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Evaluation of the ResistancePlus MG FleXible Cartridge for Near-Point-of-Care Testing of *Mycoplasma genitalium* and Associated Macrolide Resistance Mutations

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Mycoplasma genitalium is an important sexually transmitted infection that can cause acute and/or chronic urethritis in males and females, and its prevalence is approximately 16% in females and 17% in males (1). Globally, macrolide resistance is estimated to exceed 50% in most urban centers (1–6), and in our local region of Queensland, Australia, it exceeds 60% (7). PCR is routinely used for diagnosis of *M. genitalium* and macrolide resistance mutations at positions 2058 and 2059 of the 23S rRNA gene; however, the turnaround times of laboratory-based methods often exceed 24 h and so may not be sufficiently timely to inform clinical management for symptomatic patients. Here, we evaluated the performance of the ResistancePlus MG FleXible cartridge test on the Cepheid GeneXpert (hereafter termed MG-Flex), which offers the potential for near-point-of-care testing.

A bank of 181 clinical samples (*M. genitalium* positive, $n = 63$, and negative, $n = 118$) from 145 males and 36 females was used in this study. Original patient samples were stored at 4°C for 4 weeks, then at −20°C for longer-term storage. Nucleic acid samples were stored at −20°C until required. Full details of the samples are provided in Table S1 in the supplemental material. The samples had all been submitted for routine *M. genitalium* testing to Pathology Queensland (Brisbane, Australia) where they were tested using an in-house PCR that detects the *MgPa* gene of *M. genitalium* (hereafter termed in-house MgPa-PCR) (8). For the purposes of this evaluation, all samples were tested using the MG-Flex near-point-of-care assay (which detects the macrolide resistance mutations A2058T, A2058C, A2058G, and A2059G) as well as the SpeeDx ResistancePlus MG test (hereafter termed RPMG, which detects the macrolide resistance mutations A2058T, A2058C, A2058T, A2059C, and A2059G). The RPMG test is a Therapeutic Goods Administration (TGA, Australia) cleared and CE-IVD-marked (i.e., Conformité Européene-marked for in vitro diagnostic) laboratory-based test.

The MG-Flex assay was prepared per the kit instructions. Briefly, 44 μl of MG-Flex mastermix, 1 ml of neat (unextracted) clinical sample, and 10 μl of internal control were added to the FleXible cartridge (Cepheid). In some instances, swab specimens ($n = 17$; Table S1) had less than 1 ml of sample, so the volume was made up to 1 ml with sterile molecular-grade water. The RPMG assay was performed per the manufacturer's instructions using stored DNA extracts from the routine testing in the in-house MgPa-PCR.

This study was approved by the Children's Health Queensland human research ethics committee (HREC/12/QRCH/139).

The results are summarized in Table 1 and further detailed in Table S1. The MG-Flex assay detected *M. genitalium* in 61 of 63 known positive samples, providing a sensitivity

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TABLE 1 Summary of results of the RPMG and MG-Flex assays[a]

No. of samples	Gender	Sample type (no.)	In-house MgPa-PCR result	RPMG assay result	MG-Flex assay result
7	F	Cervical (3), vaginal (1), urine (3)	**Detected**	**MG detected. 23S rRNA mutations detected.**	**MG detected. 23S rRNA mutations detected.**
36	M	Urine (31), rectal (3), urethral (2)	**Detected**	**MG detected. 23S rRNA mutations detected.**	**MG detected. 23S rRNA mutations detected.**
5	F	Cervical (2), vaginal (2), urine (1)	**Detected**	**MG detected. 23S rRNA mutations not detected.**	**MG detected. 23S rRNA mutations not detected.**
13	M	Urine (11), rectal swab (2)	**Detected**	**MG detected. 23S rRNA mutations not detected.**	**MG detected. 23S rRNA mutations not detected.**
2	M	Urine (1), rectal swab (1)	**Detected**	**MG detected. 23S rRNA mutations not detected.**	MG not detected. 23S rRNA mutations not detected.
92	M	Urine (85), rectal swab (6), genital swab (1)	ND	MG not detected. 23S rRNA mutations not detected.	MG not detected. 23S rRNA mutations not detected.
24	F	Cervical (4), vaginal (2), urine (16), rectal (2)	ND	MG not detected. 23S rRNA mutations not detected.	MG not detected. 23S rRNA mutations not detected.
2	M	Rectal swab (1), urine (1)	ND	MG not detected. 23S rRNA mutations not detected.	**Invalid**

[a]Bold text represents a significant result (i.e., *M. genitalium* was detected or a result was invalid). F, female; M, male; ND, not detected.

of 96.8%. The two samples providing negative results by the MG-Flex assay (samples 62 and 63; Table S1) were both from males. For sample 62, a reduced starting volume of sample likely contributed to discordance, and in both samples, low *M. genitalium* load was observed. One hundred sixteen *M. genitalium*-negative samples were negative in the MG-Flex assay, while two samples provided invalid results (samples 180 and 181; Table S1), and there was insufficient sample to repeat testing. MG-Flex assay specificity for evaluable samples was therefore 100%. The detection of macrolide resistance mutations by the MG-Flex assay correlated 100% with that of the RPMG assay, with the exception of two samples providing negative results for *M. genitalium* in the MG-Flex assay (samples 62 and 63; Table S1). The overall agreement between the RPMG and MG-Flex assays was 98.9%, with a kappa value of 0.98. The detection limit was also assessed by testing 10-fold dilutions of an *M. genitalium*-positive sample, and the MG-Flex test was able to reliably detect an additional dilution over the RPMG assay (Table S1).

In summary, we found that the MG-Flex cartridge test was highly sensitive and specific for the detection of *M. genitalium* and 23S rRNA mutations.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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