e.g. Buckeye Lake) interfered with the analyses.
- Delta delta Ct analyses suggested that use of SPC assay results in the calculation model was effective in adjusting recovery estimates to the acceptable range in many of these interfering samples.
- Method 1609 with undiluted extracts passed the control assay criteria at a lower rate but, when outside the accepted spike recovery range, the delta delta Ct estimates from these analyses were nearly always high rather than low.
- Method 1609 (and 1611) with diluted extracts passed the EPA control assay criteria at a higher rate but delta delta Ct recovery estimates were below 50% in a higher number of sample analyses passing these controls.

19

Possible future guidelines for site evaluations (based on this study)



20

Towards Field-Portable Instrumentation for Real-Time Water Quality Monitoring Using Digital Droplet PCR

Kevan Yamahara, PhD
Monterey Bay Aquarium Research Institute

Abstract

The release of the 2012 Recreational Water Quality Criteria allows beach managers to utilize quantitative PCR (qPCR) for routine water quality monitoring. While methods used to assess water quality have advanced, techniques for automating the process have lagged; few technologies exist that fully automate the water quality monitoring process from sample collection to delivery of quantitative results. The Environmental Sample Processor (ESP) is one tool that may enable researchers and beach managers to monitor beach water quality in an autonomous manner. Current development of the ESP system is designed to allow for in-situ sample collection, sample lysis, and continuous flow digital droplet PCR (ddPCR) to quantify the Enterococci 23rDNA gene and other source tracking targets. Processes performed using the new ESP system, including sample collection, DNA extraction, and ddPCR quantification, are shown to be equivalent to traditional laboratory methods using real-time qPCR for quantification of enterococci. Quantification of enterococci gDNA by the continuous flow ddPCR instrument developed during the course of this project is positively correlated with quantifications using the BioRad ddPCR instrument (slope = 0.72, R2 = 0.99, p=0.0001). The evolving ESP/ddPCR technology may provide a new platform for conducting water quality monitoring tests that can be packaged in a portable, field-deployable unit, reducing sample handling and complex assay standardization associated with traditional qPCR.

Biosketch

Dr. Kevan Yamahara is a research specialist at the Monterey Bay Aquarium Research Institute (MBARI) in Moss Landing, California. He earned his doctorate in environmental engineering and science at Stanford University, where his dissertation focused on the fate and transport of fecal indicators and pathogens in California beach sands. At MBARI, he focuses on developing new technologies for biological monitoring of the marine environment. Dr. Yamahara is currently developing field-portable instrumentation for monitoring fecal indicators and source-tracking markers and autonomous vehicle instrumentation to detect environmental DNA of marine phytoplankton and vertebrates.

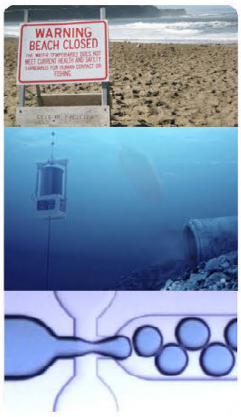
Towards Field-Portable Instrumentation for Real-Time Water Quality Monitoring

Kevan Yamahara, Andrew Hatch, Joshua Steele, Cody Youngbull, John Griffith, Christopher Scholin

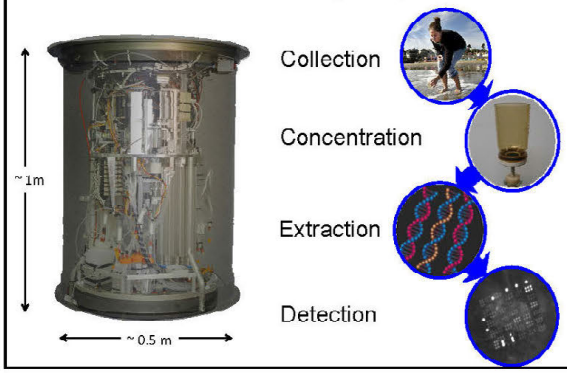
MBARI Monterey Bay Aquarium Research Institute · ASU · SCCWRP

Outline

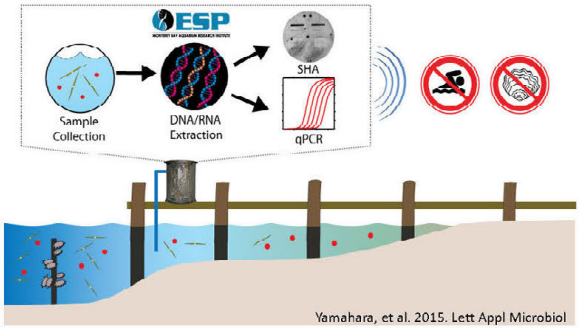
- Environmental Sample Processor (ESP)
- Proof of concept study for water quality monitoring
- New sensor development



