qPCR Monitoring Data and the Proposed Criteria Revisions

One Local Lab's Journey Towards Implementation

Great Lakes Beach Association Conference
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Proposed US Standards – Freshwater

- **Health effect data correlated to culture (*E. coli* and enterococci) and qPCR (enterococci)**
- **Culture**
  - *E. coli*
    - GM of 126 cfu per 100 mL (30 – 90 days)
    - STV of 235 cfu per 100 mL measured using EPA Method 1603, or any other equivalent method that measures culturable *E. coli* (i.e. IDEXX or similar)
  - Enterococci
    - GM of 33 cfu per 100 mL (30 – 90 days)
    - STV of 61 cfu per 100 mL measured using EPA Method 1600
- **qPCR**
  - Enterococci using EPA Method A
    - GM of 475 CCE per 100 mL
    - STV criterion of 1,000 CCE per 100 mL

Alternative Method/Indicator Combinations

- ***5.2.3 Alternative Criteria Based on Novel Indicators or New Analytical Methods, without Site-Specific Epidemiological Studies***
  - *E. coli* by qPCR, for example
  - Approval if scientifically defensible and protective of the recreational use
  - Consistent and predictable site-specific performance
  - Describes a common level of water quality
  - Consistent over a recreational season for both EPA-approved and proposed alternative indicator-method combination.

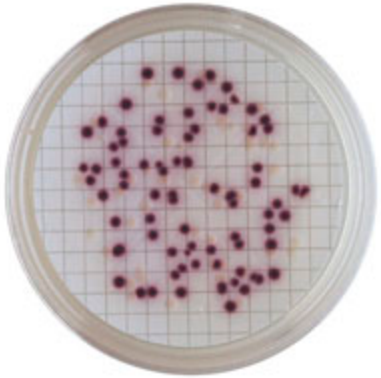
Way back in 2005 Racine was thinking about rapid methods…

qPCR was under consideration for environmental use and also making it's appearance in the clinical arena

WE HAD QUESTIONS…

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

- QPCR differs from traditional culture-based assays in that it measures all DNA:
  - live cells
  - dead cells
  - non-culturable cells
  - free DNA



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

Correlation to Current Methods

- Do QPCR CCE correlate to currently approved methods?
  - Beach status (open/advisory/closed)
  - Actual counts
  - Is there diurnal variation and would it preclude early morning sampling?
  - Could we still composite sample?

Composite Sampling

- Physical combining of multiple sub-samples into a single sample
  - Is it valid to composite?
  - Does the composite value fall within the range if individual values?
  - Would a composite sample mask a true elevation?
- Increases spatial coverage
- Maintains analytical costs
  - May be particularly useful when considering qPCR

qPCR, 2005 – 2006

- Acquired Cepheid Smart Cycler using county CRI and PHEP grant funds
  - Increase public health capacity for molecular testing
  - Ability to accept routine testing as overflow site
- Trialed US EPA Taqman *Entercoccus* assay (pre Method A) vs. Method 1600
  - Composite sample analysis
    - North Beach
    - 4 transects
    - 12, 24, 36, and 48 inch depth zones
- Initial EPA validation study participant (2006)

2005, QPCR - Mean vs. Composite

Depth Zone	QPCR Mean	QPCR Composite	Method 1600 Mean	Method 1600 Composite
12”	121.48	156.94	35.6	56
24”	64.42	74.54	12.3	3
36”	14.17	5	6.2	10
48”	23.25	32.36	5.5	19

QPCR

all NB comp:	Comp= 5	Mean= 67

CULTURE

all NB comp:	Comp = 37	Mean = 22

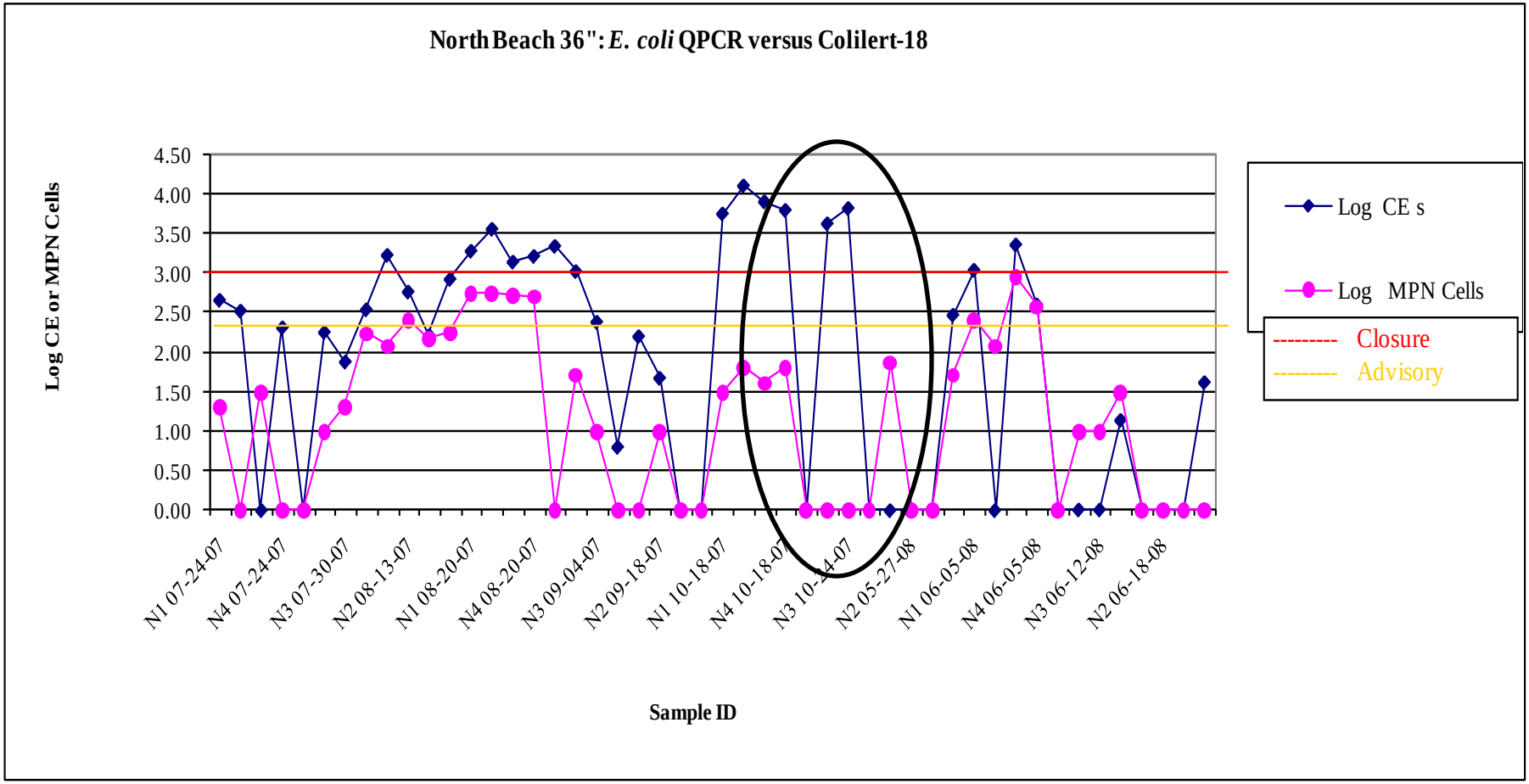
Initial Results Looked Promising

We Decided to Do Some Comparative Testing in Conjunction with Routine Regulatory Monitoring

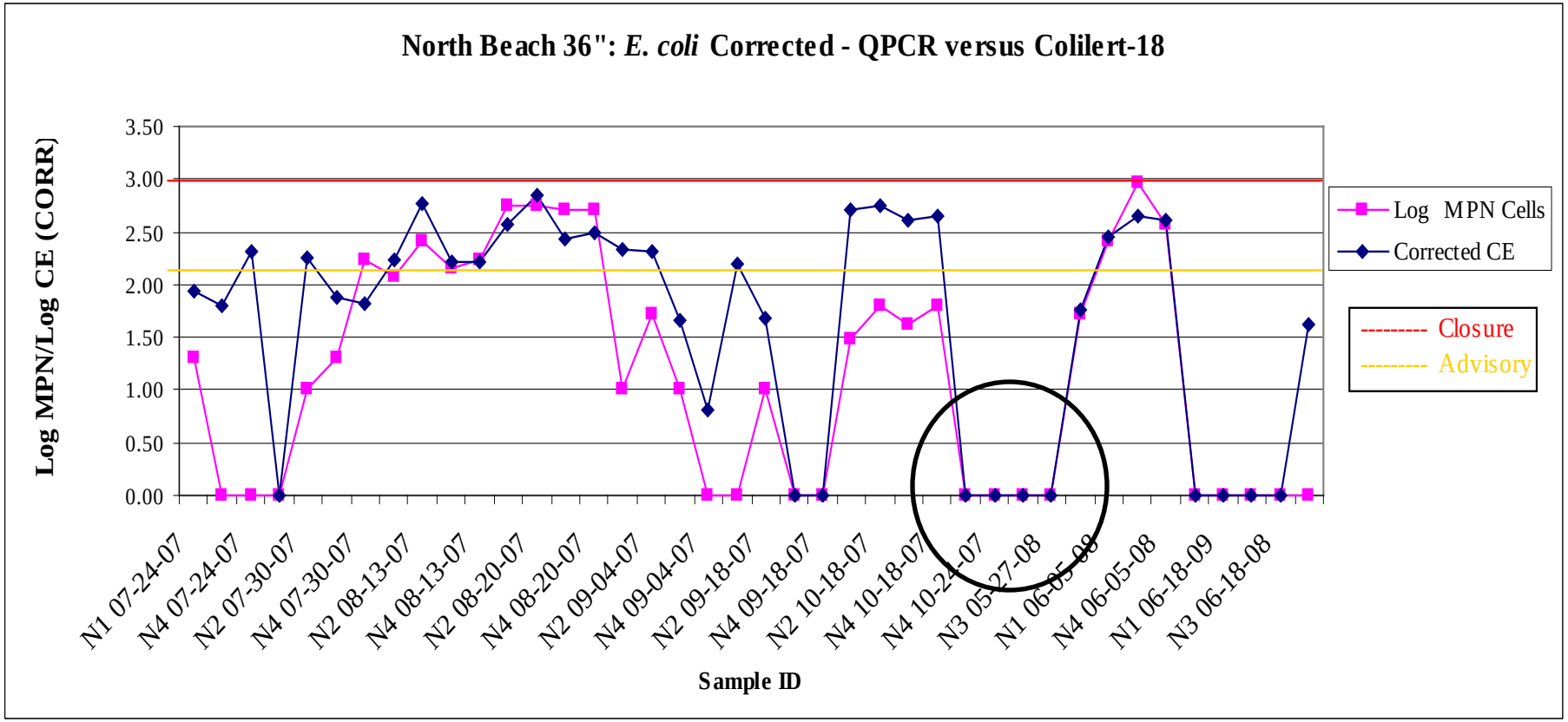
Ambient or Environmental DNA

- Does ambient background DNA exist?
- Is it in sufficient quantity to interfere with regulatory results?
- Can we determine when these conditions are likely to occur?
- Are they associated with environmental conditions?
  - Precipitation, wave height, turbidity
- Can they be corrected for?

Uncorrected for Background DNA



Corrected for Background DNA



Correlation to "Beach Status" – *E. coli* (2007 – 2008)

Sample ID	Log CE s	Log MPN Cells	Corrected CE	Sample ID	Log CE s	Log MPN Cells	Corrected CE
N1 07-24-07	2.66	1.30	1.94	N1 10-18-07	3.44	1.49	2.72
N2 07-24-07	2.53	0.00	1.81	N2 10-18-07	3.47	1.80	2.75
N3 07-24-07	0.00	1.49		N3 10-18-07	3.33	1.61	2.61
N4 07-24-07	2.32	0.00	2.32	N4 10-18-07	3.37	1.80	2.65
N1 07-30-07	0.00	0.00	0.00	N1 10-24-07	0.00	0.00	0.00
N2 07-30-07	2.26	1.00	2.26	N2 10-24-07	2.70	0.00	1.98
N3 07-30-07	1.88	1.30	1.88	N3 10-24-07	2.86	0.00	2.14
N4 07-30-07	2.54	2.24	1.82	N4 10-24-07	0.00	0.00	0.00
N1 08-13-07	3.05	2.08	2.23	N1 05-27-08	0.00	1.87	0.00
N2 08-13-07	2.77	2.41	2.77	N2 05-27-08	0.00	0.00	0.00
N3 08-13-07	2.22	2.16	2.22	N3 05-27-08	0.00	0.00	0.00
N4 08-13-07	2.93	2.24	2.21	N4 05-27-08	2.48	1.72	1.76
N1 08-20-07	3.29	2.75	2.57	N1 06-05-08	3.18	2.41	2.46
N2 08-20-07	3.56	2.75	2.84	N2 06-05-08	0.00	2.08	-0.72
N3 08-20-07	3.15	2.72	2.43	N3 06-05-08	3.37	2.96	2.65
N4 08-20-07	3.22	2.70	2.50	N4 06-05-08	2.61	2.58	2.61
N1 09-04-07	3.05	1.00	2.33	N1 06-12-08	0.01	0.00	0.01
N2 09-04-07	3.03	1.72	2.31	N2 06-12-08	0.01	1.00	0.01
N3 09-04-07	2.39	1.00	1.67	N3 06-12-08	0.01	1.00	0.01
N4 09-04-07	0.80	0.00	0.80	N4 06-12-08	1.14	1.49	1.14
N1 09-18-07	2.20	0.00	2.20	N1 06-18-09	0.00	0.00	0.00
N2 09-18-07	1.68	1.00	1.68	N2 06-18-08	0.00	0.00	0.00
N3 09-18-07	0.00	0.00	0.00	N3 06-18-08	0.00	0.00	0.00
N4 09-18-07	0.00	0.00	0.00	N4 06-18-08	1.62	0.00	1.62

