Prospective Evaluation of the ResistancePlus™ MG:

Simultaneous Detection of *Mycoplasma genitalium* (MG) and Azithromycin Resistance Mutations

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Disclosure

SpeeDx is the developer and manufacturer of the *ResistancePlus*™ assay evaluated in this study

Mycoplasma genitalium (MG) clinical association

General population

- Detected in 1-3.3%
- Exact burden of disease is unknown

Men

- Strongly associated with non-gonococcal urethritis (NGU) (6-50%)
- Other conditions: Proctitis, Balanitis, Chronic prostatitis, Acute epididymitis

Women

- Strongly associated with cervicitis (5-20%)
- Other conditions: Endometritis, Pelvic inflammatory disease, Preterm birth

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Clinical management of MG

Empiric treatment of NGU & cervicitis

- **Doxycycline:** poor efficacy, microbiological cure rate of MG is 30-40%
- **Azithromycin:** higher efficacy, 1 g single dose has 85% cure rate

Decreasing overall cure rates with azithromycin due to MG resistance

- Where 1 g azithromycin is used to treat NGU, macrolide resistance is ~30-40%

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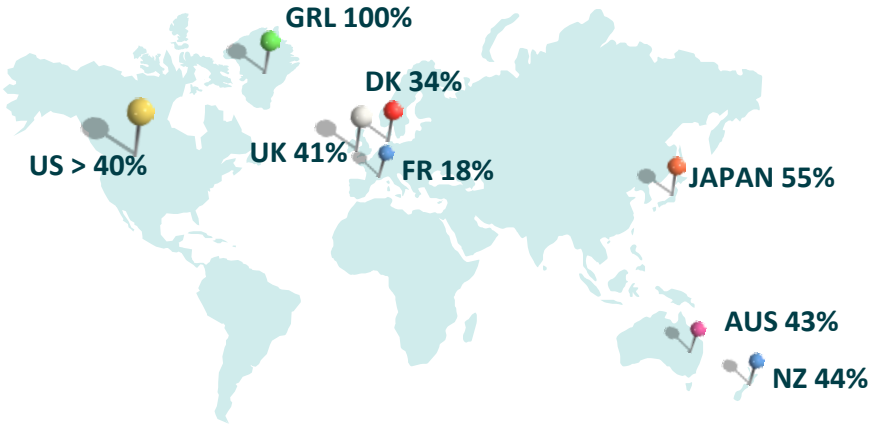
Macrolide resistance is an emerging global issue



Bissessor et. al., Clin. Infect. Dis. 2015;60(8):1228-36
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Le Roy, Emerg. Inf. Dis, 2016;22(9):1677-9.

Macrolide resistance in MG

Azithromycin resistance is associated with mutations in 23S rRNA gene

- Prevent azithromycin binding and inhibiting translation
- A2058G, A2059G, A2058T, A2058C, A2059C (*E. coli* numbering)
  - Confer high MICs or treatment failure
- 82% of treatment failures showed presence these mutations prior to treatment
  - 18% showed a mutation post-treatment

Bissessor et. al., Clin. Infect. Dis. 2015;60(8):1228-36

MG testing

SpeeDx

MG detection

- In house qPCR tests and recent commercially available CE marked tests

Macrolide resistance detection (23S rRNA mutation)

- Methods for mutation detection
  - Sequencing – Costly and generally not convenient for routine diagnostics
  - High resolution melt analysis (HRMA) – Separate assay to MG detection, not easy to analyse
  - Fluorescence resonance energy transfer (FRET) – Lacking in sensitivity

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CE IVD

- Single well qPCR assay
- Detects MG
- Detects 5 of the most common 23S rRNA macrolide resistance mutations

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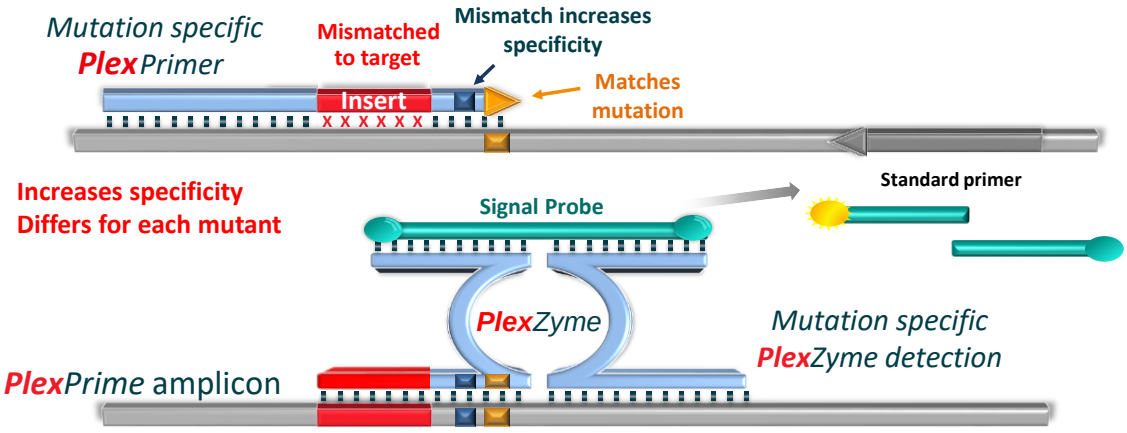
PlexPrime™ for mutation detection

