Development And Validation Of Quantitative Synthetic Molecular Standards For *Chlamydia trachomatis* Lymphogranuloma Venereum (LGV) Serovars L1, L2, And L3

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Background and Introduction

Chlamydia trachomatis is a Gram-negative, obligate intracellular pathogen responsible for the majority of bacterial sexually transmitted infections. There are currently 15 serovars of *C. trachomatis*, which are defined by their tissue tropism; serovars A, B, Ba, and C-K are generally restricted to mucosal tissues, while serovars L1, L2, and L3 infect lymphatic tissues. Serovars L1, L2, and L3 are associated with a long-term infection of the lymphatic system known as lymphogranuloma venereum (LGV). Without proper treatment, LGV can result in the obstruction of lymph flow and chronic swelling of genital tissues. Therefore, the rapid and accurate diagnosis of this disease during the early stages of infection is essential for timely and proper treatment.

While culture-based approaches can be used in the diagnosis of LGV, they are typically time consuming, labor intensive, and require BSL-3 facilities. PCR-based methods provide a highly sensitive and rapid alternative screening approach; however, the development and validation of these assays are dependent on the availability of high-quality reference materials. To address this need, ATCC designed and developed quantitative synthetic molecular standards for *C. trachomatis* serovars L1, L2, and L3. Here, we used a proprietary strategy to incorporate genes typically targeted in various assays for bacterial detection and identification. The synthetic construct for each serovar contains genes most relevant to that particular strain, and may encode for a variety of membrane proteins, virulence factors, or a variety of other sequences. As proof-of-concept, the *C. trachomatis* LGV1-3 standards were quantified using Droplet Digital™ PCR (ddPCR™; Bio-Rad) and validated via published qPCR assays.[1,2]

ATCC Synthetic Molecular Standards

BSL-1 | **Quantitative** | **Stabilized**

ATCC Part Number	Product Description
ATCC® BAA-4001SD™	Quantitative Synthetic *Chlamydia trachomatis* LGV Type 1 DNA
ATCC® BAA-4002SD™	Quantitative Synthetic *Chlamydia trachomatis* LGV Type 2 DNA
ATCC® BAA-4003SD™	Quantitative Synthetic *Chlamydia trachomatis* LGV Type 3 DNA

Applications

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

References

1. Schaeffer A and Henrich B. Rapid detection of *Chlamydia trachomatis* and typing of the Lymphogranuloma venereum associated L-Serovars by TaqMan PCR. BMC infectious diseases 8: 56, 2008. PubMed: 18447917
2. Verweij SP, *et al*. Lymphogranuloma venereum variant L2b-specific polymerase chain reaction: insertion used to close an epidemiological gap. Clinical Microbiology and Infection 17(11): 1727-1730, 2011. PubMed: 21895856

Materials and Methods

Quantitative Synthetic DNA
Using a proprietary method, we designed synthetic DNA constructs for *C. trachomatis* LGV1 (ATCC® BAA-4001SD™), *C. trachomatis* LGV2 (ATCC® BAA-4002SD™), and *C. trachomatis* LGV3 (ATCC® BAA-4003SD™). These standards comprise fragments from various conserved regions of bacterial genomes: *C. trachomatis* LGV1 includes fragments from MOMP, 16S rRNA, *pmpH, dnaB*, putative virulence plasmid integrase regions, and conserved hypothetical virulence plasmid protein and *C. trachomatis* LGV2 and LGV3 include fragments from MOMP, 16S rRNA, *pmpH,* and *dnaB* regions. Following their construction, the standards were authenticated via next-generation sequencing and then quantified via droplet digital PCR.

