Advantages of QPCR Methods for Water Quality Monitoring

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QPCR

How this works

What this does for us

Progress in Michigan

Quantitative Polymerase Chain Reaction

Detect a specific DNA code

Make copies of it

Measure it immediately

How Does PCR Work?

Denaturation

- DNA helixes are pulled apart into single strands

Annealing

- Primers bind to specific locations on the single strands of DNA

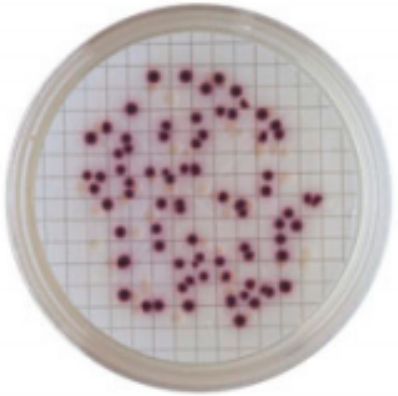
Elongation/Extension

- Facilitated by enzymes that attach to the primers, nucleotide bases are added to the free strand, forming a double-stranded DNA

Remember QPCR is *not* Measuring the Same Thing as a Culture…

- QPCR differs from traditional culture-based assays in that it measures all DNA:

DNA from
live & dead
microbes
vs
live (growing)
microbes



- Culture assays only measure cells possessing the ability to grow on the selective media you are using

Advantages

Faster

Detecting, Copying, and Measuring DNA happens in hours rather than days

Epidemiology

Results more closely tied with reported illnesses

U.S. EPA Rec Water Quality Criteria

2012 criteria based on Epidemiological Study

Table 5. Beach Action Values (BAVs).

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	OR	60 cfu
E. coli – culturable (fresh)[b]	235 cfu		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.

Table 6. Values for qPCR in marine and fresh waters.

Element	Estimated Illness Rate (NGI): 36/1,000 primary contact recreators Magnitude GM (cce per 100 mL)	STV (cce per 100 mL)	OR	Estimated Illness Rate (NGI): 32/1,000 primary contact recreators Magnitude GM (cce per 100 mL)	STV (cce per 100 mL)
qPCR[a]	470	2,000		300	1,280
Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a 10 percent excursion frequency of the selected STV magnitude in the same 30-day interval.					

[a] EPA *Enterococcus* spp. Method 1611 for qPCR (U.S. EPA, 2012b).

Advantages

Specific

Identification based on DNA, specific to host

Molecular Source Tracking

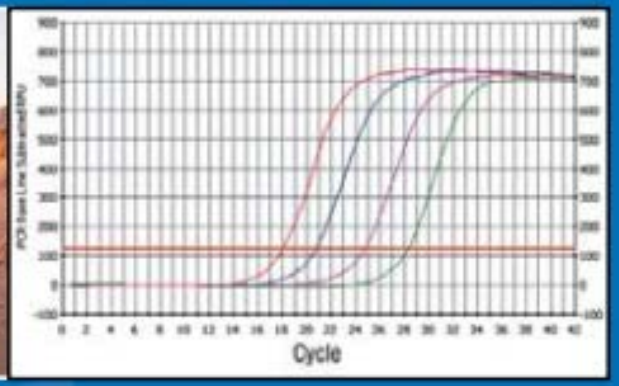
(e.g., human, dog, bird, cow, algal toxins, pathogens, and …)

EPA
United States
Environmental Protection
Agency

Human Microbial Source Tracking with qPCR:
Method Standardization Update and Research Activites

Orin C. Shanks

Laboratory Technical Information Group 2016
EPA Region 2



https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=528418

Office of Research and Development
National Risk Management Research Laboratory, Water Supply and Water Resources Division

April 25, 2016

Waterborne pathogens

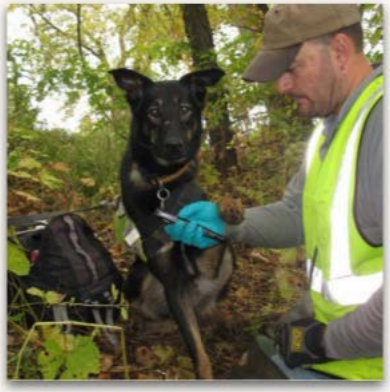
Pathogen		Disease	Effects
Bacteria	*Escherichia coli* (enteropathogenic)	Gastroenteritis	Vomiting, diarrhea, death in susceptible populations
	Helicobacter pylori	Gastritis	Diarrhea. Peptic ulcers are a long-term sequela.
	Legionella pneumophila	Legionellosis	Acute respiratory illness
	Leptospira	Leptospirosis	Jaundice, fever (Weil's disease)
	Pseudomonas	Infections in immunocompromised individuals	Urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, and a variety of systemic infections
	Salmonella typhi	Typhoid fever	High fever, diarrhea, ulceration of the small intestine
	Salmonella	Salmonellosis	Diarrhea, dehydration
	Shigella	Shigellosis	Bacillary dysentery
	Vibrio cholerae	Cholera	Extremely heavy diarrhea, dehydration
	Yersinia enterolitica	Yersinosis	Diarrhea
Protozoans	*Balantidium coli*	Balantidiasis	Diarrhea, dysentery
	Cryptosporidium	Cryptosporidiosis	Diarrhea
	Entamoeba histolytica	Ameobiasis (amoebic dysentery)	Prolonged diarrhea with bleeding, abscesses of the liver and small intestine
	Giardia lamblia	Giardiasis	Mild to severe diarrhea, nausea, indigestion