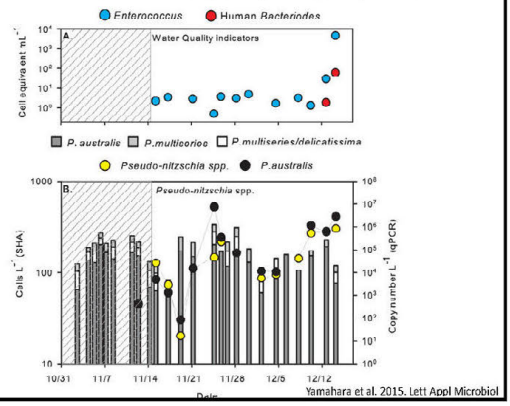
2nd Generation Environmental Sample Processor (ESP)



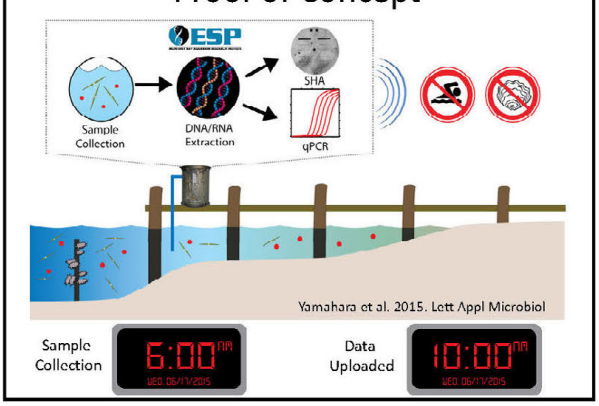
Proof of Concept



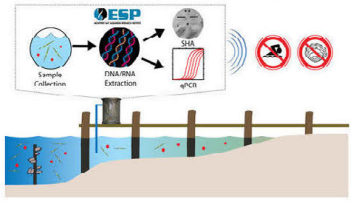
Santa Cruz Wharf, CA, Deployment



Proof of Concept

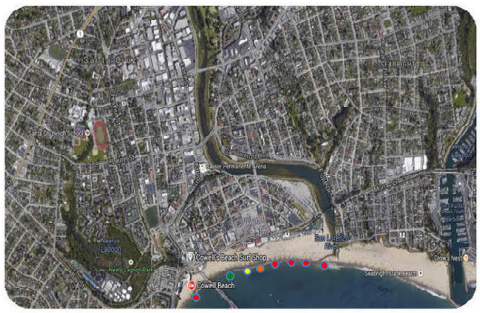


Proof of Concept



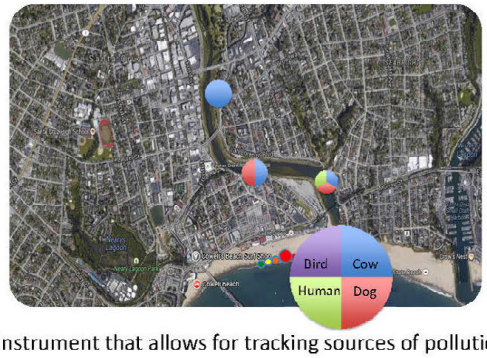
- Quantification of BOTH fecal indicators and harmful algae from the same sample
- Sample to Results in ~ 4 hours
- Limitations of size and portability

The Next Conceptual Idea



Instrument to survey a number of locations and to determine "hot spots"

The Next Conceptual Idea



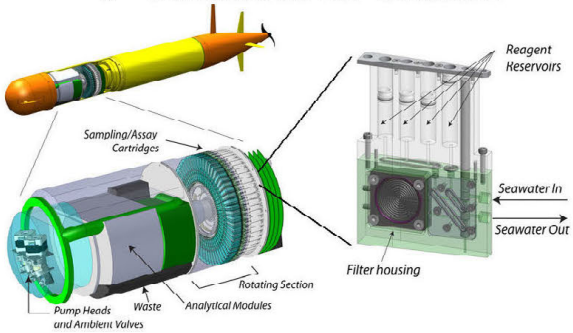
Instrument that allows for tracking sources of pollution

Instrument Design Criteria

- Tracking sources of contamination requires mobility
  - Engineering design for a hand-carry instrument
  - **Modular design** – separate sample collection and detection

