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

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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. burnetii* (ATCC® BAA-400000SD™). 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

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<thead>
<tr>
<th>BSL-1</th>
<th>Quantitative</th>
<th>Stabilized</th>
<th>ISO 13485</th>
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<tr>
<td>ATCC® No.</td>
<td>Description</td>
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<tr>
<td>BAA-400000SD™</td>
<td>Quantitative Synthetic <em>Coxiella burnetii</em> DNA</td>
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<tr>
<td>VR-3268SD™</td>
<td>Quantitative Synthetic Nipah virus RNA</td>
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<td>VR-3268SD™</td>
<td>Quantitative Synthetic Lassa virus RNA</td>
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Applications

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

References & Acknowledgements

3. Thank you to the Molecular BioProduction and Molecular Laboratory Testing Service (MTLS) team, and all those involved in the production and launch of these synthetic molecular standards.

Material and Methods

Quantitative Synthetic DNA

The quantitative synthetic DNA was designed and synthesized using a proprietary method. The preparation includes fragments from the outer membrane protein (com), ascorbate dehydrogenase (ido), transposase (IS111A), 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/RT-qPCR 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.

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 R² 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.

Extension of the Approach to Support Biodefense Research

Figure 1. qPCR assay to verify the functionality of synthetic molecular standards. A, B: Amplification plots and C, D: standard curves were generated with the Lassa and Nipah virus molecular standards, respectively. The qRT-PCR assay was performed using a published primer and probe set, and protocol.1

Figure 2. Absolute quantification of Lassa and Nipah virus via digital PCR. One-dimensional (OD) amplification plots of positive and negative digital PCR (dPCR) replicates for BSL-1 Lassa and BSL-2 Nipah virus. The dPCR results were generated using digital PCR, using the same primers and probes from the published qPCR assay. Digital PCR was performed as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 20 sec and 60°C for 1 min, and enzyme deactivation at 65°C for 10 min. Data were analyzed using the QuantaSoft software.

Figure 3. Absolute quantification of *Coxiella burnetii* via digital PCR. (A) One-dimensional (OD) amplification plots of positive and negative digital PCR replicates for BSL-1 Lassa and BSL-2 Nipah virus. The dPCR results were generated using digital PCR, using the same primers and probes from the published qPCR assay. Digital PCR was performed as follows: initial denaturation at 60°C for 10 min, followed by 40 cycles of 94°C for 20 sec and 56°C for 1 min, and enzyme deactivation at 65°C for 10 min. Data were analyzed using the QuantaSoft software.