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Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment

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ABSTRACT

Despite wide application to beach water monitoring and microbial source identification, results produced by quantitative PCR (qPCR) methods are subject to bias introduced by reliance on quantitative standards. Digital PCR technology provides direct, standards-free quantification and may potentially alleviate or greatly reduce other qPCR limitations such as difficulty in multiplexing and susceptibility to PCR inhibition. This study examined the efficacy of employing a duplex droplet digital PCR (ddPCR) assay that simultaneously quantifies Enterococcus spp. and the human fecal-associated HF183 marker for water quality assessment. Duplex ddPCR performance was evaluated side-by-side with qPCR and simplex ddPCR using reference material and 131 fecal and water samples. Results for fecal and water samples were highly correlated between ddPCR and simplex qPCR (coefficients > 0.93, $p < 0.001$). Duplexing Enterococcus and HF183 in qPCR led to competition and resulted in non-detection or underestimation of the target with low concentration relative to the other, while results produced by simplex and duplex ddPCR were consistent and often indistinguishable from one another. ddPCR showed greater tolerance for inhibition, with no discernable effect on quantification at inhibitor concentrations one to two orders of magnitude higher than that tolerated by qPCR. Overall, ddPCR also exhibited improved precision, higher run-to-run repeatability, similar diagnostic sensitivity and specificity on the HF183 marker, but a lower upper limit of quantification than qPCR. Digital PCR has the potential to become a reliable and economical alternative to qPCR for recreational water monitoring and fecal source identification. Findings from this study may also be of interest to other aspects of water research such as detection of pathogens and antibiotic resistance genes.