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RT-qPCR (ISC-RT-qPCR) Method for Detection of Human Norovirus in Food and Environmental Sample

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Human noroviruses (HuNoVs) are the main cause of acute non- bacterial gastroenteritis worldwide. In this analysis, a novel in situ mediated viral receptor RT-qPCR (ISC-RT-qPCR) was used to detect HuNoVs in oysters and compared to the standard RT-qPCR system. Ten HuNoVs RT-PCR positive and 5 negative clinical samples from patients with gastroenteritis were used to equate the specificity and sensitivity of the ISC- RT-qPCR with that of the RT-qPCR assay. ISC-RT-qPCR had a one-log and two-log increase in sensitivity relative to the RT- qPCR studies for genotype I (GI) and GII, respectively. HuNoV concentrations in oyster tissues have been studied in artificially inoculated oysters. GI HuNoVs could be found in all tissues of inoculated oysters by both ISC-RT-qPCR and RT-qPCR. GII HuNoVs could only be found in gills and gastrointestinal glands by both methods. The number of viral genomic copies (vgc) calculated by ISC-RT-qPCR was comparable to RT- qPCR for the identification of GI and GII HuNoVs in inoculated oysters. Thirty-six samples of oyster from the local market were checked for both HuNoVs. More HuNoVs could be found in retail oysters by ISC-RT-qPCR. The GI HuNoV detection levels for gills, digestive glands and residual tissues were 33.3, 25.0 and 19.4 per cent for ISC-RT-qPCR; and 5.6, 11.1 and 11.1 per cent for RT-qPCR, respectively. The GII HuNoV detection rate in gills was 2.8 per cent by ISC-RT- qPCR; no GII HuNoV was found in these oysters by RT-qPCR. Overall, all results have shown that ISC-RT-qPCR is a promising method for the detection of HuNoVs in oyster samples.

Both studies involving clinical samples were conducted in the BSL-2 laboratory. Live stool samples were diluted in a 1:20 suspension in phosphate-buffered saline (PBS, pH 7.2, NaCl 137.0 mmol / L, KCl 2.7 mmol / L, Na2HPO4 10.0 mmol / L, KH2PO4 2.0 mmol / L), clarified by low-speed centrifugation (3,000 rpm) for 5 min, and processed as viral stocks at −80 ° C. Each sample was tested using RT-PCR with JV12/13 primers. The RT-PCR products have been sequenced by the Genewiz Bio- Technology Co. Ltd (Suzhou, China, USA).

Two chosen samples were used to compare the ISC-RT-qPCR and RT- qPCR sensitivities for GI and GII HuNoV and used for oyster inoculation. RT-qPCR was performed with extracted viral RNA followed by reverse transcription and qPCR amplification with the same primer sample sets used for ISC-RT-qPCR as mentioned above. For the RNA extraction process, RNA was extracted from 100.0 μL of clinical and oyster samples using the manufacturer's protocol RNA extraction package. The extracted RNA was air-dried and dissolved in 10.0 μL of diethyl-pyrocarbonate (DEPC) treated water prior to the reverse transcription response. The tissues of each oyster were divided into gills (G), digestive glands (D), including stomach, digestive diverticula, and residual tissues (O). PBS was applied to each sample (approximately 2.0 g) at a ratio of 4:1 followed by homogenization with the homogenizer (AES Chemunex, France) at 12,000 rpm for 1 min. Homogenized samples were combined with equivalent quantities of glycerol (50.0%) and processed at −80 ° C for potential use.

The identification of HuNoVs from food samples other than oysters has been difficult. Many infected food samples contained far fewer HuNoVs than oysters. Complicated processes are needed to concentrate viruses, release viral genomes, and extract RT-PCR inhibitors from different food matrixes. The ISC-RT-qPCR method could simplify steps in the concentration of viruses, viral extraction, removal of RT-PCR inhibitors with a higher sensitivity than the conventional RT- qPCR assay and have a high potential for use in food samples other than oysters. We are currently in the process of checking whether this approach could be used for the identification of HuNoVs in industrial and environmental samples.