High levels of *Mycoplasma genitalium* antibiotic resistance are observed in Australia

Bone S[1], Tan LY[1], Walker S[1], Su J[2], Murray G[2], Bradshaw C[3], Fairley C[3], Garland S[2], Mokany E[1] and Tabrizi S[2]

[1]SpeeDx Pty Ltd, Cicada Innovations, Sydney, Australia, [2]The Royal Women's Hospital, Melbourne, Australia, [2]Melbourne Sexual Health Centre, Melbourne, Australia

Background

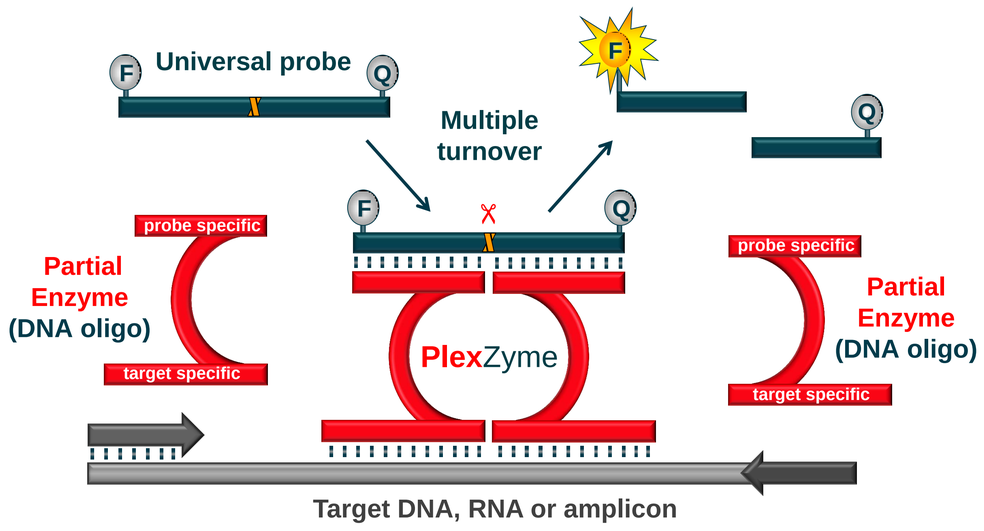
Mycoplasma genitalium (MG) is an emerging STI, strongly associated with nongonococcal urethritis and cervicitis. However, treatment of MG is complicated due to antibiotic resistance to the standard treatment, azithromycin. Moxifloxacin (fluoroquinolone) can be used as a second-line antibiotic.[1]

European IUSTI guidelines on MG infections and management of non-gonococcal urethritis strongly recommend NAAT testing for MG and screening for macrolide resistance, since this can provide clinical advantage and inform on the most appropriate therapy.[2]

The *Resistance*Plus™ MG kit (CE-IVD, SpeeDx) has been developed as a single well assay for the simultaneous detection of MG and five mutations in the 23S rRNA gene associated with azithromycin resistance.

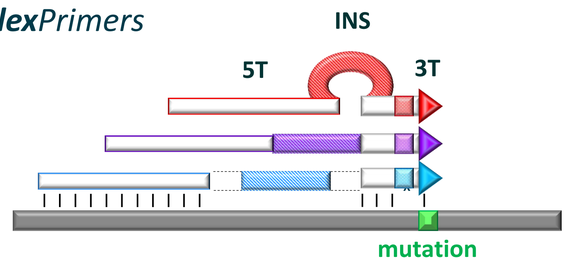
PlexPCR™ technology for NAATs

- **PlexZyme™ detection in qPCR is highly specific & sensitive**
- **Superior performance in multiplex**



