



Training the Next Generation of Water Quality Professionals: Lessons Learned from Workshops on Rapid, qPCR-based Methods

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Background

2012 RWQC document released

- Revised criteria for water quality monitoring so that public health protected similarly in both coastal and fresh waters.
- An early-alert approach (BAV) to use to quickly issue swimming advisories for the public.
- Use of predictive water quality models and sanitary surveys to identify sources of pollution and to develop criteria for specific beaches.
- **Allow states to use new rapid testing method (qPCR) on a site specific basis**

Background

- Water quality labs traditionally perform microbiological techniques...not PCR
- No formalized training provided by EPA to implement use of rapid methods





Rapid methods approved by EPA

- Rapid methods can be defined as having a result in 4 hours or less from the time the assay is initiated. SAME DAY REPORTING.
 - Currently EPA approved culture methods take 18 to 24 hours for a result
- Methods 1609 and 1611 for measuring enterococcus by qPCR
- *E. coli* qPCR at fresh water beaches in Wisconsin, Ohio, and Michigan



Demonstration projects

- Summer 2010 Orange County used lyophilized Enterococcus (SampleReady –non-EPA method)
- Summer 2011 LA County and City of LA used EPA method 1611
- Labs of varying expertise (experienced, some experience, no experience)
- Preceded by a week of classroom/lab instruction followed 3 weeks of trial implementation
- Inhibited samples not used for health warnings
- Training conducted by experienced lab personnel from SCCWRP and UNC



Training

- PCR theory
- qPCR methods for measuring enterococcus
- Using cell based standards for reporting a CE
- Common reference material provided by UNC
- Pipetting efficiency
- Developed simple worksheet for input of results
- Machines programed by SCCWRP –no user settings were changed by monitoring personnel



Training

- After the initial “learning curve”, inexperienced users were performing with same accuracy as experienced users and were able to get results out as quickly

Sequence	Everyday	Some qPCR	No qPCR
Sampler Out	7:00 am	6:56 am	7:04 am
Sampler Return	8:04 am	7:44 am	7:48 am
qPCR plate in	9:52 am	9:40 am	9:30 am
qPCR plate out	10:49 am	10:58 am	10:36 am
Data reported	11:22 am	11:07 am	10:55 am

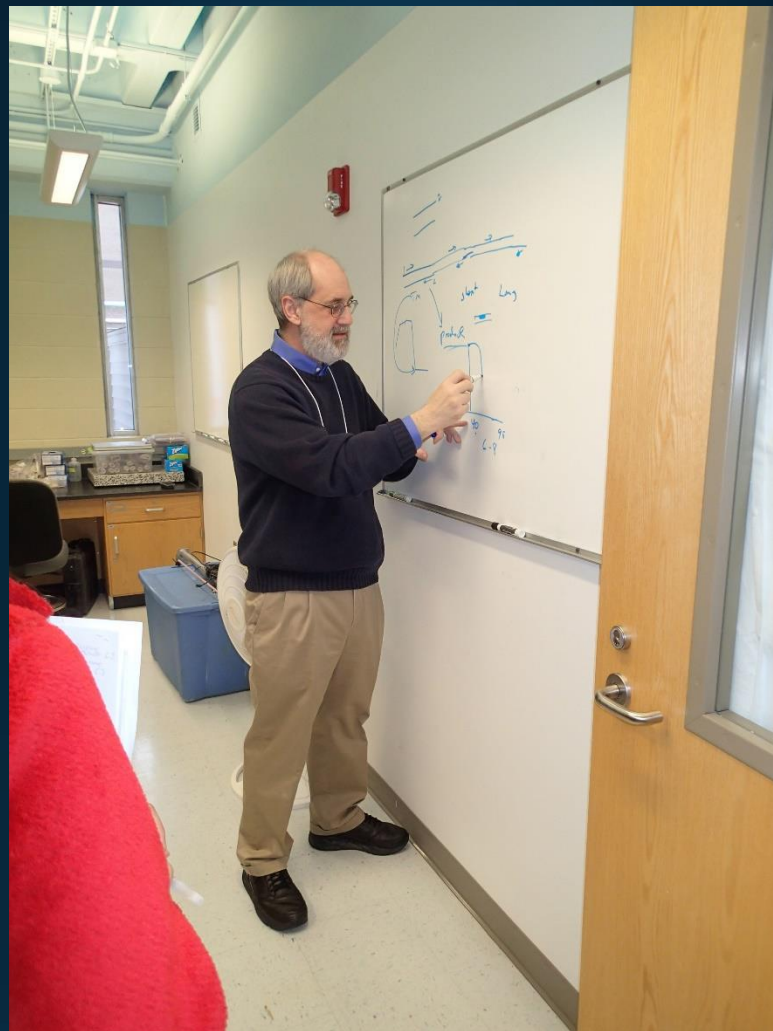
Molecular Training Facility (MTF)