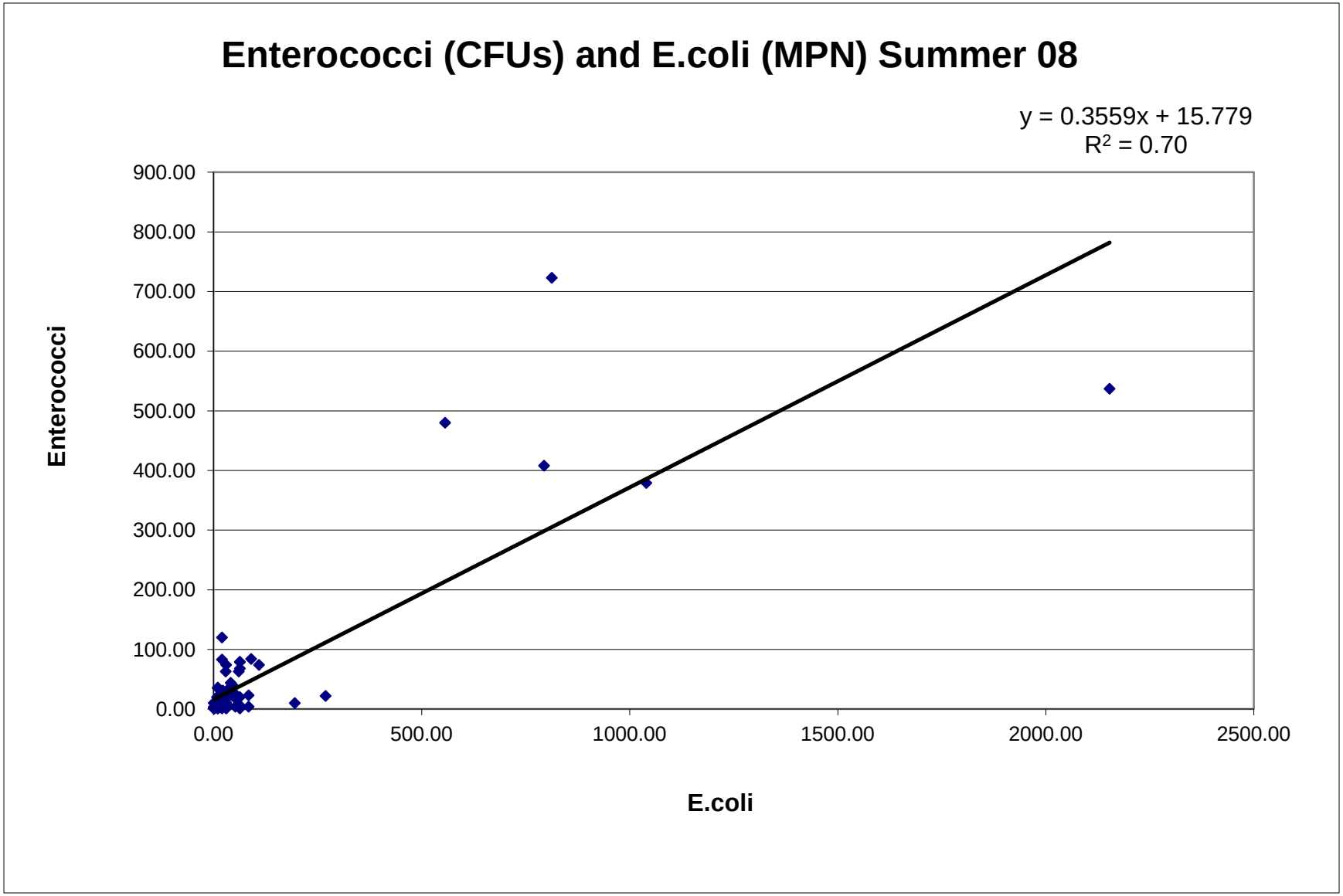
Numerical/Regulatory Agreement – *E. coli* (2008)

2008 *E. coli*, QPCR vs. Colilert-18 - Composite Samples, 1.0 m								
Date	QPCR Prediction (Status, CE)				Colilert-18 (Status, MPN)			
	North		Zoo		North		Zoo	
6/30/2008	open	**10-30**	open	**<10**	open	**41**	open	**10**
7/1/2008	open	**20-50**	open	10-30	open	**10**	open	10
7/2/2008	open	<10-10	open	**<10-10**	open	<10	open	**31**
7/3/2008	open	**50-75**	**open**	**<10-20**	open	**213**	**advisory**	**419**
7/7/2008	open	<10-20	open	<10-35	open	20	open	20
7/8/2008	open	<10-20	open	20-50	open	10	open	41
7/9/2008	open	<10-30	open	<10-20	open	<10	open	<10
7/10/2008	open	**<10-20**	open	<10-20	open	**63**	open	<10
7/11/2008	open	10-50	open	**<10-30**	open	20	open	**41**
7/14/2008	open	<10-20	open	<10-30	open	<10	open	<10
7/15/2008	open	10-30	open	<10-30	open	10	open	10
7/16/2008	open	<10-30	open	<10-20	open	<10	open	<10
7/17/2008	open	<10-30	open	<10-10	open	20	open	<10
7/18/2008	open	<10-20	open	<10-20	open	<10	open	<10
7/21/2008	open	10-40	open	<10-40	open	30	open	30
7/22/2008	open	<10-20	open	<10-20	open	10	open	10
7/23/2008	open	<10-20	open	<10-20	open	<10	open	10
7/24/2008	open	<10-30	open	<10-20	open	<10	open	<10
7/25/2008	open	<10-20	open	<10-20	open	20	open	<10
7/28/2008	open	10-20	open	<10-20	open	10	open	<10
7/29/2008	open	<10-20	open	<10-20	open	<10	open	<10
7/30/2008	open	<10-30	open	<10-30	open	31	open	<10
7/31/2008	open	10-30	open	<10-30	open	20	open	<10

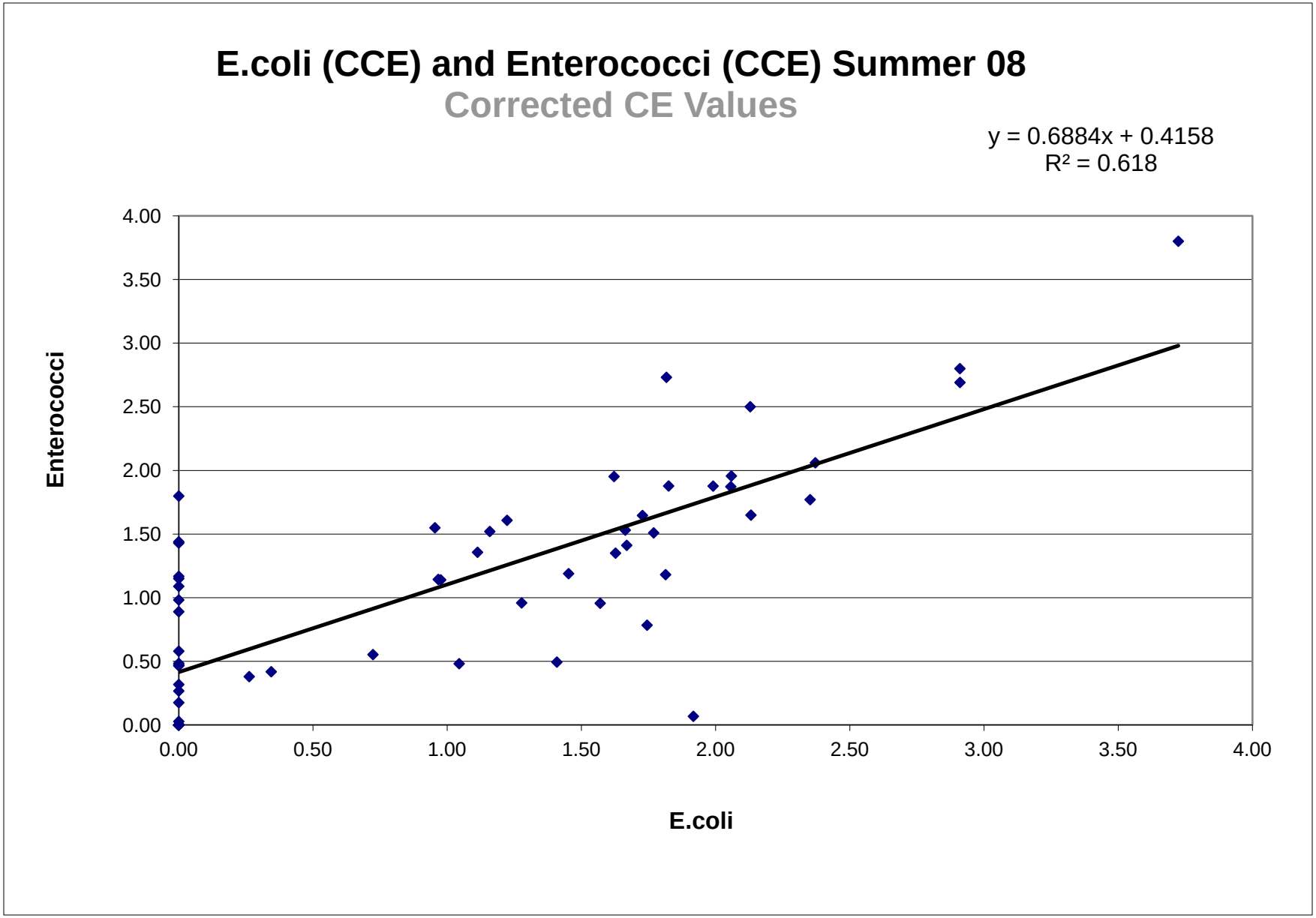
Numerical/Regulatory Agreement – *E. coli* (2008)

2008 *E. coli*, QPCR vs. Colilert-18 - Composite Samples, 1.0 m								
	QPCR Prediction (Status, CE)				Colilert-18 (Status, MPN)			
Date	North		Zoo		North		Zoo	
8/1/2008	open	10-50	open	<10-20	open	20	open	<10
8/4/2008	open	100-250	advisory	813	open	195	advisory	794
8/5/2008	open	10-30	open	30-70	open	20	open	63
8/6/2008	open	50-100	advisory	250-350	open	63	advisory	269
8/7/2008	open	30-60	open	**<10-40**	open	52	open	**52**
8/8/2008	open	30-60	open	<10-20	open	63	open	20
8/11/2008	open	10-30	open	10-30	open	31	open	10
8/12/2008	open	20-50	open	<10-20	open	30	open	10
8/13/2008	open	30-70	open	23-30	open	63	open	31
8/14/2008	open	35-80	open	<10-20	open	84	open	10
8/15/2008	open	10-30	open	20-40	open	10	open	20
8/18/2008	open	20-50	open	<10-20	open	30	open	<10
8/19/2008	open	70-120	open	40-70	open	109	open	63
8/20/2008	open	10-30	open	40-70	open	20	open	63
8/21/2008	open	<10-20	open	20-30	open	<10	open	20
8/22/2008	open	<10-20	open	**<10-30**	open	10	open	**52**
8/25/2008	**closed**	**>1000**	**closed**	**>1000**	**open**	**52**	**open**	**20**
8/26/2008	open	<10-20	open	<10-20	open	20	open	10
8/27/2008	open	<10-20	open	<10-20	open	10	open	<10
8/28/2008	open	20-40	open	30-60	open	31	open	63
8/29/2008	open	**<10-20**	open	20-40	open	**31**	open	20
9/2/2008	open	<10-30	open	<10-20	open	<10	open	<10

Correlation between Indicators - Cx



Correlation between Indicators - CCE



Results - 2008

- Enterococci – composite samples
  - 22 sampling days
  - Application of corrective factor when indicated
  - 98% agreement for regulatory action
  - 64% agreement for numerical value
- *E. coli* – composite samples
  - 46 sampling days
  - Application of corrective factor when indicated
  - 98% agreement for regulatory action
  - 87% agreement for numerical value

