The detection of Mycoplasma genitalium was evaluated on 1,080 urine samples by the use of a Panther instrument. Overall sensitivity, specificity, positive predictive values, and negative predictive values were 100%, 99.4%, 93.6%, and 100%, respectively. Detection of M. genitalium by the use of the Panther transcription-mediated amplification assay offers a simple, accurate, and sensitive platform for diagnostic laboratories.

Mycoplasma genitalium is a common cause of nongonococcal urethritis (NGU) in men and currently accounts for 10% to 35% of NGU cases globally (1, 2). In women, it has been associated with cervicitis, pelvic inflammatory disease, and infertility (1, 3–7) and studies have also suggested that it plays an important role in HIV acquisition and transmission (8, 9). The exact rates of M. genitalium prevalence have been reported in a limited number of studies, with prevalences of 0.8% to 2.3% among young women and 1.1% to 6.9% among young men (10–14).

As this organism is highly fastidious and slow growing, culture is not feasible for diagnosis and is performed in only a small number of laboratories worldwide for research purposes. Diagnosis relies on nucleic acid amplification tests (NAAT); however, as there have been limited commercial assays available, most laboratories have utilized in-house NAATs, utilizing quantitative PCR (qPCR) assays with various targets (15–21). For some time, a research-use-only transcription-mediated amplification assay for detection of M. genitalium (MG-TMA) has been available from Hologic (Bedford, MA, USA) for use on either the manual or automated TIGRIS DTS system; however, this has recently been introduced onto the Panther platform utilizing the open-channel software feature, with the manufacturer package insert indicating a sensitivity of 0.01 CFU/ml (equivalent to 0.004 copies per reaction). The assay has received the CE mark for in vitro diagnosis (CE-IVD) in Europe and is becoming accredited through other regulatory bodies for utilization in diagnostic laboratories. In this study, we evaluated the performance of the MG-TMA on the Panther platform for the detection of M. genitalium 16S rRNA by comparison to three assays: an alternative 16S rRNA target assay (Alt-TMA) available on Panther and two previously described PCR assays, one targeting a 78-bp region of the 16S rRNA, was also tested on the Panther platform. All results with a relative light unit (RLU) value of 50,000 were interpreted as positive, with strict procedures being followed to avoid specimen contamination and carryover.

An additional 1-ml aliquot was centrifuged for 10 min at 10,000 × g and the pellet resuspended in 200 μl of phosphate-buffered saline. The resuspended pellet was subsequently extracted using MagNA Pure 96 (Roche Diagnostics GmbH, Penzberg, Germany) and a DNA and viral nucleic acid small-volume kit (Pathogen Universal 200 protocol). Transcribed DNA was eluted into a final volume of 10 μl: 5-μl aliquots were utilized in two qPCR assays targeting the 16S rRNA gene (20) and the MgPa gene (18). Both qPCR assays were run on a Roche LC480 real-time PCR instrument using Sensi-FAST Probe No-ROX chemistry (Bioline, Alexandria, NSW, Australia).

A gold standard for the patient infection status was determined by utilizing a consensus of two of the three results (not including the test being evaluated) for each sample tested. Sensitivity and specificity and positive, negative, and overall percent agreement


Editor: A. J. McAdam, Boston Children's Hospital

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TABLE 1 Comparison of detection of M. genitalium to detection by the consensus expanded gold standard

