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Effect of platform, reference material, and quantification model on enumeration of *Enterococcus* by quantitative PCR methods

Yiping Cao, Mano Sivaganesan[1], Julie Kinzelman[2], A. Denene Blackwood[3], Rachel T. Noble[3], Richard A. Haugland[4], John F. Griffith and Stephen B. Weisberg

[1]*United States Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH*

[2]*City of Racine, Racine, WI*

[3]*University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, NC*

[4]*United States Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH*

ABSTRACT

Quantitative polymerase chain reaction (qPCR) is increasingly being used for the quantitative detection of fecal indicator bacteria in beach water. QPCR allows for same-day health warnings, and its application is being considered as an option for recreational water quality testing in the United States (USEPA, 2011. EPA-OW-2011-0466, FRL-9609-3, Notice of Availability of Draft Recreational Water Quality Criteria and Request for Scientific Views). However, transition of qPCR from a research tool to routine water quality testing requires information on how various method variations affect target enumeration. Here we compared qPCR performance and enumeration of enterococci in spiked and environmental water samples using three qPCR platforms (Applied Biosystem StepOnePlus™, the BioRad iQ™5 and the Cepheid SmartCycler® II), two reference materials (lyophilized cells and frozen cells on filters) and two comparative CT quantification models (ΔCT and ΔΔCT). Reference materials exerted the biggest influence, consistently affecting results by approximately 0.5 log(10) unit. Platform had the smallest effect, generally exerting <0.1 log(10) unit difference in final results. Quantification model led to small differences (0.04-0.2 log(10) unit) in this study with relatively uninhibited samples, but has the potential to cause as much as 8-fold (0.9 log(10) unit) difference in potentially inhibitory samples. Our findings indicate the need for a certified and centralized source of reference materials and additional studies to assess applicability of the quantification models in analyses of PCR inhibitory samples.