Your Sewer District . . . **Keeping our Great Lake great.**

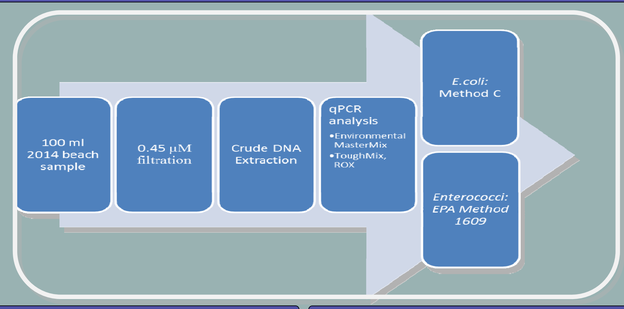
Make It Tough: A qPCR MasterMix Comparative Study

Introduction

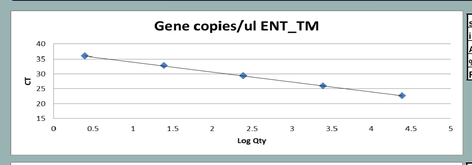
The water quality of bathing beaches in Northeast Ohio is determined by the microbial concentration typically, the concentration of *E. coli* and/or *Enterococci.* The standard methods used to analyze for FIBs (fecal indicator bacteria) take 24 hours to obtain results. The use of rapid methods allows for real time results within 3-4 hours. Since 2007, Analytical Services has been testing various real-time quantitative polymerase chain-reaction (qPCR) chemistries to discover an assay which will closely correlate to the standard culture based methods, reduce environmental inhibitory effects, and ensure accuracy for predictive model impact.

The use of qPCR methodology has become a critical component of the beach monitoring program at the Northeast Ohio Regional Sewer District (NEORSD). The results from the qPCR analysis have been used as a stand-alone predictor as well as a variable in predictive modeling, to determine the water quality of bathing beaches for public notification. Since timely public notification is of paramount importance, qPCR provides faster results so the public can make informed decisions about whether to refrain from recreational beach use based on data from that day and not the previous day results obtain from standard culture based methods.

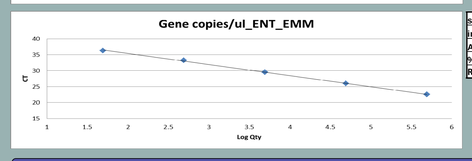
Method/Process



Enterococci calibration curves

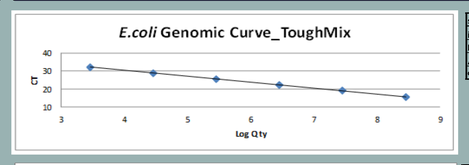


slope:	3.3388
intercept:	37.303
AF:	1.995088056
% Efficiency:	99.30%
R2:	R2 =0.9998

