Implementation of *E. coli* qPCR-based Method for Water Quality Monitoring Case Study: Challenges and Lessons learned

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Introduction

Quantitative polymerase chain reaction (qPCR) methods provide same-day enumeration of fecal indicator bacteria (FIB) in recreational waters. However, successful implementation requires extensive knowledge of the analytical procedure, rigorous staff training, and possible reorganization and reconstruction of physical laboratory space to optimize work flow. Hence, the widespread implementation of qPCR may be limited by the amount of resources and guidance available to first time users. In this case study, the qPCR-proficient City of Racine Health Department (RHD) (WI, USA) provided implementation guidance, staff training, and result evaluation (*E. coli*/ qPCR at three public bathing beaches) to the Wilmette Water Plant (WWP) (IL, USA), a facility with no previous experience in rapid molecular techniques.

Objective

- To successfully implement a qPCR-based *E. coli* method into routine, regulatory monitoring at three recreational beaches utilizing a facility with no previous experience in rapid molecular methods.

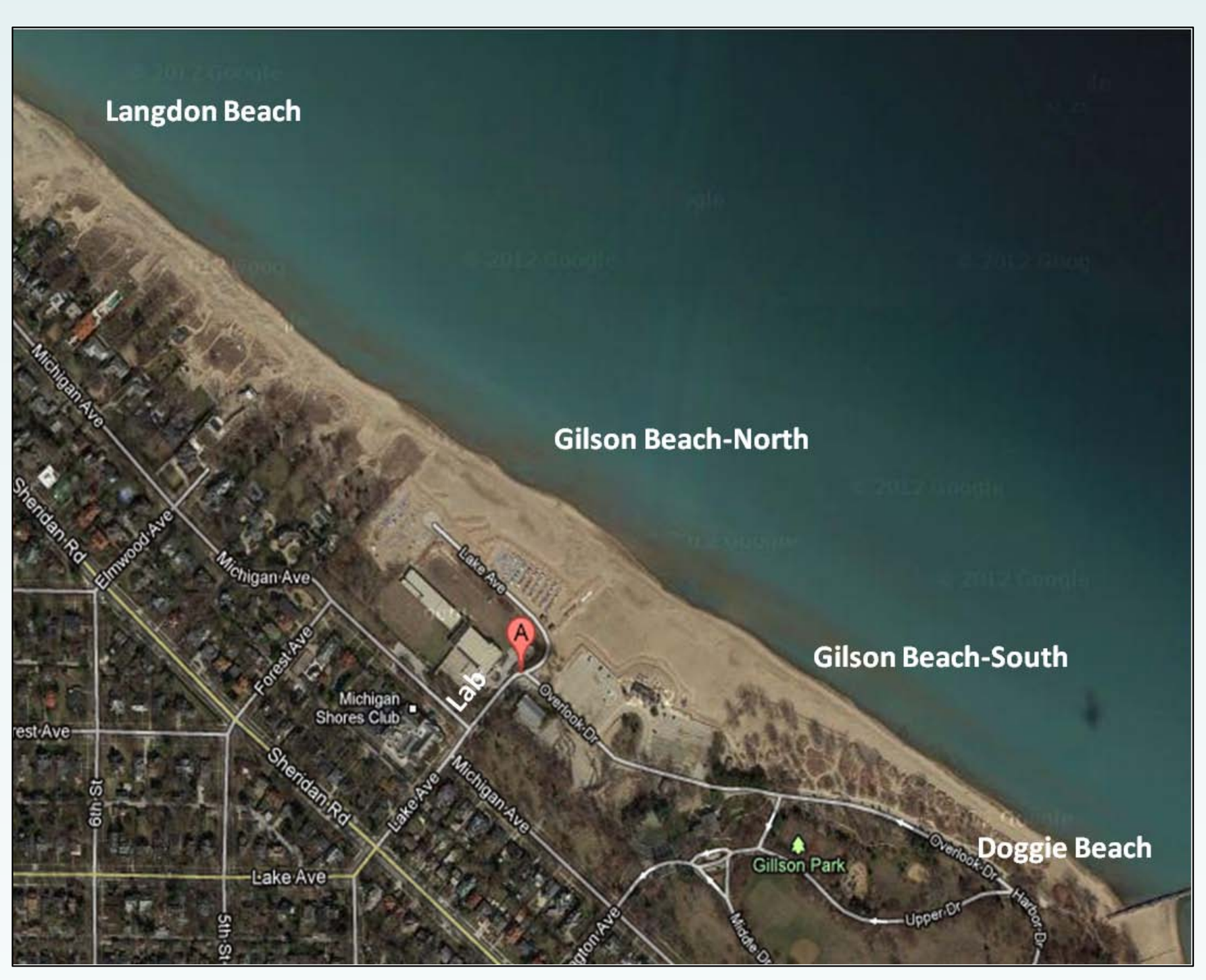
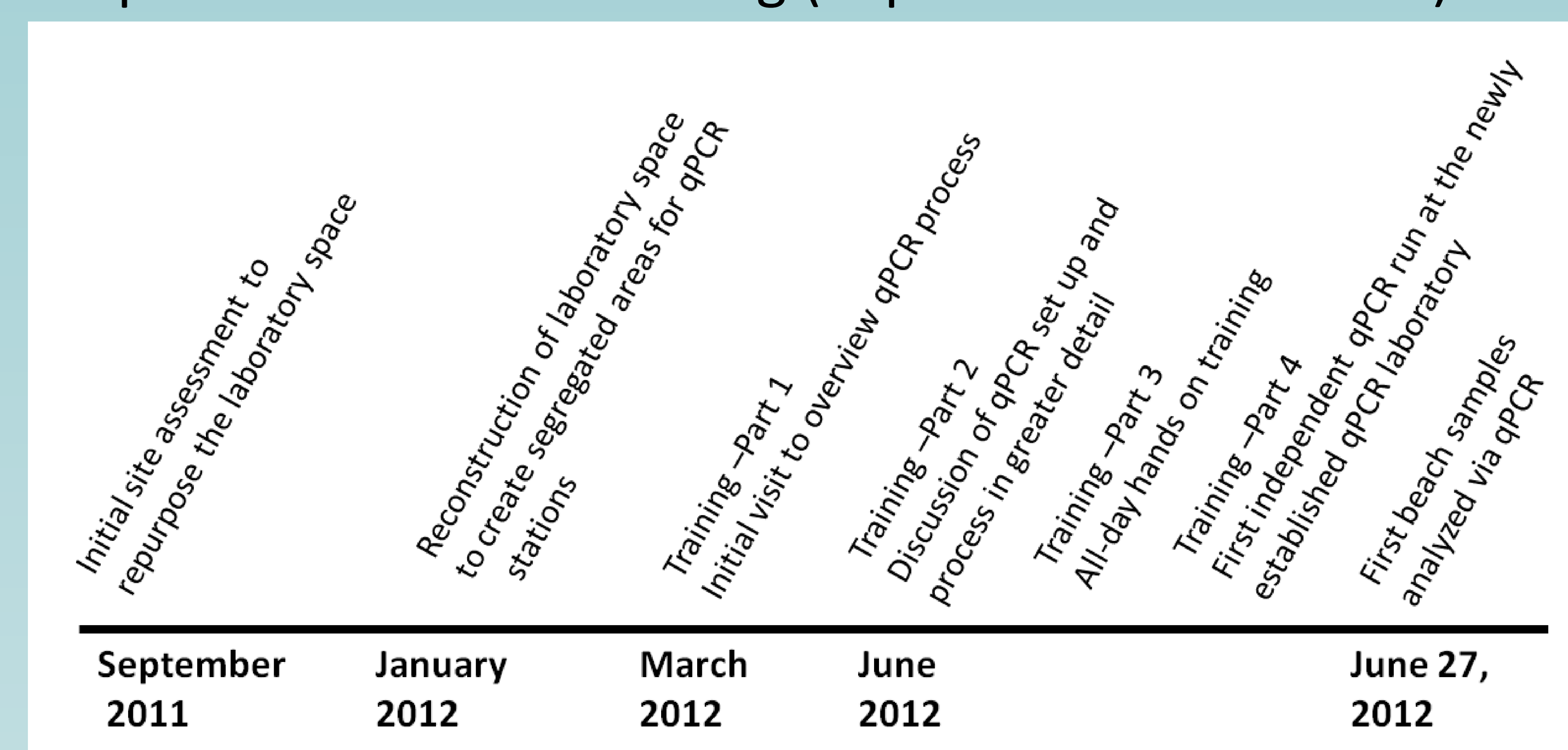


Figure 1. Wilmette Water Plant Laboratory, Village of Wilmette, IL, USA (A) and three public bathing beaches (Langdon, Gilson-N and Gilson-S).

