Clinical performance studies for *ResistancePlus*® MG

Contents

1 Product description

The ***Resistance****Plus*® MG kit simultaneously detects *M. genitalium* and 5 mutations at positions 2058 and 2059 in the 23S rRNA gene (*E. coli* numbering) that are associated with resistance to azithromycin (macrolide-based antibiotic). The ***Resistance****Plus*® MG kit is a 1-well real-time PCR multiplex consisting of 3 readouts. Readout 1 indicates the presence or absence of *M. genitalium* through detection of the MgPa gene; Readout 2 indicates the presence of a A2058G, A2059G, A2058T, A2058C or A2059C mutation in the 23S rRNA gene; and Readout 3 is an internal control to monitor extraction efficiency and qPCR inhibition. The ***Resistance****Plus*® MG kit utilises ***Plex****Zyme*® and ***Plex****Prime*® for specificity and superior multiplexing capability. The assay is validated on samples extracted using the MagNA Pure 96 System (Roche), MICROLAB STARlet IVD (Hamilton), QIAsymphony® SP (QIAGEN), NUCLISENS® easyMAG® (Biomérieux) and real-time detection on the Roche LightCycler® 480 Instrument II (LC480 II), the Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx) and the Bio-Rad CFX96™ IVD (CFX96 IVD) and CFX96 Touch™ (CFX96 Touch) Real-time PCR Detection Systems.

2 Intended use

The ***Resistance****Plus*® MG kit is a qualitative multiplexed in vitro diagnostic real-time PCR test for the identification of *M. genitalium* and detection of 5 mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, and A2059C, *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 kit may be used with the following specimen types: male and female urine, and anal, rectal, cervical, endocervical, vaginal, urethral, penile, penile meatal and pharyngeal swabs, 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 kit 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.

3 Contact information

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4 Clinical Study 1 - Royal Women's Hospital (RWH), Melbourne, Australia (LC480 II)

A prospective-retrospective clinical study was conducted at Royal Women's Hospital (RWH), Melbourne, Australia. Samples were collected from May 2016-June 2016 and based on the clinical laboratory results, 111 *M. genitalium* positive and 100 consecutive *M. genitalium* negative samples were selected for inclusion in the study. The 211 samples consisted of 84 urine, 7 anal swabs, 1 urogenital swab (no site specified (nss)), 1 rectal swab, and 1 urethral swab from men, and 33 urine, 33 cervical swabs, 16 endocervical swabs, 14 vaginal swabs, 13 high vaginal swabs, and 8 urogenital swabs (nss) from women. To determine performance of the ***Resistance****Plus*® MG kit, *M. genitalium* detection was compared to the clinical laboratory results from a well-established 16S rRNA qPCR used for routine diagnostics at RWH[1], and 23S rRNA mutant detection was compared to Sanger sequencing[2]. The ***Resistance****Plus*® MG kit was performed on the LC480 II, after sample extraction on the MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Universal Pathogen 200 protocol. For *M. genitalium* detection, a composite reference was used for discordant samples using a third qPCR reaction targeting the MgPa gene[3]. For 23S rRNA mutant detection, Sanger sequencing was taken as the true result. Resolved results and sensitivity and specificity of the ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 1**. Two specimens were excluded as the Internal Control result was invalid (1 female urine and 1 male urine). 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 2**. The 23S rRNA mutation analysis is shown in **Table 3**.

Table 1. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 1)

		***M. genitalium* detection 16S rRNA qPCR**			**23S rRNA mutant detection Sequencing**	
		Positive	**Negative**		**Mutant**	**Wild type**
***ResistancePlus*® MG**	**Positive**	106	0	**Mutant detected**	68	2
	Negative	4	99^	**Mutant not detected**	2	31
Sensitivity		96.4% (95% CI 91.0-99.0%)		**Sensitivity**	97.1% (95% CI 90.1-99.7%)	
Specificity		100.0% (95% CI 96.3-100.0%)		**Specificity**	93.8% (95% CI 79.2-99.2%)	

