

E. coli qPCR as an alternative method for Great Lakes beach monitoring

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Presentation Outline

- U.S. EPA Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods
- U.S. EPA 2015 Great Lakes Beaches study
- U.S. EPA draft Method C: *E. coli* by qPCR
- Comparison of *E. coli* qPCR densities with *E. coli* culture count densities
- Comparison of *E. coli* qPCR densities with enterococci qPCR densities
- Summary and preliminary conclusions



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

- EPA-820-R-14-011, available at:
<https://www.epa.gov/sites/production/files/2015-11/documents/sitespecific-alternative-recreational-indicators-methods.pdf>
- Objective of document: provide support materials for developing site-specific alternative water quality criteria for new fecal indicator methods that EPA has not validated and issued
- Metrics evaluated:
 - Index of Agreement (IA)
 - Pearson's correlation coefficient squared (R-squared)

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

- Index of Agreement (IA):

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

- Where x represents density of the microorganism determined by an approved EPA method (EPA Method 1600, 1603 or 1611 or equivalent), and y represents the density of the microorganism determined by the alternative method. Also in the equation, i is a counter, N (sample size) is the total number of data points in the data set, and are the averages of the x and y data sets, respectively, and IA varies from 0 to 1 (Willmott and Wicks, 1980) with 1 being perfect agreement. Guidance for doing calculations is provided in the document.



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

- IA assesses both additive and proportional differences between the alternative and EPA's indicator/methods.
- Data sets with an $IA \geq 0.7$ have acceptable agreement.
- If IA is ≥ 0.7 , you can use the alternative indicator/method together with the numerical criteria values for the corresponding EPA indicator/method published in EPA's 2012 Recreational Water Quality Criteria.

