Presentation/Poster Board Number: 023

Development and Validation of a Quantitative Synthetic Molecular Standard for *Coxiella burnetii*

Britany Tang, BS, Shirshendu Saha, MS*, Michael Geimer, BS, Kyle Young, MBA, and Dev Mittar, PhD* | ATCC, Manassas, VA 20110

Background and Introduction

Q fever is a highly infectious zoonotic disease caused by *Coxiella burnetii*, a group B biological warfare agent found worldwide that infects humans and a wide range of domestic and wild animals. This disease is associated with reproductive disorders in animals; in humans, symptoms can be mild or severe and may progress to pneumonia or chronic complications such as endocarditis, meningoencephalitis, and osteomyelitis. To control zoonotic transmission, accurate and sensitive detection is critical. Currently, real-time PCR assays are routinely used to ensure rapid detection and quantification; however, the accuracy and reproducibility of these assays are limited by the lack of precisely quantified controls. While genomic DNA can be used as a standard for these assays, *C. burnetii* is difficult to cultivate as it is a slow-growing obligate intracellular pathogen that requires BSL-3 facilities. To address this need, ATCC developed a quantitative synthetic molecular standard for *C. burnetti* (ATCC® BAA-4000SD™). As a proof-of-concept, we tested the functionality of the standard via qPCR with a published primer and probe set, and protocol.[1] To make high-containment pathogens more accessible, this approach was also extended to Nipah virus and Lassa virus.[2]

ATCC Synthetic Molecular Standards

BSL-1	Quantitative	Stabilized	ISO 13485

ATCC® No.	Description
BAA-4000SD™	Quantitative Synthetic *Coxiella burnetii* DNA
VR-3269SD™	Quantitative Synthetic Nipah virus RNA
VR-3268SD™	Quantitative Synthetic Lassa virus RNA

Applications

- Generation of a standard curve for quantitative PCR/ RT-PCR
- Positive control for PCR/ RT-PCR assays
- Assay verification and validation studies
- Monitor assay-to-assay and lot-to-lot variation
- Molecular diagnostics assay development

References & Acknowledgements

1. De Bruin A, *et al*. Detection of *Coxiella burnetii* in complex matrices by using multiplex quantitative PCR during a major Q fever outbreak in The Netherlands." Appl. Environ. Microbiol. 77(18): 6516-6523, 2011.
2. Jensen KS, *et al*. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLOS ONE 13(6): e0199534, 2018.
3. Thank you to the Molecular BioProduction and Molecular Laboratory Testing Service (MLTS) teams, and all those involved in the production and launch of these synthetic molecular standards.

Material and Methods

Quantitative Synthetic DNA
The synthetic standard was designed and synthesized using a proprietary method. The preparation includes fragments from the outer membrane protein (com1), isocitrate dehydrogenase (icd), transposase (IS1111A), DNA gyrase subunit A (gyrA), and superoxide dismutase (sodB) regions. The standard was then validated via next-generation sequencing and quantified via Droplet Digital™ PCR (ddPCR; Bio-Rad).

qPCR/ qRT-PCR Assay
qRT-PCR assays were performed on the CFX96™ Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's instructions with slight modifications.

Digital PCR Assay
Digital PCR assays were performed according to the manufacturer's instructions on the QX200™ droplet reader and QuantaSoft™ software 1.7.4.0917 (Bio-Rad) for droplet generation and data analysis.

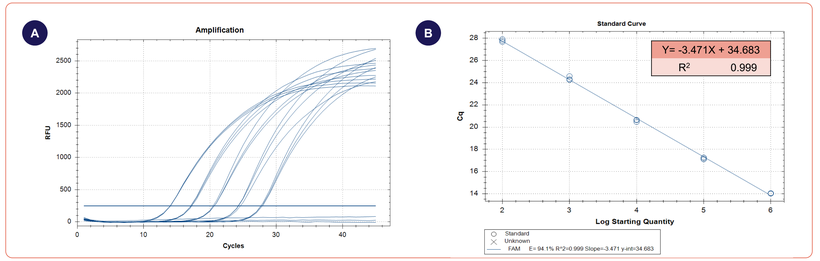

Figure 1. qPCR assay to verify the functionality of the synthetic molecular standard. An (A) amplification plot and (B) standard curve were generated with the *C. burnetii* synthetic molecular standard. The qPCR assay was performed as previously described.[1] Cycling conditions were 50°C for 2 min and 95°C for 2 min, followed by 45 cycles of 95°C for 15 sec and 60°C for 30 sec,. A standard curve was generated by using serial ten-fold dilutions that ranged from 1 × 10^2 to 1 × 10^6 copies/reaction. The DNA standard was tested in triplicate.

