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A comparison study of Colilert and qPCR methods at Pere Marquette Beach, Muskegon County, MI

Safiya Best
Grand Valley State University, bestsa@mail.gvsu.edu

Richard Rediske
Grand Valley State University, redisker@gvsu.edu

Molly Lane
Grand Valley State University, lanemol@mail.gvsu.edu

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Safiya Best, Dr. Richard Rediske and Molly Lane

Annis Water Resources Institute

Abstract

Pere Marquette Beach serves as the primary attraction for tourism and coastal recreation in Muskegon, MI. Because beaches attract many people daily, it is important to monitor beach water quality for pathogens that may cause waterborne disease. Molecular-based methods are emerging as replacements for culture-based techniques for monitoring beaches. Culture-based methods require 18-hour incubation while Quantitative Polymerase Chain Reaction (qPCR) can yield results in two hours. My research examines the correlation between the culture-based Colilert-18® method and qPCR measurements of *E. coli* at a Lake Michigan beach in Muskegon County, MI. While Colilert 18 is a defined substrate method and measures culturable cells, the qPCR method quantifies both living and nonliving DNA. Regression analysis (R^2) was used to correlate analytical results and the Index of Agreement (IA) was employed to evaluate method comparability. This research demonstrates the equivalency of both methods for *E. coli* measurements at Pere Marquette beach (R^2 = 0.8012; IA = 0.71). The significant positive difference between the methods suggests that current guidelines for beach warnings and closures need to be revised to reflect the presence of nonviable DNA/cells in beach water. This study was important for assessing the applicability of qPCR for providing same-day results for pathogens at local beaches.

Introduction

Beaches often attract tourists to visit a community due to the wealth of recreational opportunities they provide. Tourism is important to building strong economies in coastal cities. A typical beach-goer spends $13.13 within 10 miles of a beach per visit (*Murray et al.*, 2001). A single beach advisory can cost, on average, $100,000 in lost revenue to businesses within a 10-mile radius of the beach (*Jentes*, 2000). As the number of visitors to recreational waters increases, the risk of exposure to waterborne pathogens also rise. Being exposure to pathogenic bacteria in water can cause illnesses such as skin irritations, respiratory infections and gastrointestinal (GI) illness (*Seyfried et al.*, 1985; *Wade et al.*, 2008).

One of the important functions of the Public Health is to monitor beaches for pathogens from the release of fecal matter. The presence of fecal contamination has been shown to increase the risk of contracting Recreational Water Illnesses (RWI's) (Seyfried et al., 1985). Because fecal microbial contamination poses a risk to public health, recreational water-quality guidelines were implemented by the Environmental Protection Agency (EPA) under the authority of the Clean Water Act, Section 304a (USEPA, 1986). The EPA guideline for no contact advisory is 300 MPN/100 mL. A commonly used bacteria indicator for beach quality assessment is *Escherichia coli* and it is found in the lower intestine of warm-blooded animals in high concentrations (Whitman et al., 1999). Potential sources of fecal contamination at bathing beaches include agriculture runoff, vessel wastewater discharge, and animal waste, untreated sewage water, leaking septic systems and treated municipal wastewater (Colford *et al.*, 2007). Although there are many pathogens associated with fecal bacteria, testing for all of them would create a financial and regulatory burden. To provide rapid and low cost test results, it is

important to select representative indicator bacteria for screening and monitoring. E. coli is a reliable indicator of fecal contamination due to its high concentration in the intestine relative to pathogens and its correlation with waterborne diseases (Whitman et al., 1999) The guidelines for potential pathogens in freshwater systems are set based on the probability of developing a GI illness when *E. coli* is present (Edberg *et al*., 2000). There also are large ranges of potential illnesses caused by pathogenic *E. coli* strains, from mild GI symptoms (i.e. nausea) to the fatal hemolytic uremic syndrome (Soller et al., 2010). In 2011-2012, there were 20 illness outbreaks from fresh water contact and 7 from strains of *E. coli* alone that were linked to untreated recreational waters, in (Hlavsa et al., 2015). Clearly, water monitoring and testing are vital tools in protecting the public health.

