Clinical performance studies for *ResistancePlus*® MG FleXible

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1 Intended use

The ***Resistance****Plus*® MG FleXible assay is a qualitative multiplexed *in vitro* diagnostic real-time PCR test for the identification of *M. genitalium* and detection of mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, *Escherichia coli* numbering), that are associated with resistance to azithromycin (macrolide antibiotic). It is intended to aid in the diagnosis of *M. genitalium* and detects mutations associated with azithromycin resistance in *M. genitalium* and should be used in conjunction with clinical and other laboratory information.

The ***Resistance****Plus*® MG FleXible assay may be used with the following specimen types: male and female urine, vaginal swab, cervical swab, rectal swab, urethral swab, from symptomatic and asymptomatic patients. Negative results do not preclude *M. genitalium* infections and do not provide confirmation of azithromycin susceptibility as there may be other mechanisms of treatment failure. The ***Resistance****Plus*® MG FleXible assay is intended to be used in professional settings such as hospitals, or reference or state laboratories. It is not intended for self-testing, home use, or point of care use.

The ***Resistance****Plus*® MG FleXible assay is performed on Cepheid GeneXpert® Instrument Systems.

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3 Clinical Study 1 - STD Laboratory, University of Alabama, Birmingham, Alabama, USA

A prospective-retrospective clinical study was conducted at the STD Laboratory, University of Alabama, Birmingham, Alabama, USA. Samples were collected from September 2018 - March 2019, and based on an in-house *M. genitalium* pdhD real-time PCR (performed at Johns Hopkins Center for the Development of Point-of-Care Sexually Transmitted Diseases, Baltimore, Maryland, USA), 21 *M. genitalium* positive and 54 consecutive *M. genitalium* negative samples were selected for inclusion in the study. The 76 specimens consisted of 38 male urine (collected in Xpert® Urine Specimen Collection Kit) and 38 vaginal swabs (collected in Xpert® Vaginal/Endocervical Specimen Collection kit). To determine performance of the ***Resistance****Plus*® MG FleXible kit, *M. genitalium* detection was compared to the pdhD real-time PCR result, and 23S rRNA mutant detection was compared to Sanger sequencing. The sensitivity and specificity of the ***Resistance****Plus*® MG FleXible kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 2**. One specimen (vaginal swab) was reported as Invalid by ***Resistance****Plus*® MG FleXible and was excluded from analysis. Analysis of 23S rRNA mutation detection only includes samples where the mutant status could be determined. Analysis of results in accordance to specimen type is shown in **Table 3**. The 23S rRNA mutation analysis is shown in **Table 4**.

Table 2. Clinical evaluation of *ResistancePlus*® MG FleXible

		Reference *M. genitalium* detection (pdhD qPCR)			Reference 23S rRNA mutant detection (Sanger Sequencing)	
		MG Positive	MG Negative		Mutant	Wild type
***ResistancePlus*® MG FleXible**	**MG Positive**	21	2	**Mutant**	13	0
	MG Negative	0	52	**Mutant not detected**	1	7
	Total	21	54	**Total**	14	7
Sensitivity		100.0% (95% CI 83.9 – 100.0%)		**Sensitivity**	92.9% (95% CI 66.1 – 99.8%)	
Specificity		96.3% (95% CI 87.3 – 98.7%)		**Specificity**	100.0% (95% CI 59.0 – 100.0%)	

Table 3. Clinical result analysis in accordance to specimen type[#]

Specimen	Expected MG negative	Expected MG positive, 23S rRNA wild type	Expected MG positive, 23S rRNA mutant
Male urine	33/33	1/1	3/4[1]
Female vaginal swab	22/22	6/6	10/10

[#] Samples were included in this analysis if they had a valid result from both the reference test and ***Resistance****Plus*® MG FleXible

[1] 1 sample was incorrectly called *M. genitalium* detected, 23S rRNA mutant not detected

