thermo scientific

Preparation and Evaluation of MS2 Phage-Like Particles (PLP) as Full Process Quality Control for Molecular Assay

Cuijuan Tie, Dru Kutulas, Liang-Chun Liu, and Hui Wang. Clinical Diagnostics Division, Fremont, CA, Thermo Fisher Scientific

ABSTRACT

Introduction: Full process quality control (QC) material plays a crucial role in development of molecular assays for the detection of viral and cellular RNA targets; allowing evaluation of the entire workflow from nucleic acid extraction, amplification, through detection and data analysis. However, sourcing and maintaining the QC materials derived from clinical specimens is severely hampered by biosafety concerns, high cost, limited availability, and transport/trade issues, etc. To overcome these challenges, a recombinant MS2-PLP (MS2 Phage-Like Particle) carrying multiple pathogen target sequences, has been developed. Synthetic, highly customizable, non-infectious MS2-PLPs that are GMP-friendly, and cost effectively produced could constitute invaluable tools to support molecular diagnostic assay development, validation, and routine performance and quality monitoring.

Methods: Target-specific MS2-PLPs were generated through one-plasmid-driven expression system. First, heterologous RNA sequences from multiple target genes were expressed *in vivo* and encapsulated by MS2 viral coat protein, rendering the enclosed RNA resistant to ubiquitous nucleases. Next, the MS2-PLPs were purified and formulated into different matrices to mimic real patient samples. Finally, RNAs were extracted and quantified by digital PCR. Platform compatibility was examined by testing various extraction methods and several PCR instruments. The shelf-life was determined by accelerated and real-time stability. In use studies were assessed to determine freeze-thaw stability.

Results: Different target-specific MS2-PLPs were constructed using the one-plasmid-driven expression method, whereby one or more target sequences from various pathogens, covering over 2000 nucleotides, were successfully incorporated into the system. These targets were qualified and quantified by qPCR and digital PCR. Matrix formulations such as VTM (viral transportation medium) vs. Human plasma were evaluated and demonstrated that MS2-PLP is stable in both matrixes with comparable results. In addition, the accelerated stability results supported a shelf-life of up to 12 months when stored at -20˚ C. Real-time stability studies are confirmed, and all tested time points have passed stability criteria. The freeze-thaw stability study results support up to six freeze-thaw cycles after first use.

Conclusions: The MS2-PLPs were successfully generated to be used as full process QC materials to monitor the entire testing process for assay development, validation, and routine performance evaluation. The multiplexed target sequence design, incorporating several different pathogenic targets, allows MS2-PLP usage when native clinical specimens are inaccessible, highly infectious, or expensive. Moreover, MS2-PLP can be utilized as patient-like QC materials not only for viral and bacterial pathogens detection, but also for hot spot detection in sequencing-based assays for the cancer diagnostic field.

INTRODUCTION: Design and Development

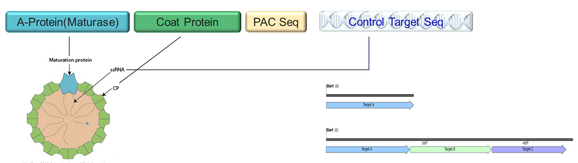
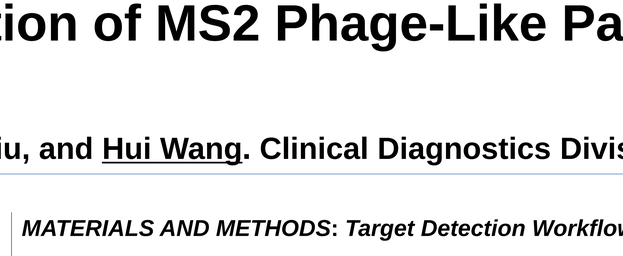


Figure 1. MS2-PLP Packaging Representative Diagram. Either one or multiple target genes can be designed in the control target sequence region.

MATERIALS AND METHODS: Target Detection Workflow

Figure 2. Workflow of MS2-PLP RNA Extraction, Detection by qPCR or Droplet Digital PCR and Data Analysis: KingFisher™ automated extraction instrument was used for RNA extraction. Both qPCR and Droplet digital PCR platform were performed with primers and probes targeting to target sequence to confirm and quantify each target.