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

^ The ***Resistance****Plus*® MG kit detected 1 true *M. genitalium* negative using composite reference, table represents resolved results

Table 2. Clinical result analysis in accordance to specimen^ (Clinical Study 1)			
Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	28/28	8/10[1]	41/42[1]
Female urine	12/13	11/11	4/6[2]
Cervical swab	21/21	5/5	7/7[3]
Endocervical swab	10/10	3/3	3/3[4]
Vaginal swab	8/8	1/1	2/2[5]
High vaginal swab	9/9	1/1	4/4[6]
Male anal swab	3/3	0/0	5/5[7]
Female swab (nss)	5/5	2/2	1/1[8]
Male swab (nss)	0/0	0/0	1/1[9]
Male rectal swab	1/1	0/0	0/0
Male urethral swab	1/1	0/0	0/0

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

^ 2 female urine, 3 male urine, 1 vaginal swab excluded as sequencing failed and mutant status could not be determined

[1] Male urine: 2 *M. genitalium* wildtype miscalled as *M. genitalium* mutant detected, 18 A2058G, 20 A2059G, 3 A2058T correctly detected; 1 A2058G miscalled as *M. genitalium* not detected

[2] Female urine: 1 A2058G, 3 A2059G correctly detected; 2 A2059G miscalled as *M. genitalium* detected, mutant not detected

[3] Cervical swab: 1 A2058G, 6 A2059G correctly detected

[4] Endocervical swab: 2 A2059G, 1 A2058T correctly detected

[5] Vaginal swab: 3 A2058G, 1 A2059G correctly detected

[6] High vaginal swab: 2 A2059G correctly detected

[7] Male anal swab: 1 A2058G, 3 A2059G, 1 A2058T correctly detected.

[8] Female swab (no site specified (nss)): 1 A2059G correctly detected

[9] Male swab (nss): 1 A2059G correctly detected

Table 3. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 1)	
Reference result^	***ResistancePlus*® MG result**
Wild type	31/33[1]
A2058G	24/25[2]
A2059G	39/41[3]
A2058T	5/5

^ For *M. genitalium* positive samples only

[1] Wild type: 2 Male urine miscalled as *M. genitalium* mutant detected

[2] A2058G: 1 Male urine miscalled as *M. genitalium* not detected

[3] A2059G: 2 Female urine miscalled as *M. genitalium* mutant not detected

5 Clinical Study 2 - Royal Women's Hospital (RWH), Melbourne, Australia (7500 Fast)

A subset of the extracted specimens from study 1 were run on the 7500 Fast. Results were compared to the clinical result from the 16S rRNA qPCR (Twin 2011) and Sanger sequencing (Twin 2012). Discordant samples for *M. genitalium* detection were re-tested with the 16S rRNA qPCR (Twin 2011) due to suspected sample degradation. Resolved results and sensitivity and specificity of the ***Resistance****Plus*® $MG_{(550)}$ kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 4**. Analysis of 23S rRNA mutation detection only includes samples where the mutant status could be determined.

Table 4. Clinical evaluation of the *ResistancePlus*® $MG_{(550)}$ kit (Clinical Study 2)

		***M. genitalium* detection 16S rRNA qPCR**				**23S rRNA mutant detection Sequencing**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG**	**Positive**	99	0^		**Mutant detected**	62	0
	Negative	2	81[#]		**Mutant not detected**	5	30
Sensitivity		98.0% (95% CI 93.0-99.8%)			**Sensitivity**	92.5% (95% CI 83.4-97.5%)	
Specificity		100.0% (95% CI 95.6-100.0%)			**Specificity**	100.0% (95% CI 88.4-100.0%)	

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (E. coli numbering); Wild type – absence of mutation in these positions

^ The ***Resistance****Plus*® $MG_{(550)}$ kit detected 1 true *M. genitalium* positive using reference test, table represents resolved results

[#] The ***Resistance****Plus*® $MG_{(550)}$ kit detected 10 samples true *M. genitalium* negatives using reference test, table represents resolved results

