1 **Title**: Evaluation of the ResistancePlus® MG FleXible cartridge for near point-of-care testing
2 of *Mycoplasma genitalium* and associated macrolide resistance mutations

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10 **Running title**: Evaluation of ResistancePlus® MG FleXible

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16 **Text.**

17 *Mycoplasma genitalium* is an important sexually-transmitted infection that can cause acute
18 and/or chronic urethritis in males and females, and its prevalence is approximately 16% in
19 females and 17% in males (1). Globally, macrolide resistance is estimated to exceed 50% in
20 most urban centres (1-6), and in our local region of Queensland, Australia, exceeds 60% (7).
21 PCR is routinely used for diagnosis of *M. genitalium* and macrolide resistance mutations at
22 positions 2058 and 2059 of the 23S rRNA gene, however, the turnaround times of laboratory-

based methods often exceed 24hrs, 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 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 were 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 Supplementary Table 1. 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 in-house-MgPa-PCR) (8). For the purposes of this evaluation, all samples were tested using the MG-Flex near point-of-care assay (detects the macrolide resistance mutations A2058T, A2058C, A2058G and A2059G) as well as the SpeeDx ResistancePlus® MG test (hereafter 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 laboratory-based test.

The MG-Flex assay was prepared as 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; Supplementary Table 1) 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 as per manufacturer's instructions using stored DNA extracts from the routine testing in the in-house-MgPa-PCR.

The results are summarised in Table 1 and further detailed in Supplementary Table 1. The MG-Flex assay detected *M. genitalium* in 61 of 63 known positive samples, providing a sensitivity of 96.8%. The two samples providing negative results by the MG-Flex assay (Samples 62 & 63, Supplementary Table 1) 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. 116 *M. genitalium*-negative samples were negative in the MG-Flex assay, while two samples provided invalid results (Samples 180 & 181, Supplementary Table 1) 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 the *M. genitalium* in the MG-Flex assay (Samples 62 & 63, Supplementary Table 1). 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 ten-fold dilutions of a *M. genitalium* positive sample and the MG-Flex test was able to reliably detect an additional dilution over the RPMG assay (supplementary Table 1).

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.

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102 **Table 1.** Summary of results across RPMG and MG-Flex assays

No. of samples	Gender (M/F)	Sample type (number)	In-house-MgPa-PCR	RPMG assay	MG-Flex assay
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

103 nd = not detected