Table 4. Clinical result analysis according to 23S rRNA mutation

Reference result	***ResistancePlus*® MG FleXible result**
Wild type	7/7
A2059G	8/8
A2058G	5/6[1]

[1] 1 sample was incorrectly called *M. genitalium* detected, 23S rRNA mutant not detected

4 Clinical Study 2 - Statens Serum Institut, Denmark

A retrospective clinical study was performed at Statens Serum Institut (SSI), Copenhagen, Denmark, to evaluate the performance of the ***Resistance****Plus*® MG FleXible kit for the detection of *M. genitalium* and azithromycin resistance-associated mutations in characterised, clinical UTM collected samples and neat urine, tested on the GeneXpert® (Cepheid) platform.

Samples were initially collected for routine MG testing at SSI. Remnant samples were used to compare performance of the ***Resistance****Plus*® MG FleXible assay to an in-house, validated MgPa PCR assay for MG detection and Sanger sequencing for 23S rRNA status. Samples were collected from Denmark and Sweden between 2009 - 2015 and included a total of 60 specimens, of which 34 were collected from males and 26 from females. The collected samples included 11 cervical swabs, 7 vaginal swabs, 2 urethral swabs and 40 urines. Swab specimens were collected in the Copan UTM 3mL collection kit (cat no. 306C, conical tube filled with 3mL UTM™ medium packaged with one regular FLOQSwab™, sterile) and urine specimens were collected as neat urine in a sterile collection cup. 5 samples (2 male urines, 1 female cervical swab and 2 female vaginal swabs) were excluded from further analysis as they were aborted during testing or invalid and could not be repeated.

The overall sensitivity and specificity of the ***Resistance****Plus*® MG FleXible kit for *M. genitalium* detection was 95.8% and 96.8%, respectively, and for 23S rRNA mutant detection was 100.0% and 100.0%, respectively (**Table 5**). Analysis of results in accordance to specimen type is shown in **Table 6**. The 23S rRNA mutation analysis is shown in **Table 7**.

Table 5. Clinical evaluation of the *ResistancePlus*® MG FleXible

		Reference *M. genitalium* detection (MgPa PCR assay)	
		MG Positive	**MG Negative**
***ResistancePlus*® MG FleXible**	**MG Positive**	23	1
	MG Negative	1	30
	Total	24	31
Sensitivity		95.8% (95% CI 78.9 – 99.9%)	
Specificity		96.8% (95% CI 83.3 – 99.9%)	

	Reference 23S rRNA mutant detection (Sanger Sequencing)	
	Mutant	**Wild type**
Mutant	12	0
Mutant not detected	0	11
Total	12	11
Sensitivity	100.0% (95% CI 73.5 – 100.0%)	
Specificity	100.0% (95% CI 71.5 – 100.0%)	

Table 6. Clinical result analysis in accordance to specimen type[#]

Specimen	Expected MG negative	Expected MG positive, 23S rRNA wild type	Expected MG positive, 23S rRNA mutant
Cervical swabs	8/8	-	2/2
Vaginal swabs	5/5	-	-
Urethral swabs	-	1/1	1/1
Male urine	12/12	8/8	9/10[1]
Female urine	5/6[2]	2/2	-

[#] Samples were included in this analysis if they had a valid result from both the reference test and ***Resistance****Plus*® MG FleXible kit

[1] Male urine: 1 sample was incorrectly called *M. genitalium* not detected.