6 Clinical Study 3 - Canterbury Health Laboratories (CHL), Christchurch, New Zealand

A retrospective clinical study was conducted at Canterbury Health Laboratories (CHL), Christchurch, New Zealand on characterised, archived samples from 2010-2016, consisting of 103 *M. genitalium* positive and 61 *M. genitalium* negative samples, collected with the multi-Collect Specimen Collection Kit (Abbott). The 164 samples consisted of 110 urine and 4 rectal swabs from men, and 11 urine, 17 cervical swabs, 15 vaginal swabs, 1 urethral swab, 1 urethral/vaginal swab, 1 vaginal/cervical swab and 4 samples from unknown sites from women. To determine performance of the ***Resistance****Plus*® MG kit, *M. genitalium* detection was compared to the clinical laboratory result from a well-established MgPa qPCR, which is also used for routine diagnostics at CHL (Jensen 2004), and 23S rRNA mutant detection was compared to Sanger sequencing (Jensen 2008). The ***Resistance****Plus*® MG kit was performed on the LC480 II, after sample extraction on the MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Universal Pathogen 200 protocol. For *M. genitalium* detection, the routine MgPa test was repeated for discordant samples. For 23S rRNA mutant detection, Sanger sequencing was taken as the true result. The sensitivity and specificity of the ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 5**. Five samples were excluded as the Internal Control result was invalid. 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 6**. The 23S rRNA mutation analysis is shown in **Table 7**.

Table 5. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 3)

		***M. genitalium* detection 16S rRNA qPCR**			**23S rRNA mutant detection Sequencing**	
		Positive	**Negative**		**Mutant**	**Wild type**
***ResistancePlus*® MG**	**Positive**	90	0	**Mutant detected**	61	1
	Negative	7	67^	**Mutant not detected**	6	22
Sensitivity		92.8% (95% CI 85.7-97.1%)		**Sensitivity**	91.0% (95% CI 81.5-96.6%)	
Specificity		100.0% (95% CI 94.6-100.0%)		**Specificity**	95.6% (95% CI 79.7-99.9%)	

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

^ The ***Resistance****Plus*® MG kit detected 7 true *M. genitalium* negatives, table represents resolved results

Table 6. Clinical result analysis in accordance to specimen (Clinical Study 3)

Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	45/45	17/18[1]	38/47[1]
Female urine	4/4	1/1	6/6[2]
Cervical swab	5/5	3/3	8/9[3]
Vaginal swab	6/6	1/1	8/8[4]
Male rectal swab	3/3	0/0	0/1[5]
Female (unknown site)	1/1	1/1	1/2[6]
Female urethral swab	1/1	0/0	0/0
Urethral/vaginal swab	1/1	0/0	0/0
Vaginal/cervical swab	1/1	0/0	0/0

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

[1] Male urine: 1 *M. genitalium* wild type miscalled as *M. genitalium* mutant detected, 4 A2058G, 32 A2059G, 1 A2058T, 1 A2058C, 1 A2059C, correctly detected; 1 A2058G, 1 A2059G and 1 A2059C miscalled as *M. genitalium* not detected, 3 A2058G and 2 A2059G miscalled as *M. genitalium* mutant not detected

[2] Female urine: 2 A2058G, 4 A2059G correctly detected

[3] Cervical swab: 3 A2058G, 4 A2059G, 1 A2058C correctly detected; 1 A2059G miscalled as *M. genitalium* not detected

[4] Vaginal swab: 1 A2058G, 7 A2059G correctly detected

[5] Male rectal swab: 1 A2059G miscalled as *M. genitalium* not detected

[6] Female (unknown site): 1 A2059G correctly detected; 1 A2059G miscalled as *M. genitalium* mutant not detected

Table 7. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 3)	
Reference result^	***ResistancePlus*® MG result**
Wild type	22/23[1]
A2058G	10/13[2]
A2059G	47/50[3]
A2058T	1/1
A2058C	2/2
A2059C	1/1

^ For *M. genitalium* positive samples only

[1] Wild type: 1 Male urine miscalled as *M. genitalium* mutant detected

[2] A2058G: 3 Male urine miscalled as *M. genitalium* mutant not detected

[3] A2059G: 2 Male urine miscalled as *M. genitalium* mutant not detected, 1 Female sample (unknown site) miscalled as *M. genitalium* mutant not detected

