Development of the ResistancePlus GC Test to Inform Ciprofloxacin Treatment of Gonorrhoea

Litty Tan
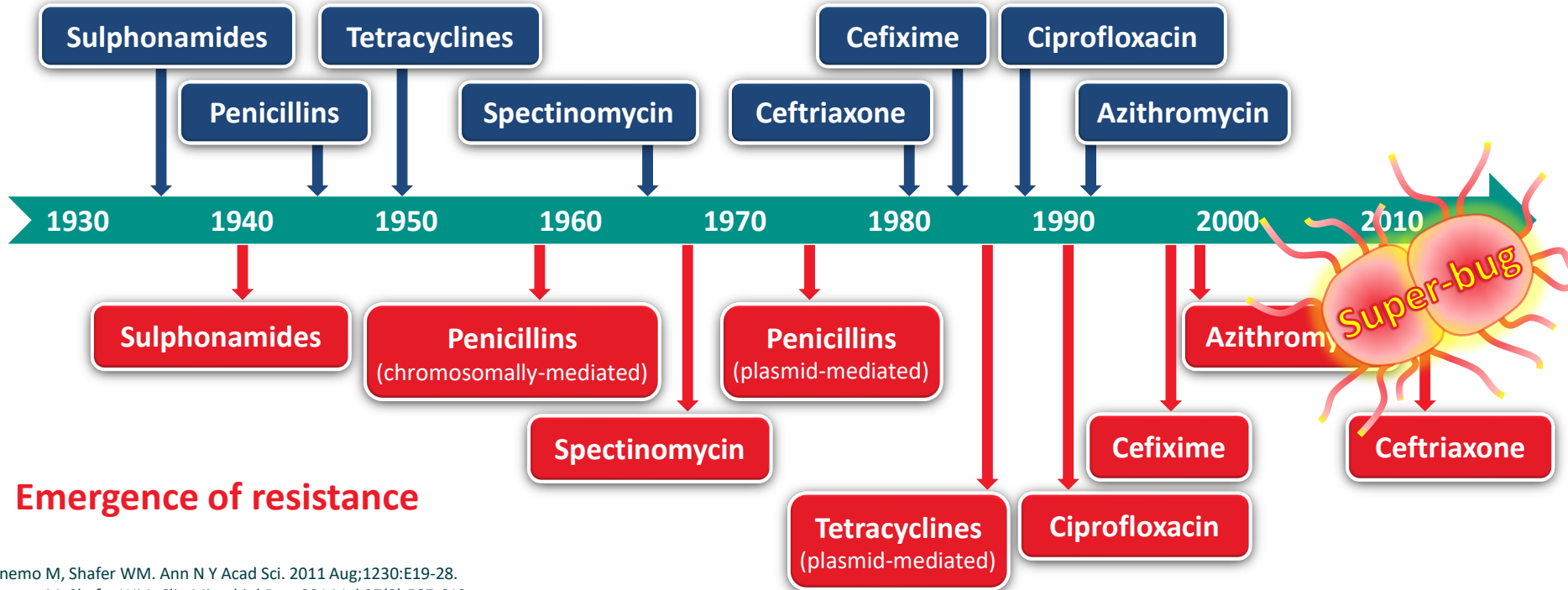
Director of R&D

littyt@speedx.com.au

Evolution of Antimicrobial Resistance (AMR) in Neisseria gonorrhoeae (GC)

SpeeDx

Antibiotics used for treatment

| Sulphonamides | Tetracyclines | | Cefixime | Ciprofloxacin |

| Penicillins | Spectinomycin | Ceftriaxone | Azithromycin |

1930 1940 1950 1960 1970 1980 1990 2000 2010

Sulphonamides

Penicillins (chromosomally-mediated)

Penicillins (plasmid-mediated)

Azithrom...

Super-bug

Spectinomycin

Cefixime

Ceftriaxone

Emergence of resistance

Tetracyclines (plasmid-mediated)

Ciprofloxacin

Unemo M, Shafer WM. Ann N Y Acad Sci. 2011 Aug;1230:E19-28.
Unemo M, Shafer WM. Clin Microbiol Rev. 2014 Jul;27(3):587-613.