- NC Biotechnology Center President's Initiative Award and partnership with Marine Bio-Technologies Center of Innovation
- **Goal: prepare the next generation workforce in rapid, molecular diagnostics**
 - recreational water quality
 - aquaculture
 - food safety
 - drinking water



First Workshop Objectives

- Bridge the gap between RWQC and implementation of rapid methods
- Teach other supporting concepts and provide resources to promote molecular method success





Workshop structure

- March 10th -15th, 2013
- Train participants to perform water quality monitoring for Enterococcus using EPA methods 1609 and 1611 and other qPCR methods for Enterococcus and *E. coli*.
- Train participants on multiple thermal cycler formats
- Exposure to different mastermix formulations
- Technology transfer to Molecular Source Tracking
- MIQE guidelines
- QA/QC, PCR theory, dCt/ ddCT

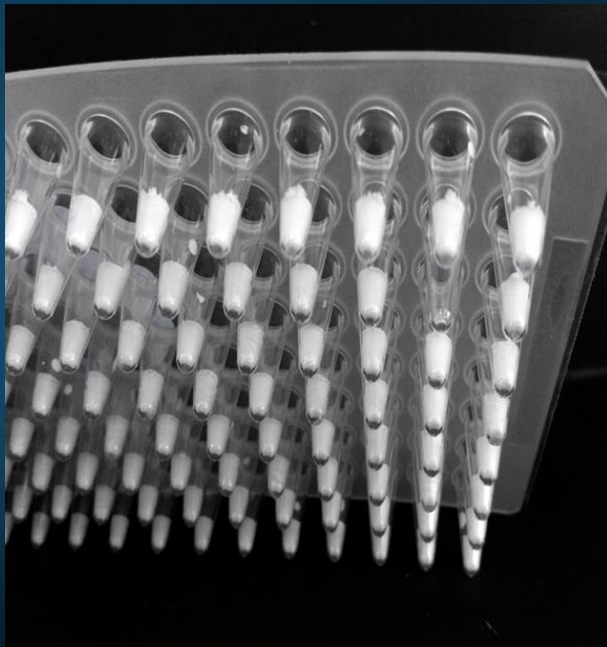


EPA Methods 1609 and 1611

- Enterococcus (Haugland et al 2005, EPA RWC, 2012)
- Recommended method
 - LifeTechnologies Universal or Environmental MasterMix, primers and FAM/TAMRA probe
 - LifeTechnologies StepOnePlus

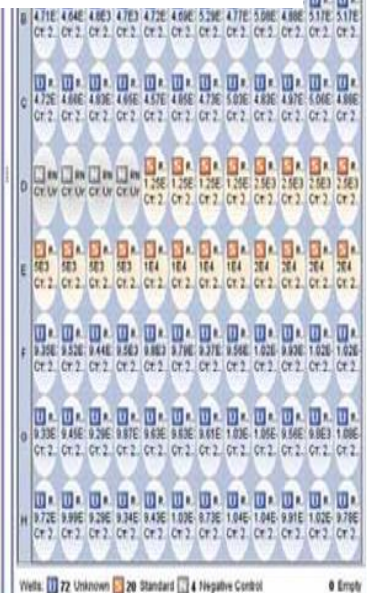
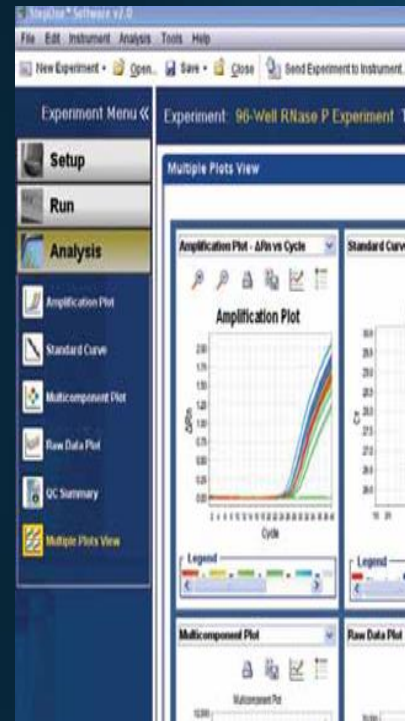
E. coli SampleReady qPCR (BioGx)