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result category</th>
<th>Positive</th>
<th>Negative</th>
<th>Total no.</th>
<th>% sensitivity/% specificity (95% CI)</th>
<th>% positive predictive value/% negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG-TMA</td>
<td>Positive</td>
<td>88</td>
<td>6b</td>
<td>94</td>
<td>100 (95.8–100)/99.4 (98.7–99.8)</td>
<td>93.6 (86.6–97.6)/100 (99.6–100)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>986</td>
<td>986</td>
<td>100 (95.8–100)/99.4 (98.7–99.8)</td>
<td>93.6 (86.6–97.6)/100 (99.6–100)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>88</td>
<td>992</td>
<td>1,080</td>
<td>100 (95.8–100)/99.4 (98.7–99.8)</td>
<td>93.6 (86.6–97.6)/100 (99.6–100)</td>
</tr>
<tr>
<td>Alt-TMA</td>
<td>Positive</td>
<td>88</td>
<td>7'</td>
<td>95</td>
<td>100 (95.8–100)/99.3 (98.6–99.7)</td>
<td>92.6 (85.4–97.0)/100 (99.6–100)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>985</td>
<td>985</td>
<td>100 (95.8–100)/99.3 (98.6–99.7)</td>
<td>92.6 (85.4–97.0)/100 (99.6–100)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>88</td>
<td>992</td>
<td>1,080</td>
<td>100 (95.8–100)/99.3 (98.6–99.7)</td>
<td>92.6 (85.4–97.0)/100 (99.6–100)</td>
</tr>
<tr>
<td>MgPa qPCR</td>
<td>Positive</td>
<td>84</td>
<td>7d</td>
<td>91</td>
<td>91.3 (83.6–96.1)/99.3 (98.6–99.7)</td>
<td>92.3 (84.8–96.6)/99.2 (98.4–99.7)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8'</td>
<td>981</td>
<td>989</td>
<td>91.3 (83.6–96.1)/99.3 (98.6–99.7)</td>
<td>92.3 (84.8–96.6)/99.2 (98.4–99.7)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>92</td>
<td>988</td>
<td>1,080</td>
<td>91.3 (83.6–96.1)/99.3 (98.6–99.7)</td>
<td>92.3 (84.8–96.6)/99.2 (98.4–99.7)</td>
</tr>
<tr>
<td>16S qPCR</td>
<td>Positive</td>
<td>82</td>
<td>0</td>
<td>82</td>
<td>89.1 (80.9–94.7)/100 (99.6–100)</td>
<td>100 (95.6–100)/99.0 (98.2–99.5)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10f</td>
<td>988</td>
<td>998</td>
<td>89.1 (80.9–94.7)/100 (99.6–100)</td>
<td>100 (95.6–100)/99.0 (98.2–99.5)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>92</td>
<td>988</td>
<td>1,080</td>
<td>89.1 (80.9–94.7)/100 (99.6–100)</td>
<td>100 (95.6–100)/99.0 (98.2–99.5)</td>
</tr>
</tbody>
</table>

a Consensus results for M. genitalium detection represent positivity for 2 of 3 tests (not including the test being evaluated) and were used as the expanded gold standard to compare test performances.
b All 6 results were negative by 16S and MgPa qPCRs; 4 of the 6 were Alt-TMA positive.
c All 7 results were negative by 16S and MgPa qPCRs. All were positive by MG-TMA.
d All 7 results were negative by MG-TMA, Alt-TMA, and 16S assay. All gave high quantification cycle (Cq) values (over 40).
e All 8 results were positive by MG-TMA and Alt-TMA, with only 2 being positive by 16S assay. All had high Cq values (over 40) on 16S qPCR.
f All 10 results were positive by MG-TMA and Alt-TMA, with only 5 being positive by MgPa.

and 95% confidence intervals were calculated by comparison to the gold standard.

Overall, 1,080 consecutive urine samples collected from men and women over the course of 3 months were evaluated using MG-TMA, and the results were compared to those obtained with the gold standard. MG-TMA showed sensitivity and specificity of 100% and 99.4%, respectively, and positive and negative predictive values of 93.6% and 100%, respectively (Table 1). The comparison of the two Aptima assays showed a very high correlation (kappa [K] = 0.97; 95% confidence interval [CI], 0.93 to 1.00). Comparison of MG-TMA results to the consensus results showed a very good correlation, with a kappa of 0.95 (95% CI, 0.93 to 0.99). The M. genitalium assay performed on the Panther platform integrated well with the laboratory procedures, allowing rapid testing and the possibility of rapid and accurate reporting using integration with the laboratory information system.

Comparing the results from the two TMA assays to the consensus results, 4 of 6 isolates with discordant results were positive in both TMA assays and were from asymptomatic male patients. This most likely reflects the higher analytical sensitivity of TMA than qPCR. The two samples that were positive on MG-TMA and negative on Alt-TMA came from asymptomatic female patients and may have had lower copy numbers.

This was the first study evaluating the MG-TMA on the Panther platform reported to date and the only one to have used four assays for comparisons. It showed remarkable concordance between the assays and tight confidence intervals around the estimates. The study involved predominantly symptomatic men and fewer women, most of whom were asymptomatic and pregnant, so future studies should sample nonpregnant and symptomatic women. Notwithstanding this limitation, the data suggest that the Aptima assay, performed on the automated Panther platform, offers a simple, accurate, and sensitive method for use by diagnostic laboratories for detection of this important pathogen.

ACKNOWLEDGMENT

Hologic is the developer and manufacturer of the evaluated assay and supplied the detection kits utilized for this study.

REFERENCES


2202 jcm.asm.org  Journal of Clinical Microbiology  August 2016 Volume 54 Number 8