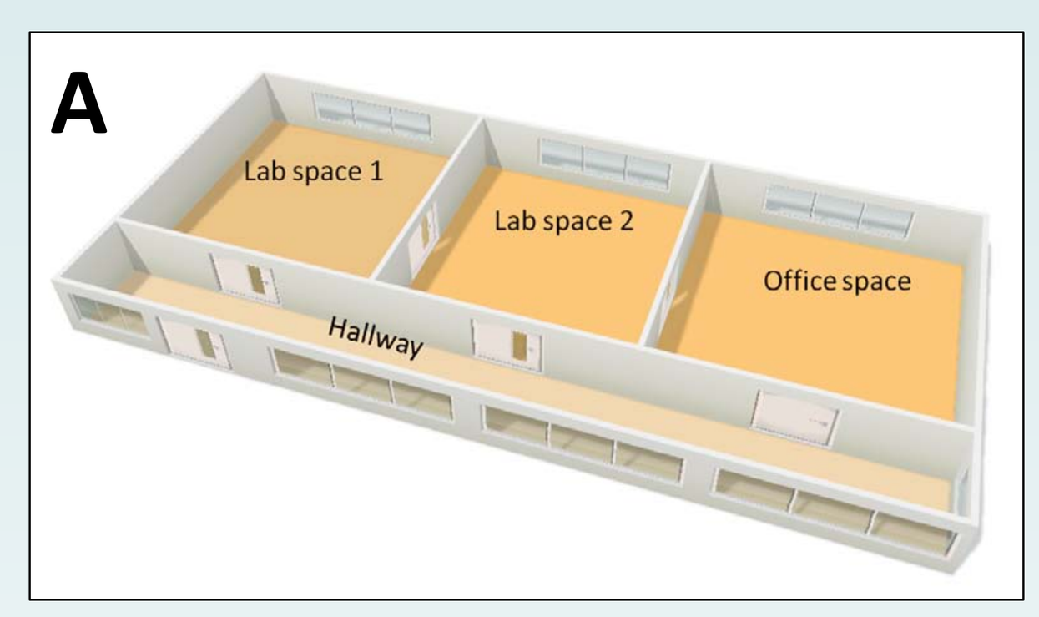
Approach

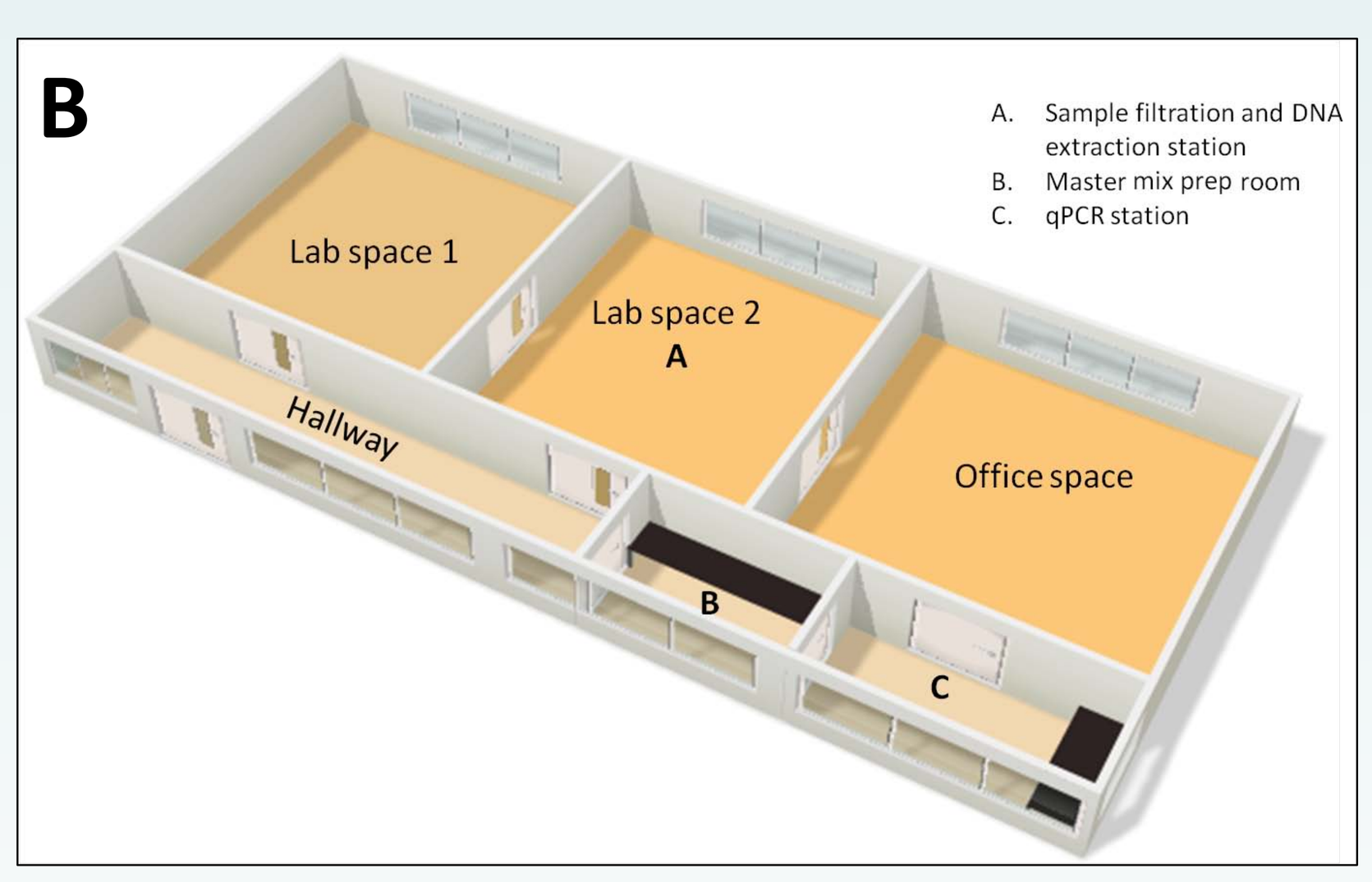
- Site assessment, reconstruction, instrument and supply acquisition and staff training (Sept. 2011 – June 2012)



- Samples collected by lifeguards and analyzed by culture and qPCR (June 27 - Aug. 28, 2012)
  - Crude DNA extract (1:5 dilution), BioGx Smart Beads™, Cepheid Smart Cycler® II platform
  - IDEXX Colilert®-18
- Beach Sanitary Survey (BSS) data collected (July 20 – Aug. 31, 2012)
- Split samples testing performed using single source of reagents (*E. coli* calibrators and SPC, provided by RHD) (Oct. 2012).

Figure 2. Laboratory space before (A) and after construction (B). Repurposed space includes segregated stations for unidirectional workflow.





Results

Site	Pearson's correlation coefficient (*r*)	ANOVA (P-value)	Beach management decision agreement (%)
Gilson Beach North	0.264	0.767	74.3%
Gilson Beach South	0.079	0.491	74.3%
Langdon Beach	0.059	0.165	79.3%

Table 1. Relationship between culture and qPCR methods. Log 10 E. coli CCE/100 ml (qPCR) and MPN/100 ml (culture).

Site	Total number of sampling events	Number of inhibition events	Frequency of inhibition (%)
Gilson Beach North	39	4	10.3
Gilson Beach South	39	4	10.3
Langdon Beach	36	7	19.4

Table 2. Frequency of inhibition events by site.

