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Testing of General and Human-Associated Fecal Contamination in Waters

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ABSTRACT

qPCR has become increasingly popular for microbial water quality testing because it is faster, more specific, and more flexible than culture-based methods. However, qPCR method limitations such as quantification bias introduced by reliance on standards and susceptibility to PCR inhibitors are major obstacles for implementation in water testing. This is because water testing requires accurate quantification of rare targets and because environmental waters often contain PCR inhibitors. Digital PCR offers the opportunity to maintain qPCR's advantages over culture-based methods while ameliorating two of qPCR's major limitations: the necessity to run standard curves and high susceptibility to inhibition. Here we describe a complete method for simultaneous testing for a general microbial water quality indicator (*Enterococcus* spp.) and a human-associated fecal marker in environmental waters. The complete method includes water sampling and filtration to capture bacteria, DNA extraction from bacteria captured on the filter, and droplet digital PCR to quantify the genetic markers from bacteria indicative of general and human-associated fecal contamination.