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Development of a Probe Hybridization Method to Facilitate Detection of Noroviruses in Oysters

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Abstract

Centers for Disease Control (CDC) reports that at least 50% of all foodborne outbreaks of gastroenteritis are due to noroviruses (NoV). Since NoV is mainly transmitted through the fecal-oral route and the infectious dose may be as low as 10 viral particles, the risk of infection after consumption of raw or improperly cooked seafood or after exposure to contaminated water is considered high. Although highly sensitive methods to detect NoV using RT-PCR are already available, isolation of either NoV RNA or virions from shellfish remains a cumbersome process. We developed a new hybridization method to extract NoV RNA from contaminated shellfish that is much faster compared to existing methods. Using the new method, NoV detection includes three basic steps: an initial extraction of total RNA using TRIZol, followed by isolation of NoV RNA using biotinylated DNA probe hybridization and then NoV detection by TaqMan RT-PCR. With oyster (*Crassostrea virginica*) homogenate spiked with 100 PCR detection units (PDU) of NoV, the virus can be detected with C_T values at about 30. Compared to published methods that require an initial virus purification step, the new method is much faster, requiring approximately 3 hr compared to at least 8 hr using conventional methods. Coupled with TaqMan RT-PCR, the new method can be used to detect NoV in contaminated oysters and clams (*Corbicula fluminea*) within 8 hr. The detection limit was 100 PDU of NoV in spiked oyster tissue samples. The method has been successfully used to detect NoV in oysters artificially contaminated in the laboratory and in rare cases, oysters collected from the field.

Goal

Develop a faster method to detect noroviruses in shellfish using real-time PCR

Problems with existing methods

1. Most of the extracted RNA belongs to the oyster
2. Isolation of virus prior to RNA extraction takes too much time

Approach used

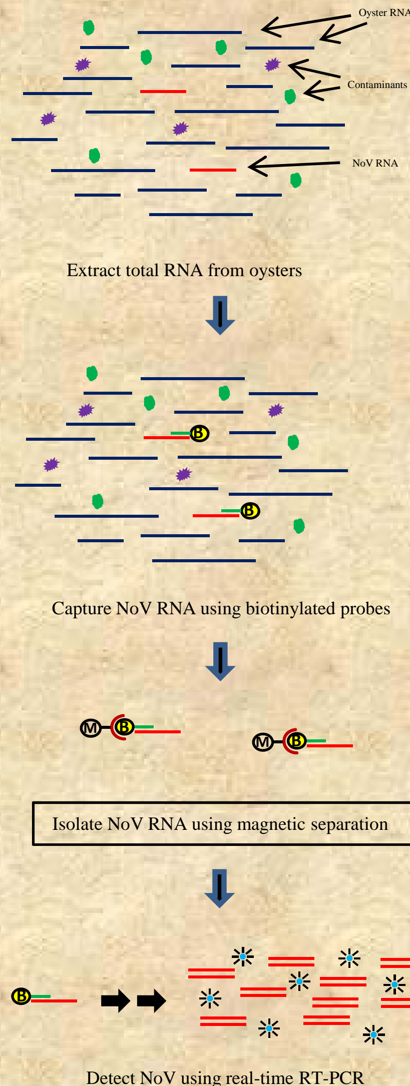
1. Extract total RNA from oyster
2. Enrich for norovirus RNA using probe hybridization
3. Detect noroviruses using real-time RT-PCR

Probes and primer sequences

NoV RNA capture probe	Biotin-TCGACGCCATCTTCATTCACA
[1]Forward primer - JJV2F	CAAGAGTCAATGTTTAGGTGGATGAG
[1]Reverse primer - COG2R	TCGACGCCATCTTCATTCACA
[1]qPCR probe - RING2-TP	FAM-TGGGAGGGCGATCGCAATCT-BHQ

[1]Kageyama et al. 2003. J Clin Microbiol 41:1548-1557

Assay Procedure



Extract total RNA from oysters

Capture NoV RNA using biotinylated probes

Isolate NoV RNA using magnetic separation

Detect NoV using real-time RT-PCR

Assay Optimization

NoV RNA can be captured quickly

	Duration	C_t values
Hybridization step	1 min	28.5
	15 min	27.7
	1 h	28.1
	2 hr	28.2
Capture step	1 min	32.5
	10 min	28.9
	30 min	28.1
	1 h	28.3

Circles: Recommended times

Assay condition variables

Biotinylated probe conc	0.1, 0.5 & **2*** pmol
Magnetic bead amount	10, 50 & **100** µg
Salt conc	0.4 , **0.8** & 1 M
Amounts of shellfish tissue homogenate used	**100** & 250 µL

*Bold: optimum conditions

The detection limit of the assay is 100 PDU

Amount of NoV spiked in oyster samples		C_t values
1 PDU	Sample A	Not detected
	Sample B	Not detected
10 PDU	Sample A	Not detected
	Sample B	Not detected
50 PDU	Sample A	Not detected
	Sample B	Not detected
100 PDU	Sample A	31.4
	Sample B	27.9

Results

Assay is more sensitive compared to two published methods

Experiments	Ct values using 3 different assays		
	Method A[1]	Method B[2]	This method
1	Not detected	32.6	22.2
	Not detected	33.3	22.2
2	Not detected	29.5	26.2
	Not detected	29.6	25.8
3	Not detected	30.6	28.3
	Not detected	31.1	26.0

[1]Baert et al. 2007 Lett Appl Microbiol 44: 106-111
[2]Beuret et al. 2003 Appl Environ Microbiol 69: 2292-2297

NoV detected in contaminated oysters

	Exposure duration	Sample	C_t values
Oysters exposed to NoV in the lab (2 x 10^3 PDU per L)	1 day	1	35.5
	1 day	2	29.9
	1 day	3	35.6
	3 days	1	Not detected
	3 days	2	32.6
	3 days	3	33.5
Natural oysters	Unknown	1	40.8
	Unknown	2	38.4

Summary

1. A more rapid method to prepare samples for NoV detection in oysters was developed using probe hybridization to isolate NoV RNA.
   - NoV RNA can be isolated more rapidly - 3 hr vs. 8 hr using other methods
   - NoV can be detected in 8 hr
   - Detection limit is 100 PDU
2. The method was used successfully to detect NoV in laboratory contaminated and natural oysters.
3. The method is the more rapid and sensitive than published methods that we tried.

E-mail us if you want to try the method

Acknowledgements

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