7 Clinical Study 4 - Vall d'Hebron University Hospital (HUVH), Barcelona, Spain

A retrospective clinical study was performed at Vall d'Hebron University Hospital (HUVH), Barcelona, Spain, to evaluate the performance of the ***Resistance****Plus*® $MG_{(675)}$ kit for the detection of *M. genitalium* and azithromycin resistance-associated mutations in retrospective samples collected between December 2017-April 2018. Samples included 92 *M. genitalium* positive and 108 consecutive *M. genitalium* negative specimens, collected using the DeltaSwab ViCUM® (Deltalab, Spain) for swabs or Vacumed® Urine (FL medical, Italy) for male and female urine. The 200 samples consisted of 46 urine, 30 vaginal swabs, 30 urethral swabs, 40 cervical swabs, 8 pharyngeal swabs and 46 rectal swabs. Samples were extracted with the STARlet IVD (Hamilton) and run on the CFX96 IVD (Bio-Rad) instrument. To assess the performance, *M. genitalium* detection was compared to Allplex™ STI Essential (Seegene) as well as to the ***Resistance****Plus*® MG kit (SpeeDx) on the LC480 II for both *M. genitalium* detection and 23S rRNA status. The sensitivity and specificity of the ***Resistance****Plus*® $MG_{(675)}$ kit for *M. genitalium* detection compared to Allplex™ STI Essential (Seegene) is shown in **Table 8**. The sensitivity and specificity of the ***Resistance****Plus*® $MG_{(675)}$ kit for *M. genitalium* detection was 100.0% (95% CI 95.9-100.0%) and 97.4% (95% CI 92.4-99.5%), respectively and for 23S rRNA mutant detection was as shown in **Table 9**. Analysis of results in accordance to specimen type is shown in **Table 10**.

Table 8. Comparison of *ResistancePlus*® $MG_{(675)}$ kit with Allplex™ STI essential (Clinical Study 4)

		***M. genitalium* detection Allplex™ STI Essential**	
		Positive	**Negative**
***ResistancePlus*® $MG_{(675)}$**	**Positive**	89	1
	Negative	3	107
Sensitivity		96.7% (95% CI 90.8-99.3%)	
Specificity		99.1% (95% CI 94.95-100.0%)	

Table 9. Clinical evaluation of the *ResistancePlus*® $MG_{(675)}$ kit (Clinical Study 4)

		***M. genitalium* detection *ResistancePlus*® MG (LC480 II)**			**23S rRNA mutant detection[#] *ResistancePlus*® MG (LC480 II)**	
		Positive	**Negative**		**Mutant detected**	**Mutant not detected**
***ResistancePlus*® $MG_{(675)}$**	**Positive**	87	3	**Mutant detected**	42^	0
	Negative	0	110	**Mutant not detected**	2	42*
Sensitivity		100.0% (95% CI 95.9-100.0%)		**Sensitivity**	95.5% (95% CI 84.5-99.4%)	
Specificity		97.4% (95% CI 92.4-99.5%)		**Specificity**	100.0% (95% CI 91.6-100.0%)	

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

^ The ***Resistance****Plus*® $MG_{(675)}$ kit detected 1 true M. genitalium mutant using reference test, table represents resolved results

* The ***Resistance****Plus*® $MG_{(675)}$ kit detected 1 true M. genitalium negative using reference test, table represents resolved results

[#] 1 sample excluded from analysis as this was sequenced as mixed wild-type and mutant

Table 10. Clinical result analysis in accordance to specimen (Clinical Study 4)			
Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	26/26	5/5	15/15
Male urethral swab	15/15	3/3	11/12[1]
Female cervical swab	16/16	11/11	2/3[3]
Female vaginal swab	20/20	15/15	5/5
Male rectal swab	19/22[1]	5/5	8/8
Female rectal swab	7/7	3/3	0/0
Male pharyngeal swab	5/5	0/0	1/1
Female pharyngeal swab	2/2	0/0	0/0