Source: EPA's *National Beach Guidance and Performance Criteria for Grants*

Canine Scent Tracking

Environmental Canine Services

Karen and Logan



Scott and Sable

Aryn and Crush

Stephanie and Kona

Dan and Abbey

Laura and Kenna

Should I consider QPCR?

- Do you monitor a beach or river or lake?
- Have you closed a beach due to high *E. coli*?
- How often do you close a beach?
- How far away is the beach from a lab?
- Do you want results in 4 hours?
- Do you want lab equipment that can identify sources of fecal contamination?

What do I need for QPCR?

- Proximity of lab to beach
- Lab willing to dedicate staff to perform qPCR method (consistency)
- Training, preferably “wet-lab, hands-on”
- Equipment Costs
  - $50k if lab is already well equipped
  - $100K if lab has no equipment

What do we have in Michigan?

- E. coli Method
- Trained Staff

EPA
United States
Environmental Protection
Agency

Method C: *Escherichia coli* in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR)

June 2015

Our Network of Michigan qPCR Labs

Marquette Area Wastewater Treatment Plant
Lake Superior State University
Northwest Michigan Regional Lab
NPS- Sleeping Bear Dunes
Central Michigan Health District
Ferris State University
Saginaw County Dept of Public Health
Saginaw Valley State University
Grand Valley State University
Hope College
Kalamazoo County Health & Community Services
Michigan State University
USGS- Lansing
Oakland County Health Department
Oakland University

2016 Validation Study

- 22 participating Laboratories
  - 11 public health, municipal, regional and Governmental laboratories
  - 11 college and University laboratories
  - Varying levels of training and experience

- Two Phases in the study
  - Phase 1: Standards and calibrator sample analyses, Proficiency demonstration
  - Phase 2: Unknown water sample analyses and *E. coli* target sequence quantification

- Total of 54 Blinded water samples analyzed in common by each lab in Phase 2
  - Water samples collected from 6 sites (2 inland and 4 great Lakes)
  - Each collected sample divided into 3 subsamples (1 ambient and 2 *E. coli* spike levels or ambient and 2 lake water dilutions)
  - 3 replicates of each subsample analyzed in duplicate by each lab

- 21 Laboratories completed the study (phase 1 and phase 2)

Enterococci Multi-lab Validation Study

Journal of Microbiological Methods 123 (2016) 114–125

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters

Richard A. Haugland [a,*], Shawn Siefring [a], Manju Varma [a], Kevin H. Oshima [a], Mano Sivaganesan [b], Yiping Cao [c], Meredith Raith [c], John Griffith [c], Stephen B. Weisberg [c], Rachel T. Noble [d], A. Denene Blackwood [d], Julie Kinzelman [e], Tamara Anan'eva [e], Rebecca N. Bushon [f], Erin A. Stelzer [f], Valarie J. Harwood [g], Katrina V. Gordon [g], Christopher Sinigalliano [h]

[a] U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, USA
[b] U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH, USA
[c] Southern California Coastal Water Research Project Authority, Costa Mesa, CA, USA
[d] Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC, USA
[e] City of Racine Health Department, Racine, WI, USA
[f] U.S. Geological Survey, Columbus, OH, USA
[g] Department of Integrative Biology, University of South Florida, Tampa, FL, USA
[h] National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratories, Ocean Chemistry Division, Miami, FL, USA

Announced Monday, March 14, 2016

Next Steps

- Compare results in 2016
  - qPCR *E. coli* vs. Colilert (Michigan Labs)
  - qPCR *E. coli* vs. qPCR Enterococci (USEPA)

- Collect and Compare results in 2017
  - qPCR *E. coli* vs. Colilert (Michigan Labs)
  - qPCR *E. coli* vs. qPCR Enterococci (USEPA)

Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods

EPA-820-R-14-011

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Health and Ecological Criteria Division