Considering the health risks and economic impacts associated with *E. coli* exposure, it is important to test, and report contaminated waters as quickly and accurately as possible. Two U.S. EPA approved methods to quantify *E. coli* densities in water samples are membrane filtration and Colilert-18®. Membrane filtration is described in U.S. EPA Method 1603 (US EPA, 2006). Samples are filtered, exposed to a culture medium, and then incubated for 22 ± 2 h. E. coli colonies were then counted based on a color change induced by the culture medium in a technique. The IDEXX Colilert-18® reagents in conjunction with the Quanti-Tray/2000 (Crane et al., 2006) is classified as a defined substrate method and requires 18 hours to detect both total coliforms and *E. coli* in water. The Colilert-18® method uses two color indicators O-Nitrophenyl-β-D-galactopyranoside (ONPG) and 4- methylumbelliferyl-β-D-glucuronide (MUG), to detect coliforms (Crane et al., 2006). The β-galactosidase enzyme metabolizes ONPG, enabling a yellow color change for identification. Fluorescence is created when *E. coli* metabolizes MUG by using the β-glucuronidase enzyme, which can be detected by UV light.

Although the Colilert method separates and identifies nonconiforms from the target organisms, there are molecular based methods that can provide similar resolution in a more rapid format. Quantitative PCR (also known as real-time PCR) is a method of quantifying the amount of target DNA found in samples. It has become an important method in medical, forensic, and environmental biology due to its specificity and speed of analysis.

The use of qPCR as a replacement method for Colilert 18® has been validated repeatedly (Haughland et al., 2005; Whitman et al., 2010) and in 2014, the EPA submitted a draft method for the use of qPCR to measure *E. coli* in water samples (US EPA, 2014a). Unlike the Colilert-18® method, no incubation is needed, considerably speeding up sample turn-around time. qPCR allows for a more rapid detection in about four hours, it also lessens the risk of pathogen exposure by providing same day results. Using the qPCR method makes it possible to sample and assess *E. coli* levels prior to peak beach usage times. Increased turbidity has a marked effect on detection failure with qPCR methods (Siefring et al., 2008) and therefore, additional research is needed on qPCR's responsiveness to varying water body types. Furthermore, a qPCR assay is different from substrate methods in that it quantifies both viable and non-viable target cells in addition to DNA fragments (Varma, 2009). It also is hypothesized that the concentration of *E. coli* cells detected by the qPCR method will be higher than the concentration detected using the Colilert-18® method. Tributaries are suspected as a sources of non-viable bacterial cells and DNA fragments resulting in elevated fecal Indicator Bacteria (FIB) counts and are a source of turbidity (Malin et al. 2000).

We investigated the correlation between Colilert-18® and qPCR Draft Method C in bathing beach samples collected at Pere Marquette Park in Muskegon, MI. Statistical methods including R^2 and the Index of Agreement (Willmott et al., 2012) were used to examine method

comparability. Consistently elevated bacterial counts due to quantitative differences between methods would prompt the need for changes to current water-quality guidelines. These results will be useful in developing water quality standards for Great Lakes Beaches.

Experimental

Study Site

Pere Marquette is a large city beach, located on Lake Michigan in Muskegon, MI shown in Figure 2. It is located immediately to the south of the Muskegon River channel and is one block away from a special pet-friendly beach (Kruse Beach). The surrounding area for Pere Marquette Beach also includes sand dunes, residential property and businesses. This beach was chosen because of previously recorded high *E. coli* counts (MDEQ 2018).

Figure 1. Pere Marquette Beach, Muskegon, MI.

