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 L1, L2, and L3 into a plasmid containing a rRNA, pmpH, virulence plasmid protein and several 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**

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<tr>
<th>BSL-1</th>
<th>Quantitative</th>
<th>Stabilized</th>
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<tr>
<td>ATCC® BAA-4001SD™</td>
<td>Quantitative Synthetic Chlamydia trachomatis LGV Type 1 DNA</td>
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<tr>
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<td>ATCC® BAA-4003SD™</td>
<td>Quantitative Synthetic Chlamydia trachomatis LGV Type 3 DNA</td>
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**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

**Materials and Methods**

**Quantitative Synthetic DNA**

Using a proprietary method, we designed synthetic DNA constructs for C. trachomatis LGV1 (ATCC® BAA-4001SD™), LGV2 (ATCC® BAA-4002SD™), and 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, plasmid, and other diagnostic regions; C. trachomatis LGV2 includes fragments from MOMP, 16S rRNA, pmpH, dnaB, and other diagnostic regions; and C. trachomatis LGV3 includes fragments from MOMP, 16S rRNA, pmpH, dnaB, and other diagnostic 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 (Bio-Rad) for droplet generation and data analysis.

**Results**

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 R² values. Taken together, these standards provide well-characterized controls for viral detection and quantification.

**Conclusion**

References