[2] Female urine: 1 sample was incorrectly called *M. genitalium* detected, 23S rRNA mutation detected

Table 7. 23S rRNA mutation analysis

Reference result	***ResistancePlus*® MG FleXible result**
Wild type	11/11
A2059G	6/7[1]
A2058G	6/6

[1] 1 sample was incorrectly called *M. genitalium* not detected

5 Clinical Study 3 - University of Queensland Centre for Clinical Research, Brisbane, Australia

A retrospective clinical study was conducted at University of Queensland Centre for Clinical Research (UQCCR), Australia, to evaluate the performance of the ***Resistance****Plus*® MG FleXible kit using dry swab samples collected by cobas® PCR media collection kit (Roche, cat no. 06466281190) and neat urine samples collected in a sterile collection cup. Samples were collected for routine MG testing at Pathology Queensland, and then sent to UQCCR for resistance testing. Remnant samples were tested to compare performance of the ***Resistance****Plus*® MG FleXible assay to the diagnostic result, which consisted of a MgPa real-time PCR assay for MG detection and ***Resistance****Plus*® MG (performed on the ABI 7500 Fast Dx) for 23S rRNA mutation detection. Samples were collected between September 2017 – May 2019 and included a total of 186 specimens, of which 149 were collected from males and 37 from females. The 186 samples consisted of 9 cervical swabs, 6 vaginal swabs, 19 rectal swabs, 2 urethral swabs, 1 genital swab site unspecified and 149 urines. 2 samples (1 male urine and 1 male rectal swab) were excluded from further analysis as they were invalid and could not be repeated.

The overall sensitivity and specificity of the ***Resistance****Plus*® MG FleXible kit for *M. genitalium* detection was 97.1% and 99.1%, respectively, and for 23S rRNA mutant detection was 100.0% and 100.0%, respectively (**Table 8**). Analysis of results in accordance to specimen type is shown in **Table 9**.

Table 8. Clinical performance of the *ResistancePlus*® MG FleXible

		Reference *M. genitalium* detection (MgPa PCR assay)			Reference 23S rRNA mutant detection (RPMG (7500 Fast Dx))[#]	
		MG Positive	**MG Negative**		**Mutant**	**Wild type**
***ResistancePlus*® MG FleXible**	**MG Positive**	66	1	**Mutant**	43	0
	MG Negative	2	115	**Mutant not detected**	0	18
	Total	68	116	**Total**	43	18
Sensitivity		97.1% (95% CI 89.8 – 99.6%)		**Sensitivity**	100.0% (95% CI 91.8 – 100.0%)	
Specificity		99.1% (95% CI 95.3 – 99.9%)		**Specificity**	100.0% (95% CI 81.47 – 100.0%)	

[#] 5 MG positive samples (4 male rectal swabs and 1 female vaginal swab) were excluded from 23S rRNA gene analysis as they had no available 23S rRNA gene reference result

Table 9. Clinical result analysis in accordance to specimen type[#]

Specimen	Expected MG negative	Expected MG positive, 23S rRNA wild type	Expected MG positive, 23S rRNA mutant
Male urine	84/85[1]	11/11	31/31
Female urine	16/16	1/1	3/3
Male rectal swabs	6/6	2/2	3/4[2]
Female rectal swabs	2/2	-	-
Cervical swabs	4/4	2/2	3/3
Vaginal swabs	2/2	2/2	1/1
Male urethral swabs	-	-	2/2
Male genital swab	1/1	-	-

[#] Only samples with a reference result were included in this analysis

[1] Male urine: 1 sample was false positive *M. genitalium* detected, 23S rRNA mutation not detected

[2] Male rectal swabs: 1 sample was false negative *M. genitalium* not detected

6 Clinical Study 4 - Melbourne Sexual Health Centre & Royal Women's Hospital, Melbourne, Australia

A retrospective clinical study was conducted at Melbourne Sexual Health Centre (collection site) and Royal Women's Hospital (study site), Melbourne, Australia, to evaluate the performance of the ***Resistance****Plus*® MG FleXible kit for the detection of *M. genitalium* and azithromycin resistance-associated mutations from dry swabs (cervicovaginal and anal) tested on the GeneXpert® IV (Cepheid) platform.