Sampling Methods

Sampling occurred one time per week, over six consecutive weeks. Samples were collected from north, center and south swim area, approximately one foot below the surface of the water. The three samples were collected in separate 500-mL sterile HDPE sampling bottle and individually ran. Samples were collected and stored on ice until transported back to the lab for analysis. The three 500 mL samples were composited into a single sterile HDPE container. A 100-mL aliquot was taken from the composited sample used for analysis by the IDEXX Colilert-18® method, and the other 200-mL used for qPCR.

Colilert-18®. The Colilert-18® method was performed according to EPA Method 1604 (U.S. EPA, 2002). A 100mL aliquot of each composited beach water sample was tested using the IDEXX Quanti-Tray/2000. The substrate powder was added immediately when the samples were brought to the lab, and then incubated at 35°C for 18 h. The Colilert Quanti-Tray®/2000 were exposed to long-wave UV light and blue fluorescent wells were counted as positive. The number of positive wells was MPN/100 mL.

qPCR. Water samples for qPCR analysis were analyzed according to EPA Draft Method C (Each 100 ml water sample was filtered through a 47mm diameter/45-µm pore size polycarbonate filter. It was rinsed with phosphate buffered saline (PBS) buffer solution. The filter assemblies were pre-sterilized and sterilized forceps were used to fold the filter four times and placed in a DNA extraction tube that contained glass beads. While working in a laminar flow hood, DNA extraction fluid was added to each screw-top tube, followed by bead milling and two rounds of centrifuging.) At the end of this process, there was approximately 100-mL of clean, DNA-extracted sample supernatant for use in the analysis. In addition, quality control (QC)

filters were prepared for each analysis run. Three calibrators, two filter blanks, a reference matrix spike and a matrix spike were treated in the same manner as water samples Preparation of *E. coli* and salmon DNA master mix also were conducted in a laminar flow hood. A 20-µL aliquot of *E. coli* master mix (TaqMan) was loaded into the top 48 wells of the 96-well plate and 20- µL of salmon DNA master mix was loaded into the bottom 48 wells. Samples and QC aliquots were added in duplicate to both top and bottom wells. The sample tray was placed into the Applied Biossystems StepOnePlus™ Real-Time PCR instrument. A series of 40 cycles of heating and cooling were used for DNA separation and recombination and the results of cycle yield an exponential reaction product. The accompanying software package calculates a cycle threshold value (C_t) for each sample based on a predetermined threshold level. The C_t will give rise to the genomic equivalent using a Microsoft Excel spreadsheet created by the U.S. EPA.)

The DNA strands were amplified through a series of steps including breaking the helix apart by denaturation, annealing the strands by recombining the DNA and using the dNTPs to form the complementary strand of DNA. The TaqMan reagents copy the strands. The reaction starts with a primer and probe attached to a quencher and fluorescent dye. The probe anneals to the sample DNA downstream from the forward primer. As the DNA extends, the probe was cleaved. When the fluorophore and quencher were no longer near each other, the fluorophore fluoresced. This determined the amount of target DNA present. After the process was complete, there is double the amount of DNA compared to the start of the cycle as shown in Figure 2.