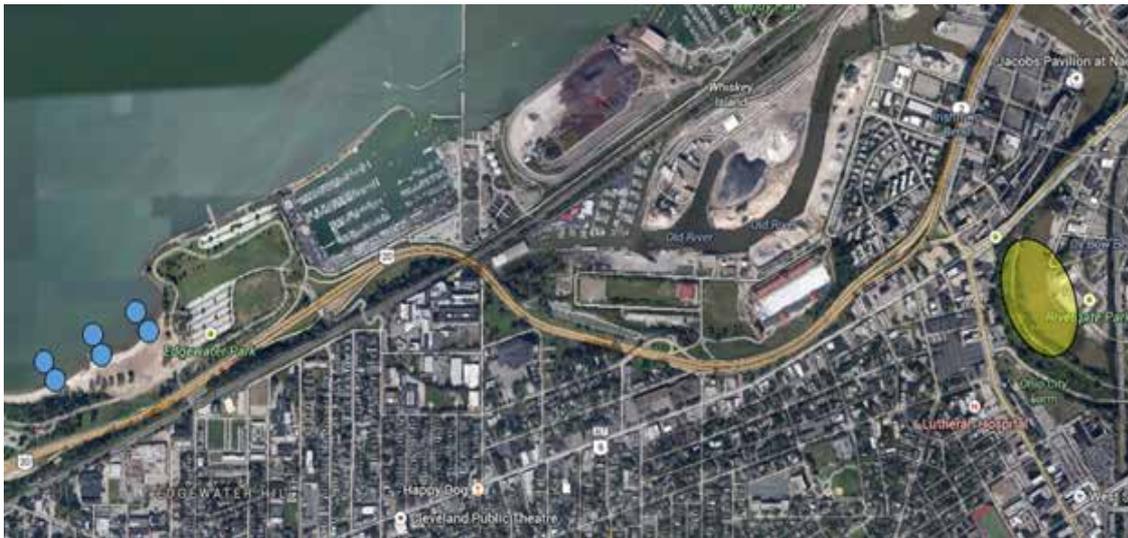


EPA Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods

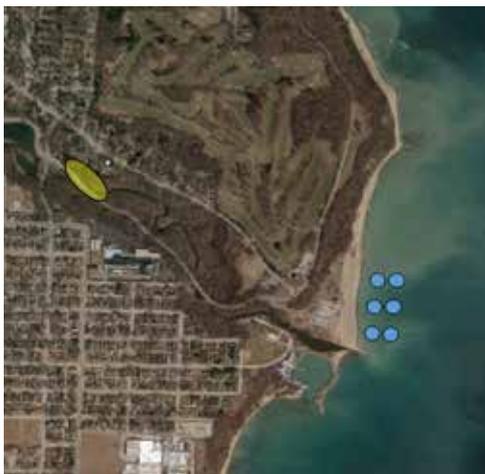
- Pearson's correlation coefficient squared (R-squared):
 - Calculate and plot data using any standard data analysis software (Excel, R). Guidance for calculations is also provided.
- Calculate the R-squared value, if the IA threshold is not passed.
 - R-squared measures proportional differences between two data sets, but not additive differences.
 - Data sets with R-squared ≥ 0.6 have acceptable agreement.
- Acceptable agreement suggests a new numerical limit can be calculated, but the value will likely differ from the existing criteria.
 - Guidance for establishing new numerical limits is provided in the document.

EPA 2015 Great Lakes Beaches study

- Three urban beaches likely POTW impacted.
- Blue circles denote approximate beach water sampling locations (composited from the six locations).
- Yellow ellipses indicate general locations for sampling stations on nearby rivers.
- Sample were also collected from historic beach sampling sites (not shown).



Edgewater Beach, Cleveland, OH



Grant Park Beach,
S. Milwaukee, WI



Washington Park Beach,
Michigan City, IN

EPA 2015 Great Lakes Beaches study

- Study objectives included:
 - Determination of occurrence and densities of Coliphage fecal indicators using standard and improved methods
 - Evaluation of predictive water quality models
 - Development and evaluation of fate and transport models
 - Epidemiological analyses (Washington Park only)
 - Evaluation of E. coli qPCR methods vs. accepted culture and enterococci qPCR methods using Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods (this presentation)

ORIGINAL ARTICLE

Comparison of quantitative PCR assays for *Escherichia coli* targeting ribosomal RNA and single copy genes

E.C. Chern¹, S. Siefring¹, J. Paar², M. Doolittle³ and R.A. Haugland¹

Letters in Applied Microbiology 52, 298–306 © 2011 The Society for Applied Microbiology

- Method C assay targets 23S ribosomal RNA gene (same as Methods 1611 and 1609 for enterococci)
- Method C uses Environmental Master Mix (same as Method 1609 for enterococci)
- Method C uses same delta-delta Ct quantitative model as in updated Methods 1611.1 and 1609.1 for enterococci to calculate target gene copy densities
- Method C cell or genome equivalents (GEQ) are based on seven 23S gene copies/*E. coli* genome for comparisons with culture (15 copies/per cell equivalent is used in enterococci Methods 1611.1 and 1609.1 – value based on 2003-2007 NEEAR epi studies)

Comparison of E. coli qPCR GEQ densities with E. coli culture (Colilert®) MPN densities

	N>LOD/N	Mean Ecoli log10 MPN	Mean Ecoli log10 GEQ	*Index of Agreement	*R-squared
Edgewater Beach Composite	66/67	2.24	2.92	0.58	0.38
Edgewater Beach Historic	66/67	1.88	2.67	0.51	0.27
Edgewater Beach All Samples	132/134	2.06	2.80	0.56	0.37
Edgewater, River	67/67	2.58	3.17	0.84	NA
Grant Park Beach Composite	68/68	1.73	2.69	0.61	0.60
Grant Park Beach Historic	67/68	1.68	2.70	0.55	0.46
Grant Park Beach All Samples	135/136	1.70	2.69	0.58	0.53
Grant Park, River	70/70	2.96	3.50	0.69	0.42
Washington Park Beach Composite	64/68	1.81	2.46	0.63	0.66
Washington Park Beach Historic	63/68	1.86	2.51	0.66	0.69
Washington Park Beach All Samples	127/136	1.83	2.55	0.62	0.66
Washington Park, River	63/68	2.99	3.35	0.83	NA



