Computational Design of a Synthetic Molecular Standard for Human Parechovirus 3

Poster # 10

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Synthetic Human Parechovirus 3 RNA (ATCC® VR-3260SD™)

Advantages	Applications
• Manufactured and authenticated with ISO13485:2016 compliance	• Generation of a standard curve for quantitative RT-PCR
• BSL-1 ready-to-use control	• Positive control for RT-PCR assays
• No shipping restrictions	• Assay verification and validation studies
• Quantitative format	• Monitor assay-to-assay and lot-to-lot variation
• Stabilized RNA	• Molecular diagnostics assay development

Background & Introduction

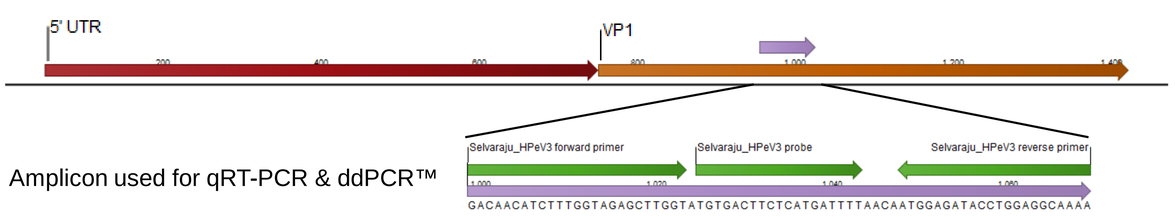
Human parechovirus 3 (HPeV3) has been increasingly identified in cases of aseptic meningitis among neonates and young infants less than 1 year of age, and is associated with paralysis, viral-like sepsis, central nervous system (CNS) infection, and sudden death. Because these clinical manifestations are similar to those associated with enterovirus infections, HPeV3 infections are often misdiagnosed, which in turn results in poor patient outcome. Therefore, molecular detection assays that provide a rapid and accurate diagnosis of HPeV3 are critical for ensuring prompt and appropriate treatment. Due to its sensitivity and quick turnaround time, the preferred method for the detection of RNA viruses is quantitative reverse-transcription PCR (qRT-PCR), which relies on the generation of a standard curve that is prepared using a quantitative viral RNA standard. To this end, we have designed, developed, and quantified a synthetic molecular standard for HPeV3 that is compliant with ISO 13485. This preparation is supported by stringent quality control analyses to ensure product identity, stability, and functionality with molecular applications, making it an ideal control for assessing assay performance and ensuring accurate and reproducible results. In the following proof-of-concept study, the HPeV3 synthetic molecular standard was quantified using Droplet Digital™ PCR (ddPCR™; Bio-Rad) and validated via qRT-PCR using published primers.[1]

Computational Design Strategy

Reference Sequence: HPeV3 Strain BJ-C317 Genome

- Genome sequence data collection
- Multiple sequence alignment
- Phylogenetic analysis
- Sequence editing and assembly
- Artificial RNA synthesis
- Sequence verification and authentication via next-generation sequencing
- Stabilization of RNA
- Absolute quantification via ddPCR™ assay
- Validation using qRT-PCR assay

Synthetic Design



Amplicon used for qRT-PCR & ddPCR™

Primer and Probe Sequences[1]

Forward Primer Sequence (5' to 3'): GAC AAC ATC TTT GGT AGA GCT TGG T
Reverse Primer Sequence (3' to 5'): TTT TGC CTC CAG GTA TCT CCA T
Probe Sequence (5' to 3'): TGT GAC TTC TCA TGA TTT T