[1] Male rectal swab: 3 *M. genitalium* negative miscalled as *M. genitalium* positive

[2] Male urethral swab: 1 *M. genitalium* 23S rRNA mutation positive miscalled as *M. genitalium* 23S rRNA mutation negative

[3] Female cervical swab: 1 *M. genitalium* 23S rRNA mutation positive miscalled as *M. genitalium* 23S rRNA mutation negative

8 Clinical Study 5 - Royal Women's Hospital (RWH), Melbourne, Australia (Aptima® collection)

A retrospective clinical study was conducted at Royal Women's Hospital (RWH), Melbourne, Australia using Aptima® collected urine and swabs from June 2017-November 2017. Matched patient specimens consisted of 98 *M. genitalium* positive and 87 consecutive *M. genitalium* negative, collected as neat urine (routine sample) or with the Aptima® Urine Specimen Collection kit (Hologic), or as dry swab (routine sample) or with the Aptima® Unisex Swab Specimen Collection kit (Hologic). The 185 samples consisted of 122 urine, 18 rectal swabs, 15 cervical swabs, and 25 vaginal swabs. To determine the performance of Aptima® collected samples with the ***Resistance****Plus*® MG kit, *M. genitalium* and 23S rRNA mutant detection was compared to the clinical diagnostic results obtained from the ***Resistance****Plus*® MG kit (SpeeDx) using the routine sample. Testing of Aptima® collected samples was performed on the LC480 II, after sample extraction on the MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Viral NA Universal LV 1000 protocol. Clinical diagnostic results from RWH, obtained from a matched diagnostic sample tested with the ***Resistance****Plus*® MG kit (SpeeDx), was taken as the true result for *M. genitalium*. For the 23S rRNA mutant detection, the result was compared to the diagnostic result and Sanger sequencing.

The sensitivity and specificity of the ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 11**. 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 12**.

Table 11. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 5)

		***M. genitalium* detection** ***ResistancePlus*® MG (routine sample)**			**23S rRNA mutant detection** ***ResistancePlus*® MG (routine sample)**	
		Positive	**Negative**		**Mutant**	**Wild type**
***ResistancePlus*® MG (with 1ml Aptima sample)**	**Positive**	94	3	**Mutant detected**	65	0
	Negative	4	84	**Mutant not detected**	1*	28
Sensitivity		95.9% (95% CI 89.9-98.9%)		**Sensitivity**	98.5% (95% CI 91.8-100.0%)	
Specificity		96.6% (95% CI 90.3-99.3%)		**Specificity**	100.0% (95% CI 87.7-100.0%)	

* Sample could not be sequenced

Table 12: Clinical result analysis in accordance to specimen type (Clinical Study 5)

Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Urine	50/52[1]	21/22[1]	45/48[1]
Cervical swab	11/11	1/1	3/3
Vaginal swab	14/15[2]	3/4[2]	6/6
Rectal swab	9/9	3/3	5/6[3]
Anal swab	0/0	0/0	5/5

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, A2059C positions (*E.coli* numbering); Wild type – absence of mutation in these positions

[1] Urine: 2 *M. genitalium* negatives miscalled as *M. genitalium* wild type and mutant respectively; 1 *M. genitalium* wild type miscalled as *M. genitalium* negative; 2 *M. genitalium* mutants miscalled as *M. genitalium* wild type, 1 *M. genitalium* mutant miscalled as *M. genitalium* negative

[2] Vaginal swab: 1 *M. genitalium* negative miscalled as *M. genitalium* wild type; 1 *M. genitalium* wild type miscalled as *M. genitalium* negative

