Poster # 1106

Development of Improved Synthetic Molecular Standards for Norovirus Genogroups I and II

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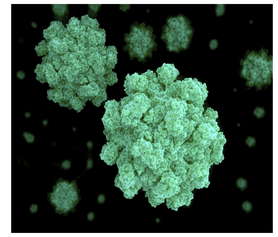


Background & Introduction

- Noroviruses (NoV) are the most common cause of epidemic gastroenteritis accounting for 95% of viral gastroenteritis outbreaks worldwide.
- NoV are difficult to detect due to their genetic heterogeneity and unculturable nature. Quantitative Reverse Transcription PCR (qRT-PCR) is used for the detection of NoV from clinical, food, and environmental samples.
- ATCC has improved existing first-generation qualitative synthetic molecular standards for NoV Genogroups I and II (NoV-GI and NoV-GII, respectively). These improved second-generation synthetic RNA standards are synthesized under ISO 13485:2003 guidance, quantified by Droplet Digital™ PCR (ddPCR™) and stabilized by the addition of RNAstable®.
- These second-generation synthetic molecular standards contain genome fragments from the ORF-1 and ORF-2 junctions from NoV-GI and NoV-GII and an additional fragment from the VP2 region for NoV-GII strains.
- In this study, we used the second-generation synthetic molecular standards for NoV-GI and NoV-GII to generate standard curves using two different primer sets; one used by the laboratories in the CaliciNet[1], and another recommended by the European Committee for Standards (CEN/TC/WG6/TAG4) working group[2].
- Furthermore, we used the respective standard curves to quantify the working reagents for NoV-GI and NoV-GII from National Institute for Biological Standards and Control (NIBSC) using the above primer sets.

Materials and Methods

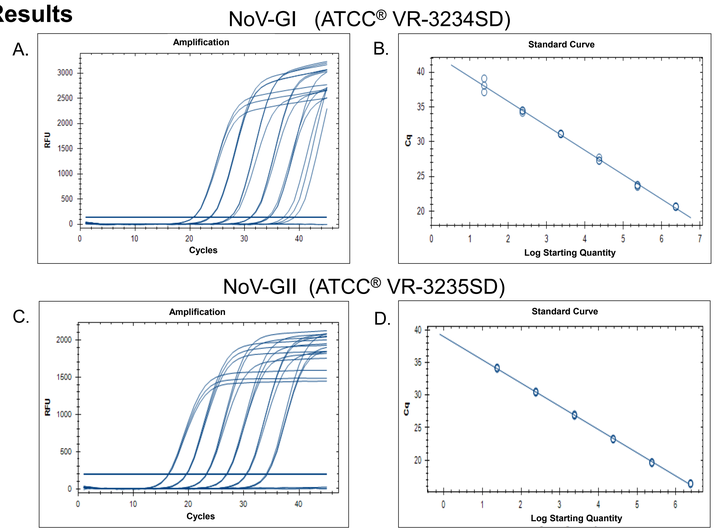
Reagents:
The following reagents were used in the qRT-PCR assays:

Second-Generation Synthetic Molecular Standards	
ATCC® No.	Description
VR-3234SD	Synthetic Norovirus GI RNA (NoV-GI)
VR-3235SD	Synthetic Norovirus GII RNA (NoV-GII)

- The first-generation synthetic molecular standards for NoV-GI and NoV-GII (ATCC® VR-3199SD and ATCC® VR-3234SD respectively) were from ATCC.
- The working reagents for NoV-GI and NoV-GII were obtained from NIBSC, UK. Viral RNA from the NoV-GI and NoV-GII working reagents was extracted using the QIAamp® Viral RNA Mini Kit (QIAGEN®). Viral RNA was assayed as undiluted samples and diluted 1:10 and 1:100 for the qRT-PCR assay. Each dilution was run in triplicate wells.

qRT-PCR assay:
qRT-PCR assays were performed using the CFX96™ Real-Time PCR Detection System (Bio-Rad). The primer and probe sets from the Real-Time RT-PCR assay used by laboratories in CaliciNet[1] and an assay recommended CEN/TC/WG6/TAG4 group[2] were employed to examine each individual NoV genotype using the following conditions. Cycling conditions for all primer sets were 50ºC for 15 min and 95ºC for 2 min, followed by 45 cycles of 95ºC for 15 sec and 60ºC for 60 sec for both the NoV-GI and NoV-GII assays. Standard curves were generated using serial ten-fold dilutions of the synthetic RNA standards, ranging from 20 copies to 2 x 10^6 copies/reaction. RNA samples and standards were tested in triplicate. The relative fluorescence unit (RFU) baseline threshold was set and genome copy numbers calculated using CFX Manager™ 3.0 Software (Bio-Rad).

Results



E.

Primer and Probe		NoV-GI	NoV-GII
CaliciNet Assay[1]	Slope	-3.513	-3.577
	R^2	0.996	1.00
CEN/TC/WG6/TAG4 Assay[2]	Slope	-3.692	-3.625
	R^2	0.987	0.998

Figure 1. Generation of Standard Curves from Second-Generation Synthetic Molecular Standards for NoV-GI and NoV-GII. A & C.) Amplification plots and **B & D.)** standard curves generated using the second-generation NoV-GI and NoV-GII synthetic molecular standards (ATCC® VR-3234SD and ATCC® VR-3235SD, respectively) with the CaliciNet primer and probe set[1]. **E.)** The slope and R^2 values generated using the second-generation NoV-GI and NoV-GII synthetic molecular standards with the primer and probe sets from the Real-Time RT-PCR Assay CaliciNet[1] and CEN/TC/WG6/TAG4[2] group.