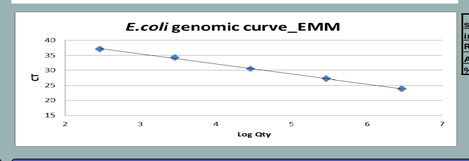


slope:	3.4708
intercept:	42.34
AF:	1.8811801
% Efficiency:	94.14%
R2:	0.9988

E.coli calibration curves



slope	3.291
intercept	43.475
R2	0.9996
AF	2.03859
% Efficiency	101.31%



slope	3.3676
intercept	45.619
R2	0.9985
AF	1.969474
% Efficiency =	98.13%

Enterococci Quality Control Data

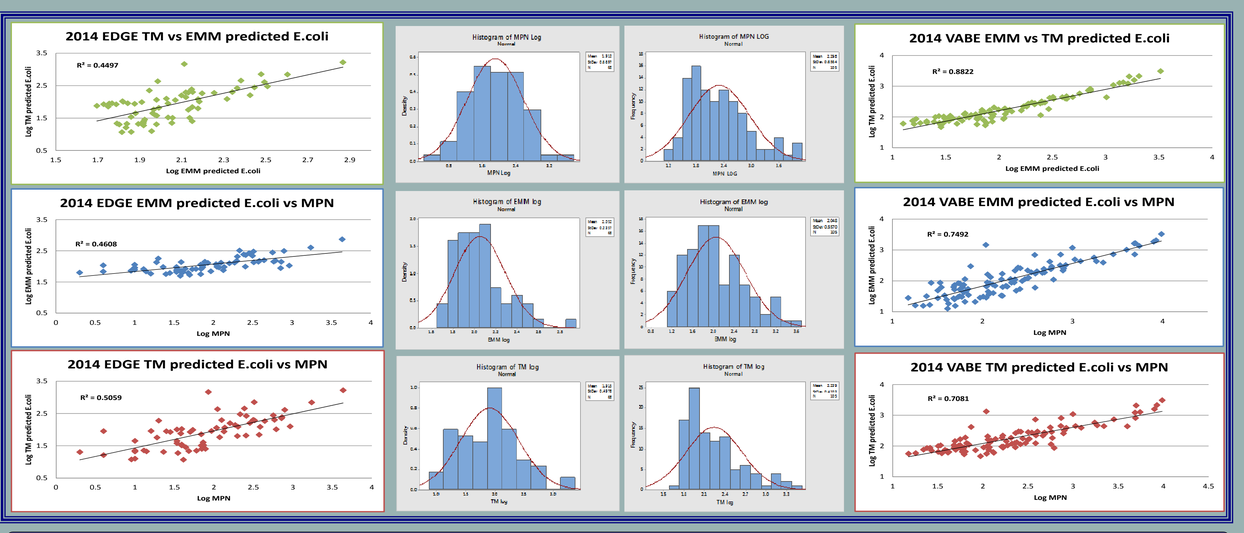
Beach	Assay	# sketa inhibited	# IAC inhibited	# (+) PBS BLK	# (+) NTC
EDGE	ToughMix	19/121	0/121	1/7	0/7
EDGE	EMM	22/121	0/121	3/6	0/6
VABE	ToughMix	5/120	1/120	1/7	0/7
VABE	EMM	6/120	0/120	2/6	1/6

E.coli Quality Control Data

Beach	Assay	# sketa inhibited	# IAC inhibited	# (+) PBS BLK	# (+) NTC
EDGE	ToughMix	2/120	0/120	2/6	2/6
EDGE	EMM	10/120	1/120	4/6	4/6
VABE	ToughMix	0/120	0/120	1/6	0/6
VABE	EMM	0/120	0/120	5/6	2/6