WHO Global Action Plan for AMR GC

- Increase awareness on correct antibiotic use
- Effective prevention, diagnosis and control
- Monitor treatment failures
- Strengthen AMR surveillance
- Effective drug regulations and prescription policies
- Newer molecular methods for monitoring and detecting AMR
- Alternative effective treatment regimens

Global action plan
to control the spread and impact of
antimicrobial resistance in *Neisseria gonorrhoeae*

World Health Organization

Molecular detection of genetic markers for resistance/susceptibility could allow 'older' drugs to be used for gonorrhoea treatment

GC Susceptibility to Ciprofloxacin
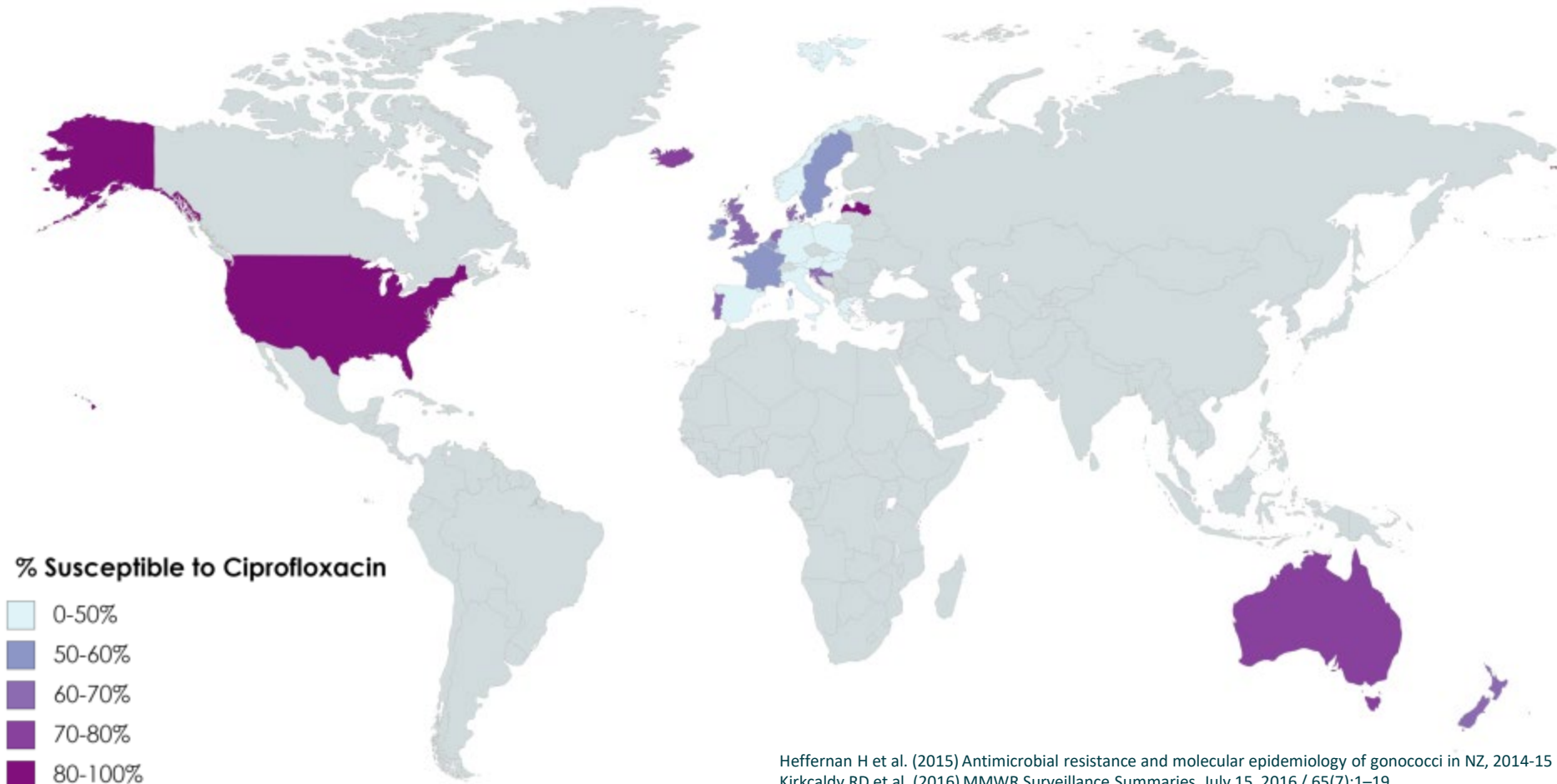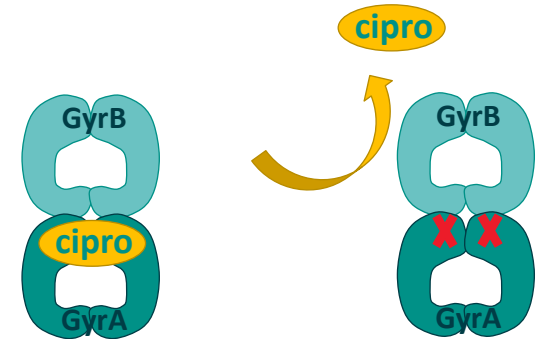