Cervicovaginal swabs and anal swabs were prospectively collected from patients presenting with a STI syndrome (pelvic inflammatory disease, urethritis and proctitis) or sexual contacts of MG positive patients. From each patient providing a swab specimen, an additional swab of the same bodily site was obtained for the purpose of this study. Samples were selected, retrospectively, for inclusion in this study once the diagnostic result (comparator method) was known. Samples were tested at the Royal Women's Hospital with the ***Resistance****Plus*® MG FleXible kit and compared to the reference test, ***Resistance****Plus*® MG (performed on the LightCycler® 480 II) for both detection of *M. genitalium* and 23S rRNA mutations.

Samples were collected between May 2019 to September 2019 and included a total of 90 swab specimens. Specimen types included 9 cervical swabs,18 vaginal swabs, 21 anal swabs and 42 cervicovaginal swabs. All dry swab samples were collected using Copan FLOQSwab (cat no. 552C) collection kit. Out of the 90 swab samples tested with the ***Resistance****Plus*® MG FleXible test, there was 1 cervicovaginal swab with an error (1.1% of total); caused by a probe check failure. Additionally, 1 vaginal sample was called invalid due to the background slope in the internal control channel exceeding ADF limits, resulting in an invalid rate of 1.1%. These 2 samples are excluded from the performance calculations below.

The performance of the ***Resistance****Plus*® MG FleXible assay for all swab samples before discrepant testing is summarised in **Table 10** below.

Table 10: Performance of *ResistancePlus*® MG FleXible assay with all swab samples

***M. genitalium* detection**		**Reference test (RPMG (LC480 II))**			**23S rRNA mutation detection**	**Reference test (RPMG (LC480 II))**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG FleXible**	**Positive**	36	2		**Mutant detected**	24	2
	Negative	3	47		**Mutant not detected**	2	8

The 2 false mutant samples were sequenced to determine the true result. Both of these discrepant samples (1 anal swab and 1 cervicovaginal swab) were shown to be true 23S mutant samples (A2059G) by sequencing. The 2 false wild-type samples (cervicovaginal swabs) were also sequenced, with 1 shown to be a true mutant (A2059G) and the other failed to sequence.

The performance of the ***Resistance****Plus*® MG FleXible assay for all swab samples after discrepant testing is summarised in **Table 11** below.

Table 11: Performance of *ResistancePlus*® MG FleXible assay with all samples (after discrepant testing)

***M. genitalium* detection**		**Reference test (RPMG (LC480 II))**			**23S rRNA mutation detection[%]**	**Reference test (RPMG (LC480 II))**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG FleXible**	**Positive**	36	2		**Mutant detected**	26	0
	Negative	3	47		**Mutant not detected**	2	8
Sensitivity		92.3% (95% CI 79.1 – 98.4%)			**Sensitivity**	92.9% (95% CI 76.5 – 99.1%)	
Specificity		95.9% (95% CI 86.0 – 99.5%)			**Specificity**	100.0% (95% CI 63.1-100.0%)	

[%] 23S rRNA mutation detection performance calculated for samples positive for MG by both methods

The performance of the ***Resistance****Plus*® MG FleXible across all specimen types is summarised in **Table 12**.

Table 12. Clinical result analysis in accordance to specimen type

Specimen	Expected *M. genitalium* negative	Expected *M. genitalium* positive, 23S rRNA wild type	Expected *M. genitalium* positive, 23S rRNA mutant
Vaginal swab	16/16	-	1/1
Cervical swab	9/9	-	-
Cervicovaginal swab	11/13[1]	9/10[2]	15/18[3]
Anal swab	11/11	0/1[4]	9/9

[1] 2 cervicovaginal swabs were reported as MG detected, 23S mutation not detected by the ***Resistance****Plus*® MG Flexible test

[2] 1 as MG not detected by the ***Resistance****Plus*® MG Flexible test

[3] 2 cervicovaginal swabs was reported as MG detected, 23S mutation not detected and 1 as MG not detected by the ***Resistance****Plus*® MG Flexible test

[4] 1 anal swab was reported as MG not detected by the ***Resistance****Plus*® MG Flexible test