[3] Rectal swab: 1 *M. genitalium* mutant miscalled as *M. genitalium* negative

9 Clinical Study 6 - University of Queensland Centre for Clinical Research (UQCCR), Australia

A retrospective clinical study was conducted at University of Queensland Centre for Clinical Research (UQCCR), Australia, using cobas® x480 extracts from urine and swab samples collected from February 2017-February 2019. Specimens consisted of 85 *M. genitalium* positive and 84 *M. genitalium* negative extracts, originally collected as neat urine or with the cobas® PCR media collection kit (Roche) and extracted on the cobas® x480 (cobas® 4800, Roche) instrument using the "Full Workflow" and "CT/NG" protocol, without addition of SpeeDx Internal Control Cells. The 169 extracts consisted of 28 rectal swabs, 13 vaginal swabs, 5 high vaginal swabs, 15 cervical swabs, 1 ectocervical swab, 5 urethral swabs, 5 pharyngeal swabs, 1 penile swab, 1 penile meatal, 1 mouth swab, as well as 83 male and 11 female urine specimens.

To determine the performance of cobas® extracts with the ***Resistance****Plus*® $MG_{(550)}$ kit, *M. genitalium* detection was compared to the routine diagnostic result (MgPa PCR assay (Trembizki *et al.*, 2017)) and 23S rRNA mutant detection was compared to Sanger sequencing. The ***Resistance****Plus*® $MG_{(550)}$ kit was performed on the ABI 7500 Fast Dx, with master mix prepared using the ***Plex****PCR*® Amplification control as described in **Section** Error! Reference source not found.. The sensitivity and specificity of the ***Resistance****Plus*® $MG_{(550)}$ kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 13**. 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 14**. The 23S rRNA mutation analysis is shown in **Table 15**.

Table 13: Clinical evaluation of the *ResistancePlus*® $MG_{(550)}$ kit (Clinical Study 6)

		***M. genitalium* detection MgPa qPCR**			**23S rRNA mutant detection Sanger Sequencing**	
		Positive	**Negative**		**Mutant**	**Wild type**
***ResistancePlus*® $MG_{(550)}$**	**Positive**	80	0	**Mutant detected**	49^	0
	Negative	5	84	**Mutant not detected**	0	25
Sensitivity		94.1% (95% CI 86.8-98.1%)		**Sensitivity**	100.0% (95% CI 92.8-100.0%)	
Specificity		100.0% (95% CI 95.7-100.0%)		**Specificity**	100.0% (95% CI 86.3-100.0%)	

^ 1 vaginal sample returned a mixed wild-type/A2059G sequencing result which was correctly identified as mutant by the ***Resistance****Plus*® $MG_{(550)}$ assay

Table 14. Clinical result analysis in accordance to specimen (Clinical Study 6) #			
Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	42/42	13/13	26/27[1]
Female urine	6/6	1/1	3/3[2]
Cervical swab	5/5	6/6	2/2[3]
Ectocervical swab	1/1	-	-
Vaginal swab	1/1	1/2	7/7[4^]
High vaginal swab	2/2	2/2	1/1[5]
Male rectal swab	17/17	1/1	7/8[6]
Female rectal swab	1/1	-	-
Male urethral swab	3/3	-	2/2[7]
Male pharyngeal	5/5	-	-
Penile swab	-	1/1	-
Penile meatal swab	-	-	1/1[8]
Male mouth swab	1/1	-	-

6 samples were excluded as sequencing failed and true 23S status could not be determined, including: 2 cervical, 2 urine, 1 vaginal and 1 rectal sample

[1] Male urine: 8 A2058G, 3 A2058T and 15 A2059G correctly identified; 1 A2058T was incorrectly identified as *M. genitalium* not detected

[2] Female urine: 2 A2058G and 1 A2059G correctly identified

[3] Cervical swab: 2 A2058G correctly identified

[4] Vaginal swab: 3 A2058G, 2 A2058T and 1 A2059G correctly identified; ^ 1 vaginal swab was identified as a mixture WT/A2059G

[5] High vaginal swab: 1 A2059G correctly identified

[6] Male rectal swab: 5 A2059G, 1 A2058T and 1 A2058G correctly identified; 1 A2058G was incorrectly identified as *M. genitalium* not detected

[7] Male urethral swab: 2 A2059G correctly identified

[8] Penile meatal swab: 1 A2059G correctly identified

Table 15. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 6)	
Reference result^	***ResistancePlus*® MG result**
Wild type	25/26[1]
A2058G	16/17[2]
A2059G	27/27[3]
A2058T	6/7[4]
A2058C	-
A2059C	-