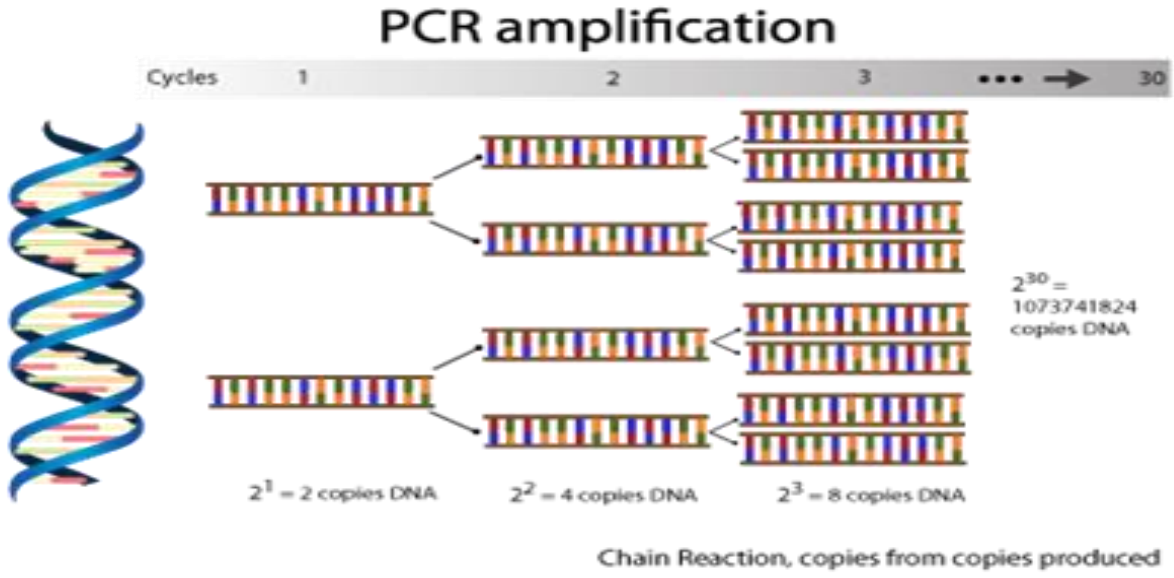

Figure 2. The amplification of DNA strands for each cycle using qPCR method (http://www.gmotesting.com/Testing-Options/Genetic-analysis).

At the end of the run, the accompanying software package calculated a cycle threshold value (CT) for each sample based on a predetermined threshold level, which gives rise to an genomic equivalent using a Microsoft Excel spreadsheet created by USEPA.

Statistical Methods.

Linear regression and the Index of Agreement (Wilmont et al., 2012) were used to analyze the test results and determine the comparability of methods. The Mann-Whitney Rank Sum Test was used to determine if the results of the qPCR results were significantly higher than Colilert-18® measurements. The Index of Agreement calculation is shown in Figure 3.

$$IA = 1 - \frac{\frac{1}{N}\sum_{i=1}^{N}\left(x_i - y_i\right)^2}{\frac{1}{N}\sum_{i=1}^{N}\left(\left|x_i - \overline{x}\right| + \left|y_i - \overline{x}\right|\right)^2}$$

Figure 3. The Index of Agreement calculation formula (Wilmont et al., 2012). X and Y represent the results of Colilert-18® and qPCR methods)

False positive (Type I Error) represents rejection of the true null hypothesis where Colilert-18 results would not have resulted in beach closure (< 300 MPN/100 mL) and qPCR results would trigger a closure (> 300 GE/100). False negative (Type II Error) represents failure to reject a false null hypothesis where Colilert-18 results would trigger a beach closure (> 300 MPN/100 mL) and qPCR results keep the beach open (< 300 GE/100).

Results

The results varied widely for both the Colilert method and the qPCR method (Table 1).

Table 1. Beach Conditions and Colilert-18® and qPCR results for water samples collected at Pere Marquette Park, Muskegon, MI 2018.

Date	Location	Wind Speed Direction	# birds	Rain Events	People at the Beach	Colilert (MPN/100 mL)	qPCR (GE/100mL)
6/12/2018	North	4 mph SW	65	<48 hours	1	2420	4411
	Center					229	299
	South					50	261
6/13/2019	North	7 mph E	70	<72 hours	15	30	274
	Center					365	934
	South					40	155
6/19/2018	North	5 mph SE	46	<24 hours	3	34	934
	Center					222	1688
	South					48	155
6/26/2018	North	8 mph SE	100	<48 hours	0	548	931
	Center					166	346
	South					4	131
7/5/2018	North	7 mph W	102	>72 hours	2	125	346
	Center					157	931
	South					86	408
7/9/2018	North	8 mph SW	100	>72 hours	5	32	248
	Center					45	265
	South					22	40

The 6/12/2018 contained the highest results for both qPCR and Colilert. This may be due to a sewage leak on a tributary of the Grand River that occurred prior to sampling and presence of a SW wind that would push water to the north along the coast. Pere Marquette was resampled the next day with a SE wind and the results were below closure levels. The two highest samples

occurred at Station 1 near the channel seawall (Figure 1). The lowest levels of E. coli occurred at the south location, furthest from the seawall.