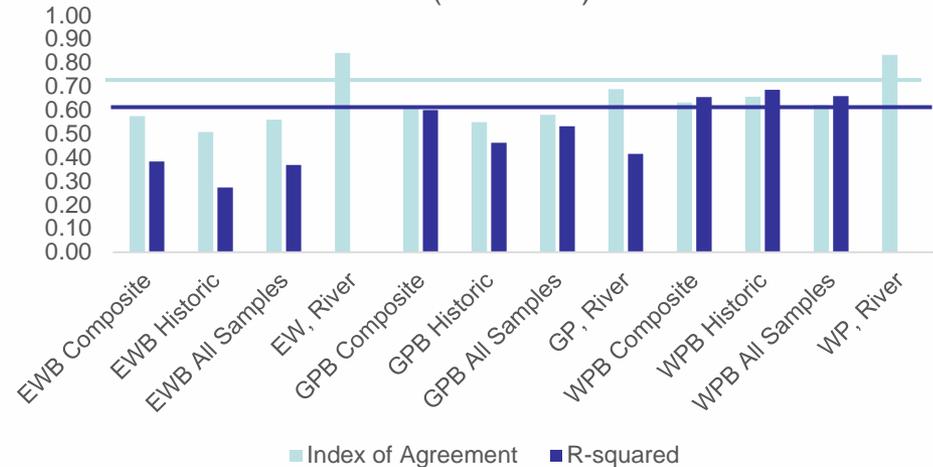
Comparison of E. coli qPCR gene copy densities with enterococci qPCR gene copy densities

	N>LOD/N	Mean Ent log10 copies	Mean Ecoli log10 copies	Index of Agreement	R-squared
Edgewater Beach Composite	67/67	3.50	3.76	0.76	NA
Edgewater Beach Historic	64/67	3.12	3.53	0.73	NA
Edgewater Beach All Samples	131/134	3.31	3.63	0.75	NA
Edgewater, River	70/70	4.03	4.07	0.96	NA
Grant Park Beach Composite	68/68	3.49	3.52	0.81	NA
Grant Park Beach Historic	67/67	3.30	3.54	0.79	NA
Grant Park Beach All Samples	135/135	3.40	3.54	0.80	NA
Grant Park, River	75/75	4.59	4.41	0.90	NA
Washington Park Beach Composite	132/132	3.11	3.44	0.72	NA
Washington Park Beach Historic	129/131	3.11	3.56	0.68	0.44
Washington Park Beach All Samples	261/263	3.11	3.50	0.70	NA
Washington Park, River	132/132	4.62	4.18	0.72	NA

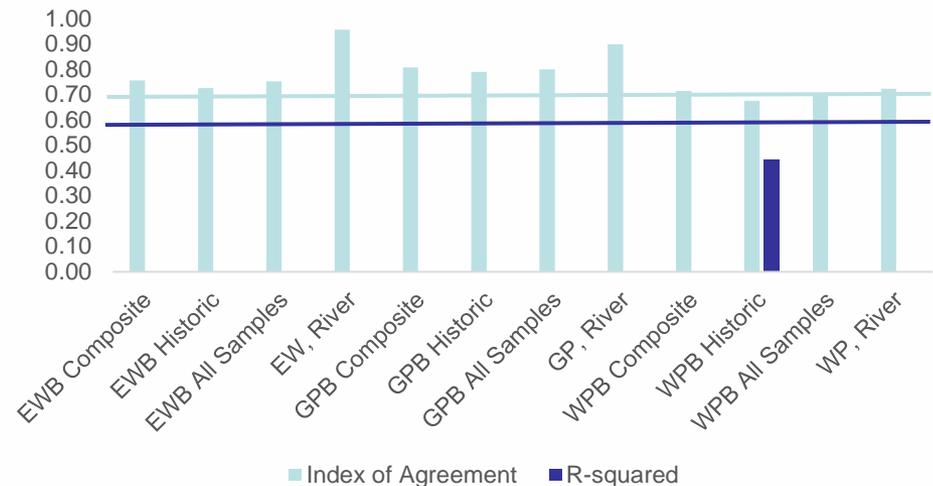
Summary and preliminary conclusions

- The majority of sites failed to meet the Index of Agreement threshold of ≥ 0.7 in comparisons between E.coli culture counts and Method C qPCR GEQ (generally due to lower culturable densities)
- WP sites and the GP composites passed the R-squared threshold of ≥ 0.6 which should make them eligible for developing new water quality criteria values for qPCR
- All sites (except WP historic) met the Index of Agreement threshold in comparisons between Ent (Method 1609) and E.coli (Method C) qPCR.
- These sites should be eligible for applying the established Ent qPCR water quality criteria to Method C

E.coli log10 MPN (Colilert®) vs E.coli log10 GEQ (Method C)



Ent log10 copies (Method 1609) vs E.coli log10 copies (Method C)



Contact Information

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