2009 - 2010

Correlation w/o correction

Composite sampling

North Beach (2009) – *E. coli* vs. Enterococci

	C-18	*mEI*
C-18	1	
mEI	**0.910535**	1

Zoo Beach (2009) – E. coli vs. Enterococci

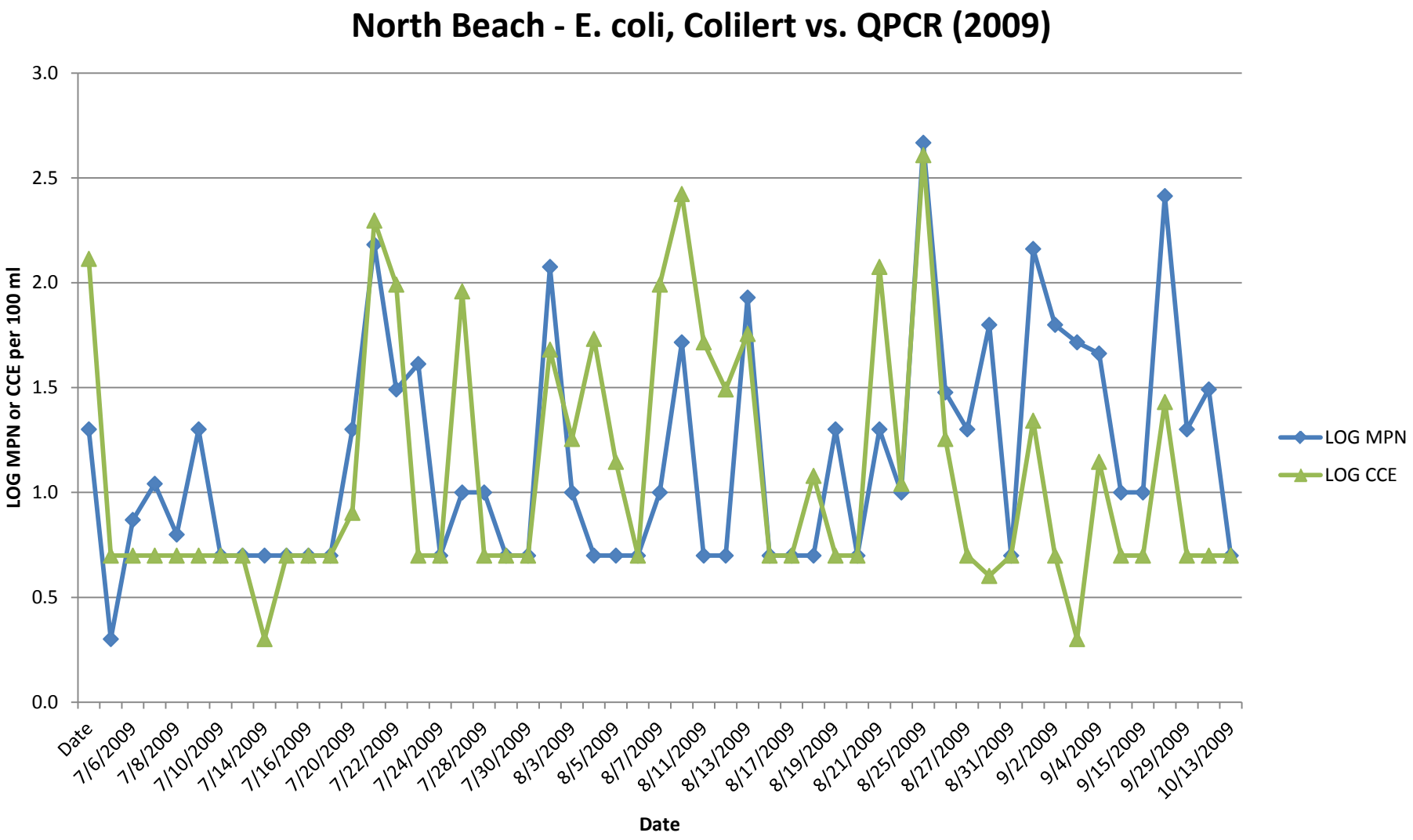
	C-18	*mEI*
C-18	1	
mEI	**0.726051**	1

Results – 2009

- North Beach
  - 53 sampling days
  - Numerical match, Colilert-18 vs. QPCR = 68%
  - Regulatory agreement = 96%*
    - 1 Type I and 1 Type II error
- Zoo Beach
  - 52 sampling days
  - Numerical match, Colilert-18 vs. QPCR = 81%
  - Regulatory Agreement = 96%*
    - 2 Type II errors

North Beach - 2009

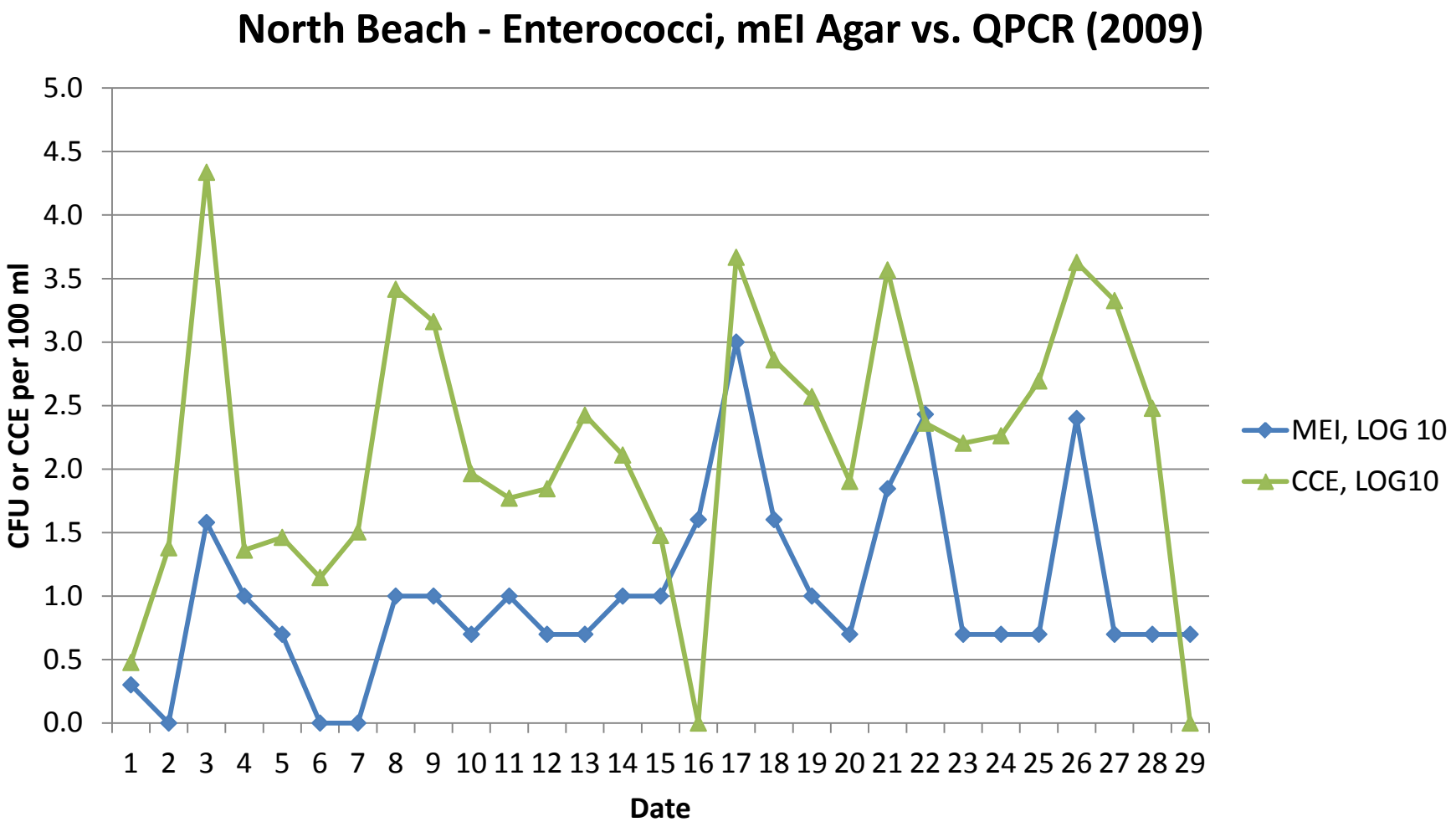
Date	Colilert-18	CCE-Uncorrected	CCE-UNC, MEAN	Date	Colilert-18	CCE-Uncorrected	CCE-UNC, MEAN
7/2/2009	**20**	119 - 140	**130**	8/6/2009	<10	11 - 17	14
7/6/2009	2.0	0	0	8/7/2009	<10	0	0
7/7/2009	7.4	0	0	8/10/2009	**10**	80 - 117	**98**
7/8/2009	11.0	0	0	8/11/2009	**52**	220 - 310	**265**
7/9/2009	6.3	0	0	8/12/2009	<10	0 - 52	26
7/10/2009	**20**	0	**0**	8/13/2009	<10	0 - 31	16
7/13/2009	<10	0	0	8/14/2009	**85**	0 - 57	**28**
7/14/2009	<10	0	0	8/17/2009	<10	0	0
7/15/2009	<10	0 - 2	1	8/18/2009	<10	0	0
7/16/2009	<10	0	0	8/19/2009	<10	8 - 17	12
7/17/2009	<10	0	0	8/20/2009	**20**	0	**0**
7/20/2009	<10	0	0	8/21/2009	<10	0	0
7/21/2009	20	0 - 8	4	8/24/2009	**20**	115 - 123	**119**
7/22/2009	152	93 - 304	198	8/25/2009	10	0 - 11	6
7/23/2009	31	32 - 164	98	8/26/2009	465	322 - 489	406
7/24/2009	**41**	0	**0**	8/27/2009	30	2 - 34	18
7/27/2009	<10	0	0	8/28/2009	20	0	0
7/28/2009	10	0 - 91	46	8/31/2009	**63**	2 - 7	**4**
7/29/2009	10	0	0	9/1/2009	<10	0	0
7/30/2009	<10	0	0	9/2/2009	**145**	16 - 28	**22**
7/31/2009	<10	0	0	9/3/2009	**63**	0	**0**
8/3/2009	**119**	44 - 52	**48**	9/4/2009	**52**	0 - 2	**1**
8/4/2009	10	7 - 29	18	9/8/2009	46	6 - 22	14
8/5/2009	<10	0 - 54	27	9/15/2009	10	0	0