The Colilert-18® concentration results were compared to the qPCR CT values (Figure 4) and a significant positive correlation was determined (R^2 = 0.8012). The Index of Agreement

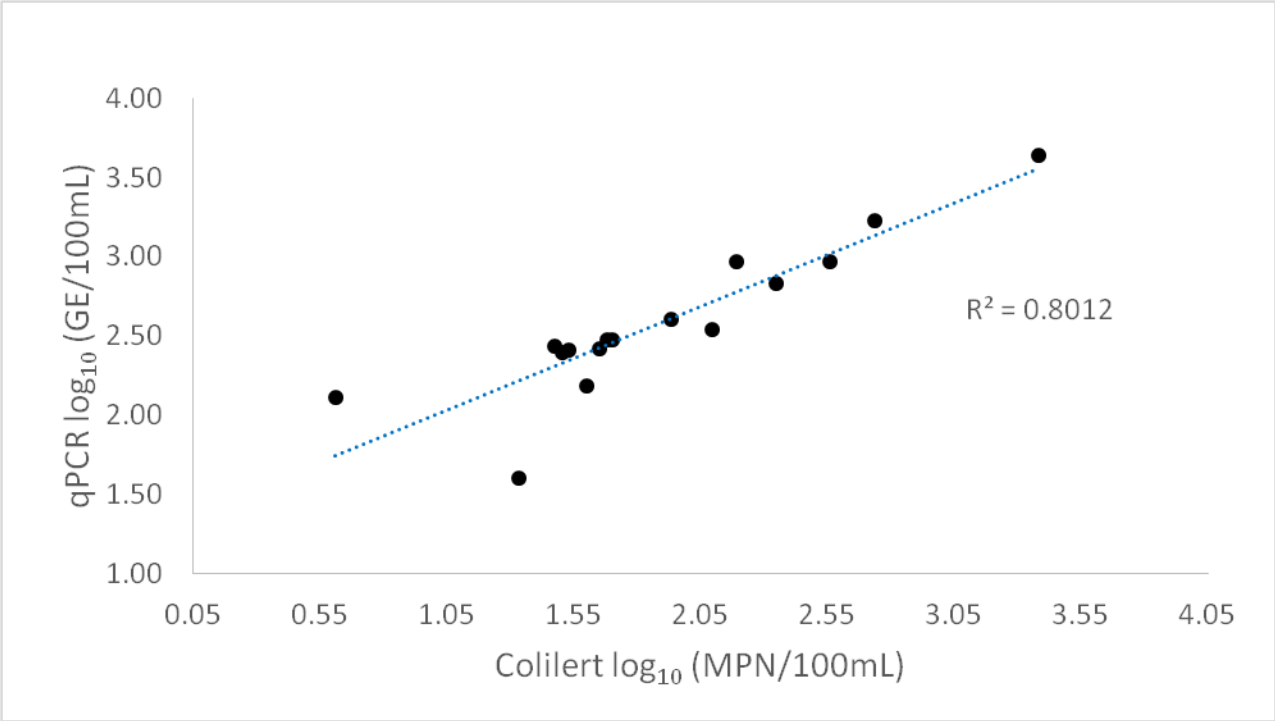

Figure 2. Comparison of Colilert and qPCR Method Samples from Pere Marquette Beach, Muskegon, Michigan 2018.

was 0.71. The *E. coli* concentrations measured by the qPCR method were significantly higher than the Colilert results (p=0.001). No Type II Error was present indicating the qPCR did not yield false negative results. For 6 of the 18 samples, qPCR results exceeded 300 GE/100 mL while Colilert-18® were below 300 cfu/100 mL, indicating 33% occurrence of false positive data at the current EPA guideline.

