

**DEVELOPMENT AND APPLICATION OF MOLECULAR TECHNIQUES FOR  
THE DETECTION OF HUMAN ENTERIC VIRUSES IN ENVIRONMENTAL  
SAMPLES**

**BY**

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## ABSTRACT

### DEVELOPMENT AND APPLICATION OF MOLECULAR TECHNIQUES FOR THE DETECTION OF HUMAN ENTERIC VIRUSES IN ENVIRONMENTAL SAMPLES

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Shellfish and water have been implicated in the transmission of viral diseases. National surveillance data on waterborne and foodborne illnesses underestimate the public health significance of these viruses. The focus of this research was to evaluate, develop, and apply molecular techniques to detect human enteric viruses in environmental samples.

The initial study evaluated a nucleic acid hybridization assay using a <sup>32</sup>P labeled cDNA probe to detect poliovirus in clams. Clams (*Mercenaria mercenaria*) were harvested from Narragansett Bay, Rhode Island. Poliovirus was detected in clams from the conditionally approved area in 3 of 9 collections and in the prohibited area in 4 of 9 collections. Once in the prohibited area, the coliform standards for water and shellfish were acceptable, although poliovirus was detected by the hybridization probe assay. This study demonstrated that nucleic acid hybridization can be used to detect

enteric viruses, but was limited by its degree of sensitivity.

There was a need to increase the sensitivity of viral diagnostics. Two alternative molecular tools were developed. First was the T7 polymerase-based amplification system designed to indirectly amplify target RNA without reverse transcriptase. The T7 amplification system contained a viral recognition sequence, a T7 polymerase recognition sequence and a detection sequence to generate RNA transcripts. Efficacy of the amplification sequence was measured by  $^{32}\text{P}$  incorporation in the RNA transcripts. Results demonstrated that the T7 amplification sequence was functional by its ability to produce radiolabeled RNA transcripts.

The second system developed was a nucleic acid isolation procedure that used magnetic bead technology. A biotinylated oligonucleotide probe was hybridized to poliovirus-RNA in solution. Streptavidin-coated magnetic beads were used to isolate the RNA-oligonucleotide hybrid. This procedure could recover viral RNA suitable for amplification by reverse transcription-polymerase chain reaction (RT-PCR). This technique was used to recover viral RNA from concentrated groundwater samples. Results indicated that this capture system was effective in both concentrating, and purifying poliovirus RNA while removing environmental RT-PCR inhibitors. A detection sensitivity of one plaque-forming unit in 250  $\mu\text{l}$  of a concentrated groundwater sample was routinely attained.