96% Regulatory Agreement

Enterococci

NORTH	***LOG cfu***	*CCE*	*LOG/CCE*
LOG cfu	1		
CCE	0.362849411	1	
LOG/CCE	**0.50710848**	0.587393	1

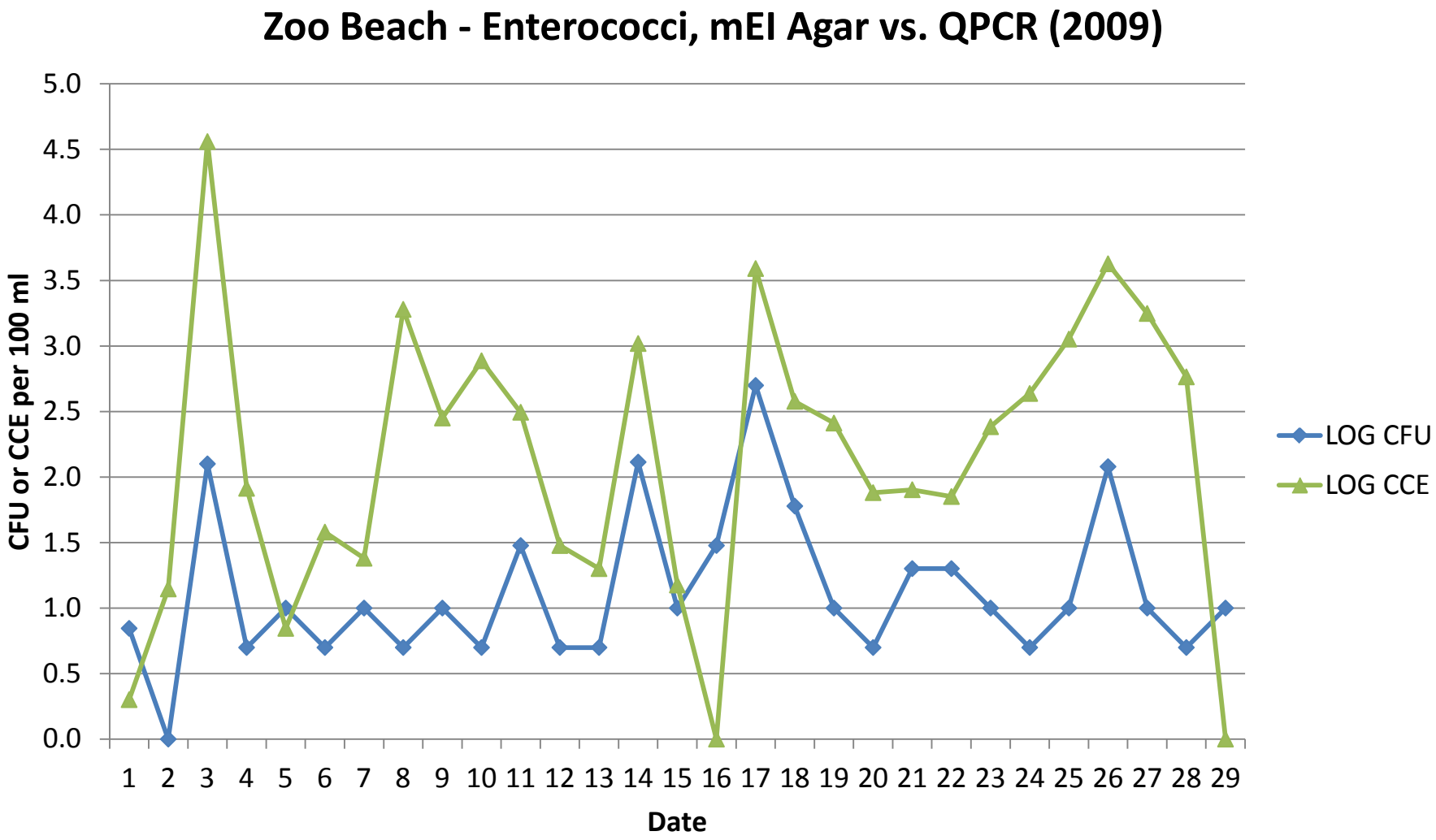


Zoo Beach - 2009

Date	Colilert-18	CCE-Uncorrected	CCE-UNC, MEAN	Date	Colilert-18	CCE-Uncorrected	CCE-UNC, MEAN
7/2/2009	**689**	309 - 449	**379**	8/6/2009	<10	0	0
7/6/2009	1.0	0	0	8/7/2009	**85**	0	**0**
7/7/2009	7.4	0	0	8/10/2009	<10	0 - 6	3
7/8/2009	8.5	0	0	8/11/2009	**10**	111 - 244	**178**
7/9/2009	1.0	0	0	8/12/2009	<10	0	0
7/10/2009	74	39	39	8/13/2009	**<10**	64 - 144	**104**
7/13/2009	30	54 - 75	64	8/14/2009	269	401	401
7/14/2009	<10	0 - 23	12	8/17/2009	20	0 - 29	14
7/15/2009	**63**	0 - 11	**6**	8/18/2009	<10	0 - 4	2
7/16/2009	<10	0	0	8/19/2009	<10	0	0
7/17/2009	<10	0	0	8/20/2009	**63**	6	**6**
7/20/2009	<10	0	0	8/21/2009	**20**	0	**0**
7/21/2009	**132**	64 - 71	**68**	8/24/2009	<10	0	0
7/22/2009	85	29 - 86	58	8/25/2009	10	0 - 3	2
7/23/2009	10	12 - 106	59	8/26/2009	313	256 - 271	264
7/24/2009	<10	0	0	8/27/2009	20	0	0
7/27/2009	<10	0	0	8/28/2009	**30**	0	**0**
7/28/2009	<10	8 - 40	24	8/31/2009	**20**	0 - 1	**1**
7/29/2009	<10	0	0	9/1/2009	<10	0	0
7/30/2009	<10	0 - 2	1	9/2/2009	**31**	0	**0**
7/31/2009	<10	0	0	9/3/2009	<10	0	0
8/3/2009	**404**	160 - 302	**231**	9/4/2009	20	0 -1	1
8/4/2009	<10	0 - 14	7	9/8/2009	6	0 - 5	3
8/5/2009	<10	0	0	9/15/2009	10	0	0

Enterococci

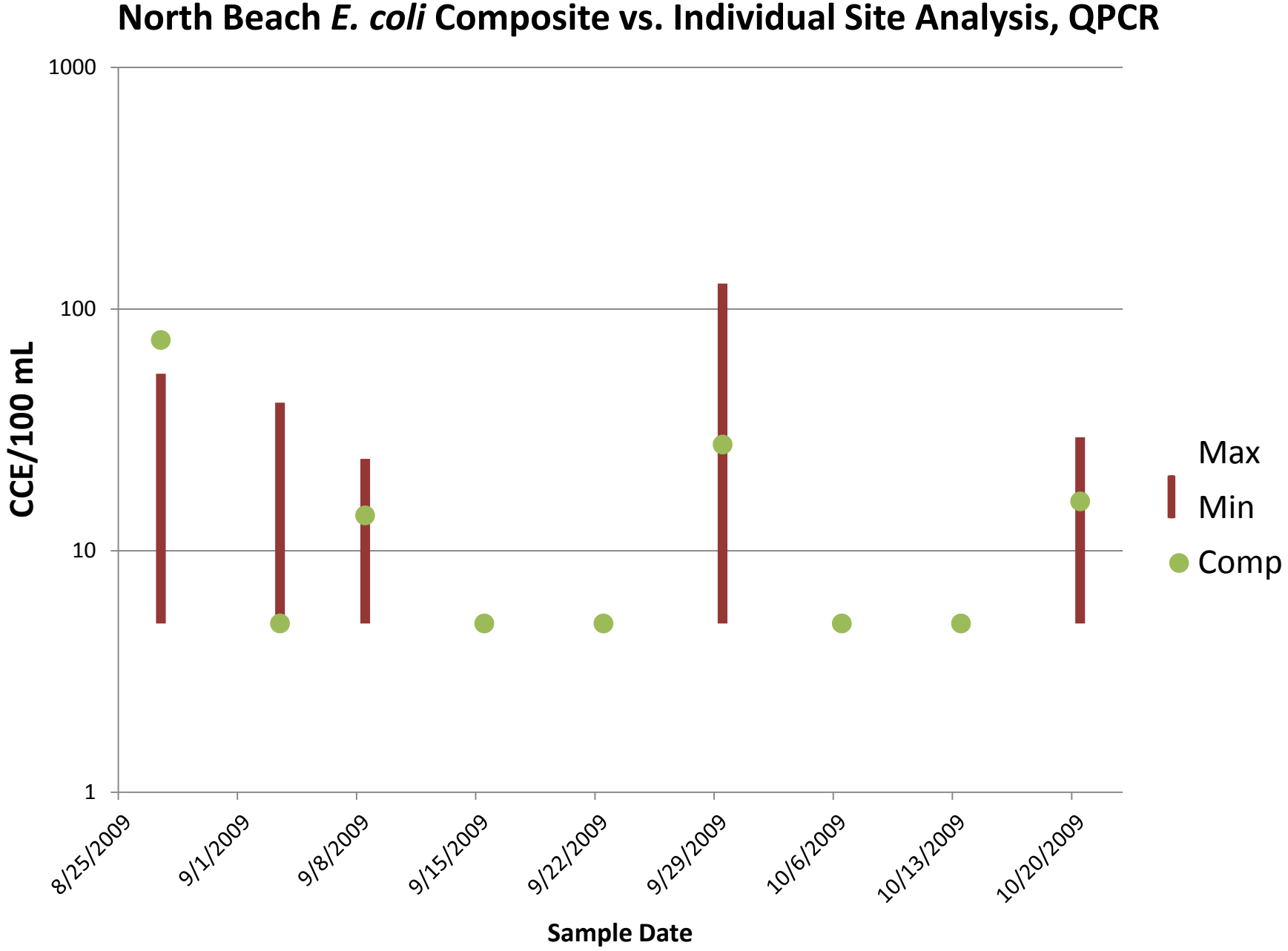
ZOO	***LOG,CFU***	*CCE*	*LOG/CCE*
LOG/CFU	1		
CCE	0.419861	1	
LOG/CCE	**0.462341**	0.517691	1



Composite Sampling

- August 2009 – June 2010
  - No mean difference in *E. coli* concentration across beach transects, i.e. valid to composite samples
  - 40 sampling events across two beaches
  - Composite samples fell within the range of the individual samples 92% of the time
  - The use of composite sampling would have made no difference with respect to regulatory action

- Compositing samples represents a significant cost savings


North Beach E. coli Composite vs. Individual Site Analysis, QPCR
1000
100
10
1
CCE/100 mL
8/25/2009
9/1/2009
9/8/2009
9/15/2009
9/22/2009
9/29/2009
10/6/2009
10/13/2009
10/20/2009
Sample Date
Max
Min
Comp

Results – 2010

- ***E. coli***
- North Beach
  - 87 sampling days
  - Numerical match, Colilert-18 vs. QPCR = 66%
  - Regulatory Agreement = 98%*
    - 1 Type I and 1 Type II error
- Zoo Beach
  - 88 sampling days
  - Numerical match, Colilert-18 vs. QPCR = 73%
  - Regulatory Agreement = 86%*
    - 12 Type I errors
    - *Cluster of 8 errors likely due to analyst error

2010 QPCR Implementation Pilot Study

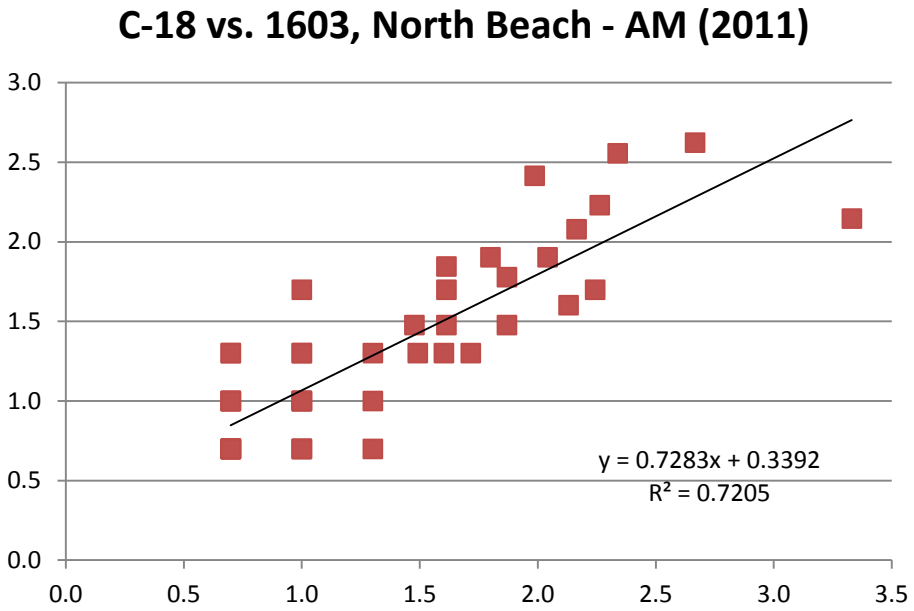
- July 23 – September 7, 2010
- Parallel to similar effort in Orange County, CA
- Daily management decisions made same-day using QPCR
- Confirmed the following morning by culture
- Non-consensus or inhibition defaulted to previous day's *E. coli* results as determined by the culture-based method
- Same management decisions would have been made for the duration of the pilot study, regardless of analytical method

2011 Final Pre-Implementation Study

- Establish relationship to NEEAR health effects
  - Enterococci and *E. coli* by culture
- Establish relationship between qPCR and culture
  - Enterococci by culture and qPCR
  - *E. coli* by culture and qPCR
- Determine if sampling time was reflective of water quality
- Examine regulatory action agreement
  - Choice of indicator
  - Do results make sense in light of sanitary survey data
- Look at qPCR vs. Virtual Beach

Correlation: culture to culture

There is not perfect agreement, even between two culture-based methods



Anova: Single Factor

SUMMARY

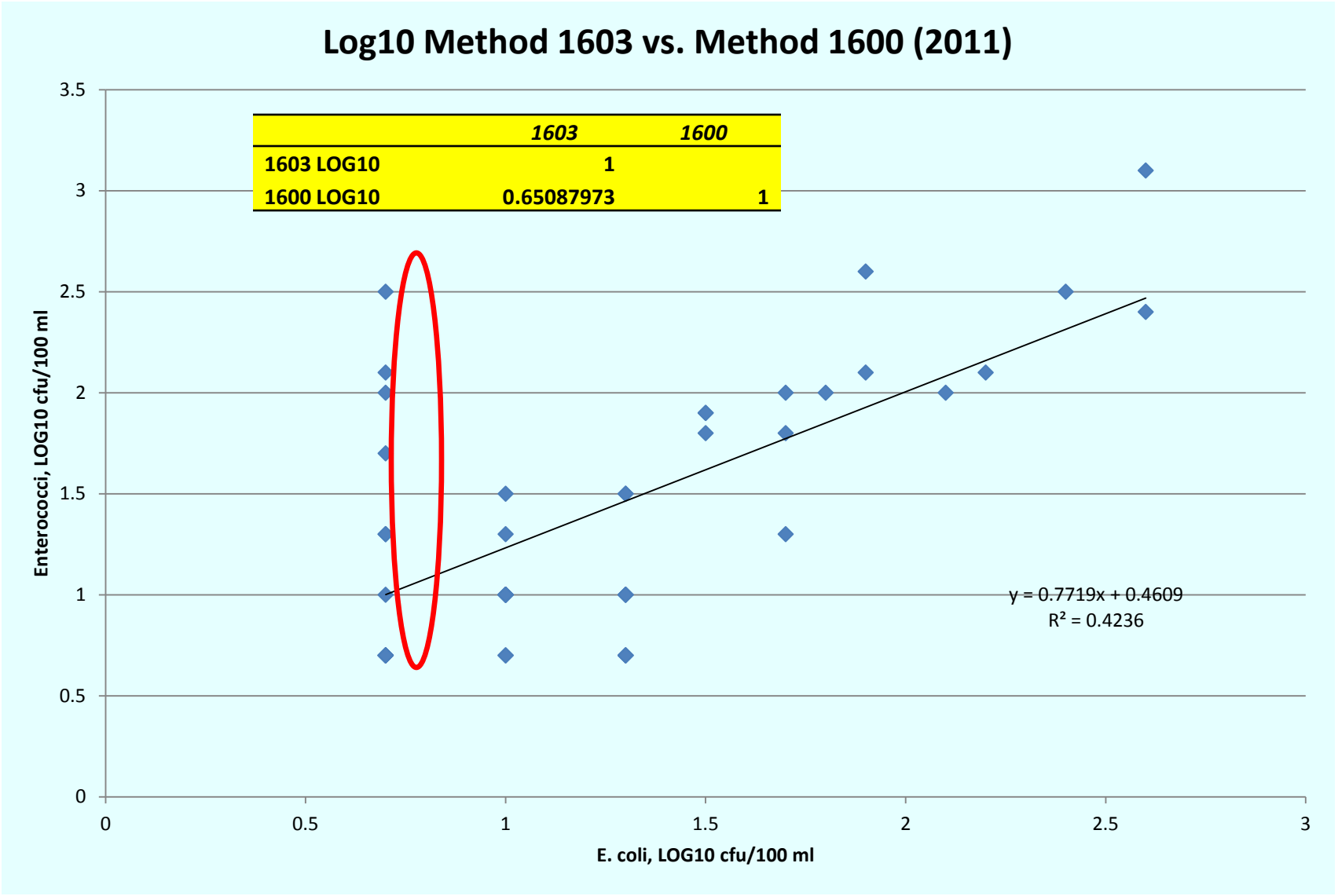
Groups	*Count*	*Sum*	*Average*	*Variance*
Colilert-18	46	4388	95.39130435	102011.8879
Method 1603	46	2350	51.08695652	7865.458937