Discussion

The EPA guidelines recommend the Index of Agreement (IA) to be ≥ 0.7 for method equivalency (US EPA, 2014b). If the IA is below 0.70, R^2 must be > 0.6 for the verification of the qPCR method. The index of agreement and R^2 suggests that these two methods can be used interchangeably. This has been verified by literature as well. According to Dorevitch et al., monitoring multiple beaches using qPCR methods can generate precise and accurate data for timely public notifications regarding beach water quality (Dorevitch et al, 2017). There were no false negatives with respect to the 300 (MPN/100 mL) limits for Beach Advisory, which suggests that Pere Marquette beach would not meet body contact standards by qPCR and exceed safe levels by Colilert-18®.results predicting beach closure. According to the data, there are six false positives, suggesting that Pere Marquette beach would have been closed or under and advisory on six occasions when Colilert-18® predicted safe conditions. The data for qPCR are significantly higher than Colilert results because qPCR quantifies live and dead cells. This allows the results to vary widely in number. According to Liu et al, *E. coli* bacteria tend to survive longer in more turbid water and cold water than clear and warm water (Liu et al., 2006). Possible errors of this research include measuring error of pipetting and drifting of the instrument. These may have been the reason for some data samples failing and being undetectable by the qPCR instrument.

Although the RT-qPCR method that was used for this research, there are other methods for using PCR. This includes Reverse Transcriptase PCR, Multiplex-PCR, Nucleic Acid Based Sequence Amplification, etc. Although qPCR has limited experience with the performance in a broad range of environmental conditions, the method stills serves as one of the most timely and most efficient techniques for qualifying bacteria (Sivaganensan et al., 2014). It has been

recommended to perform site-specific analysis of the method's performance before being used for beach notification programs (Sivaganensan et al., 2014). Another application that this research could be used for is to determine the source of fecal bacteria when it is applied to human and animal strains of *E. coli* (Silva and Domingues, 2015). Identifying the fecal pathogens is important in assessing and eliminating the bacteria from contaminating the beach water (Liu et al, 2017).

Conclusion

According to the data, the qPCR results for Pere Marquette were consistently higher than Colilert results. Since the beach is south of the Muskegon Lake Channel and north of the Grand River, these sources may contribute additional nonviable bacteria cells that result in elevated results. The Index of Agreement and R^2 were consistent with EPA the acceptance values for Pere Marquette Data indicating method equivalency. There were no false negatives and four false positives, which indicate that there were 6 times where qPCR exceeded 300 GE/100 mL and Colilert results, were below. This would result in an unnecessary beach advisory. The data for two samples failed and were omitted due to possible analyst error and inhibition by organic material. These results should be confirmed with additional monitoring data over multiple years.

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References

Cabelli, V. J., Dufour, A. P., McCabe, L. J., & Levin, M. A. (1982). Swimming-associated gastroenteritis and water quality. *American journal of epidemiology*, *115*(4), 606-616.

Colford Jr, J. M., Wade, T. J., Schiff, K. C., Wright, C. C., Griffith, J. F., Sandhu, S. K., ... & Weisberg, S. B. (2007). Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology, 27-35.

Congress, U. S. (2000). Beaches environmental assessment and coastal health act of 2000. Public Law, 102-284.

Crane, R., Jackson, C. B., Sedlacek, R., & Walker, D. (2006). EPA approves new test procedures for the analysis of microbiological pollutants in wastewater and sludge. *Loveland, CO: Hach Company. Available online at http://www. hach. com/fmmimghach*.