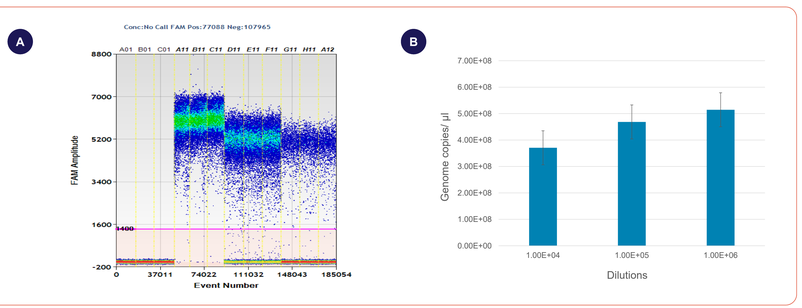

Figure 2. Absolute quantification of *C. burnetii* via digital PCR. (A) One-dimensional (1D) amplitude scatter plots of positive and negative digital PCR droplet reactions for 3 dilutions. (B) Average genome copy number/µL per dilution. *C. burnetii* was quantified by digital PCR by using the same primers and probe from the published qPCR assay.[1] Digital PCR was performed as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles at 94°C for 30 sec and 55°C for 1 min, and enzyme deactivation at 98°C for 10 min. Droplets were analyzed in the QX200 droplet reader. Data were analyzed with QuantaSoft software.

Extension of the Approach to Support Biodefense Research

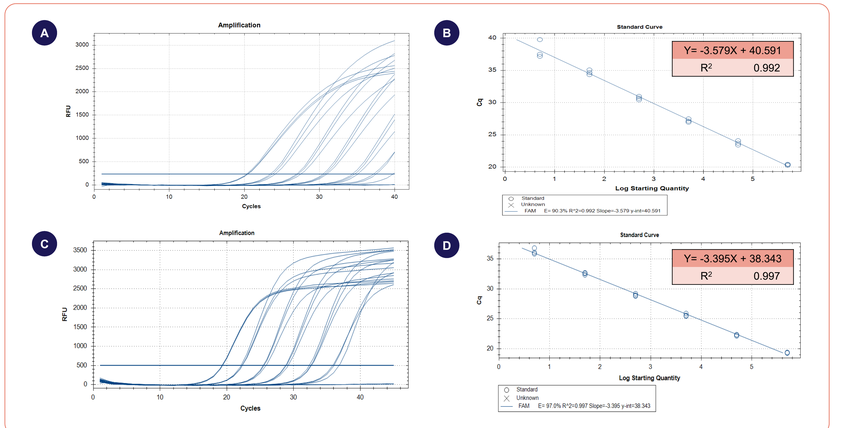

Figure 3. qRT-PCR assay to verify the functionality of synthetic molecular standards. (A, C) Amplification plots and (B, D) standard curves were generated with the Lassa and Nipah virus molecular standards, respectively. The qRT-PCR assay was performed using an assay designed in-house for Lassa virus and a published qRT-PCR assay for Nipah virus.[2] Cycling conditions were 50°C for 15 min and 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec for the Lassa virus standard, and 50°C for 15 min and 95°C for 2 min, followed by 45 cycles of 95°C for 15 sec and 58°C for 1 min for the Nipah virus standard.

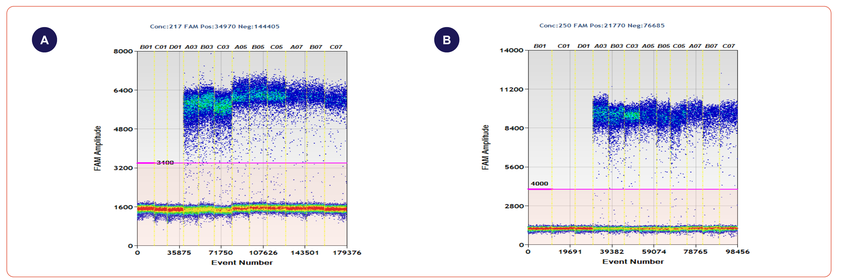

Figure 4. Absolute quantification of Lassa and Nipah virus via digital PCR. One-dimensional (1D) amplitude scatter plots of positive and negative digital PCR droplet reactions for (A) Lassa and (B) Nipah virus. Lassa and Nipah virus were quantified by digital PCR by using the same primers and probe from the in-house assay and published qRT-PCR assay.[2] Digital PCR for Lassa virus was performed as follows: reverse transcription at 50°C for 60 min, enzyme activation at 95°C for 10 min, followed by 40 cycles at 95°C for 30 sec and 60°C for 1 min, and enzyme deactivation at 98°C for 10 min. ddPCR for Nipah virus was performed as follows: reverse transcription at 50°C for 60 min, enzyme activation at 95°C for 10 min, amplified 40X at 95°C for 30 sec and 58°C for 1 min, and enzyme deactivation at 98°C for 10 min. Droplets were analyzed in the QX200 droplet reader. Data were analyzed with QuantaSoft software.

Conclusions

Overall, our data demonstrate that the synthetic standard for *C. burnetii* can be used as a control for the development, verification, and validation of assays for bacterial detection and quantification. Further, this standard is compatible with numerous published assays and exhibits minimal variability as shown by the slope and R^2 values. Taken together, quantitative synthetic standards are well-characterized, accessible controls for molecular diagnostic assays, and our data supports the extension of this approach to other pathogens that are unsuitable for routine nucleic acid extraction, such as those that are fastidious, uncultivable, or require high-containment.

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** sales@atcc.org **Web:** www.atcc.org