Enhanced specificity and superior multiplexing



Mutation specific *PlexPrime* AND Mutation specific *PlexZyme* detection

Patent: WO 2013/123552

ResistancePlus™ MG

Sensitivity and specificity

	Target	
Channel 1	MG detection (MgPa)	
Channel 2	23S rRNA mutation	A2058G
		A2059G
		A2058T
		A2058C
		A2059C
Channel 3	Internal Control	

ResistancePlus™ MG
Sensitivity and specificity

	Target		Sensitivity (copies/reaction)
Channel 1	MG detection (MgPa)		10
Channel 2	23S rRNA mutation	A2058G	12
		A2059G	10
		A2058T	10
		A2058C	10
		A2059C	15
Channel 3	Internal Control		n/a

	Specific against other targets
Targets	*M. hominis, U. parvum, U. urealyticum, C. trachomatis, N. gonorrhoeae, T. vaginalis, S. pneumoniae, M. pneumoniae, M. gallisepticum, E. coli, E. faecalis, P. aeruginosa, K. pneumoniae*, HSV-1, HSV-2

Prospective study: *ResistancePlus*™ MG
Sample cohort

- 1089 samples received Nov 2015-Dec 2015 at RWH (Melbourne, Australia)
  - Royal Women's Hospital, Melbourne Sexual Health Centre, and external sites
  - Symptomatic and asymptomatic males and females

	Urine/ urethral swab	Anal swab	Cervical/ vaginal swab	Sample numbers
Male	354	34	n/a	388
Female	203	2	496	701
Total	557	36	496	1089

Prospective study: *ResistancePlus*™ MG
M. genitalium detection

		In house qPCR (16S rRNA)		
		+	-	Total
SpeeDx	+	64	0	64
	-	1	1024	1025
	Total	65	1024	1089

	%	95% CI
Sensitivity	98.5	91.7 to 99.9
Specificity	100.0	99.6 to 100.0
PPV	100.0	94.4 to 100.0
NPV	99.9	99.5 to 100.0

- **MG prevalence = 6.0%**
  - Male 10.8% (42/388)
  - Female 3.3% (23/701)

Prospective study: *ResistancePlus*™ MG
23S rRNA mutant detection

		Sanger Sequencing		
		Mutant	Wild type	Total
SpeeDx	Mutant	38	1	39
	Wild type	0	25	25
	Total	38	26	64*

	%	95% CI
Sensitivity	100.0	90.8 to 100.0
Specificity	96.2	80.4 to 99.9
PPV	97.4	86.5 to 99.9
NPV	100.0	86.3 to 100.0

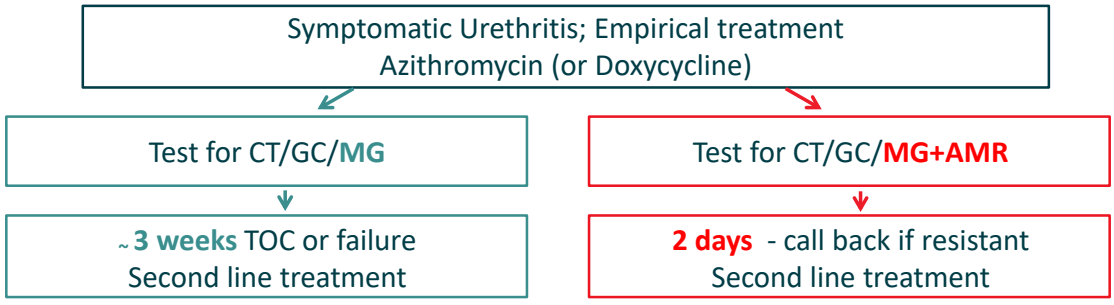
* Only includes MG positive samples by both methods

- **MG 23S rRNA mutant prevalence = 63.1%**
  - Male 81.0% (34/42)
  - Female 30.4% (7/23)

Highly multiplexed test with excellent clinical sensitivity and specificity

Submitted for TGA Approval

Improved patient care with *ResistancePlus*™ MG



- Patient has MG+AMR ~ 6 weeks
- Community spread of AMR MG

- **Improve patient management and clinical outcomes**
- **Limit spread of AMR**

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ResistancePlus™ assays in development

- MG fluoroquinolone resistance
- Gonorrhoea macrolide resistance

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MG and emerging resistance

Fluoroquinolones

- Moxifloxacin often used as second line treatment
  - 12% treatment failure in Australia
- Resistance linked to clustered mutations in ***parC***
  - High levels of resistance linked to a **G248T** mutation
  - Other parC mutations in the literature include **A247C**, **G259A/C/T**

ResistancePlus™ MG – Fluoroquinolone resistance

Preliminary clinical testing

	Target	
Channel 1	MG detection (MgPa)	
Channel 2	parC mutation	G248T
Channel 3	parC mutations	G247C
		G259A
		G259T
		G259C
Channel 4	Internal Control	

MG detection: 100% sensitivity (18/18)

MG parC mutations: 91% sensitivity (10/11) & 100% specificity (7/7)

For further queries:

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