- Approved for monitoring of freshwater beaches by EPA on a site specific basis
- Currently being used in Wisconsin, Ohio, and Michigan



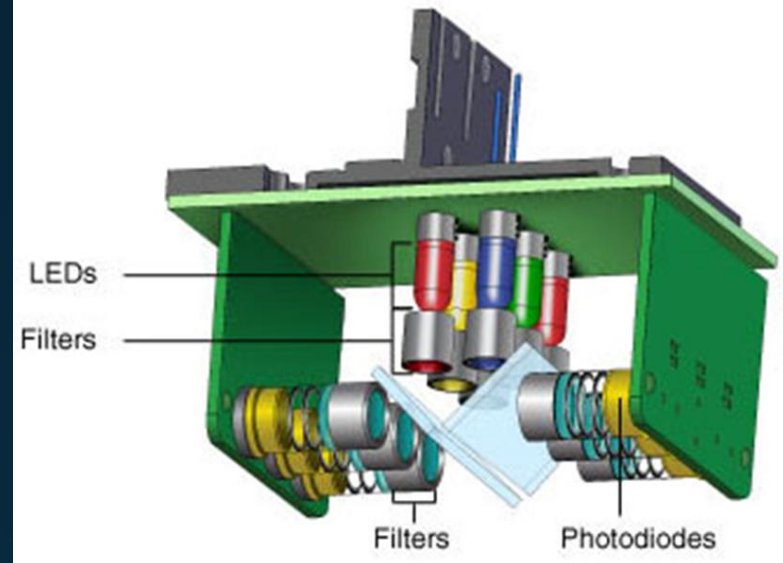
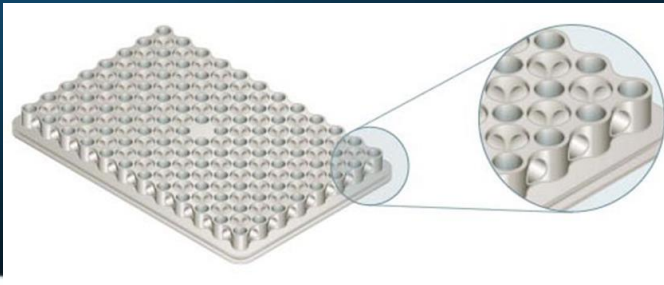
Life Technologies StepOne Plus

- 3-color/48-well (StepOne™) or 4-color/96-well (StepOnePlus™) •
- Long-life LED-based FAM™/SYBR®Green I, VIC®/JOE™, and ROX™
- Features VeriFlex™ Block technology, which combines six independently controllable Peltier blocks
- Uses reference dye (ROX) to control for discrepancies across 96 well plate



BioRad CFX Touch

- Six filtered LEDs and six filtered photodiodes
- Thermal gradient feature identifies optimal annealing temperature in a single run.
- Reduced-mass sample block fast ramping and settling produce the shortest time to target temperature available in a thermal cycler.