Sample Collection/Processing

3rd Generation ESP Solution

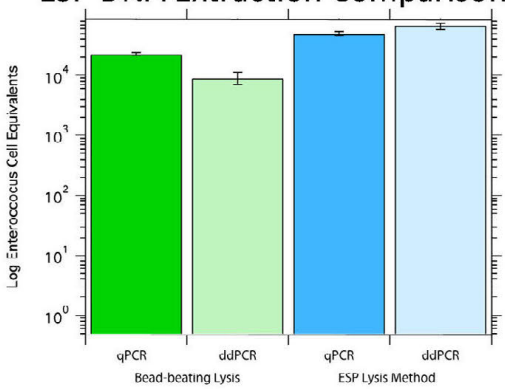


- Same engineering concepts, different form factor

Prototype 3rd Generation ESP

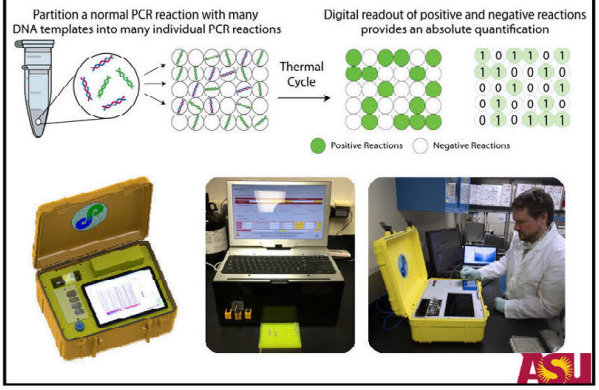
- 3rd Generation (3G) ESP technology
  - Sample Collection and Processing
    - Preservation and In-situ Lysis
  - Digital PCR (ddPCR) or Surface Plasmon Resonance (SPR)

ESP DNA Extraction Comparison

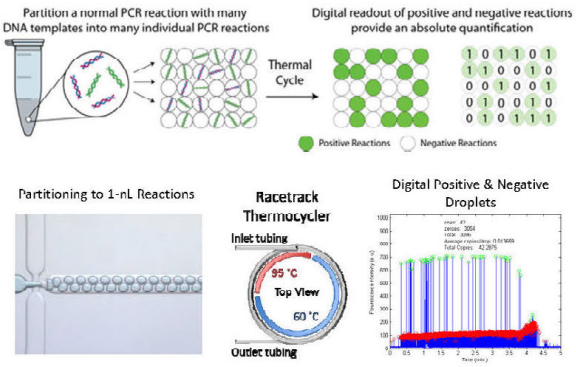


Analyte Detection

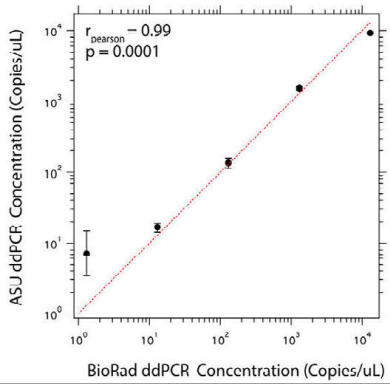
ASU Droplet Digital PCR Module



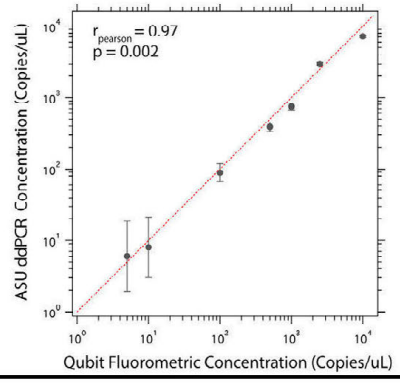
Droplet Digital PCR Module



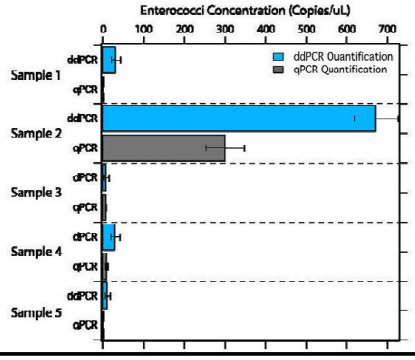
ddPCR Quantification of Enterococcus



ddPCR Quantification of HF183



Quantification of Environmental Samples Using ESP Methods



Conclusions and Next Steps

- The *challenge of portable biological sensors* for water quality monitoring is *sample acquisition and processing* for downstream analyses
- *Modular designs may allow for greater flexibility* for detecting/quantifying intended targets (e.g. ddPCR for DNA, cELISA for toxins)
  - New analytical detection methods are being developed all the time
- Field sampling trials will begin later this year in 2016

