Comparison of Two *Mycoplasma genitalium* Real-Time PCR Detection Methodologies

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Established in-house quantitative PCR (qPCR) assays to detect the *Mycoplasma genitalium* adhesion protein (MgPa) and the 16S rRNA gene were found to be comparable for screening purposes, with a kappa value of 0.97 (95% confidence interval [CI], 0.94 to 1.01) and no difference in bacterial load quantified ($P = 0.4399$).

Limited knowledge exists regarding the epidemiology of *Mycoplasma genitalium* in the general population (3, 9, 12). In the absence of adequate and reliable culture and approved commercial assay techniques, most laboratories use in-house nucleic acid amplification tests (NAATs) for detection of this bacterium. Quantitative PCR (qPCR) assays have been designed for a variety of *M. genitalium* targets (2, 5, 7, 8, 14, 15, 17, 18, 20, 22), though the most cited qPCR assays are the one described by Jensen et al., which targets a 78-bp region of the *M. genitalium* genome (10). Hardick et al. described a multiplex assay that incorporated both the MgPa and 16S rRNA gene qPCR assays (11) and found that the 16S rRNA target did not detect 59 of 607 samples (9.7%) in which MgPa was detected. Lack of detection by the 16S rRNA gene component could possibly be attributed to competition when the two targets were multiplexed, and the authors recommended further testing in singleplex reactions.

To investigate the issue of varying sensitivities between the MgPa and 16S rRNA gene assays, an initial experiment was carried out to determine the detection limit of each assay. A clinical sample equivalent to 1,200 copies/µl of *M. genitalium* was diluted 1:4 to extinction and run in triplicate. Each assay consisted of 5 µl template in a 20-µl reaction on the LightCycler 480 real-time PCR system (Roche Diagnostics), using PCR conditions as described previously (8, 22). The MgPa assay was able to detect ≥6 copies/reaction of *M. genitalium* in three replicate reactions, and the 16S rRNA gene assay detected ≥23 copies per reaction. Both assays were able to detect *M. genitalium* down to a single copy although in only one of three replicate reactions each. Further analysis on a separate clinical sample diluted to approximately six copies per reaction was carried out with 12 replicates. The MgPa assay detected *M. genitalium* in eight of these reactions (mean quantification cycle [Cq] = 39.50; standard deviation [SD] = 0.94), and the 16S rRNA gene assay detected *M. genitalium* in seven (mean Cq = 39.37; SD = 0.80).

Testing was then carried out on 845 self-collected vaginal swab samples (from 761 individuals) obtained as part of the Chlamydia Incidence and Re-Infection Rates Study (CIRIS) (21), which consisted of specimens collected at the recruitment and at a 12-month follow-up. Sample processing and DNA extraction were as described previously (19). Each sample was initially screened for the 16S rRNA gene target; then samples were stored at −30°C for a median of 25 months (average = 22.2; range = 1 to 29) before subsequent testing for the MgPa gene and retesting with the 16S RNA gene assay in cases where the assays gave discordant results. Nine DNA samples tested negative or yielded a low human β-globin gene signal, indicating inadequate sampling, and were removed from subsequent analyses. Also removed from the study were three DNA samples from different patients in which *M. genitalium* was detected by the 16S rRNA assay (with 15, 68, and 129 copies per reaction; Table 1. Clinical cohort screening sample results

<table>
<thead>
<tr>
<th>16S rRNA result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>38</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>793</td>
<td>795</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>793</td>
<td>833</td>
</tr>
</tbody>
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Cq = 40.48, 38.06, and 35.22, respectively) but which tested negative with both the MgPa gene and 16S rRNA gene assay after being kept in storage at −30°C for 16 to 26 months. Further repeated testing failed to generate PCR amplicons, indicating that either multiple freeze-thaw cycles or extended storage at −30°C was likely to have resulted in degradation of DNA samples. The potentially detrimental effect of freezing on M. genitalium samples and DNA has been reported in other studies (4, 13), and work in our own laboratory has shown levels of M. genitalium DNA to significantly reduce after three freeze-thaw cycles (data not shown). In future studies, freezing of samples destined for M. genitalium testing is not recommended, to avoid potential degradation.

Of the 40 samples found to be positive by one or both methods (Table 1), 38 (88.4%) were positive by both assays and 2 (4.7%) were positive by the MgPa assay only. Based on these results, the sensitivity and specificity of the 16S rRNA gene assay compared to those of the MgPa were 95.0% (95% confidence interval [CI], 0.831 to 0.994) and 100%, respectively (1). Kappa analysis of these two methods gave a score of 0.973 (95% CI, 0.936 to 1.010), indicating an “almost perfect” agreement between these two assays (16). The two discordant samples in this study with M. genitalium detected only by the MgPa assay were from the same patient but at different time periods (recruitment and at a 12-month follow up), with concentrations of 81 and 86 copies per reaction (Cq values = 37.06 and 36.98, respectively).

Of the 38 samples that were positive for M. genitalium by both assays, only one sample showed evidence of slight degradation between retesting, showing a reduction from 411 copies per reaction initially with the 16S rRNA gene assay (Cq = 33.16) to fewer than 10 copies per reaction when retested with both assays 26 months later (Cq = 40.0 to 40.3). With the remaining 37 samples, the calculated concentrations for the MgPa assay ranged from 9 copies to 2.6 × 10^5 copies per reaction (mean = 3.0 × 10^3; SD = 5.5 × 10^3), while results for the 16S rRNA assay ranged from 34 copies to 1.7 × 10^5 copies per reaction (mean = 2.5 × 10^3; SD = 4.1 × 10^3). Using a paired t test, the concentrations given for each sample were not statistically different overall (P = 0.44). This was confirmed by Deming regression analysis (6) giving a near-perfect line of best fit (Fig. 1).

In summary, the MgPa and 16S rRNA gene assays appear equally suitable for the detection of M. genitalium in vaginal swabs. Further work is warranted to determine how effective these methods are for other clinical specimen types and to identify the reason(s) why two samples, from the same patient, gave rise to amplicons in the MgPa assay and not the 16S rRNA gene assay. These data will inform clinicians and researchers regarding the comparative performances of the two commonly used in-house assays for the detection of M. genitalium and are of particular relevance as routine testing and screening for the bacterium become increasingly common.

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