



Evaluation of a *gyrA* Real-Time PCR Test for Guiding Ciprofloxacin Therapy of Gonorrhoea

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Acknowledgements

SpeedX – Samantha Ebeyan, Corey Oostendorp, Madeline Windsor, Simon Erskine, Elisa Mokany, Colin Denver
University of Queensland Centre for Clinical Research – David Whiley, Lebogang Mhango, Amanda Bordin, Ella Trembizki

Background



Antimicrobial resistant *Neisseria gonorrhoeae* (GC)

- Gonorrhoea is treated empirically, however resistance has developed to all previously recommended therapies
- Limited alternate therapies; New antimicrobials are not yet available

New strategy: Individualised patient treatment guided by antibiotic susceptibility information

- NAAT detection of genetic markers for resistance/susceptibility are feasible for routine diagnostics
- Resistance guided therapy is now recommended in Australia for the management of *Mycoplasma genitalium*¹

Re-introduce GC ciprofloxacin treatment based on *gyrA* genotype

- gyrA* S91F/WT genotype has >98% Sensitivity/Specificity compared to Ciprofloxacin Resistance/Susceptibility^{2,3,4}
- >70% susceptible in USA⁵, Australia⁶, NZ⁷; ~12-74% susceptible in Europe⁸

Aims:

- Analytical and clinical evaluation of the ResistancePlus GC test
 - Real-time PCR test for simultaneous detection of GC and *gyrA* markers for ciprofloxacin susceptibility/resistance

ResistancePlus GC		
	Channel	Target
1 Well	1	<i>N. gonorrhoeae</i> (<i>opa</i>)
	2	<i>N. gonorrhoeae</i> (<i>porA</i>)
	3	<i>gyrA</i> S91 wild type
	4	<i>gyrA</i> S91F mutation
	5	Internal Control

1) Australian STI Management Guidelines for Use in Primary Care 5) MMWR Surveillance Summaries. July 15, 2016 / 65(7):1–19





2) Trembizki, E. et al. Lancet Infect. Dis. 16, 1005–1006 (2016). 7) Australian Gonococcal Surveillance Programme Annual Report, 2017

3) Grad, Y. H. et al. J Infect Dis. 2016 Nov 15;214(10):1579–1587. 6) Antimicrobial resistance and molecular epidemiology of gonococci in NZ, 2014–15

4) Harris, S. R. et al. Lancet Infect. Dis. 18, 758–768 (2018).

8) Gonococcal antimicrobial susceptibility surveillance in Europe, 2015. Stockholm: ECDC;2017

ResistancePlus GC (beta) Analytical Performance

-  Analytical sensitivity
 - GC gyrA S91 wildtype – 15 geq/reaction
 - GC gyrA S91F mutant – 15 geq/reaction
-  Inclusivity
 - WHO strains (B, C, F, G, K, L, M, N, P), H041, FC428, A8806
-  Analytical specificity
 - 100% specificity:
 - *Neisseria spp.* and other organisms found in genital/throat/rectal sites
-  Interference
 - No interference at 3x LOD in the presence of *Neisseria spp.*

ResistancePlus GC (beta)

GC clinical isolates - UQ Centre for Clinical Research

-  Australian isolates (top 70 most common genotypes from 2012)

		WGS				Ciprofloxacin AST	
		S91F mut	S91 WT			Resistant (R)	Susceptible (S)
SpeedX	S91F mut	28	0	SpeedX	S91F mut	27	1*
	S91 WT	0	42		S91 WT	0	42
	Total	28	42		Total	27	43
Sensitivity		100.0% (95% CI 87.7-100.0%)		Sensitivity		100.0% (95% CI 87.7-100.0%)	
Specificity		100.0% (95% CI 91.4-100.0%)		Specificity		97.7% (95% CI 87.7-99.9%)	

* LS – less susceptible (S91F mutation by WGS)

High concordance to gyrA genotype & ciprofloxacin R/S phenotype

ResistancePlus GC (beta) Clinical Performance

Cobas extracts - UQ Centre for Clinical Research



- GC detection compared to GC clinical results (Cobas+in-house qPCR confirmation)
- gyrA detection compared to in-house gyrA qPCR

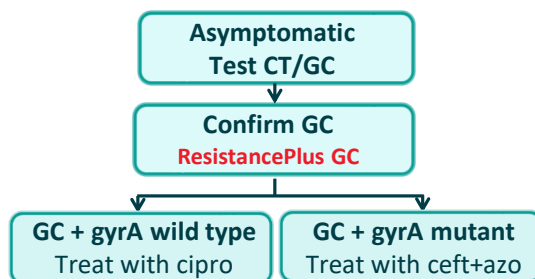
Cobas Specimens		GC detection		gyrA detection	
Specimen Type	Sample Numbers	Sensitivity	Specificity	Sensitivity	Specificity
Genital swabs*	181 pos & 180 neg	96.1% (95% CI 92.1-98.4%)	100.0% (95% CI 97.9-100.0%)	100.0% (95% CI 73.5-100.0%)	100.0% (95% CI 91.0-100.0%)
Pharyngeal swabs	81 pos & 110 neg	98.8% (95% CI 93.3-100.0%)	99.1% (95% CI 95.0-100.0%)	100.0% (95% CI 73.5-100.0%)	100.0% (95% CI 91.0-100.0%)
Urine^	27 male & 18 female	100.0% (95% CI 92.1-100.0%)	not tested	100.0% (95% CI 39.8-100.0%)	100.0% (95% CI 69.2-100.0%)
Rectal Swab^	15 male & 1 female	93.8% (95% CI 69.8-99.8%)	not tested	100.0% (95% CI 47.8-100.0%)	100.0% (95% CI 89.4-100.0%)

* Swabs - 166 cervical, 106 vaginal, 27 penile, 49 male urethral, 13 not specified

ResistancePlus GC – Future work



- Expected date for CE marking/TGA (Nov 2018 submission)
 - Cobas extracts, Aptima Sample, BD Viper sample
- Potential Implementation Pathway



- GRAND2 clinical trial