^ For *M. genitalium* positive samples only

[1] Wild type: 1 vaginal swab miscalled as *M. genitalium* not detected

[2] A2058G: 1 rectal swab miscalled as *M. genitalium* not detected

[3] A2059G: 1 vaginal swab mixed wild-type/A2059G correctly identified as *M. genitalium*, 23S mutation detected

[4] A2058T: 1 male urine swab miscalled as *M. genitalium* not detected

10 Clinical Study 7 - Microbiological Diagnostic Unit Public Health Unit (MDU), Victoria, Australia

A retrospective clinical study was conducted at Microbiological Diagnostic Unit Public Health Unit (MDU), Victoria, Australia, using dry swabs and neat urine collected from October 2018-January 2019. Specimens consisted of 59 *M. genitalium* positive and 31 *M. genitalium* negative samples, including 15 anal swabs, 19 vaginal swabs, 2 high vaginal, 8 cervical, 1 urethral swab, as well as 45 male urine specimens.

The ***Resistance****Plus*® MG kit was performed on the LC480 II, after sample extraction on the QIAsymphony SP (QIAGEN) instrument using the DSP Virus/Pathogen Mini kit and the Complex200_V6_DSP protocol. Results were compared to the routine diagnostic results obtained from the ***Resistance****Plus*® MG kit (SpeeDx) using samples extracted on the MagNA Pure 96 Instrument (MP96). For discordant results, a 16S rRNA qPCR (Twin 2011) test was performed for *M. genitalium* detection, and Sanger sequencing (Twin 2012) was performed for 23S rRNA mutant detection. The sensitivity and specificity of the ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 16**. 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 17**.

Table 16: Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 7)

		***M. genitalium* detection *ResistancePlus*® MG (MP96)**			**23S rRNA mutant detection *ResistancePlus*® MG (MP96)**	
		Positive	**Negative**		**Mutant**	**Wild type**
***ResistancePlus*® MG (QIAsymphony SP)**	**Positive**	54	0[%]	**Mutant detected**	28	1[#]
	Negative	1[*]	34	**Mutant not detected**	1[#]	22
Sensitivity		98.2% (95% CI 90.3-100.0%)		**Sensitivity**	96.6% (95% CI 82.2-99.9%)	
Specificity		100.0% (95% CI 89.7-100.0%)		**Specificity**	95.7% (95% CI 78.1-99.9%)	

[*] The ***Resistance****Plus*® MG kit detected 6 true *M. genitalium* negative samples which were positive with reference test, table represents resolved results

[%] The ***Resistance****Plus*® MG kit detected 2 true *M. genitalium* positive samples which were negative with reference test, table represents resolved results

[#] 2 discordant urine samples could not be resolved as sequencing failed

Table 17. Clinical result analysis in accordance to specimen (Clinical Study 7) [#]

Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male Urine	17/17	9/9	12/14[1]
Female Urine	1/1	1/2[2]	1/1
Cervical swab	3/3	2/2	3/3
Vaginal swab	8/8[#]	7/7	3/3
High vaginal swab	1/1	1/1	-
Male Anal swab	4/4	2/2	8/8
Male urethral swab	-	-	1/1

[#] 1 vaginal swab was excluded as it produced an invalid result with the ***Resistance****Plus*® MG kit

[1] Male urine: 1 *M. genitalium* 23S rRNA wild type was incorrectly identified as *M. genitalium* not detected; 1 *M. genitalium* 23S rRNA mutant was incorrectly identified as *M. genitalium* detected, 23S mutation not detected

[2] Female urine: 1 incorrectly identified as *M. genitalium* detected, 23S rRNA mutation detected

11 Clinical Study 8 - Multi-centre clinical study

The clinical performance of the ***Resistance****Plus*® MG assay was established in a prospective, multi-centre clinical study. Specimens were collected from 10 geographically diverse sites which included family planning, STD clinics, HIV clinics, obstetrics/gynecology (OB/GYN) clinics, and primary care clinics. A total of 1097 male urine and 1289 female vaginal (either self-collected or clinician-collected) swab samples, from symptomatic and asymptomatic subjects were included in the study.