PlexPrime™ enables mutation-specific detection & amplification

Mutation-specific multiplexed *PlexPrimers*



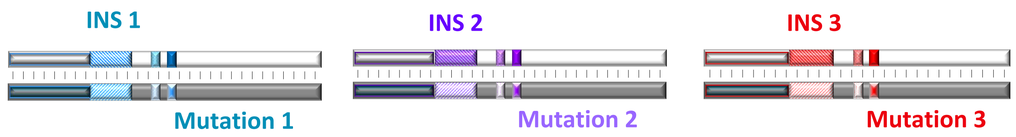
INS - unique sequence & conformation

5T - bind different target regions

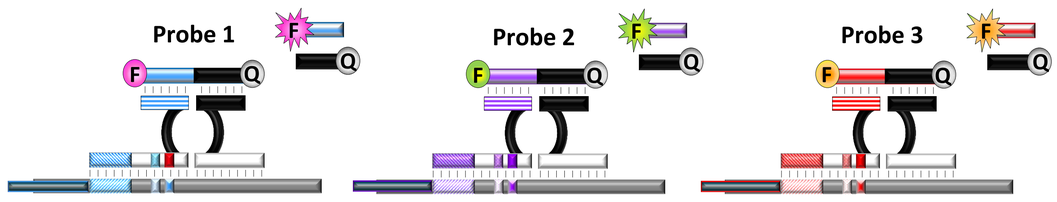
3T - match to mutation

REDUCE COMPETITION & INCREASE SPECIFITY

***PlexPrime* amplicons are distinctly different**



Mutation-specific *PlexZyme* detection



Superior multiplex capacity for clustered mutations

References: 1.Jensen & Bradshaw (2015) BMC Infectious Diseases, doi:10.1186/s12879-015-1041-6 2.http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf

3. Tabrizi et al (2016) PLoS One, doi: 10.1371/journal.pone.0156740

4. Tabrizi et al (2017) J Clin Microbiol, doi: 10.1128/JCM.02312-16

Evaluation study

- The *Resistance*Plus™ MG kit (SpeeDx) was evaluated in a prospective study on 1089 consecutive urine/urethral swabs, cervical/vaginal swabs and anogenital swab samples in symptomatic and asymptomatic male and female patients.
- Clinical specimens came from patients of Melbourne Sexual Health Centre and the Royal Women's Hospital, Melbourne, Australia. Specimens were tested at the Royal Women's Hospital with the *ResitancePlus*™ MG assay (SpeeDx Pty Ltd) as described in Tabrizi et al (2016) Plos One & Tabrizi et al (2017) JCM.
- Results were compared to an in-house qPCR test for MG detection and sequencing of positives to determine 23S rRNA mutation status

Results

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

Sensitivity 98.5%
(95% CI: 91.7-99.9%)
Specificity 100.0%
(95% CI: 99.6-100.0%)
Disease prevalence 6.0%

23S rRNA mutation detection		HRMA + Sequencing +	-	Total
SpeeDx	+	38	1	39
	-	0	25	25
	Total	38	26	64

Sensitivity 100.0%
(95% CI: 90.8-100.0%)
Specificity 96.2%
(95% CI: 80.4-99.9%)
Resistance prevalence 63.1%

Potential to guide treatment based on the detection of mutations associated with azithromycin resistance

Benefits of antibiotic resistance testing

- Detection of antibiotic resistance by qPCR can provide timely actionable information to allow personalised treatment and ensure faster cure rates and limit the spread of resistance.

In development

***PlexPCR*™ *N. gonorrhoeae ResistancePlus*™ kit**

N. gonorrhoeae genes; porA and opa
Macrolide resistance; 23S rRNA A2059G and C2611T

***PlexPCR*™ *M. pneumoniae ResistancePlus*™ kit**

M. pneumoniae gene; CARDS
Macrolide resistance; 23S rRNA A2058G, A2058C, A2059G & A2062G

***PlexPCR*™ *Carbapenemase ResistancePlus*™ kit**

Targets genes NDM, VIM, OXA, IMP and KPC

Conclusions

- The *Resistance*Plus™ MG assay demonstrated excellent clinical performance for the simultaneous detection of MG and assessment of mutations in the 23S gene associated with azithromycin resistance.
- Implications: The *Resistance*Plus™ MG assay could be useful for surveillance efforts where MG is high in incidence and azithromycin treatment failures are reported or expected.

SpeeDx

***PlexPCR*™ is a flexible, rapid & cost-effective technology for multiplexed detection of targets and genetic variants**

If you are interested in multiplexing your assay and/or wanting to achieve specific single base discrimination contact **info@speedx.com.au** or for more information about ***PlexPrime*™** & ***PlexPCR*™** technology visit **www.speedx.com.au**