



Optimizing Molecular Methods to Detect Human Caliciviruses in Environmental Samples (Water Research Foundation Report)

Jan Vinje

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Among the human caliciviruses, noroviruses are the most common cause of acute gastroenteritis in humans. Exposure to contaminated water is considered a significant health risk because of the low minimal infectious dose. Many published molecular methods are based on the detection of noroviruses in stool samples and limited data is available on their limit of detection. This project attempted to develop novel and rapid real-time RT-PCR based methods for the sensitive detection of noroviruses in environmental water samples. The objectives of this project were to (1) develop and evaluate a carbohydrate ligand-binding assay for the purification of noroviruses from reverse transcriptase-polymerase chain reaction (RT-PCR) interfering substances; (2) develop a real-time RT-PCR assay for noroviruses; (3) compare and evaluate previously published and recently developed primer pairs targeting different regions of the norovirus genome in a conventional RT-PCR assay; and (4) use the developed methods for concentration, purification, and molecular detection to examine environmental samples (e.g., shellfish, source and finished water, sewage). In this study, the research team developed and evaluated novel methods for the concentration, detection, and genotyping of noroviruses from complex environmental matrices including (1) a novel assay based on H-type 1 histo-bloodgroup carbohydrate bound to magnetic beads, (2) a broadly reactive one-step TaqMan® RT-PCR assay for the detection of GI and GII noroviruses, and (3) hemi-nested conventional RT-PCR assay for genotyping of low-copy number of noroviruses. These methods were applied on naturally contaminated shellfish, raw and finished water, and sewage. The results indicated that the TaqMan® real-time RT-PCR assay is a superior method for rapid and simple monitoring of environmental waters for noroviruses.

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