Acknowledgements

ESP
Chris Scholin
James Birch
Douglas Pargett
Scott Jensen
Brent Roman
Chris Preston
Roman Marin
Bill Ussler
MBARI Technicians

ASU School of Earth & Space Exploration
Arizona State University
Cody Youngbull
Andrew Hatch
Andrew Larson
Tathagata Ray
Kelly Lintecum
Greg Wells

SCCWRP
Steve Welsberg
John Griffith
Joshua Steele
Blythe Layton

the David & Lucile Packard Foundation
MBARI Monterey Bay Aquarium Research Institute
Gordon and Betty Moore Foundation
NSF
SCCWRP
NASA

Question & Answer Session

Question 1
(Unknown): How long does it take for a digital droplet?

Answer 1
Kevan Yamahara: It's about the same time as for the qPCR [quantitative polymerase chain reaction] system; we could reduce the number of cycles so we are looking into that.

Comment 1
(Unknown): Rumor was that it takes 5 hours for results with digital qPCR.

Comment 1 (follow-up)
Kevan Yamahara: No, it is probably less than an hour.

Question 2
(Unknown): How do you keep the integrity of the sample once you launch it? When the sample goes from point A to point B, how do you make sure the second site doesn't have the carryover from the first site?

Answer 2
Kevan Yamahara: We have looked at how to flush the system out. We let it sit for 15 to 20 minutes, then flush it with a solution, and are working on a handoff system between cartridge handling (based on bleach or other solution).

Answer 2 (follow-up)
John Griffith: We work closely with EPA. It's not ready for prime time, but in the upcoming year it will be comparable to regular qPCR. We'll communicate with EPA as usual.

Question 3
Steve Weisberg: For Shannon Briggs. I find this session to be gratifying. I took a look back at prior beach conferences. I looked back at the needs back then, then how we started developing the newer technologies to respond to those needs, then how we started getting more specific, then getting into application and learning from the challenges. It is great to see the transition from concept and methodology to the application. But, what is next? You put effort and resources into training these laboratories in qPCR, but who is watching you? Shannon, you invested a lot in this equipment, and it could be replaced in a few years. Was this a good time to make the investment?

Answer 3
Shannon Briggs: Yes things have evolved. The certification process has changed. We're not near drinking water yet; we discussed this last night. The site-specific document that came out in 2014 is a bit of a guidance that proves we are doing something right. But it's a day-by-day thing. Kevan's stuff looks very promising. This thing landed on us by chance—the connection started because of a public meeting. But, yes, I have 5 years to make it work.

Question 4
Suzanne Young: For the extraction methods for DNA, is everyone using kits?

Answer 4
Abhilasha Shrestha: It was a crude extraction for us.

Answer 4 (follow-up)
Kevan Yamahara: Ours was crude with a DNA sequence. We used a gene extraction kit.

Answer 4 (follow-up)
Rich Haugland: Ours was also crude.

Question 4 (follow-up)
Suzanne Young: So, there is a time lag if you need to do additional dilutions or spike controls, or add on more assays. There is a difference between EPA methods and more practical or applied methods.

Answer 4 (follow-up)
Rich Haugland: Site characterization, look at your site to see if you can get good results. The control assay or spike control assay maybe could be done. Need to characterize your site as part of the decision process.

Question 5
Keri Kazcor: For inhibition, is that more in marine waters? What is causing it and what can be done?

Answer 5
Marek Kris: We have a beach on the north shore. Should have groundwater; why is there brown water in Hawaii? Had a lot of salinity. I think it's mostly an issue in freshwaters impacted by human sources. So, dilute the sample to deal with inhibition. In Hawaii we are trying to do slow speed centrifugation. We think the speed is a factor. Not sure what else.

Question 5 (follow-up)
Keri Kazcor: Are you sure there wasn't a great correlation between culture and qPCR?

Answer 5 (follow-up)
Abhilasha Shrestha: If you look at the same water samples you see a correlation. But you don't see it with today's qPCR results, and yesterday's sample. Your results can vary within 6 hours and even more so within 24 hours of the culture results.

Question 6
Mark Sobsey: All of the presentations were about bacteria. I'm curious if anyone is applying these methods to coliphage. They have the short-term advantage, and can be detected in low numbers. Are any of you working on coliphage molecular detection? If interested, come by my poster where I present a new method.

Answer 6
Shannon Briggs: We have a researcher doing molecular qPCR work. You have to have a very expensive filter.

Comment 6
Mark Sobsey: No, there are other really simple ways.

Answer 6 (follow-up)
Shannon Briggs: We are looking at viruses in beach water.