The ***Resistance****Plus*® MG assay was run on samples extracted on the NucliSENS® easyMAG® and real-time PCR performed on the Applied Biosystems® 7500 Fast Dx instrument. Performance was compared to a validated in-house pdhD real-time PCR assay for *M. genitalium* detection and sanger sequencing for 23S rRNA mutation detection. Analysis of 23S rRNA mutation detection only included samples where the mutant status could be determined.

The overall sensitivity and specificity of the ***Resistance****Plus*® MG kit for *M. genitalium* detection was 90.4% and 98.1%, respectively, and for 23S rRNA mutant detection was 91.6% and 97.8%, respectively (**Table 18**). Analysis of results in accordance to specimen type is shown in **Table 19**. The 23S rRNA mutation analysis is shown in **Table 20**.

Table 18. Clinical evaluation of the *ResistancePlus*® MG kit

		Reference MG detection (pdhD real-time PCR assay)			Reference 23S mutant detection (Sanger Sequencing)	
		MG Positive	**MG Negative**		**Mutant**	**Wild type**
ResistancePlus MG (550)	**MG Positive**	170	40	**Mutant**	109	1^
	MG Negative	18	2107	**Mutant not detected**	10	44
	Total	188	2147	**Total**	119	45
	Sensitivity	90.4% (95% CI 85.3 – 94.2%)		**Sensitivity**	91.6% (95% CI 85.1 – 95.9%)	
	Specificity	98.1% (95% CI 97.5 – 98.7%)		**Specificity**	97.8% (95% CI 88.2 – 99.9%)	

^ 1 male urine (15-M5023) and 1 female swab (15-F1018) were re-sequenced and confirmed to be true mutants (the male urine was a A2058G/wild type mixture)

Table 19. 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	984/1002[1]	17/18[2]	69/75[3]
Female vaginal swab	1123/1145[4]	27/31[5]	40/56[6]

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

[1] 11 samples were incorrectly called *M. genitalium* 23S mutant not detected, and 7 were called *M. genitalium* 23S mutant

[2] 1 sample was incorrectly called *M. genitalium* 23S mutant

[3] 6 samples were incorrectly called *M. genitalium* not detected, 1 sample correctly identified as *M. genitalium* 23S mutant detected was a mixture of A2058G and wild type

[4] 15 samples were incorrectly called *M. genitalium* 23S mutant not detected, 5 samples were incorrectly called *M. genitalium* 23S mutant, and 2 were inconclusive for 23S

[5] 3 samples were incorrectly called *M. genitalium* not detected, and 1 sample was inconclusive for 23S

[6] 10 samples were incorrectly called *M. genitalium* 23S mutant not detected, 1 sample was inconclusive for 23S, and 5 were incorrectly called *M. genitalium* not detected

Table 20. Clinical result analysis according to 23S rRNA mutation	
Reference result	***ResistancePlus*® MG result**
Wild type	44/49[1]
A2059G	65/74[2]
A2058G	38/49[3]
A2058T	5/6[4]
A2059C	1/1

[1] 3 samples were incorrectly called *M. genitalium* not detected, 1 sample was false *M. genitalium* 23S mutant detected and ,1 sample 23S inconclusive

[2] 4 samples were incorrectly called *M. genitalium* not detected, 4 samples were false *M. genitalium* detected, 23S not detected and, 1 sample 23S inconclusive

[3] 5 samples were incorrectly called *M. genitalium* not detected, 5 samples were false *M. genitalium* detected, 23S not detected, 1 sample correctly identified as *M. genitalium* 23S mutant detected was a mixture of A2058G and wild type

[4] 1 samples were incorrectly called *M. genitalium* not detected

12 References

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3. Jensen JS, Bjornelius E, Dohn B, Lidbrink P. Use of Taqman 5' nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol. 2004 42:683-692.