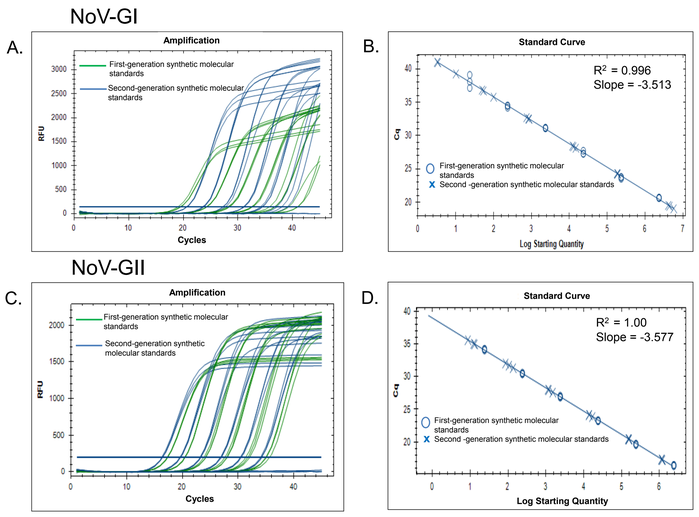


Figure 2. Comparison of First-Generation and Second-Generation Synthetic Molecular Standards. A) Amplification plot and **B)** standard curve generated using the first- and second-generation NoV-GI molecular standard ATCC® VR-3199SD and ATCC® VR-3234SD, respectively, versus the **C)** amplification plot and **D)** standard curve generated using the first- and second-generation NoV-GII synthetic molecular standards ATCC® VR-3200SD and ATCC® VR-3235SD, respectively, with the primer and probe set used by CaliciNet[1].

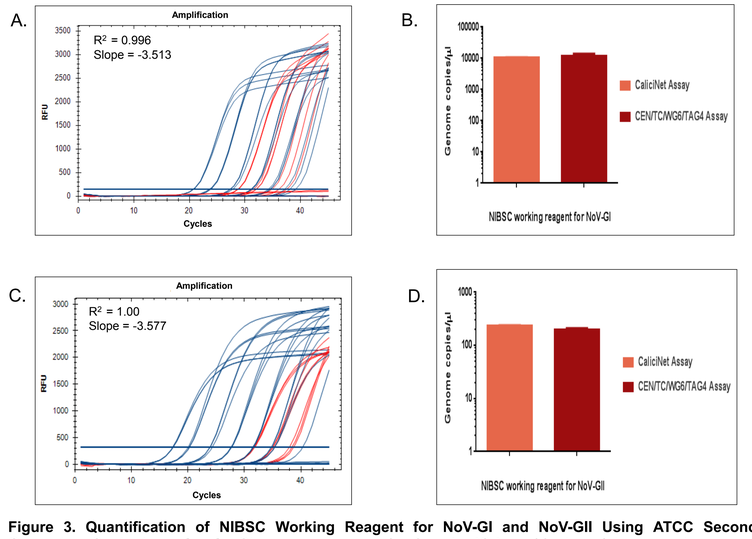


Figure 3. Quantification of NIBSC Working Reagent for NoV-GI and NoV-GII Using ATCC Second-Generation Synthetic Molecular Standards. An example of a qRT-PCR amplification plot showing the second-generation synthetic molecular standards (Blue) **A)** NoV-GI **C)** NoV-GII and the working reagent from NIBSC (Red). The NIBSC working reagent was used as an undiluted sample and diluted ten-fold twice and run in triplicate wells. **B & D)** Quantitation of the NIBSC working reagent for NoV-GI and NoV-GII samples respectively using the CaliciNet[1] and CEN/TC/WG6/TAG4 assay[2]. The average from triplicate wells from undiluted samples were used to calculate the quantities of NoV genome.

Salient features	Applications
• Fully authenticated & characterized ATCC® Genuine Nucleics • Generated under ISO 13485:2003 • Quantitative format • Functional with additional assays as compared to the first-generation synthetic molecular standards • BSL-1 ready-to-use control • Stabilized RNA	• Generation of a standard curve for qRT-PCR • Positive control for RT-PCR assays • Independent standard for validation and verification studies • Monitor assay-to-assay and lot-to-lot variation

Conclusion

The second-generation NoV synthetic molecular standards have been significantly improved over the first- generation synthetic molecular standards. The quantitative nature of these standards directly allows generation of standard curves for determination of NoV genome copies from unknown samples. Moreover, the improved design of synthetic construct provides ability to work with additional molecular assays for detection and quantification of NoV.

References

1. Vega *et al*. Novel surveillance network for Norovirus gastroenteritis outbreaks, United States. Emerg Infect Dis.17(8):1389-1395, 2011.
2. Le Guyader *et al*. Detection and quantification of noroviruses in shellfish. Appl Environ Microbiol 75(3): 618-624, 2009.

Disclaimers