% Susceptible to Ciprofloxacin

- 0-50%
- 50-60%
- 60-70%
- 70-80%
- 80-100%

Heffernan H et al. (2015) Antimicrobial resistance and molecular epidemiology of gonococci in NZ, 2014-15
Kirkcaldy RD et al. (2016) MMWR Surveillance Summaries. July 15, 2016 / 65(7);1–19
Lahra MM et al. (2017) Australian Gonococcal Surveillance Programme Annual Report, 2017

GC Mechanisms of Fluoroquinolone Resistance

- Fluoroquinolones were predominant GC treatment in 1990s
  - 2007 - No longer recommended

- Quinolones act by inhibition of DNA gyrase and topoisomerase IV
  - DNA gyrase – heterotetramer of GyrA and GyrB
  - Topoisomerase IV – heterotetramer of ParC and ParE

- Bacteria develop resistance through mutations in the QRDR (quinolone resistance determining region)
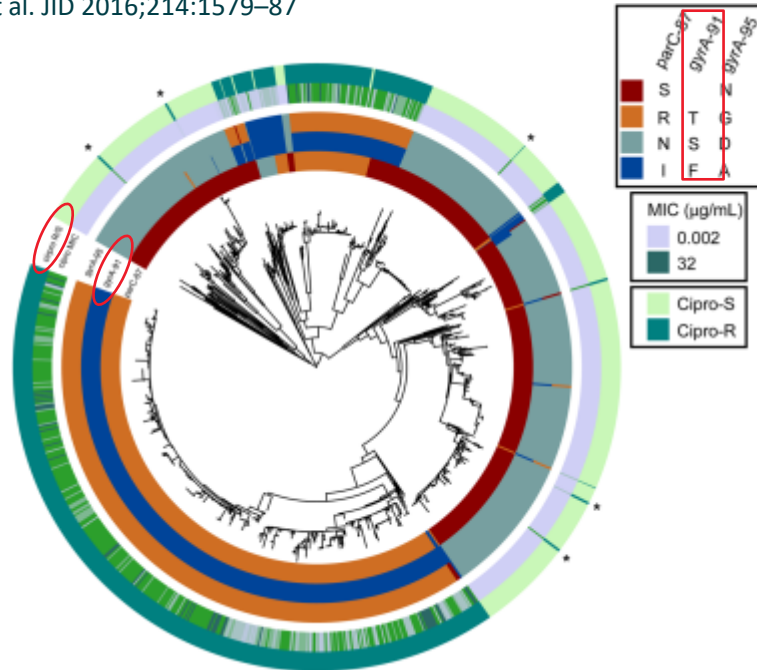 - GC: GyrA (aa positions 91, 95) and ParC (87)

GyrA Genotype Predicts Cipro Resistance in GC
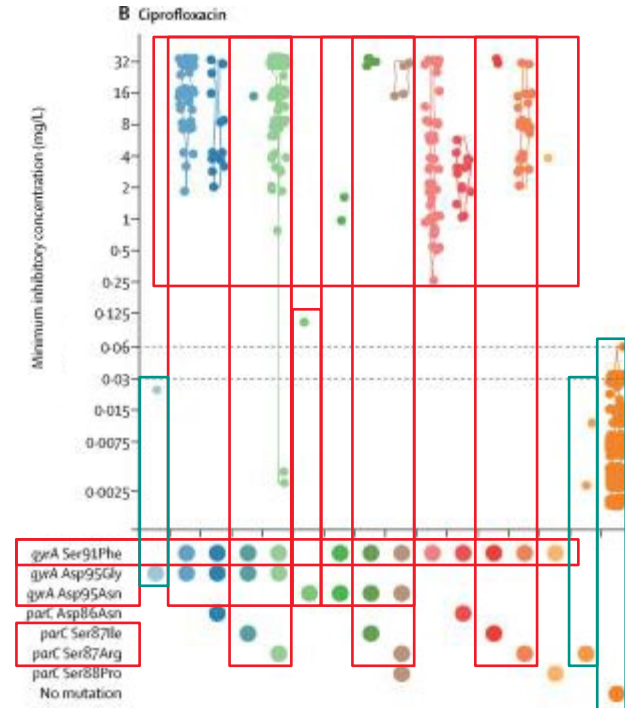
Genomic Epidemiology in US, 2000-2013 isolates