METHOD 1603 vs. Colilert-18

ANOVA

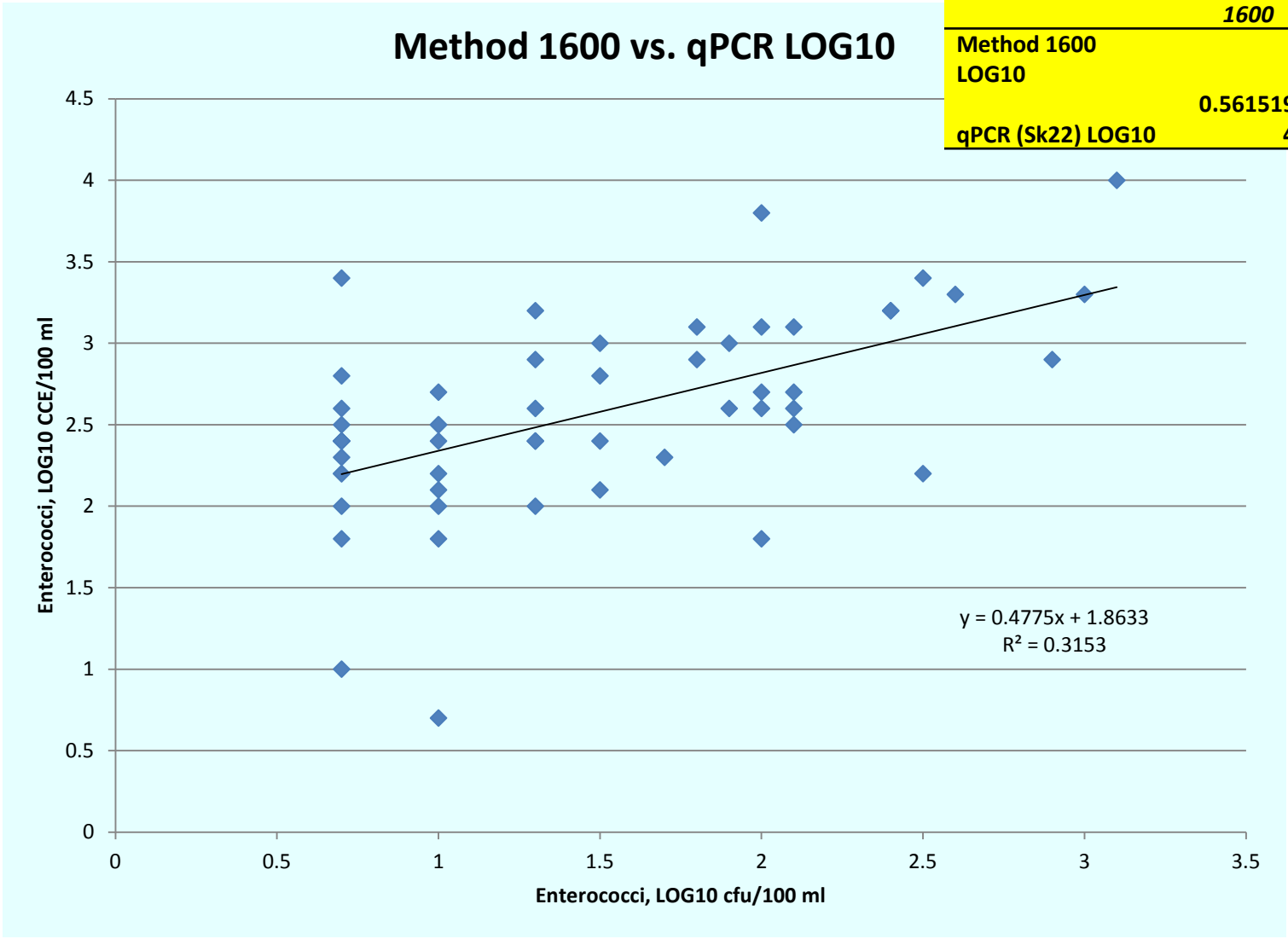
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	45146.13043	1	45146.13043	0.821755015	0.367088177	3.946875558
Within Groups	4944480.609	90	54938.67343			
Total	4989626.739	91				

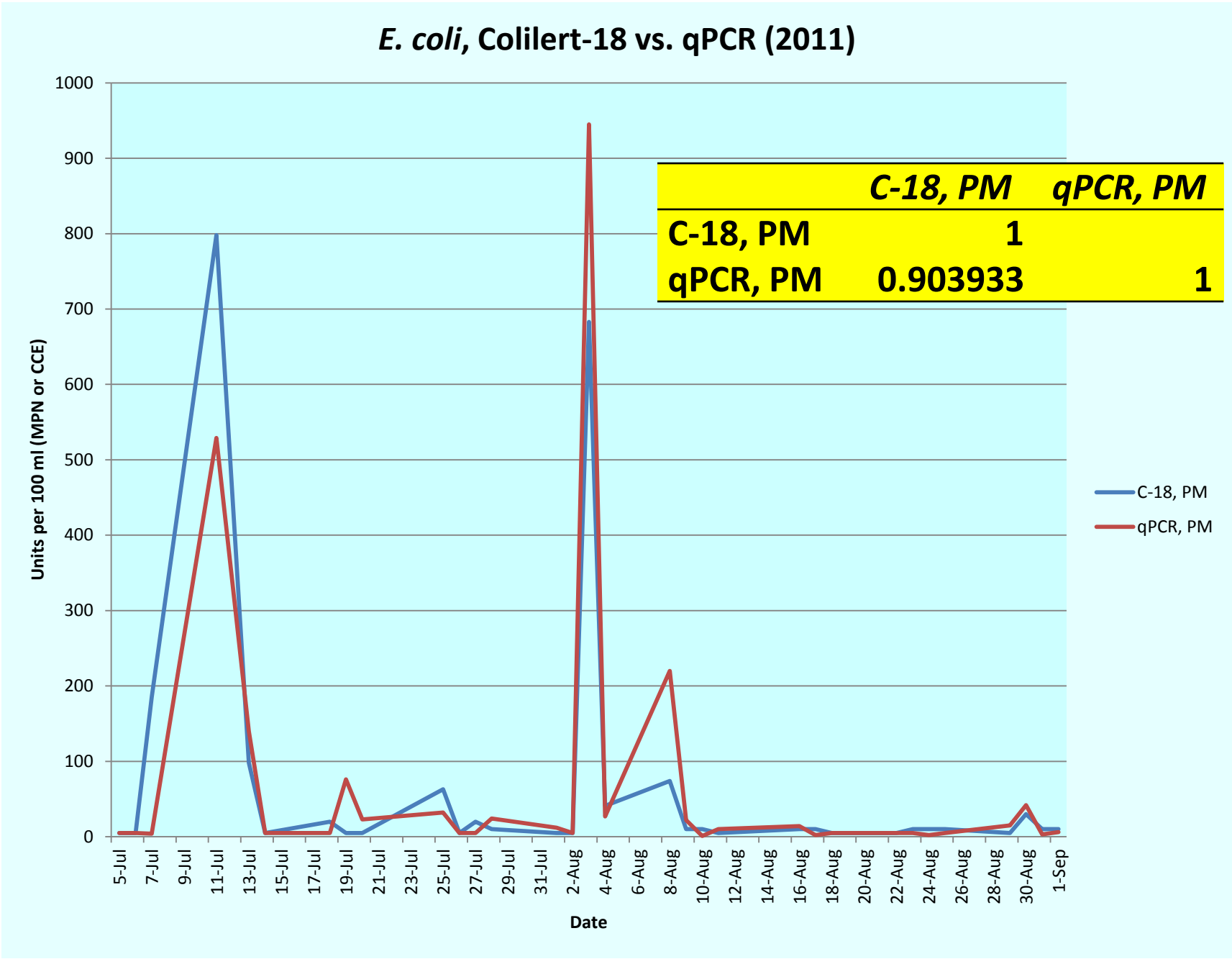
E. coli (Method 1603) and enterococci (Method 1600) were correlated when using culture-based enumeration methods



Enterococci, as enumerated by qPCR (CCE/100 ml), was positively correlated with Enterolert.

	Method 1600	*qPCR*
Method 1600 LOG10	**1**	
	0.5615192	
qPCR (Sk22) LOG10	**43**	**1**




E. coli, Colilert-18 vs. qPCR (2011)
C-18, PM qPCR, PM
C-18, PM 1
qPCR, PM 0.903933 1
Units per 100 ml (MPN or CCE)
1000
900
800
700
600
500
400
300
200
100
0
5-Jul
7-Jul
9-Jul
11-Jul
13-Jul
15-Jul
17-Jul
19-Jul
21-Jul
23-Jul
25-Jul
27-Jul
29-Jul
31-Jul
2-Aug
4-Aug
6-Aug
8-Aug
10-Aug
12-Aug
14-Aug
16-Aug
18-Aug
20-Aug
22-Aug
24-Aug
26-Aug
28-Aug
30-Aug
1-Sep
Date
C-18, PM
qPCR, PM