E.coli Method Comparison

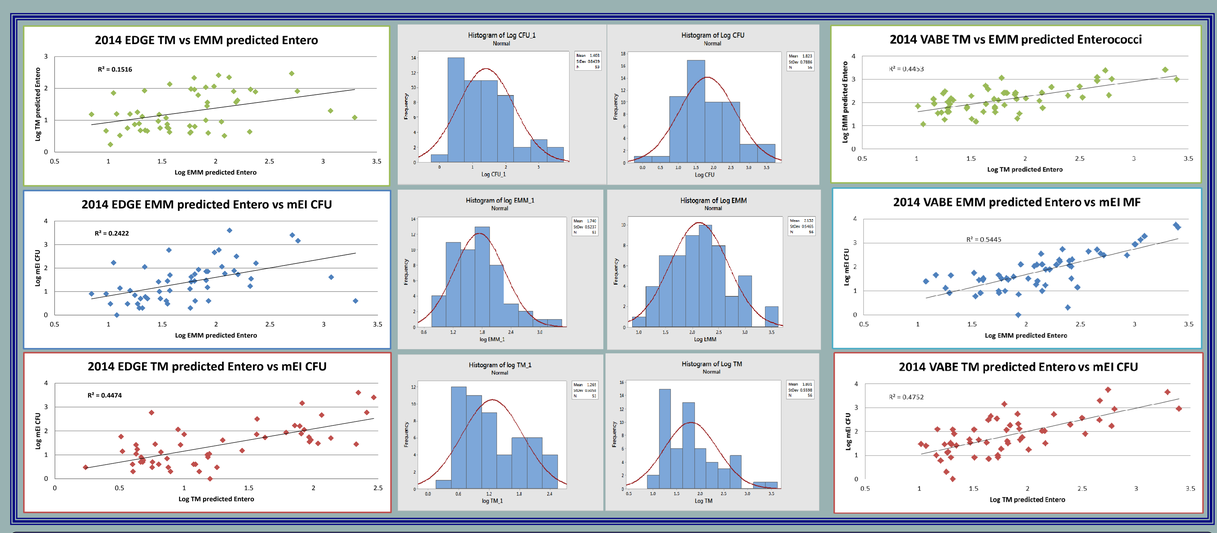
Edgewater and Villa Angela Beaches



E. Coli Results

Enterococci Method Comparison

Edgewater and Villa Angela Beaches



Enterococci Results

Villa Angela Beach *E. Coli* Environmental MasterMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	120	Accuracy / Sensitivity	88% / 71%	Specificity 96%	Total Samples	120	Accuracy / Sensitivity	70% / 32%	Specificity 79%
# Greater 235	42	75	3	30	12	# >3357	22	77	1	7	35
# Less than 235	78	CNE	False +	CE	False -	#<3357	98	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	120	Accuracy / Sensitivity	85% / 70%	Specificity 95%	Total Samples	120	Accuracy / Sensitivity	69% / 92%	Specificity 67%
# Greater 190	47	69	4	33	14	# >2026	12	72	1	11	36
# Less than 190	73	CNE	False +	CE	False -	#<2026	108	CNE	False +	CE	False -

Villa Angela Beach *E. Coli* ToughMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total # Samples	120	Accuracy / Sensitivity	86% / 64%	Specificity 97%	Total # Samples	120	Accuracy / Sensitivity	72% / 90%	Specificity 70%
#>235	42	76	2	27	15	#>3357	10	77	1	9	33
#<235	78	CNE	False +	CE	False -	#<3357	110	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	120	Accuracy / Sensitivity	85% / 66%	Specificity 97%	Total Samples	120	Accuracy / Sensitivity	75% / 93%	Specificity 73%
# Greater 190	47	71	2	31	16	#>2026	14	77	1	13	34
# Less than 190	73	CNE	False +	CE	False -	#<2026	106	CNE	False +	CE	False -

Edgewater Beach *E. Coli* Environmental MasterMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	121	Accuracy / Sensitivity	89% / 37%	Specificity 99%	Total Samples	121	Accuracy / Sensitivity	84% / 50%	Specificity 85%
# Greater 235	19	101	1	7	12	#>3357	2	101	1	1	18
# Less than 235	102	CNE	False +	CE	False -	#<3357	119	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	121	Accuracy / Sensitivity	89% / 48%	Specificity 100%	Total Samples	121	Accuracy / Sensitivity	85% / 67%	Specificity 86%
# Greater 190	23	97	0	11	13	#>2026	3	101	1	2	22
# Less than 190	97	CNE	False +	CE	False -	#<2026	118	CNE	False +	CE	False -

Edgewater Beach *E. Coli* ToughMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	121	Accuracy / Sensitivity	86% / 42%	Specificity 94%	Total Samples	121	Accuracy / Sensitivity	83% / 33%	Specificity 85%
# Greater 235	19	96	6	8	11	# >3357	3	100	2	1	18
# Less than 235	102	CNE	False +	CE	False -	#<3357	118	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total Samples	121	Accuracy / Sensitivity	91% / 57%	Specificity 100%	Total Samples	121	Accuracy / Sensitivity	80% / 50%	Specificity 81%
# Greater 190	23	97	6	13	11	#>2026	4	95	2	2	22
# Less than 190	97	CNE	False +	CE	False -	#<2026	117	CNE	False +	CE	False -

Conclusion and Summary

This comparison was done to identify a qPCR assay that correlated with the culture based reference methods, reduced inhibition in the sample matrices, had minimal residual *E. coli* contaminates in the mix, and could be used for multiple fecal indicating bacteria. The assays tested were the Environmental MasterMix and ToughMix, ROX.

The results of the comparison study indicated that ToughMix had a reduction the number of controls with elevated Ct value, as well as a reduction in the number of samples showing inhibition.

Both assays had comparable results when statistically analyzed against samples from two different beaches, and two separate organisms. The results for the ToughMix were highly correlated with and not significantly different from the culture based methods.

Based on the results of this study NEORSD has decided to utilize the ToughMix ROX assay for qPCR analysis of both *Enterococci* and *E.coli* for samples collected at recreational beaches monitored by the NEORSD.

