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

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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 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 iQTM5 and the Cepheid SmartCycler® II), two reference materials (lyophilized cells and frozen cells on filters) and two comparative CT quantification models (Δ CT and $\Delta\Delta$ CT). Reference materials exerted the biggest influence, consistently affecting results by approximately 0.5 log₁₀ unit. Platform had the smallest effect, generally exerting <0.1 log₁₀ unit difference in final results. Quantification model led to small differences (0.04 to 0.2 log₁₀ unit) in this study with relatively uninhibited samples, but has the potential to cause as much as 8-fold (0.9 log₁₀ 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.

Full Text

http://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_261_272.pdf