E. coli CCE/100 mL vs. Date of Sample (2011)

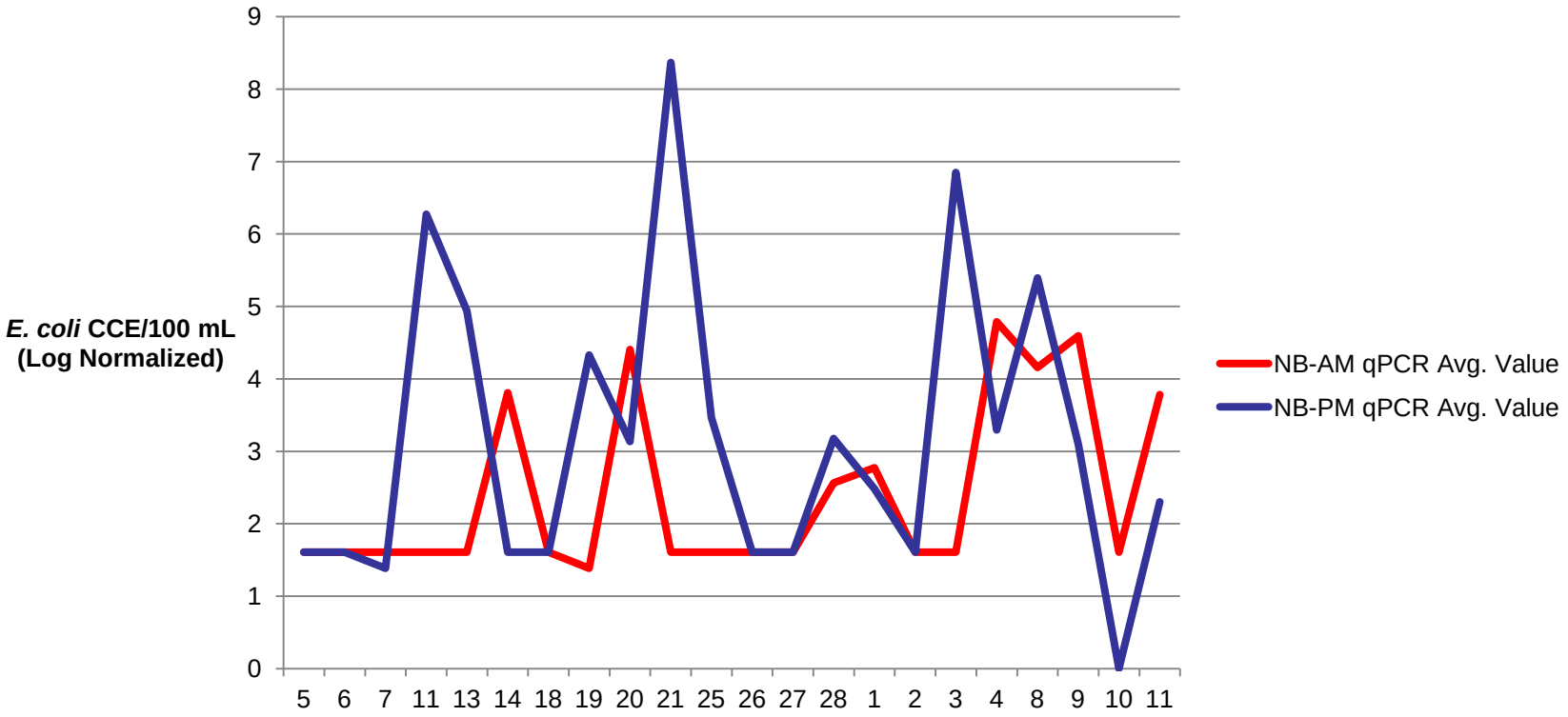


TABLE 1 - ANOVA: Single Factor, qPCR

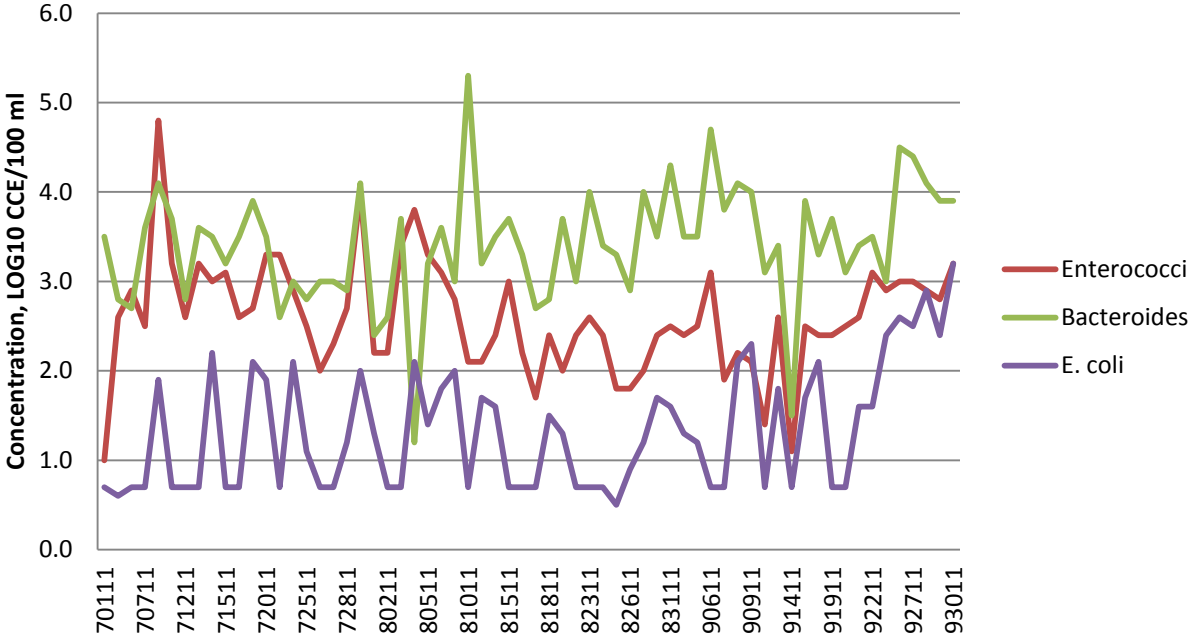
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
qPCR AM	22	53.18559135	2.41752688	1.468841003
qPCR PM	22	69.76056173	3.170934624	4.343360239

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.243855525	1	6.243855525	2.148533839	0.150153276	4.072653663
Within Groups	122.0562261	42	2.906100621			
Total	128.3000816	43				

Enterococci, Bacteroides, and *E. coli* (2011)



The use of enterococci would have resulted in significantly more exceedances.

For culture-based assays, the use of enterococci would have resulted in 7 (IDEXX Colilert-18 vs. Enterolert) to 13 (USEPA Method 1603 vs. 1600) more advisories based on proposed SVT values of 235 and 61 for *E. coli* and enterococci respectively.

For qPCR, the use of enterococci would have resulted in 10 additional exceedances based on a an SVT of 235 CCE/100 ml for *E. coli* (use of same criteria but alternative analytic method as per p. 55) and 1000 CCE/100 ml for enterococci.

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

- qPCR differs from traditional culture-based assays in that it measures all DNA:
  - live cells
  - dead cells
  - non-culturable cells
  - free DNA
- Culture assays only measure cells possessing the ability to grow on the selective media you are using
- Water body characteristics and environmental conditions may effect
  - Inhibition
  - Culture/qPCR relationship
  - Ability to use qPCR as an analytical method

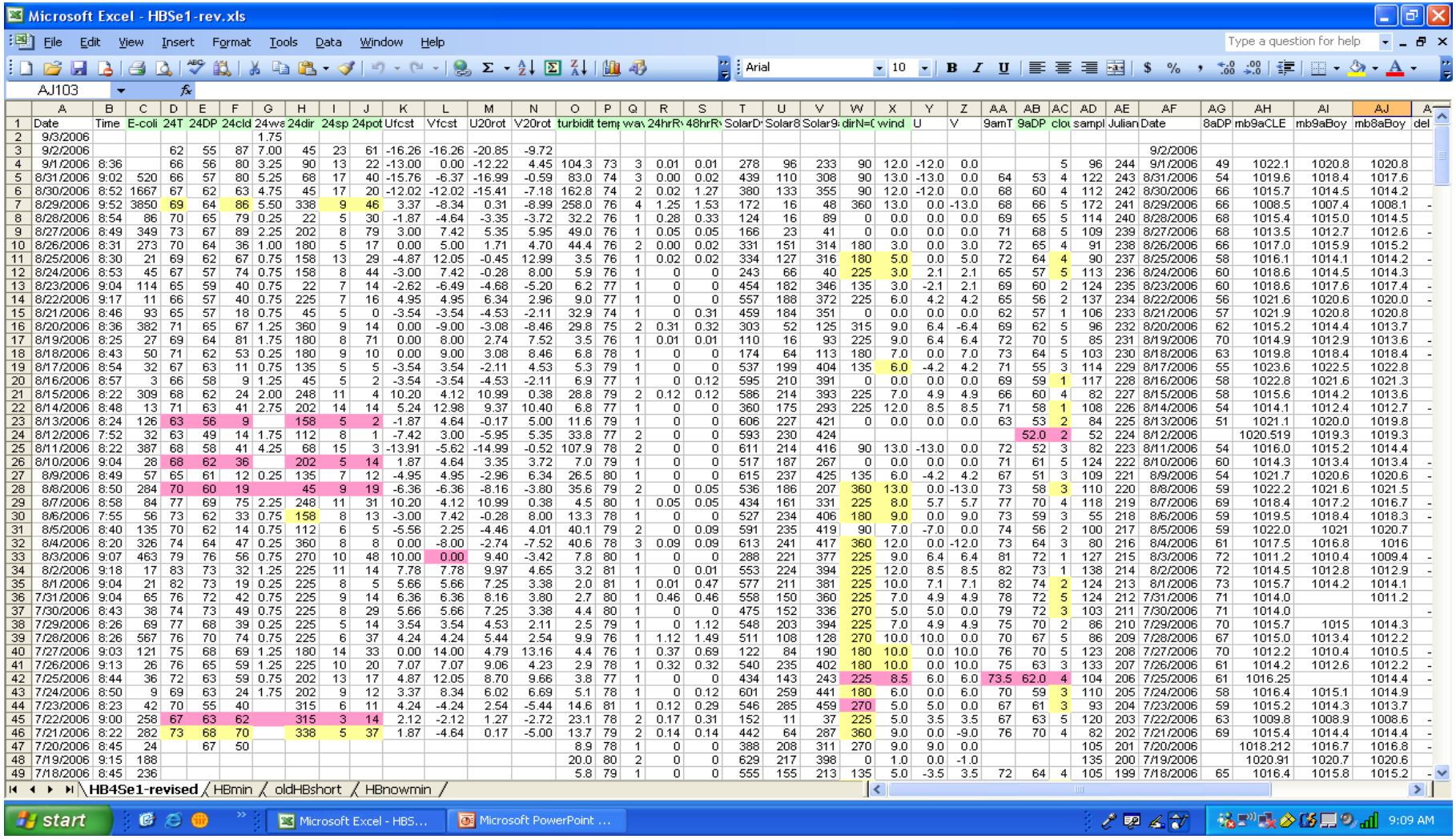
Predictive Models

- Environmental data collected as part of a Beach Sanitary Survey can be used to begin constructing a predictive model

- Virtual Beach (US EPA model)
  - http://www.epa.gov/ceampubl/swater/vb2/vb2-2-dl.html
  - Allows correlations between parameters and water quality (i.e. wave height, wind direction, rainfall, etc.)
  - Can be combined with hydrodynamic impact models to look at influence of land use on fecal loading (L-THIA)
  - Data limitations prevent full use, i.e. you will likely need multiple years of data

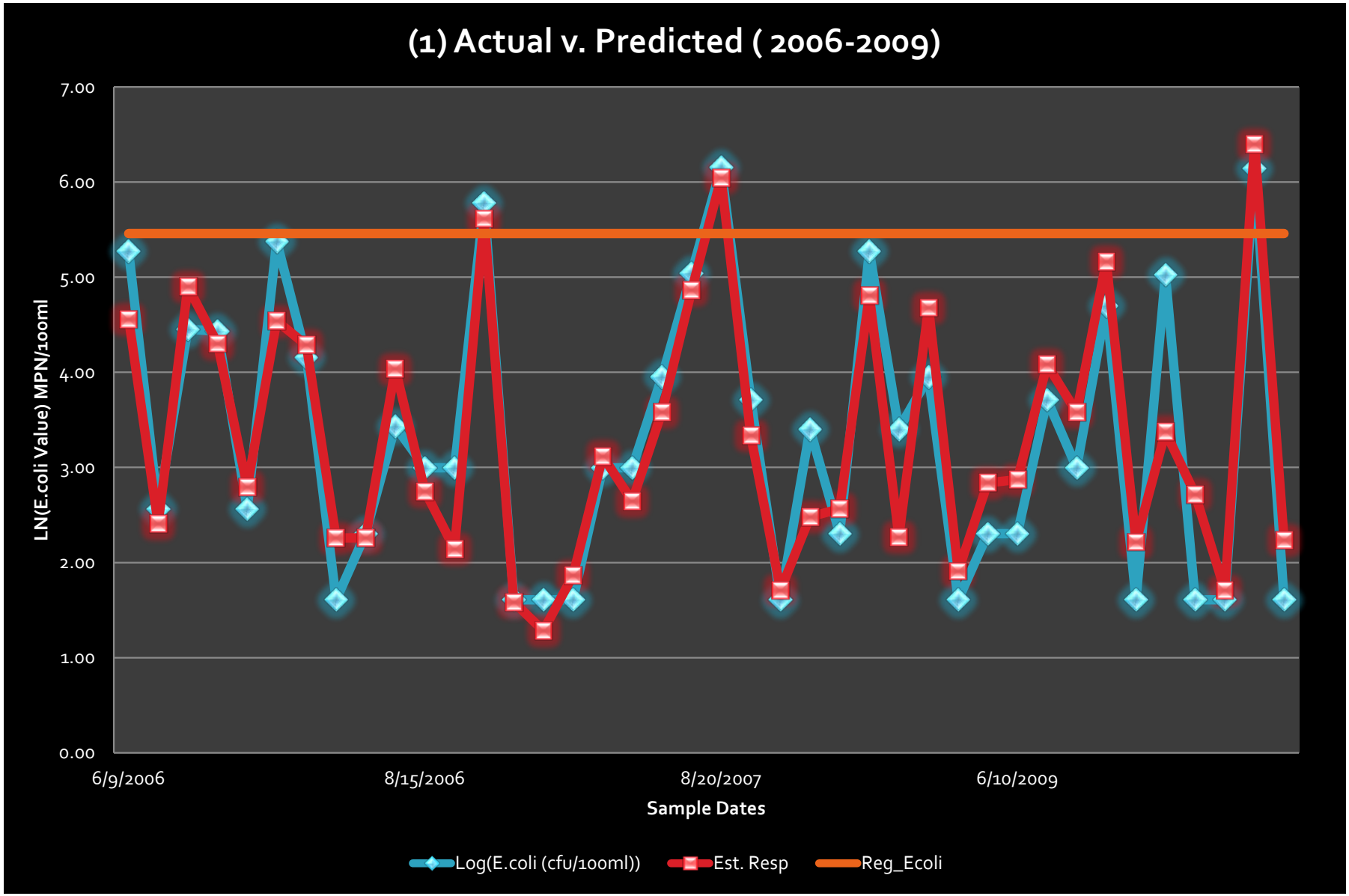
- Other models available:
  - SwimCast (Illinois), Nowcast (Ohio), Project SAFE (Indiana), Rainfall-based alerts (Wisconsin, others)