***E.coli* Assay Comparison**

Beach	comparison	Pearson Correlation	R^2	P-value
Edgewater	EMM vs TM	0.6705809	0.4497	0.0042
Edgewater	EMM vs MPN	0.6788587	0.4608	0.0360
Edgewater	TM vs MPN	0.7112917	0.5059	0.9663
Villa Angela	EMM vs TM	0.939279	0.8822	2.919E-14
Villa Angela	EMM vs MPN	0.865581	0.7492	5.650E-12
Villa Angela	TM vs MPN	0.841500	0.7081	0.1193

*EMM = Environmental MasterMix, TM = ToughMix, MPN = Most probable Number

***Enterococci* Assay Comparison**

Beach	Comparison	Pearson Correlation	R^2	P-value
Edgewater	EMM vs TM	0.3894003	0.1516	0.000001
Edgewater	EMM vs CFU	0.4921501	0.2422	0.0017
Edgewater	TM vs CFU	0.6688534	0.4474	0.1181
Villa Angela	EMM vs TM	0.6672808	0.4453	0.000001
Villa Angela	EMM vs CFU	0.7378979	0.5445	0.000062
Villa Angela	TM vs CFU	0.6893558	0.4752	0.777620

*EMM = Environmental MasterMix, TM = ToughMix, MPN = Most probable Num

Villa Angela Beach Enterococci Environmental MasterMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total # Samples	58	Accuracy / Sensitivity	81% / 100%	Specificity	Total # Samples	58	Accuracy / Sensitivity	71% / 50%	Specificity 31%
#>70	27	20	11	27	0	#>1000	20	31	0	10	17
#<70	31	CNE	False +	CE	False -	#<1000	##	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Total # Samples	58	Accuracy / Sensitivity	76% / 100%	Specificity 55%	Total # Samples	58	Accuracy / Sensitivity	78% / 100%	Specificity 70%
#>60	27	17	14	27	0	#>640	14	31	0	14	13
#<60	31	CNE	False +	CE	False -	#<640	44	CNE	False +	CE	False -

Villa Angela Beach *Enterococci* ToughMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total # Samples	58	Accuracy / Sensitivity	74% / 59%	Specificity 87%	Total # Samples	58	Accuracy / Sensitivity	69% / 62%	Specificity 81%
#>70	27	27	4	16	11	#>1000	37	17	14	23	4
#<70	31	CNE	False +	CE	False -	#<1000	21	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Total # Samples	58	Accuracy / Sensitivity	76% / 70%	Specificity 81%	Total # Samples	58	Accuracy / Sensitivity	64% / 65%	Specificity 62%
#>60	27	25	6	19	8	#>640	37	13	18	24	3
#<60	31	CNE	False +	CE	False -	#<640	21	CNE	False +	CE	False -

Edgewater Beach Enterococci Environmental MasterMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total # Samples	59	Accuracy / Sensitivity	78% / 73%	Specificity 80%	Total # Samples	59	Acc / Sensitivity	64% / 38%	Specificity 83%
# >70	15	35	9	11	4	# >1000	24	29	15	9	6
#<70	44	CNE	False +	CE	False -	#<1000	35	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total # Samples	59	Accuracy / Sensitivity	75% / 80%	Specificity 73%	Total # Samples	59	Acc / Sensitivity	63% / 37%	Specificity 84%
# >60	15	32	12	12	3	# >640	27	27	17	10	5
#<60	44	CNE	False +	CE	False -	#<640	32	CNE	False +	CE	False -

Edgewater Beach EnterococciToughMix Assay

Estimated Illness Rate (NGI): 36 per 1000 primary contact recreators: BAV

Standard Curve					Predictive Model (CCE)				
Total # Samples	59	Accuracy / Sensitivity	80% / 60%	Specificity 86%	Total # Samples	58	Accuracy / Sensitivity	81% / 60%	Specificity 95%
#>70	15	38	6	9	6	# >1000	20	36	8	12	3
#<70	44	CNE	False +	CE	False -	#<1000	38	CNE	False +	CE	False -

Estimated Illness Rate (NGI): 32 per 1000 primary contact recreators: BAV

Total # Samples	59	Accuracy / Sensitivity	81% / 67%	Specificity 86%	Total # Samples	58	Accuracy / Sensitivity	80% / 65%	Specificity 89%
#>60	15	38	6	10	5	# >640	20	34	10	13	2
#<60	44	CNE	False +	CE	False -	#<640	38	CNE	False +	CE	False -

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