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A Duplex Digital PCR Assay for Simultaneous Quantification of the *Enterococcus spp.* and the Human Fecal-associated HF183 Marker in Waters

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ABSTRACT

This manuscript describes a duplex digital PCR assay (EntHF183 dPCR) for simultaneous quantification of *Enterococcus spp.* and the human fecal-associated HF183 marker. The EntHF183 duplex dPCR (referred as EntHF183 dPCR hereon) assay uses the same primer and probe sequences as its published individual quantitative PCR (qPCR) counterparts. Likewise, the same water filtration and DNA extraction procedures as performed prior to qPCR are followed prior to running dPCR. However, the duplex dPCR assay has several advantages over the qPCR assays. Most important, the dPCR assay eliminates the need for running a standard curve and hence, the associated bias and variability, by direct quantification of its targets. In addition, while duplexing (*i.e.* simultaneous quantification) *Enterococcus* and HF183 in qPCR often leads to severe underestimation of the less abundant target in a sample, dPCR provides consistent quantification of both targets, whether quantified individually or simultaneously in the same reaction. The dPCR assay is also able to tolerate PCR inhibitor concentrations that are one to two orders of magnitude higher than those tolerated by qPCR. These advantages make the EntHF183 dPCR assay particularly attractive because it simultaneously provides accurate and repeatable information on both general and human-associated fecal contamination in environmental waters without the need to run two separate qPCR assays. Despite its advantages over qPCR, the upper quantification limit of the dPCR assay with currently available instrumentation is approximately four orders of magnitude lower than that achievable by qPCR. Consequently, dilution is needed for measurement of high concentrations of target organisms such as those typically observed following sewage spills.