SCCWRP #920

Absolute Quantification of Enterococcal 23S rRNA Gene Using Digital PCR

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ABSTRACT

We evaluated the ability of chip-based digital PCR (dPCR) to quantify enterococci, the fecal indicator recommended by the United States Environmental Protection Agency (USEPA) for water-quality monitoring. dPCR uses Poisson statistics to estimate the number of DNA fragments in a sample with a specific sequence. Underestimation may occur when a gene is redundantly encoded in the genome and multiple copies of that gene are on one DNA fragment. When genomic DNA (gDNA) was extracted using two commercial DNA extraction kits, we confirmed that dPCR could discern individual copies of the redundant 23s rRNA gene in the enterococcal genome. dPCR quantification was accurate when compared to the nominal concentration inferred from fluorometer measurements (linear regression slope = 0.98, intercept = 0.03, R^2 = 0.99, and p value <0.0001). dPCR quantification was also consistent with quantitative PCR (qPCR) measurements as well as cell counts for BioBall reference standard and 24 environmental water samples. qPCR and dPCR quantification of enterococci in the 24 environmental samples were significantly correlated (linear regression slope =1.08, R^2 of 0.96, and p value <0.0001); the group mean of the qPCR measurements was 0.19 log units higher than that of the dPCR measurements. At environmentally relevant concentrations, dPCR quantification was more precise (i.e., had narrower 95% confidence intervals than qPCR quantification). We observed that humic acid caused a similar level of inhibition in both dPCR and qPCR, but calcium inhibited dPCR to a lesser degree than qPCR. Inhibition of dPCR was partially relieved when the number of thermal cycles was increased. On the basis of these results, we conclude that dPCR is a viable option for enumerating enterococci in ambient water.