Cepheid SmartCycler II



- I-Core module is independently programmable, real time 4-channel optical reading
- Air cooled, unique tube design
- Expandable to 96
- Modular



Workshop 1

- 18 participants
 - 2 academia
 - 11 government employees
 - 2 private industry
 - 3 graduate students



Workshop 1 Highlights

- Day 1 optional PCR and pipetting bootcamp
- Day 2 Overview of qPCR, EPA presentation Q&A, prep of cell standards
- Day 3 Pipetting proficiency, LT Step One Plus presentation, standard curve 1611, method differences, collect/process std curve data and cell std culture results
- Day 4 BR CFX presentation and std curve 1611, Interference and troubleshooting lecture, collect /process std curve data
- Day 5 BioGx SC presentation and std curve SampleReady, QA/QC, EPA Q&A, collect /process std curve data, real samples
- Day 6 MIQE, MST, dct/ddct calculations, vendor show



Workshop 1

- Lecture hours
 - 24 hours in classroom
 - Includes 1.5 hours of optional PCR Boot Camp
 - Lectures taught during lunch
- Laboratory hours
 - 15.25 hours
 - Includes 3 hours of optional PCR Boot Camp



Workshop 1 Participant feedback

n=14

THE GOOD

- 94% Increased knowledge of molecular techniques

THE BAD

- 66% Felt laboratory space inadequate
- 64% Would be comfortable teaching material learned

THE UGLY

- Requested more hands on time, EPA material and presentation confusing, few participants read material beforehand



Workshop 1 Instructor feedback

- Too crowded
- Focus too broad
- Participants did not have clear grasp of data calculation worksheets, inhibition, or EPA material
- Vendor show held on Friday afternoon, but most participants did not attend
- Some participants left before workshop ended

Workshop 2

- November 3rd-8th, 2013
- 7 participants
 - 2 academia
 - 5 government labs





Workshop structure

- Train participants to perform water quality monitoring for Enterococcus using EPA methods 1609 and 1611 and other qPCR methods for Enterococcus and *E. coli*.
- Train participants on multiple thermal cycler formats
- Exposure to different mastermix formulations
- Technology transfer to Molecular Source Tracking
- MIQE guidelines
- QA/QC, PCR theory, dCt, ddCT



Workshop 2

- Lecture hours
 - 15 scheduled hours in classroom
- Laboratory hours
 - 16 + hours
- PCR Boot Camp required for all, regardless of experience



Workshop 2 Highlights

- Day 1 **PCR and pipetting Boot Camp**, prep samples and stds for week
- Day 2 Review of qPCR, EPA criteria, LT demo/hands on
- Day 3 BioGx demo/hands on, BioRad demo/hands on
- Day 4 Data analysis, dct/ddct intro, **vendor show**
- Day 5 **Invited speaker-Implementation of Rapid Methods, Panel discussion EPA policy/implementation**, real samples
- Day 6 MIQE, MST, dct/ddct calculations, troubleshooting



Workshop 2 Participant feedback

n=5

Positive feed back (rating of extremely or satisfied)

- 100% Increased knowledge of molecular techniques
- 100% Would be comfortable teaching material learned
- 100% Extremely satisfied with workshop facilities
- 100% Workshop well organized
- 100% Pleased with instructor presentation and interaction



Workshop 2 Participant feedback

- Negative feedback
 - Methods 1609 and 1611 still confusing
 - participants had trouble reading and understanding methods as written in the EPA documents
 - Dct/ddct worksheet confusing
 - Inhibition unclear



Workshop 2 Instructor Feedback

- Reduced level of participants allowed for more individualized instruction
- More focused and streamlined lectures reduced participant confusion
- Basic data calculations and analysis not clearly understood by all
- Dct/ddct worksheet and inhibition not clearly understood



Recommendations

- Reduced class size
- Individualized instruction
- Flexible format to cater to comprehension of basic concepts and varying levels of expertise
 - Multiple teaching strategies
 - Basic concepts may need to be repeated
- Hands on time
 - Laboratory
 - Instrument
 - Making mastermix and dilutions
 - Data calculations



Recommendations

- Expect the unexpected
 - Equipment failure
 - Extra reagents
 - Diagrams and clearly labeled reagents
 - Step by step instructions
- Continued support after the workshop essential
 - By email, phone calls, extra time in the lab
- EPA methods 1611 and 1609 need to be streamlined for the unexperienced user
 - Checklist or decision tree
 - Video (SCCWRP)
 - EPA tech support hotline



NEW

- EPA now offering workshop, May 12th-16th
- Teaches methods 1609 and 1611
- NO COST
- New control (plasmid std)
- Discussion of 2012 RWQC document
- No basic PCR theory will be taught
 - Impedance to those with no prior PCR experience but may benefit from rapid method



Questions?

Thanks to member of Noble lab (past and present) :

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Kellen Lauer

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