Features:



Applications:

- MS2-PLP full process QC material are developed that can used to monitor the entire testing process from sample preparation till detection for assay development, validation, and routine performance evaluation.
- MS2-PLP can be implemented to mimic native clinical specimens, especially when they are inaccessible, highly infectious, or expensive.
- MS2-PLP can be utilized as patient-like QC materials not only for viral and bacterial pathogens detection, but also for hot spot gene detection in sequencing-based assays for the cancer diagnostic field

RESULTS AND DISCUSSION:

Figure 3. Workflow for MS2-PLP Production and Purification. Plasmid containing target gene was expressed in *E. coli* and MS2-PLP was purified by gel electrophoresis using Invitrogen™ E-gel™ system and proprietary method.

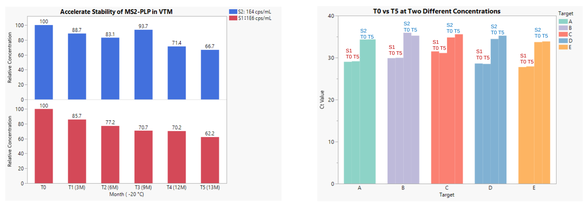


Figure 4. Accelerated Stability Study for Shelf-life Prediction.

MS2-PLP extraction was performed using KingFisher™ Apex automated extraction instrument with MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit. The accelerated stability testing was performed at 23 ºC with 2 different concentrations at 1E4 cps/mL and 1E6 cps/mL in VTM for 0, 3, 6, 9, 12, 13 months time interval equivalency using droplet digital PCR. In addition, the stability regarding different targets in the same construct were also evaluated between T0 (0 month) vs. T5 (13 months), which showed consistent results in Ct value by qPCR among all the targets.

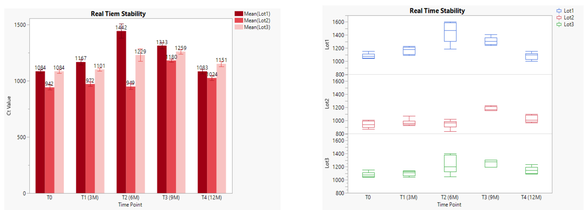


Figure 5. Real-time Stability Study.

MS2-PLP extraction was performed using KingFisher™ Apex automated extraction instrument. Two samples from 3 different lots were tested in triplicates with droplet digital PCR, the results are within the testing criteria (±20% of the original concentration) till 12 months.

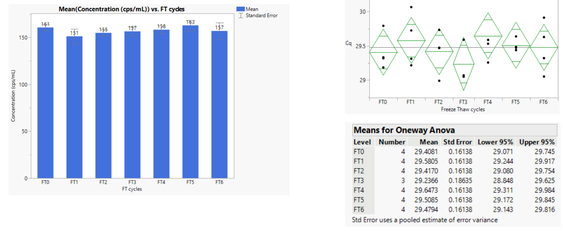


Figure 6. Freeze-Thaw Stability Study. The Freeze-thaw stability was performed to assess freeze-thaw cycles allowance for up to 6 times, and minimal of 4 hours apart to allow samples to be completely frozen. The performance was checked using both droplet digital PCR and qPCR. The acceptance criteria ensures that during the stability study, all the concentration are within ±20% of original concentration.

Figure 7. Other Matrix Evaluation by Accelerated Stability Study. MS2-PLP was spiked into Human Plasma at 2 different concentrations: 1E4 cps/mL and 1E6 cps/mL. The accelerated stability testing was performed at 23 ºC for 0, 3, 6, 9, 12, 13 months time interval equivalency using droplet digital PCR. The result showed MS2-PLP is stable in Plasma till 13 months, with ~50% decrease of the original concentration. In addition, both Plasma and VTM showed similar trend in accelerated stability.

CONCLUSIONS:

- **Either one or multiple target genes were successfully generated and detected in MS2-PLP.**
- **With above results, MS2-PLP is claimed 12 months shelf-life when stored at -20ºC based on both accelerated and real-time stability results in VTM.**
- **Freeze-thaw stability study were completed supporting that MS2-PLP is stable up to six Freeze-Thaw cycles.**
- **Matrix stability study indicated MS2-PLP is stable not only in VTM but also in Human Plasma.**

ACKNOWLEDGEMENTS

The authors would like to thank Nanette Umblas, Tami Sung, Yi Zhang, Nikita Le and Ran Hu for their help and support on the poster and project.

TRADEMARKS

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific • 46500 Kato Rd • Fremont, CA 94538 • thermofisher.com