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

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

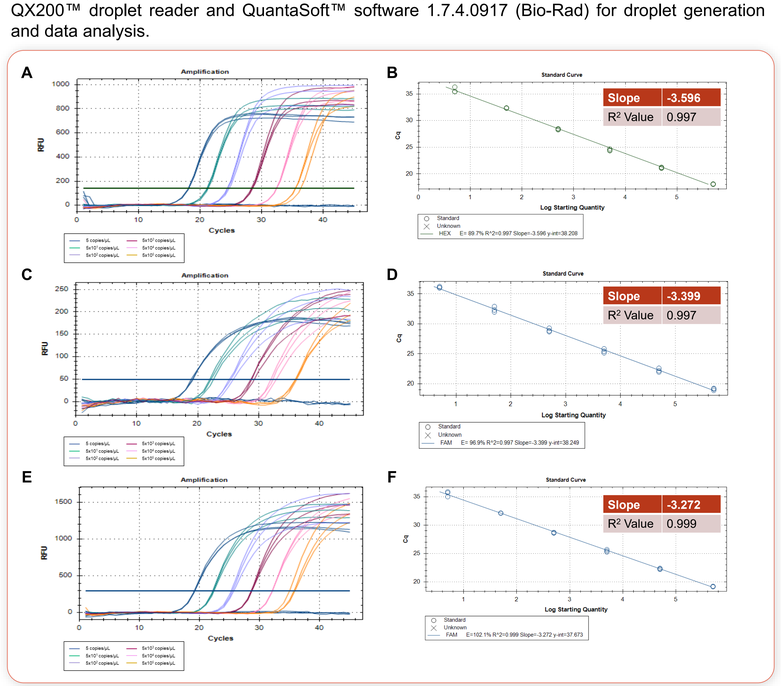

Figure 1. qPCR assay to verify the functionality of the synthetic molecular standards. (A, C, E) Amplification plots and (B, D, F) standard curves were generated with the *C. trachomatis* LGV1, LGV2, and LGV3 molecular standards, respectively. The qPCR assays were performed as previously described.[1,2] 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 1 min for all LGV serovars. Standard curves were generated by using serial 10-fold dilutions that ranged from 5 copies/µL to 5×10^4 copies/µL for all LGV serovars. The DNA standards were tested in triplicate.

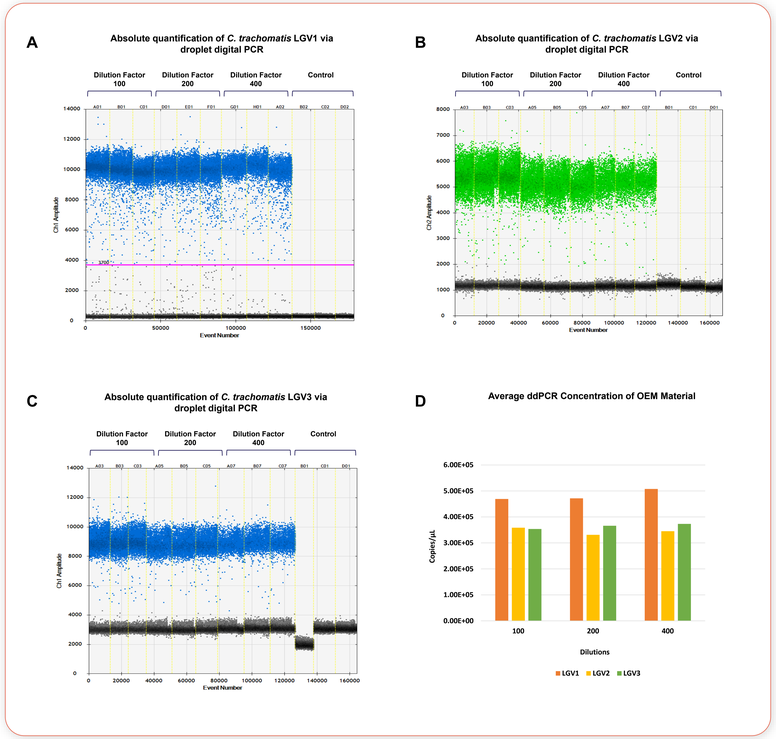

Figure 2. Absolute quantification of *C. trachomatis* LGV1, LGV2, and LGV3 via droplet digital PCR. One-dimensional (1D) amplitude scatter plots of positive and negative digital PCR droplet reactions for 3 dilutions (dilution factors 100, 200, and 400) in triplicate for (A) LGV1, (B) LGV2, and (C) LGV3. *C. trachomatis* LGV1-3 were quantified by droplet digital PCR by using the same primers and probe from the appropriate published qPCR assays.[1,2] (D) Average calculated genome equivalent copy numbers/µL per dilution of stock material. Digital PCR for all LGV serovars was performed as follows: initial denaturation at 95°C for 10 min, amplified 40× at 94°C for 30 sec and 60°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

Our proof-of-concept data demonstrates that ATCC quantitative synthetic molecular standards for *C. trachomatis* LGV1, LGV2, and LGV3 can be used as controls for assay development, verification, and validation. These standards were manufactured under ISO 13485 guidance and can be used to determine the microbial load of unknown *C. trachomatis* LGV samples through the generation of a standard curve. The standards are compatible with numerous published assays (6+, 10+, and 8+ published assays for LGV1, LGV2, and LGV3, respectively) and exhibited minimal variability as evident from the slope and R^2 values. Taken together, these standards provide well-characterized controls for viral detection and quantification.

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