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Evaluation of the SpeeDx ResistancePlus MG Diagnostic Test for *Mycoplasma genitalium* on the Applied Biosystems 7500 Fast Quantitative PCR Platform

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Mycoplasma genitalium is a sexually transmitted bacterium that causes urethritis in men and is associated with potentially serious sequelae in women (1). The levels of antibiotic resistance to the first-line (azithromycin) and second-line (moxifloxacin) treatments are high and increasing (2, 3). Two commercial nucleic acid amplification tests for the diagnosis of *M. genitalium* were recently released, the Hologic (Bedford, MA, USA) Aptima *Mycoplasma genitalium* (4) and SpeeDx (Sydney, Australia) ResistancePlus MG (5) tests.

In a single multiplexed reaction, the ResistancePlus MG assay reports both the detection of *M. genitalium* and the presence/absence of five macrolide resistance mutations in the 23S rRNA gene, facilitating individualization of antimicrobial therapy. The ResistancePlus MG test has been validated by the Roche LightCycler 480 II real-time PCR system (LC480) (5, 6). The aim of this study was to evaluate the SpeeDx ResistancePlus MG (550) diagnostic kit (Sydney, Australia) on the Applied Biosystems 7500 (ABI 7500) Fast platform, a commonly used diagnostic quantitative PCR (qPCR) platform.

A retrospective analysis was performed on samples collected from 1 May 2016 to 21 June 2016 (initially tested by 16S rRNA gene qPCR, including quantitation [7]). All positive samples ($n = 111$) and a random selection of negative samples ($n = 100$) were included in this study. Sample types included urine and swabs (anorectal, vaginal, cervical/endocervical, urethral) and originated from The Royal Women's Hospital (Melbourne, Australia), the Melbourne Sexual Health Centre, or external referrals. Samples were previously extracted on the MagNA Pure 96 (Roche) for diagnostic purposes and extracts stored at −30°C. For the test assay, reaction mixtures (20-μl final volume) were assembled with 5 μl of sample and the Plex Mastermix from the ResistancePlus MG (550) diagnostic kit, before analysis on the ABI 7500 Fast platform. Cycling consisted of 95°C for 2 min, followed by 10 cycles of 95°C for 5 s and 61°C for 30 s (−0.5°C per cycle) and 40 cycles of 95°C for 5 s and 52°C for 40 s. Detection occurred in three channels: (i) the FAM channel (FAM [6-carboxyfluorescein] dye, 495 to 520 nm) for detection of *M. genitalium* through the MgPa gene, (ii) the JOE channel (JOE [6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein] dye, 529 to 555 nm) for detection

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TABLE 1 ResistancePlus MG kit detection of *M. genitalium* and 23S rRNA gene mutations with the ABI 7500 qPCR platform[a]

PCR platform	Test result	No. of samples with *M. genitalium* 16S qPCR reference test result:		No. of samples with 23S rRNA mutation Sanger sequencing reference test result[b]:	
		+ve	−ve	Mutation +ve[c]	Mutation −ve
SpeeDx ABI 7500	+ve	99	0	62	0
	−ve	2[d]	81	5	30
Total		101	81	67	30

[a]+ve, positive; −ve, negative.
[b]Analysis of 97 *M. genitalium*-positive samples. Sequencing results for two samples were unavailable due to a failed sequencing run.
[c]Mutations ($n = 67$) included A2058G ($n = 23$), A2058T ($n = 4$), and A2059G ($n = 40$).
[d]Fewer than 10 genomes/5 μl of extract were detected in the samples when discrepant analysis was performed by repeating the in-house 16S qPCR.

of five 23S rRNA gene mutations (A2058G/C/T, A2059G/C), and (iii) the TAMRA (6-carboxytetramethylrhodamine) channel (Atto 550 dye, 554 to 576 nm) extrinsic internal control. A synthetic DNA standard of known concentration was included in all experiments. The results were compared to results from two in-house methods at the Royal Women's Hospital (16S rRNA gene qPCR [7] and Sanger sequencing of the 23S rRNA gene amplified by conventional PCR [8]) (comparator assay 1) and the ResistancePlus MG assay performed on the LC480 (comparator assay 2) (6). This study was approved by the Royal Women's Hospital Human Research and Ethics Committees.

Twenty-nine samples were disregarded due to insufficient sample volume or DNA degradation during storage (9). For the remaining 182 samples, there was high concordance for detection of *M. genitalium* organisms (98.9%) between the ResistancePlus MG assay performed on the ABI 7500 platform and by comparator assay 1 (kappa value of 0.98, 95% confidence interval [95%CI], 0.947 to 1.000), with 98.0% sensitivity and 100.0% specificity (Table 1). Two discrepant samples for *M. genitalium* detection (positive by the in-house assay, negative by the SpeeDx assay) had a low *M. genitalium* load of fewer than 10 genomes/5 μl of extract at the detection limit. For 97 positive samples (67 mutant, 30 wild-type samples), the test assay had a mutation detection sensitivity of 92.5% (95%CI, 83.4 to 97.6%) and a specificity of 100% (95%CI, 88.4 to 100%), with a high kappa value of 0.885 (95%CI, 0.787 to 0.982) (Table 1).

The clinical performance of the ResistancePlus MG assay on the ABI 7500 platform was very similar to that of the LC480 platform (comparator assay 2). Direct comparison showed agreements of 99.4% (95%CI, 0.969 to 0.999) and a κ of 0.99 (95%CI, 0.967 to 1.000) for the detection of *M. genitalium* and agreements of 94.9% (95%CI, 0.885-0.978) and a κ of 0.89 (95%CI, 0.797 to 0.985) for 23S rRNA mutation detection.

In conclusion, the ResistancePlus MG (550) kit using the ABI 7500 platform had a high sensitivity and specificity for the detection of both *M. genitalium* and 23S rRNA mutations that may confer macrolide resistance. The availability of this test on the ABI 7500 platform, which is widely used in Europe, will greatly increase the accessibility of this assay for diagnostic laboratories, further assisting clinicians in selecting suitable antibiotics for effective treatment.

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