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In situ capture of RT-qPCR (ISC-RT-qPCR) method for detection of *human norovirus* in food and environmental samples

Statement of the Problem: Human noroviruses (HuNoVs) is the major cause for the non-bacterial acute gastroenteritis worldwide. RT-qPCR is a widely used method to detect HuNoVs. However, the method is unable to enrich virus from environmental samples and to discriminate between infectious and non-infectious viruses.

Methodology & Theoretical Orientation: In this study, we explored a new ISC-RT-qPCR to concentrate HuNoV, remove inhibitors and to estimate viral infectivity. This assay was based on capturing encapsidated HuNoV by viral receptors, followed by *in situ* amplification of captured viral genomes by RT-qPCR.

Findings: We demonstrated that the ISC-RT-qPCR did not capture and enable signal amplification of heat denatured Tulane Virus (TV) and HuNoVs. We then utilized the ISC-RT-qPCR to detect HuNoV in environmental water samples and food samples for comparison against that from a conventional RT-qPCR procedure. RT-qPCR inhibitors in oyster and environmental water samples were easily removed by multiple washes in ISC-RT-qPCR. 36 oyster samples from retail markets in Shanghai were detected for HuNoV by both assays. The detection rates of GI HuNoV in gill, digestive glands, and other tissues were 33.3%, 25%, and 19.4% by ISC-RT-qPCR; and were 5.6%, 11.1% and 11.1% by RT-qPCR. The ISC-RTqPCR is more sensitive than RT-qPCR for detection of HuNoV in oysters. However, HuNoV detection rate by ISC-RTqPCR was lower for environmental water samples. From 72 RT-qPCR tested positive samples for HuNoV only 20(27.8%) of samples tested positive by ISC-RT-qPCR, suggesting that 72.2% of RT-qPCR-positive water samples were unlikely to be infectious.

Conclusion & Significance: ISC-RT-qPCR is a better estimate for infectivity of HuNoV than RT-qPCT. A better detection rate by ISC-RT-qPCR in oysters indicating likelihood of infectious HuNoV accumulated in oyster and a poor detection rate of HuNoV in environmental water by ISC-RT-qPCR indicating that majority of RT-qPCR positive samples were from non-infectious viral RNA.

Biography

Peng Tian is an internationally recognized expert in molecular virology and viral pathogenesis. He is the first to identify rotavirus NSP4 protein as a viral enterotoxin. Since joining ARS in 2002, he initiated a new virology program in the unit to develop new assay systems for HuNoV detection in food samples, new systems to measure infectivity/inactivation of HuNoV and a new model for biological accumulation of HuNoV in oysters and fresh produce. He has defined fundamental principles and developed crucial assay systems relevant to the study of HuNoV. He was the first to determine that bioaccumulation of HuNoV occurs in oysters and through the identification of ligand like molecules in lettuce for HuNoV attachment, has extended the bioaccumulation theory to fresh produce. Also, he was the first to apply HBGA as part of a method to concentrate low-titer HuNoV from food and water samples.

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