qPCR Methods for Beaches

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Michigan Beaches

1,221 Public Beaches
19 years of monitoring data
856 locations tested
About 400 sites tested annually

http://www.deq.state.mi.us/beach/
On average, 4% of samples exceed WQS
MI WQS: Daily Geometric Mean of 300 *E. coli* per 100 ml for total body contact based on culture methods that have an 18-hour delay.
qPCR

Quantitative Polymerase Chain Reaction

Polymerase chain reaction - PCR

1. Denaturation at 94-96°C
2. Annealing at ~68°C
3. Elongation at ca. 72 °C
qPCR vs Culture

Measures DNA from live and dead cells vs counts of living bacteria

Results in hours vs. days
qPCR vs Culture

Epidemiological studies conducted on enterococcus qPCR and *E. coli* culture methods

qPCR methods for Microbial/Molecular Source Tracking (MST) identify DNA of host organism (sources)
Monitoring in Michigan

2015—10 new qPCR labs added

Michigan’s qPCR lab network and list serv is created!

MIQPCR@LIST.MSU.EDU

Send request to Dreelin@msu.edu
Michigan qPCR Network

Northern Michigan University-City of Marquette
Lake Superior State University
Northwest Michigan Regional Lab
NPS- Sleeping Bear Dunes
Central Michigan Health District
Ferris State University
Saginaw County Department of Public Health
Saginaw Valley State University
Huron County
Grand Valley State University
Alma College
Kalamazoo County Health & Community Services
Michigan State University
USGS - Lansing
Oakland County Health Department
Oakland University
MSU Training Program

- Audience: laboratory professionals
- Current program: qPCR and microbial source tracking (MST)
  - 4 days
  - Hands-on training in the lab
  - Includes trainees testing their newly-learned skills with blind samples
- Planning to expand course offerings beyond current network
Faces in the labs

Nilay Sheth, Brian Scull, Rich Haugland, Chris Otto and Tiong Aw
Faces in the labs
How would you rate your confidence in your skills for completing each step of qPCR?

Confidence Level

<table>
<thead>
<tr>
<th>Step</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipetting</td>
<td>PRE: 6, POST: 6</td>
</tr>
<tr>
<td>Preparing reagents</td>
<td>PRE: 5, POST: 5</td>
</tr>
<tr>
<td>Filtration</td>
<td>PRE: 5, POST: 5</td>
</tr>
<tr>
<td>DNA extraction</td>
<td>PRE: 4, POST: 4</td>
</tr>
<tr>
<td>Running the StepOne Plus</td>
<td>PRE: 3, POST: 5</td>
</tr>
<tr>
<td>Data analysis</td>
<td>PRE: 2, POST: 4</td>
</tr>
</tbody>
</table>

PRE
POST
Evaluation of multiple laboratory performance and variability in analysis of recreational freshwaters by a rapid *Escherichia coli* qPCR method (Draft Method C)

Tiong Gim Aw a, Mano Sivaganesan b, Shannon Briggs c, Erin Dreelin d, Asli Aslan e, Samuel Dorevitch f, Abhilasha Shrestha f, Natasha Isaacs g, Julie Kinzelman h, Greg Kleinheinz i, Rachel Noble j, Rick Rediske k, Brian Scull k, Susan Rosenberg l, Barbara Weberman l, Tami Sivy m, Ben Southwell n, Shawn Siefring o, Kevin Oshima o, Richard Haugland o, p, q
Rapid Method for *E. coli*

Water Research
Available online 15 March 2019
In Press  Accepted Manuscript

Standardized data quality acceptance criteria for a rapid *Escherichia coli* qPCR method (Draft Method C) for water quality monitoring at recreational beaches

Mano Sivaganesan a, Tiong Gim Aw b, Shannon Briggs c, Erin Dreelin d, Asli Aslan e, Samuel Dorevitch f, Abhilasha Shrestha f, Natasha Isaacs g, Julie Kinzelman h, Greg Kleinheinz i, Rachel Noble i, Rick Rediske k, Brian Scull k, Susan Rosenberg l, Barbara Weberman l, Tami Sivy m, Ben Southwell n, Shawn Sieftring o, Kevin Oshima o, Richard Haugland o
Table 1. Laboratories participating in the study