Results

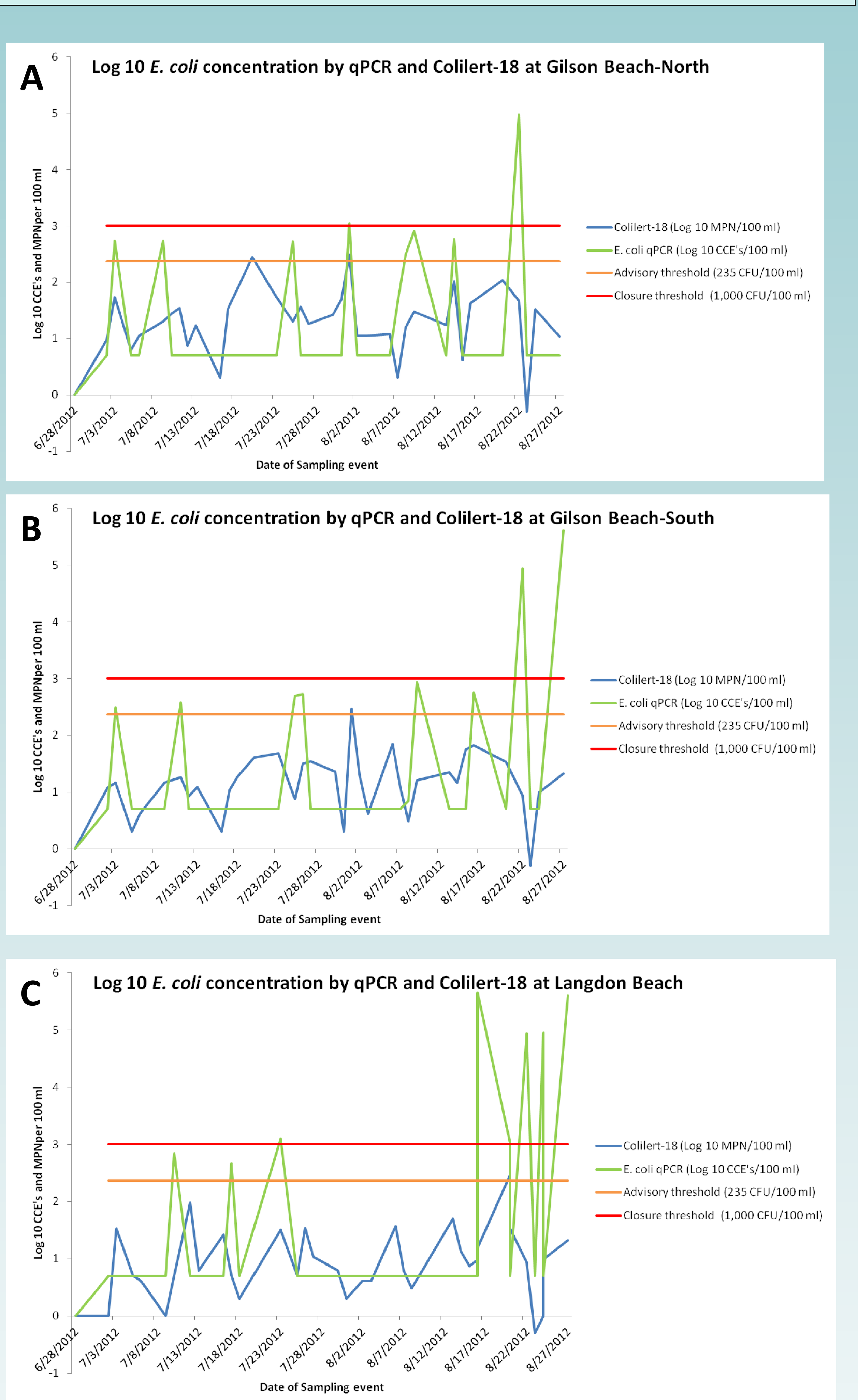


Figure 3 (A-C). Log 10 *E. coli* density in beach samples as quantified by culture and qPCR methods in relation to 1986 U.S. EPA recreational water quality criteria.

- Results showed that although numerical correlation between culture and qPCR methods was low (0.059 ≤ r ≤ 0.264) and there were fewer beach advisories and closures using the culture method (Fig. 3), overall beach management decision agreement between culture and qPCR was relatively high at all three sites (74-79%).
- Split sample testing showed no significant difference (p=0.985) in results between laboratories.
- Challenges of qPCR implementation include:
  - Frequent instances of unresolved qPCR inhibition (10-19%)
  - Disagreement between sample replicates (approaching 14%)
  - Lack of BSS data (may be informative for resolving inhibition)

Conclusion

- This case study data demonstrated that successful implementation at a laboratory with no previous experience in rapid molecular techniques is possible. However, additional training and QA/QC work is needed.

Acknowledgements

This project was supported in part by an appointment to the Internship Program at the Office of Water, U.S. Environmental Protection Agency, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and U.S. EPA.

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