VB helps turn data like these…



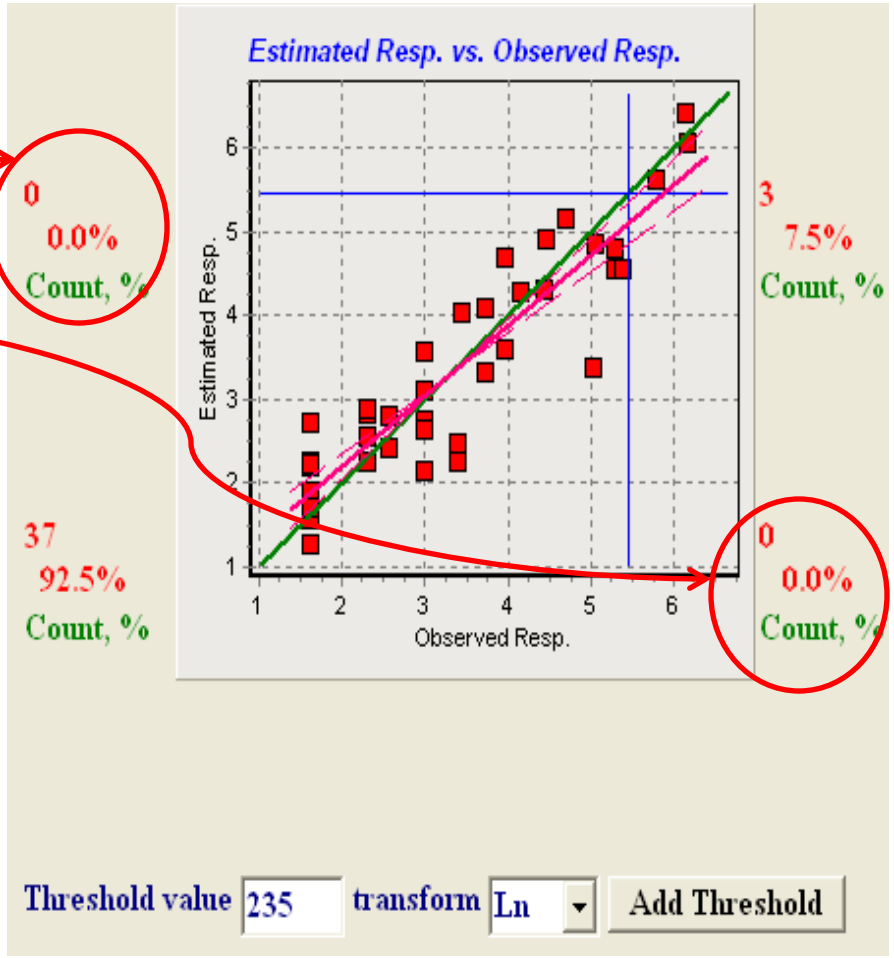
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	A
1	Date	Time	E-coli	24T	24DP	24cld	24ws	24dir	24sp	24pot	Ufcst	Vfcst	U20rot	V20rot	turbidit	temp	wav	24hrR	48hrR	SolarD	Solar8	Solar9	dirN=(	wind	U	V	9amT	9aDP	clou	sampl	Julian	Date	8aDP	mb9aCLE	mb9aBoy	mb8aBoy	del
2	9/3/2006						1.75																														
3	9/2/2006			62	55	87	7.00	45	23	61	-16.26	-16.26	-20.85	-9.72																		9/2/2006					
4	9/1/2006	8:36		66	56	80	3.25	90	13	22	-13.00	0.00	-12.22	4.45	104.3	73	3	0.01	0.01	278	96	233	90	12.0	-12.0	0.0			5	96	244	9/1/2006	49	1022.1	1020.8	1020.8	
5	8/31/2006	9:02	520	66	57	80	5.25	68	17	40	-15.76	-6.37	-16.99	-0.59	83.0	74	3	0.00	0.02	439	110	308	90	13.0	-13.0	0.0	64	53	4	122	243	8/31/2006	54	1019.6	1018.4	1017.6	
6	8/30/2006	8:52	1667	67	62	63	4.75	45	17	20	-12.02	-12.02	-15.41	-7.18	162.8	74	2	0.02	1.27	380	133	355	90	12.0	-12.0	0.0	68	60	4	112	242	8/30/2006	66	1015.7	1014.5	1014.2	
7	8/29/2006	9:52	3850	69	64	86	5.50	338	9	46	3.37	-8.34	0.31	-8.99	258.0	76	4	1.25	1.53	172	16	48	360	13.0	0.0	-13.0	68	66	5	172	241	8/29/2006	66	1008.5	1007.4	1008.1	-
8	8/28/2006	8:54	86	70	65	79	0.25	22	5	30	-1.87	-4.64	-3.35	-3.72	32.2	76	1	0.28	0.33	124	16	89	0	0.0	0.0	0.0	69	65	5	114	240	8/28/2006	68	1015.4	1015.0	1014.5	
9	8/27/2006	8:49	349	73	67	89	2.25	202	8	79	3.00	7.42	5.35	5.95	49.0	76	1	0.05	0.05	166	23	41	0	0.0	0.0	0.0	71	68	5	109	239	8/27/2006	68	1013.5	1012.7	1012.6	-
10	8/26/2006	8:31	273	70	64	36	1.00	180	5	17	0.00	5.00	1.71	4.70	44.4	76	2	0.00	0.02	331	151	314	180	3.0	0.0	3.0	72	65	4	91	238	8/26/2006	66	1017.0	1015.9	1015.2	
11	8/25/2006	8:30	21	69	62	67	0.75	158	13	29	-4.87	12.05	-0.45	12.99	3.5	76	1	0.02	0.02	334	127	316	180	5.0	0.0	5.0	72	64	4	90	237	8/25/2006	58	1016.1	1014.1	1014.2	-
12	8/24/2006	8:53	45	67	57	74	0.75	158	8	44	-3.00	7.42	-0.28	8.00	5.9	76	1	0	0	243	66	40	225	3.0	2.1	2.1	65	57	5	113	236	8/24/2006	60	1018.6	1014.5	1014.3	
13	8/23/2006	9:04	114	65	59	40	0.75	22	7	14	-2.62	-6.49	-4.68	-5.20	6.2	77	1	0	0	454	182	346	135	3.0	-2.1	2.1	69	60	2	124	235	8/23/2006	60	1018.6	1017.6	1017.4	-
14	8/22/2006	9:17	11	66	57	40	0.75	225	7	16	4.95	4.95	6.34	2.96	9.0	77	1	0	0	557	188	372	225	6.0	4.2	4.2	65	56	2	137	234	8/22/2006	56	1021.6	1020.6	1020.0	-
15	8/21/2006	8:46	93	65	57	18	0.75	45	5	0	-3.54	-3.54	-4.53	-2.11	32.9	74	1	0	0.31	459	184	351	0	0.0	0.0	0.0	62	57	1	106	233	8/21/2006	57	1021.9	1020.8	1020.8	
16	8/20/2006	8:36	382	71	65	67	1.25	360	9	14	0.00	-9.00	-3.08	-8.46	29.8	75	2	0.31	0.32	303	52	125	315	9.0	6.4	-6.4	69	62	5	96	232	8/20/2006	62	1015.2	1014.4	1013.7	
17	8/19/2006	8:25	27	69	64	81	1.75	180	8	71	0.00	8.00	2.74	7.52	3.5	76	1	0.01	0.01	110	16	93	225	9.0	6.4	6.4	72	70	5	85	231	8/19/2006	70	1014.9	1012.9	1013.6	-
18	8/18/2006	8:43	50	71	62	53	0.25	180	9	10	0.00	9.00	3.08	8.46	6.8	78	1	0	0	174	64	113	180	7.0	0.0	7.0	73	64	5	103	230	8/18/2006	63	1019.8	1018.4	1018.4	-
19	8/17/2006	8:54	32	67	63	11	0.75	135	5	5	-3.54	3.54	-2.11	4.53	5.3	79	1	0	0	537	199	404	135	6.0	-4.2	4.2	71	55	3	114	229	8/17/2006	55	1023.6	1022.5	1022.8	
20	8/16/2006	8:57	3	66	58	9	1.25	45	5	2	-3.54	-3.54	-4.53	-2.11	6.9	77	1	0	0.12	595	210	391	0	0.0	0.0	0.0	69	59	1	117	228	8/16/2006	58	1022.8	1021.6	1021.3	
21	8/15/2006	8:22	309	68	62	24	2.00	248	11	4	10.20	4.12	10.99	0.38	28.8	79	2	0.12	0.12	586	214	393	225	7.0	4.9	4.9	66	60	4	82	227	8/15/2006	58	1015.6	1014.2	1013.6	
22	8/14/2006	8:48	13	71	63	41	2.75	202	14	14	5.24	12.98	9.37	10.40	6.8	77	1	0	0	360	175	293	225	12.0	8.5	8.5	71	58	1	108	226	8/14/2006	54	1014.1	1012.4	1012.7	-
23	8/13/2006	8:24	126	63	56	9		158	5	2	-1.87	4.64	-0.17	5.00	11.6	79	1	0	0	606	227	421	0	0.0	0.0	0.0	63	53	2	84	225	8/13/2006	51	1021.1	1020.0	1019.8	
24	8/12/2006	7:52	32	63	49	14	1.75	112	8	1	-7.42	3.00	-5.95	5.35	33.8	77	2	0	0	593	230	424						52.0	2	52	224	8/12/2006		1020.519	1019.3	1019.3	
25	8/11/2006	8:22	387	68	58	41	4.25	68	15	3	-13.91	-5.62	-14.99	-0.52	107.9	78	2	0	0	611	214	416	90	13.0	-13.0	0.0	72	52	3	82	223	8/11/2006	54	1016.0	1015.2	1014.4	
26	8/10/2006	9:04	28	68	62	36		202	5	14	1.87	4.64	3.35	3.72	7.0	79	1	0	0	517	187	267	0	0.0	0.0	0.0	71	61	5	124	222	8/10/2006	60	1014.3	1013.4	1013.4	-
27	8/9/2006	8:49	57	65	61	12	0.25	135	7	12	-4.95	4.95	-2.96	6.34	26.5	80	1	0	0	615	237	425	135	6.0	-4.2	4.2	67	51	3	109	221	8/9/2006	54	1021.7	1020.6	1020.6	-
28	8/8/2006	8:50	284	70	60	19		45	9	19	-6.36	-6.36	-8.16	-3.80	35.6	79	2	0	0.05	536	186	207	360	13.0	0.0	-13.0	73	58	3	110	220	8/8/2006	59	1022.2	1021.6	1021.5	
29	8/7/2006	8:58	84	77	69	75	2.25	248	11	31	10.20	4.12	10.99	0.38	4.5	80	1	0.05	0.05	434	161	331	225	8.0	5.7	5.7	77	70	4	118	219	8/7/2006	69	1018.4	1017.2	1016.7	-
30	8/6/2006	7:55	56	73	62	33	0.75	158	8	13	-3.00	7.42	-0.28	8.00	13.3	78	1	0	0	527	234	406	180	9.0	0.0	9.0	73	59	3	55	218	8/6/2006	59	1019.5	1018.4	1018.3	-
31	8/5/2006	8:40	135	70	62	14	0.75	112	6	5	-5.56	2.25	-4.46	4.01	40.1	79	2	0	0.09	591	235	419	90	7.0	-7.0	0.0	74	56	2	100	217	8/5/2006	59	1022.0	1021	1020.7	
32	8/4/2006	8:20	326	74	64	47	0.25	360	8	8	0.00	-8.00	-2.74	-7.52	40.6	78	3	0.09	0.09	613	241	417	360	12.0	0.0	-12.0	73	64	3	80	216	8/4/2006	61	1017.5	1016.8	1016	
33	8/3/2006	9:07	463	79	76	56	0.75	270	10	48	10.00	0.00	9.40	-3.42	7.8	80	1	0	0	288	221	377	225	9.0	6.4	6.4	81	72	1	127	215	8/3/2006	72	1011.2	1010.4	1009.4	-
34	8/2/2006	9:18	17	83	73	32	1.25	225	11	14	7.78	7.78	9.97	4.65	3.2	81	1	0	0.01	553	224	394	225	12.0	8.5	8.5	82	73	1	138	214	8/2/2006	72	1014.5	1012.8	1012.9	-
35	8/1/2006	9:04	21	82	73	19	0.25	225	8	5	5.66	5.66	7.25	3.38	2.0	81	1	0.01	0.47	577	211	381	225	10.0	7.1	7.1	82	74	2	124	213	8/1/2006	73	1015.7	1014.2	1014.1	
36	7/31/2006	9:04	65	76	72	42	0.75	225	9	14	6.36	6.36	8.16	3.80	2.7	80	1	0.46	0.46	558	150	360	225	7.0	4.9	4.9	78	72	5	124	212	7/31/2006	71	1014.0		1011.2	
37	7/30/2006	8:43	38	74	73	49	0.75	225	8	29	5.66	5.66	7.25	3.38	4.4	80	1	0	0	475	152	336	270	5.0	5.0	0.0	79	72	3	103	211	7/30/2006	71	1014.0			-
38	7/29/2006	8:26	69	77	68	39	0.25	225	5	14	3.54	3.54	4.53	2.11	2.5	79	1	0	1.12	548	203	394	225	7.0	4.9	4.9	75	70	2	86	210	7/29/2006	70	1015.7	1015	1014.3	
39	7/28/2006	8:26	567	76	70	74	0.75	225	6	37	4.24	4.24	5.44	2.54	9.9	76	1	1.12	1.49	511	108	128	270	10.0	10.0	0.0	70	67	5	86	209	7/28/2006	67	1015.0	1013.4	1012.2	
40	7/27/2006	9:03	121	75	68	69	1.25	180	14	33	0.00	14.00	4.79	13.16	4.4	76	1	0.37	0.69	122	84	190	180	10.0	0.0	10.0	76	70	5	123	208	7/27/2006	70	1012.2	1010.4	1010.5	-
41	7/26/2006	9:13	26	76	65	59	1.25	225	10	20	7.07	7.07	9.06	4.23	2.9	78	1	0.32	0.32	540	235	402	180	10.0	0.0	10.0	75	63	3	133	207	7/26/2006	61	1014.2	1012.6	1012.2	-
42	7/25/2006	8:44	36	72	63	59	0.75	202	13	17	4.87	12.05	8.70	9.66	3.8	77	1	0	0	434	143	243	225	8.5	6.0	6.0	73.5	62.0	4	104	206	7/25/2006	61	1016.25		1014.4	-
43	7/24/2006	8:50	9	69	63	24	1.75	202	9	12	3.37	8.34	6.02	6.69	5.1	78	1	0	0.12	601	259	441	180	6.0	0.0	6.0	70	59	3	110	205	7/24/2006	58	1016.4	1015.1	1014.9	
44	7/23/2006	8:23	42	70	55	40		315	6	11	4.24	-4.24	2.54	-5.44	14.6	81	1	0.12	0.29	546	285	459	270	5.0	5.0	0.0	67	61	3	93	204	7/23/2006	59	1015.2	1014.3	1013.7	
45	7/22/2006	9:00	258	67	63	62		315	3	14	2.12	-2.12	1.27	-2.72	23.1	78	2	0.17	0.31	152	11	37	225	5.0	3.5	3.5	67	63	5	120	203	7/22/2006	63	1009.8	1008.9	1008.6	-
46	7/21/2006	8:22	282	73	68	70		338	5	37	1.87	-4.64	0.17	-5.00	13.7	79	2	0.14	0.14	442	64	287	360	9.0	0.0	-9.0	76	70	4	82	202	7/21/2006	69	1015.4	1014.4	1014.4	-
47	7/20/2006	8:45	24		67	50									8.9	78	1	0	0	388	208	311	270	9.0	9.0	0.0				105	201	7/20/2006		1018.212	1016.7	1016.8	-
48	7/19/2006	9:15	188												20.0	80	2	0	0	629	217	398	0	1.0	0.0	-1.0				135	200	7/19/2006		1020.91	1020.7	1020.6	
49	7/18/2006	8:45	236												5.8	79	1	0	0	555	155	213	135	5.0	-3.5	3.5	72	64	4	105	199	7/18/2006	65	1016.4	1015.8	1015.2	-

