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Evaluation of the Hologic Panther Transcription-Mediated Amplification Assay for Detection of *Mycoplasma genitalium*

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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 *M. genitalium* adhesion (MgPa) gene with sensitivity of five copies per reaction (18) and the other targeting a 517-bp region of the 16S rRNA gene with sensitivity of 10 copies per reaction (21). To our knowledge, this is the first clinical evaluation of the Panther assay for detection of *M. genitalium*.

From February to May 2015, consecutive urine samples received for *M. genitalium* testing were utilized for this evaluation. Ethical approval for this study was granted by the Royal Women's Hospital Research and Ethics Committees. The patient population included 664 men and women attending Melbourne Sexual Health Centre for management of NGU or sexual contacts of infected partners, 309 consecutive asymptomatic women from the Royal Women's Hospital undergoing screening prior to termination of pregnancy, and 107 samples referred from external laboratories. Overall, 1,080 urine samples, including 631 from men and 449 from women, were evaluated.

A 2-ml aliquot of first-void urine was transferred to Aptima urine transport medium (Hologic Inc., San Diego, CA, USA) within 24 h of collection and tested within 30 days on the Panther platform using the research-use-only version of the Aptima TMA assay for *M. genitalium* targeting an 81-bp region of 16S rRNA. A supplementary assay, Alt-TMA, targeting a different 94-bp region of 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). Extracted DNA was eluted into a final volume of 100 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

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

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

[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 (C_q) 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 C_q 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 symptomatic 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.

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