ddPCR™ for Absolute Quantification

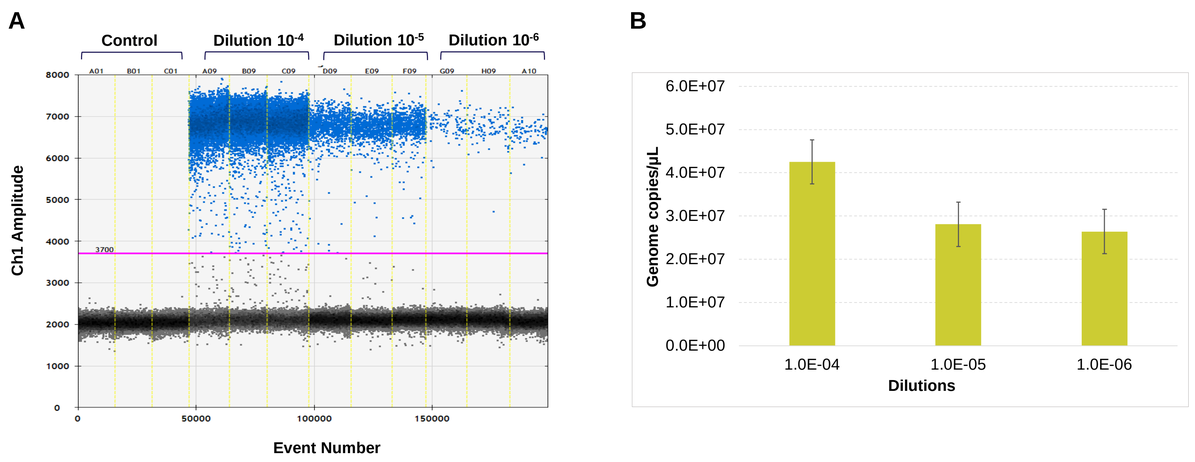


Figure 1. Absolute quantification using ddPCR™. (A) One-dimensional (1D) amplitude scatter plot of positive and negative ddPCR™ droplet reactions for 3 diluted standards (10^4 to 10^6) in triplicate. (B) Average genome copy number/µL per dilution. To ensure precise copy number, the synthetic molecular standard for HPeV3 was quantified by ddPCR™ using the same primers and probe as a published qRT-PCR assay.[1] ddPCR™ was performed as follows: initial denaturation at 95°C for 10 min, amplification over 40 cycles at 95°C for 30 sec and 57°C for 1 min, and enzyme deactivation at 98°C for 10 min. Droplets were analyzed in the QX200™ droplet reader (Bio-Rad). Data were analyzed with QuantaSoft™ software 1.7.4.0917 (Bio-Rad).

qRT-PCR Assay for Functional Testing

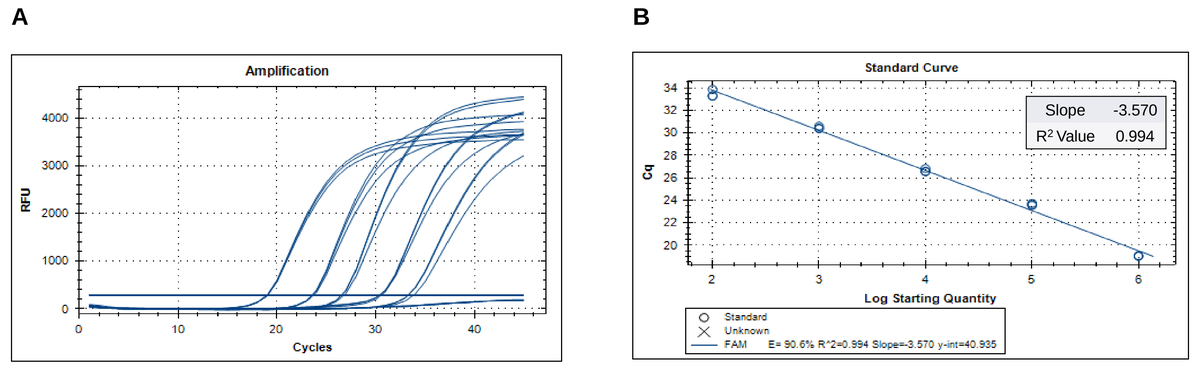


Figure 2. qRT-PCR assay to verify the functionality of the HPeV3 synthetic molecular standard. (A) An amplification plot and (B) standard curve were generated using the HPeV3 molecular standard. The qRT-PCR assay was performed as previously described[1]. Cycling conditions were 55°C for 30 min and 94°C for 2 min, followed by 45 cycles of 94°C for 15 sec and 60°C for 30 sec. The standard curve was generated using serial ten-fold dilutions, ranging from 100 to $1x10^6$ copies/µL. The RNA standard was tested in triplicate.

Conclusions

- Our data demonstrate the utility of a synthetic, quantitative RNA molecular standard for HPeV3 as a positive control in qRT-PCR assays.
- The quantitative format of the synthetic standard directly allows for the generation of a standard curve, enabling the quantification and detection of HPeV3 from unknown samples.
- This computational, synthetic approach can also be extended to other potential pathogens that are high-risk, uncultivable, or difficult-to-culture.

References

1. Selvaraju SB, *et al*. Optimization of a combined human parechovirus-enterovirus real-time reverse transcription-PCR assay and evaluation of a new parechovirus 3-specific assay for cerebrospinal fluid specimen testing. J Clin Microbiol 51(2): 452-458, 2013.

Disclaimers