December 2014

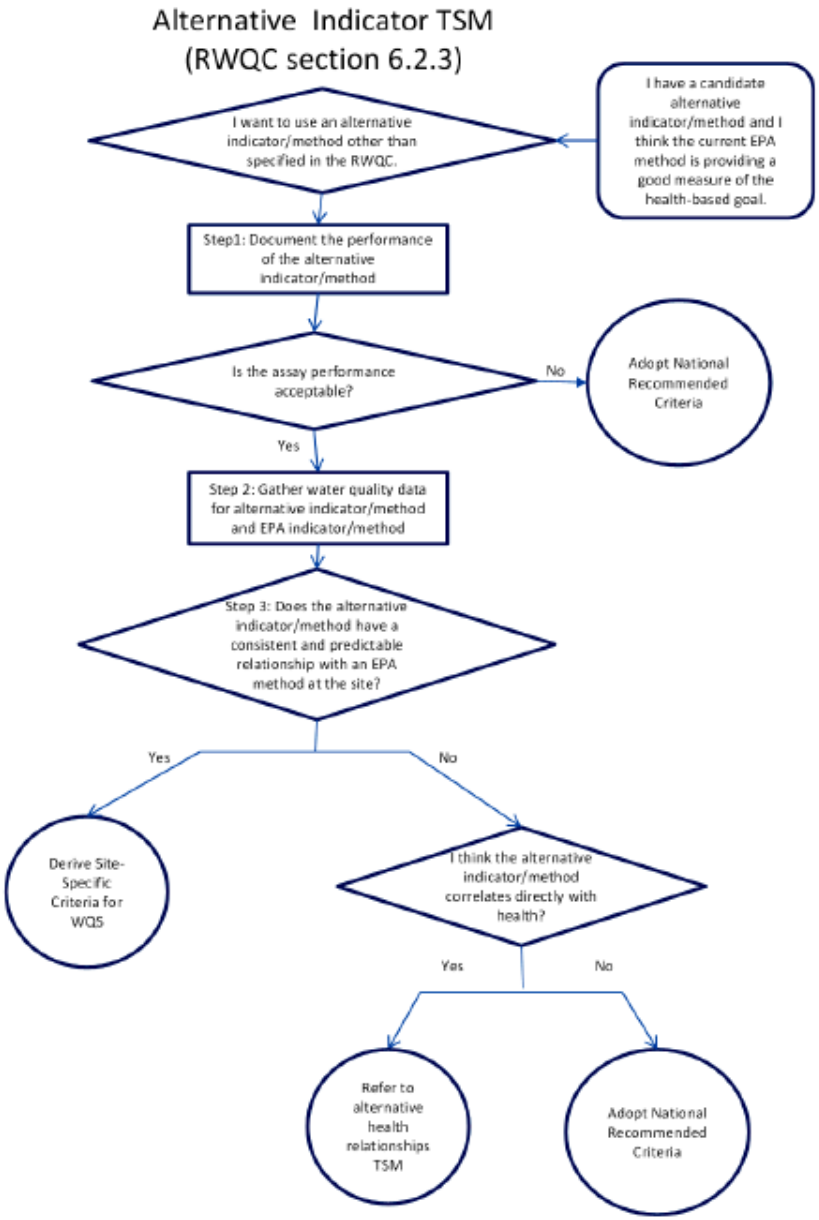


Figure 1. Flow diagram for considering approaches to alternative site-specific criteria

Great Lakes Beach Association (GLBA)
State of Lake Michigan (SOLM)
International Association of Great Lakes Research (IAGLR)

Register EARLY in case the Packers are in town!!

STATE of LAKE MICHIGAN CONFERENCE

IAGLR Presents the
2017 State of Lake Michigan Conference
November 6-9, 2017 | Green Bay, Wisconsin

IAGLR will host a series of State of Lake conferences starting with Lake Michigan in 2017. These events will blend elements from IAGLR's annual Conference on Great Lakes Research and the biennial State of Lake Michigan Conference organized under the Lake Michigan LAMP Forum for nearly two decades and held in conjunction with the Great Lakes Beach Association annual meeting.

GLBA

Goal: These conferences aim to facilitate interactions between researchers and managers on diverse topics related to issues relevant for a specific lake. The State of Lake Michigan Conference does not replace IAGLR's annual Conference on Great Lakes Research, but rather seeks to bring together Lake Michigan-specific research, policy development, management, education, and nonprofit organizations to broaden the discussion and provide diverse interaction among stakeholders.

QPCR USERS

GLBA

Great Lakes Beach Association

Working together to improve recreational beach water quality

Home About Headlines Events BEACHNET Discussion Great Lakes Beach Conference Additional Info

Beach listservs

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MIQPCR@LIST.MSU.EDU

http://www.michigan.org/blog/wp-content/uploads/Bob-Peskorse-Jr-Grand-Haven.jpeg

Bob Peskorse Jr Grand Haven

Published July 2, 2014 at 1024 × 685 in Fifteen Photos That Will Get You Dreaming of a Perfect Pure Michigan Beach Day

← Previous Next →

http://www.michigan.org/beachchallenge/

The sandy shores of Lake Michigan are just right for building a sand castle. Photo by Robert Jacobs (Honorable Mention).