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Location</th>
<th>Experience with qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Michigan District Health Department, Assurance Water Laboratory</td>
<td>Gladwin, MI</td>
<td>New</td>
</tr>
<tr>
<td>City of Racine Public Health Department</td>
<td>Racine, WI</td>
<td>Experienced</td>
</tr>
<tr>
<td>Ferris State University, Shimadzu Core Laboratory</td>
<td>Big Rapids, MI</td>
<td>New</td>
</tr>
<tr>
<td>Georgia Southern University, Department of Environmental Health Sciences</td>
<td>Statesboro, GA</td>
<td>Experienced</td>
</tr>
<tr>
<td>Grand Valley State University, Annis Water Resources Institute Health Department of Northwest</td>
<td>Muskegon, MI</td>
<td>Experienced</td>
</tr>
<tr>
<td>Michigan, Northern Michigan Regional Laboratory</td>
<td>Gaylord, MI</td>
<td>New</td>
</tr>
<tr>
<td>Kalamazoo County Health and Community Services Laboratory</td>
<td>Kalamazoo, MI</td>
<td>New</td>
</tr>
<tr>
<td>Lake Superior State University, Environmental Analysis Laboratory</td>
<td>Sault Sainte Marie, MI</td>
<td>Experienced</td>
</tr>
<tr>
<td>Marquette Area Wastewater Facility</td>
<td>Marquette, MI</td>
<td>New</td>
</tr>
<tr>
<td>Michigan State University, Department of Fisheries and Wildlife</td>
<td>East Lansing, MI</td>
<td>Experienced</td>
</tr>
<tr>
<td>Northeast Ohio Regional Sewer District Environmental and Maintenance Services Center</td>
<td>Cuyahoga Heights, OH</td>
<td>Experienced</td>
</tr>
<tr>
<td>Oakland County Health Division Laboratory</td>
<td>Pontiac, MI</td>
<td>New</td>
</tr>
<tr>
<td>Oakland University, HEART Laboratory</td>
<td>Rochester, MI</td>
<td>Experienced</td>
</tr>
<tr>
<td>Saginaw County Health Department Laboratory</td>
<td>Saginaw, MI</td>
<td>New</td>
</tr>
<tr>
<td>Saginaw Valley State University, Department of Chemistry</td>
<td>University Center, MI</td>
<td>Experienced</td>
</tr>
<tr>
<td>University of Illinois at Chicago, School of Public Health</td>
<td>Chicago, IL</td>
<td>Experienced</td>
</tr>
<tr>
<td>University of North Carolina at Chapel Hill, Institute of Marine Sciences</td>
<td>Morehead City, NC</td>
<td>Experienced</td>
</tr>
<tr>
<td>University of Wisconsin Oshkosh, Environmental Research Laboratory</td>
<td>Oshkosh, WI</td>
<td>Experienced</td>
</tr>
<tr>
<td>US NPS Sleeping Bear Dunes Water Laboratory</td>
<td>Empire, MI</td>
<td>Experienced</td>
</tr>
<tr>
<td>USEPA National Exposure Research</td>
<td>Cincinnati, OH</td>
<td>Experienced</td>
</tr>
</tbody>
</table>
300 *E. coli* per 100 ml

Local term = Number to close beach

Michigan term = Water Quality Standard

Federal term = Beach Notification Value
What number from draft Method C is equivalent to 300 *E. coli* per 100 ml?

Equivalent value? = Number to close beach

Equivalent value? = Water Quality Standard

Equivalent value? = Beach Notification Value
Quest for the equivalent qPCR Value for 300 *E. coli* MPN

108 beaches
- Paired Samples
- 3 years
- Colilert and qPCR methods
- Great Lakes beaches
- Inland Lake beaches
- Upper Peninsula
- Lower Peninsula
- Urban beaches
- Rural beaches

Map courtesy of Chris Vandenberg, SWAS
Why did it take so long?

Many Michigan beaches are too clean for statistics!!!

Took 3 years to collect sufficient data that met statistical requirements

Identifying value for all beaches based on individual values from diverse group of beaches
What did we get?

6,669 total samples from 108 beaches were collected and analyzed.
Data analysis

1. Remove “clean” beaches
   - 62 (out of 108) beaches reported at least one exceedance (one result from Colilert that was greater than 300)

2. Remove low N beaches
   - 57 beaches ≥ 10 paired quantifiable results (8 MPN < quantifiable results < 2,419 MPN)

3. Require Beach specific correlation
   - 39 Beaches > 0.6 $R^2$ with outliers out (studentized residuals).
Outliers

Advantages for removing outliers

• Tends to improve $R^2$ values
• Tends to increase number of beaches in dataset with acceptable $R^2$ values (39 vs 21)
• More data is used to develop the association (2,092 vs 920)

Disadvantage is removing individual results from beaches
Quest for the equivalent qPCR Value for 300 *E. coli* MPN

39 beaches

- Paired Samples
- 3 years
- Colilert and qPCR methods

Great Lakes beaches

- Inland Lake beaches
- Upper Peninsula
- Lower Peninsula

Urban beaches

Rural beaches
What could we use?

2,092 total samples from 39 beaches
Ordinary Least Square Model
What could we use?

2,092 total samples from 39 beaches
Ordinary Least Square Model
What number from draft Method C is equivalent to 300 \( E. coli \) per 100 ml?

1.863

Average sample exceedance rate
qPCR: 9.7 %
Colilert: 6.2 %
What are the units?

1.863 log copies per reaction

In whole numbers, that is
73 (72.9457510) copies per reaction

73 copies of DNA target sequences
of *E. coli* per reaction
What is a reaction?

Measured 73 copies of DNA target sequences of *E. coli* per reaction

Each reaction is in 5 microliters of sample
What is a reaction?

100 mL Sample

Filter

Concentrated to 600 µL

qPCR On 5 µL of concentrated sample

# DNA copies per reaction in 5 ul

\[
\frac{1.863 \log \text{DNA copies}}{\text{reaction}} = \frac{73 \text{ DNA copies}}{\text{reaction}} \times 120 = \frac{8,760 \text{ DNA copies}}{100 \text{ mL sample}}
\]
How do we use 1.863

• Application for beaches only
  – Public Health Code
• Reporting results from lab to health department
• Reporting results to public
• Reporting results on BeachGuard
• Reporting results to USEPA
Equating qPCR to MPN

BNV 1.863
1.494
2.266
Next Steps

Continue Routine Monitoring
Colilert or Draft Method C

Beaches with Exceedances
Monitoring with Microbial Source Tracking
3 Human Markers
Multiple Animal Markers
Stay Tuned

Please ask questions

More to come