DeFlorio-Barker, S., Wing, C., Jones, R. M., & Dorevitch, S. (2018). Estimate of incidence and cost of recreational waterborne illness on United States surface waters. Environmental Health, 17(1), 3.

Dorevitch, S., Shrestha, A., DeFlorio-Barker, S., Breitenbach, C., & Heimler, I. (2017). Monitoring urban beaches with qPCR vs. culture measures of fecal indicator bacteria: Implications for public notification. Environmental Health, 16(1), 45**.**

Dufour, Alfred P. "Health effects criteria for fresh recreational waters." (1984)

Edberg, S. C. L., Rice, E. W., Karlin, R. J., & Allen, M. J. (2000). Escherichia coli: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology, 88(S1), 106S-116S.

Frahm, E., & Obst, U. (2003). Application of the fluorogenic probe technique (TaqMan PCR) to the detection of Enterococcus spp. and Escherichia coli in water samples. *Journal of Microbiological Methods*, *52*(1), 123-131.

Haugland, R. A., Siefring, S. C., Wymer, L. J., Brenner, K. P., & Dufour, A. P. (2005). Comparison of Enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water research, 39(4), 559-568.

Haugland, R. A., Siefring, S., Lavender, J., & Varma, M. (2012). Influences of sample interference and interference controls on quantification of enterococci fecal indicator bacteria in surface water samples by the qPCR method. *Water research*, *46*(18), 5989-6001.

Haughland, Richard, et al. 18 Oct. 2016. National Exposure Research Laboratory, http://www.michigan.gov/documents/deq/deq-oea-tou-GLBA-qPCR-Haugland_538656_7.pdf.

Hlavsa MC, Roberts VA, Kahler AM, Hilborn ED, Mecher TR, Beach MJ, Wade TJ, Yoder, JS. (2015) Outbreaks of illness associated with recreational water — United States, 2011–2012. MMWR Morb Mortal Wkly Rep., 64(24):668-672

Holland, P. M., Abramson, R. D., Watson, R., & Gelfand, D. H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5'----3'exonuclease activity of Thermus aquaticus DNA polymerase. *Proceedings of the National Academy of Sciences*, *88*(16), 7276-7280.

Jentes, J. E., How to win back the beaches, Twine Line Newsl., 22(3), Ohio Sea Grant Coll. Program, Columbus, Ohio, 2000

Kinzelman, J. L., Singh, A., Ng, C., Pond, K. R., Bagley, R. C., & Gradus, S. (2005). Use of IDEXX Colilert-18® and Quanti-Tray/2000 as a rapid and simple enumeration method for the implementation of recreational water monitoring and notification programs. Lake and Reservoir Management, 21(1), 73-77.

Kinzelman, J., Bushon, R. N., Dorevitch, S., & Noble, R. T. (2011). *Comparative evaluation of molecular and culture methods for fecal indicator bacteria for use in inland recreational waters*. Water Environment Research Foundation.

Lavender, J. S., & Kinzelman, J. L. (2009). A cross comparison of QPCR to agar-based or defined substrate test methods for the determination of Escherichia coli and enterococci in municipal water quality monitoring programs. *Water research*, *43*(19), 4967-4979.

Liu, L., Phanikumar, M. S., Molloy, S. L., Whitman, R. L., Shively, D. A., Nevers, M. B., Rose, J. B. (2006). Modeling the Transport and Inactivation ofE. coliand Enterococci in the Near-Shore Region of Lake Michigan. Environmental Science & Technology, 40(16), 5022-5028. doi:10.1021/es060438k

Mallin, M. A., Williams, K. E., Esham, E. C., & Lowe, R. P. (2000). Effect of human development on bacteriological water quality in coastal watersheds. Ecological applications, 10(4), 1047-1056.

MDEQ, 2018. Michigan BeachGuard System. Michigan Department of Environmental Quality http://www.deq.state.mi.us/beach/BeachDetail.aspx?BeachID=385.