…into estimated *E. coli* counts



North Beach Model- Training

- $R^2 = 0.793$, n = 40
- Database covers years from 2006-2009
- No observed false positives or false negatives
- Variables include:
  - Algae Description
    - Floating or Submerged
  - # Gulls
  - Wave Height
  - Wind Speed
  - 24 hr rainfall
  - NE Winds
  - Interactive variable



2011 VB Model Pilot Study

VB Predictions vs. Colilert-18	
Correct, 42/46	91%
Incorrect, 4/46	9%
Type I, 2/46	4%
Type II, 2/46	4%

VB Predictions vs. qPCR CCE	
Correct, 43/44	98%
Incorrect, 1/44	2%
Type I, 1/44	2%
Type II, 0/46	0%

The predictive model constructed using Virtual Beach accurately estimated *E. coli* concentrations enumerated by culture-based (Colilert-18, 91%) and qPCR assays (98%).

Limitations

- Success of model is limited to the availability of quality data and the overall history of the beach

- Difficulty lies with consistency and number of parameters monitored

- Sanitary Survey data greatly improves the chances of a successful and quality model being produced
  - Greater quantity of data for each monitoring day
  - Much more detail associated with each observed parameter

Implementation - 2012

- Regulatory monitoring 5 days/week
  - Received approval as an ATP indicator/method combination in May 2012
  - 2 beaches
  - *E. coli* by qPCR (BioGx Smartbeads™: *E. coli* and Sketa)
  - Compare to culture 4 days/week
  - Compare to model 5 days/week
  - Compare *E. coli*/enterococci by culture (qPCR to follow)
    - Archived filters (Fall 2012)

Murphy's Law…sort of

SITE	Numerical Correlation (r)	Regulatory Agreement	# Advisories E. coli - Cx	#Advisories Alternative
North Beach				
Colilert vs. Enterolert	0.94	88%	5	9
Colilert vs. qPCR	0.59	89%	6	12
qPCR vs. Virtual Beach	0.19	78%		
Zoo Beach				
Colilert vs. Enterolert	0.97	96%	3	5
Colilert vs. qPCR	0.46	98%	6	7
qPCR vs. Virtual Beach	0.49	91%		

Data Handling Reality Check

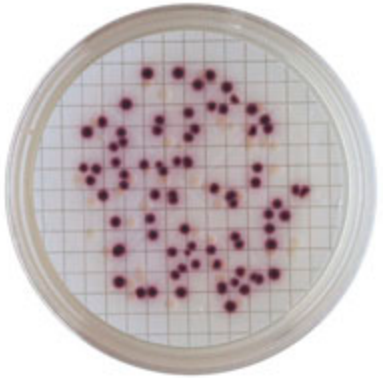
- Replicate analyses do not always agree
  - Open, advisory, closed, coin toss (?)
- Culture does not always agree with qPCR
- Results do not always make sense based on environmental conditions
- Inhibition
- False negatives w/o inhibition (algae blooms?)
- Filter blanks or NTC are contaminated
- Contamination as a result of visitors/observers not wearing PPE

Recommended Course of Action

Based on our experiences with implementation

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

- QPCR differs from traditional culture-based assays in that it measures all DNA:
  - live cells
  - dead cells
  - non-culturable cells
  - free DNA



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

Feasibility Studies

- Ability of the method to accurately characterize water quality
  - Will you use the same indicator as your culture-based assay?
  - Will the assay work with your samples?
    - Inhibition, underestimation
    - Unexplained false negatives w/o inhibition
- Turn-Around-Time
  - Will you be able to collect and process the sample in order to make it “real time”?
    - Distance of site to analytical lab
  - Do you have diurnal variation?
    - Is an early morning sample representative of water quality at your site?

Feasibility Studies

- Relationship to health effects
  - *Enterococcus* culture to *Enterococcus* qPCR
    - No need to independently establish a health relationship (NEEAR studies)
  - *E. coli* culture to *E. coli* qPCR
    - May need to independently establish intermediary relationships between culture-based methods
- Result interpretation
  - Numerical and regulatory action decision agreement
    - Environmental/meteorological conditions may influence water quality and method performance
    - Additional information may be needed (sanitary surveys)

Multiple Lines of Evidence

- Multiple lines of evidence – i.e., multiple tools per site may be necessary in order to:
  - be able to reconcile or evaluate different results from replicates
  - different results from different methods
  - analytical results that seem inconsistent with environmental conditions
- Tools = sanitary surveys, nowcast models, results of multiple analytical methods
- As with any analytical method your results need to make sense!

Beach Sanitary Surveys

- Guided data gathering at time of sample collection
- Environmental conditions
  - Wave height, turbidity, recent precipitation, presence of algal biomass
- Can be used to predict site specific inhibition
- Can be used in developing predictive models
- Can help you make sense of your data!

Environmental Data Collected – Routine/Daily Beach Sanitary Survey

- **General Beach Conditions**
  - Air temperature
  - Wind speed/direction
  - Rainfall
  - Weather condition (sunny, etc.)
  - Current speed/direction
  - Wave Height
- **Water Quality**
  - FIB concentrations
  - Water temperature
  - Water color/odor
  - Turbidity (clarity)
- **Bather Load**
  - Total number of people at beach
  - Swimmers/non-swimmers
- **Potential Pollution Sources**
  - Sources of discharge
    - Rivers, outfalls, wetlands, etc.
  - Floatables
  - Amount of debris/litter
  - Amount of algae
    - Stranded on beach
    - Floating/submerged in water
  - Presence of wildlife
    - Gull counts
    - Geese, deer, other
  - Presence of domestic animals
    - Dogs, Horses

If qPCR is Feasible…

- Choose an appropriate instrument platform
- Familiarize yourself with the method
- Select reagents and controls
- Determine QA/QC procedures
- Provide adequate staff training
- Validate the method
  - Consultation with a qPCR proficient lab recommended
- Conduct side-by-side site specific comparisons of qPCR and culture methods

Physical Space Requirements

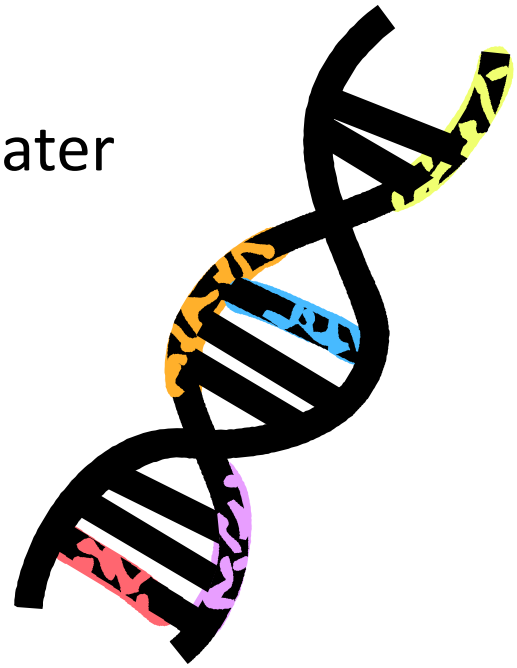
- Segregated work spaces
  - Sample filtration/DNA extraction
  - Preparation of master mix
  - Sample analysis
- Washable surfaces
  - Non-porous
  - UV disinfection, bleach
- Unidirectional workflow
- Dedicated equipment
- Dedicated PPE
- Proper disposal of DNA waste

Choosing an Instrument

- Consider which instrument platform to purchase:
  - Examine your workflow
  - Account for future uses: source tracking, drinking water, wastewater, stormwater, shellfish harvesting, other water research/monitoring?
  - Consumable costs
- Ease of use, software preference
- High throughput or flexibility?
- Balancing variability with cost
  - prepared reagents and standards are more expensive, but will dramatically reduce loss of runs due to errors

Choice of Molecular Target

- Site Specific Suitability
  - *E. coli* or enterococci for fresh water
  - Enterococci for marine water
  - Bacteroides?
- Wet chemistry or beads
  - Assay time
  - Precision/accuracy
- Sample DNA extract - pre-treatment?
  - Crude
  - Serial Dilution
  - DNA extraction or purification kits



Sample Handling & Storage

- Storing supplies
  - Calibrators, Lab prepared cells @ -80 °C
  - Controls, SPC @ 4 – 8 °C
  - DNA Reagents, product dependent
  - How long?
    - Stock salmon DNA was good for 5 years @ 4 °C
    - Lab prepared cell suspensions were good for 5 years @ -80 °C
- Storing samples
  - Fresh, analyzed w/i 4 hours of collection
  - Filters, freeze @ -80 °C
  - DNA extracts, good for 7 days @ 4 °C (up to 1% loss/day)
- Mailing samples and/or reagents
  - Degradation can occur if temp is not maintained

Reagents and Controls

- Reagents
  - Wet Chemistry or bead technology
  - Troubleshooting contamination
- Calibrators and Controls
  - Lab-prepared vs. commercial products
  - Precision (cal curves, replicate sample agreement)
  - Accuracy (calibrators, SPC)
  - Reproducibility (b/w run calibrator agreement)
    - Inter-laboratory validation studies

BioGX

QA/QC

- What QC samples should be run?
  - Method Blank (contamination during sample filtration)
  - Filter Blank (contamination in extraction process)
  - NTC (reagent contamination)
  - Calibrator (target quantification check)
  - SPC control (extraction and inhibition control)
- Number/Frequency?
- Definition of inhibition
  - >3 cycle threshold difference b/w cal-SPC and unknown-SPC (EPA definition)

QA/QC

- Field replicates vs. sample replicates
  - Sample replicates = 2 sub-samples from 1 DNA extraction
- Detection in the negative controls
  - >35 CT in more than a single NTC
  - <45 CT in a third of NTC rxns for a single MM
- Non-agreement/non-consensus
  - Replicate analyses should agree within 1 CT
  - When is it ok to average replicate CT values?
  - How do you know what the right answer is?
- Reporting out “non-detects”
  - Less than half the reciprocal of the dilution factor
  - What does that mean?

Staff Training Needs

- Aseptic technique!
- Pipetting skills
- Good laboratory practices
- Understanding of molecular concepts
  - Need comprehensive understanding of assay results
- Precision/accuracy
- Reproducibility
- Academic and hands on
  - Learn the basics
  - Observe a trained professional
    - Vendor training
    - National and regional training opportunities
    - Train the trainer (referee labs)
- Data review prior to doing it alone

Comparative Testing (Method Validation)

- **Within lab**
  - Can you continue to use the same procedures/protocols that you used for culture-based assays?
    - Time of sample collection
    - Composite sampling
  - Numerical agreement
    - Should/could you expect to see this?
  - Regulatory action agreement
    - Will you be as protective to public health and safety?
- **Between labs**
  - Inter-laboratory reproducibility studies
  - Referee labs

Method Performance

- Precision/Accuracy/Variability
  - Are the results reproducible?
- Inhibition
  - Do conditions exist which would prevent accurate quantification?
- Comparison to “gold standard”
  - How do the results compare to currently approved methods? To health risk data?
- Do the results make sense?
  - Predictive models, ambient conditions

The Way Forward

- What is the way forward for broader implementation?
- Further comparative studies
  - Know your beach
- Years of comparative data
- USE THE SANITARY SURVEY TOOL
  - Correlations b/w inhibition and ambient conditions
- Virtual Beach
  - Helps make sense of environmental conditions and analytical results

Points to Consider

- Make sure this analytical method works for you before you start the lab set up process
- Consult with proficient labs
- Exchange sample filters
- Future needs:
  - Sole source or approved QC materials
  - Accreditation
  - Laboratory Inspections
  - Staff Credentials
  - Proficiency Testing

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Thank You!

RACINE ON THE LAKE