2014 WATER MICROBIOLOGY CONFERENCE
MICROBIAL CONTAMINANTS FROM WATERSHEDS TO HUMAN EXPOSURE – May 2014

http://watermicroconference.web.unc.edu/

Cross-laboratory Comparison of a Duplex Digital PCR Assay for Simultaneous Quantification of Enterococcus spp. and Human Fecal-associated HF183 Marker

Yiping Cao[1], Meredith Raith[1], Tania Madi[2], Mauricio Larenas[2], John Griffith[1]

[1]*Southern California Coastal Water Research Project, Costa Mesa, California*
[2]*Source Molecular, Miami, Florida*

Abstract

Quantitative PCR (qPCR) provides indirect quantification by comparing unknown samples to known standards. While its speed and flexibility led to its wide use in water monitoring, the lack of reliable and consistent quantitative standards remains the biggest obstacle for qPCR applications, particularly in scenarios where comparison and integration of results across laboratories and studies are essential. Digital PCR provides direct, standards-free quantification based on limiting dilution and Poisson statistics and therefore eliminates quantification biases associated with inconsistent standards. Nevertheless, little work has been done to assess inter-laboratory consistency of digital PCR results. Here, we compared the performance of a duplex droplet digital PCR (ddPCR) assay targeting Enterococcus spp. and the HF183 marker across two laboratories. Each laboratory analyzed, in duplicate, DNA aliquots of standards, sewage and septage samples from 4 states, composite animal fecal samples for each of the 9 sources (cow, elk, deer, dog, horse, chicken, duck, goose, and raccoon) collected in 7 states, and ambient waters from southern California. Basic assay performance metrics such as linearity and detection limits of the ddPCR assay were similar between the laboratories regardless of quantification targets. For the human fecal-associated marker, both laboratories could quantify HF183 down to approximately 0.005ng sewage DNA and 0.05ng septage DNA. Neither laboratory showed cross creativity with any animal fecal DNA when tested at 2.5 ng per reaction except for two cow composite samples. The interlaboratory difference and intralaboratory variability were similarly low. The mean interlaboratory differences for Enterococcus spp. and HF183, respectively, were 0.06-0.15 and 0.004-0.17 log10 unit for various samples tested, much lower than those reported for qPCR (0.5-2 log10 unit) due to inconsistency in standards. Overall, the duplex ddPCR assay demonstrated stable performance and highly reproducible results across the two laboratories. While further studies with a larger number of laboratories would provide more information on ddPCR's reproducibility, initial evidence from this study showed that ddPCR could significantly enhance our ability to compare and integrate monitoring results across studies/sites/laboratories.