Murray, C., Sohngen, B., & Pendleton, L. (2001). Valuing water quality advisories and beach amenities in the Great Lakes. Water Resources Research, 37(10), 2583-2590.

Prüss, A. (1998). Review of epidemiological studies on health effects from exposure to recreational water. *International journal of epidemiology*, *27*(1), 1-9.

Seyfried, P. L., Tobin, R. S., Brown, N. E., & Ness, P. F. (1985). A prospective study of swimming-related illness. I. Swimming-associated health risk. American journal of public health, 75(9), 1068-1070

Siefring, S., Varma, M., Atikovic, E., Wymer, L., & Haugland, R. A. (2008). Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. *Journal of Water and Health*, *6*(2), 225-237.

Silva, D. M., & Domingues, L. (2015). On the track for an efficient detection of Escherichia coli in water: A review on PCR-based methods. Ecotoxicology and Environmental Safety, 113, 400-411.

Sivaganensan, M., Siefring, S., Varma, M., & Haugland, R. A. (2014). Comparison of Enterococcus quantitative polymerase chain reaction analysis results from Midwest US river samples using EPA Method 1611 and Method 1609 PCR reagents. Journal of microbiological methods, 101, 9-17.

Soller, J. A., Schoen, M. E., Bartrand, T., Ravenscroft, J. E., & Ashbolt, N. J. (2010). Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research*, *44*(16), 4674-4691.

U.S. EPA (1986). Ambient Water Quality for Bacteria-1986. Cincinnati, OH: Office of Water Regulations and Standards; 1986.

U.S. EPA. (2002) Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). US Environmental Protection Agency EPA-821-R-02-024. Washington DC: US Environmental Protection Agency, Office of Water.

U.S. EPA. (2006) Method 1603: Escherichia coli (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTEC); EPA-821-R-06-011; U.S. Environmental Protection Agency: Washington, DC 20460.

U.S. EPA. (2014a) Draft Method C: Escherichia coli in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR). Washington, D.C. United States Environmental Protection Agency. Office of Water.

U.S. EPA. (2014b). Overview of Technical Support Materials: A Guide to the Site-Specific Alternative Recreational Criteria TSM Documents; EPA-820-R-14-010, U.S. EPA Office of Water, Office of Science and Technology, Health and Ecological Criteria Division: Washington, D.C. https://www.epa.gov/sites/production/files/2015-11/documents/sitespecific-alternative-recreational-indicators-methods.pdf

Varma, M., Field, R., Stinson, M., Rukovets, B., Wymer, L., & Haugland, R. (2009). Quantitative real-time PCR analysis of total and propidium monoazide-resistant fecal indicator bacteria in wastewater. *Water Research*, *43*(19), 4790-4801.

Wade, T. J., Pai, N., Eisenberg, J. N., & Colford Jr, J. M. (2003). Do US Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspectives*, *111*(8), 1102.

Wade, T. J., Calderon, R. L., Brenner, K. P., Sams, E., Beach, M., Haugland, R., ... & Dufour, A. P. (2008). High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. Epidemiology, 19(3), 375-383.

Whitman, R. L., Nevers, M. B., & Gerovac, P. J. (1999). Interaction of ambient conditions and fecal coliform bacteria in Southern Lake Michigan beach waters: Monitoring program implications. Natural Areas Journal, 19(2), 166-171.

Whitman, R. L., Ge, Z., Nevers, M. B., Boehm, A. B., Chern, E. C., Haugland, R. A., ... & White, E. M. (2010). Relationship and variation of qPCR and culturable enterococci estimates in ambient surface waters are predictable. Environmental science & technology, 44(13), 5049-5054**.**

Willmott, C. J., Robeson, S. M., & Matsuura, K. (2012). A refined index of model performance. International Journal of Climatology, 32(13), 2088-2094.