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Droplet digital PCR quantification of Human Adenovirus

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Abstract

Over 90% of the waterborne illnesses are caused by enteric viruses. Because of their low infectious dose and our increased desire to use quantitative microbial risk assessment (QMRA) in water quality management, sensitive and accurate quantification tools for these viruses are needed. However, current methods based on qPCR are generally insensitive, and can be biased due to variation in standards and presence of inhibitory substances in ambient waters. Digital PCR, which is based on limiting dilution end point PCR and Poisson statistics, offers direct quantification with potentially higher sensitivity and accuracy than qPCR without the need for external standards. Here, we conducted digital PCR assays on a droplet digital PCR (ddPCR) system using published qPCR primer/probes sets targeting human Adenoviruses (HAdV). Commercial DNA standards and DNA extracts of primary influents collected over two months from five wastewater treatment plants in southern California were used for developing and evaluating the ddPCR assay. High linearity (R^2>0.98, p<0.001) was demonstrated by serial dilutions of both standard and sewage DNA and for both qPCR and ddPCR. Similar detection limits were also observed between qPCR and ddPCR assays at the per reaction level. However, ddPCR increased the effective analysis volume of DNA extracts by retrospectively merging multiple reactions using Poisson statistics and provided quantification at very low target concentrations where qPCR could not. Additionally, differential amplification efficiency was observed among standards and sewage samples. This led to biased qPCR quantification but had minimal impact on ddPCR quantification due to its binary nature (i.e. endpoint PCR). Given the lower titer of enteric viruses in environmental waters and the high genetic diversity of HAdV, the improved lower limit of quantification and accuracy by ddPCR compared to qPCR can greatly benefit monitoring for viruses and application of results to QMRA.