Grad et al. JID 2016;214:1579–87

GyrA Genotype	Predictive Value
S91F	PPV of Ciprofloxacin Resistance = 98%
S91 WT	NPV of Ciprofloxacin Susceptibility = 99%

Genomic Survey in Europe, 2013 isolates

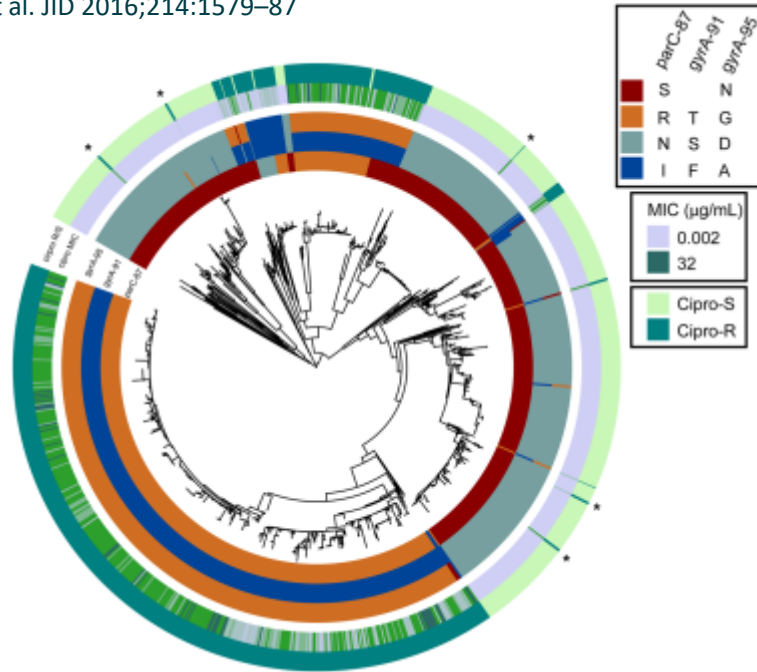Harris et al. Lancet Infect Dis, published online May 15, 2018
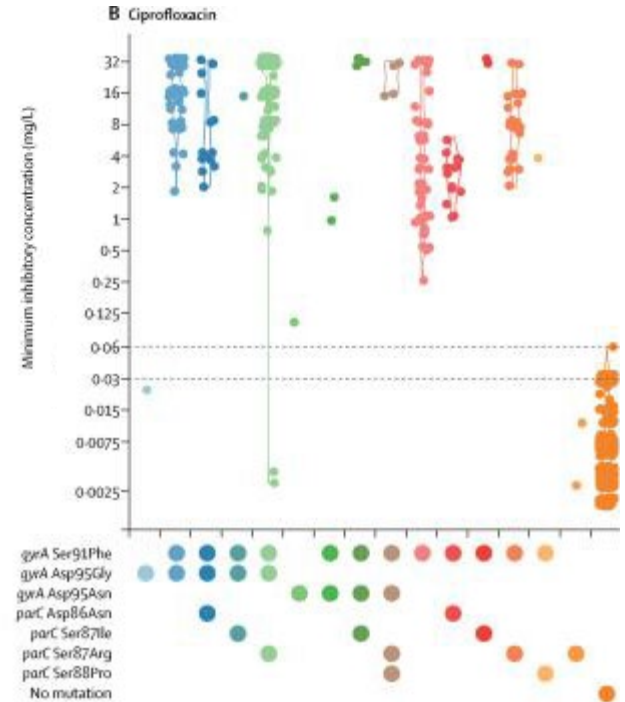
GyrA Genotype Predicts Cipro Resistance in GC

Genomic Epidemiology in US, 2000-2013 isolates

Grad et al. JID 2016;214:1579–87

Genomic Survey in Europe, 2013 isolates

Harris et al. Lancet Infect Dis, published online May 15, 2018

GyrA S91/S91F is highly predictive of ciprofloxacin susceptibility/resistance

GC Molecular Diagnostics With gyrA Genotyping Can Guide Ciprofloxacin Treatment

SpeeDx

- Advantages
  - Oral treatment preferred
  - Antibiotic stewardship of ceftriaxone
  - Utilize existing drug while new drugs are still in development

- Pooled estimate of real-time PCR gyrA genotyping tests for predicting GC susceptibility to ciprofloxacin

  Allan-Blitz et al. Sex Transm Dis. 2017 May;44(5):261-265.
  - Sensitivity: 98.2% (95% CI, 96.5–99.1%), Specificity: 98.6% (95% CI, 97.0–99.3%)

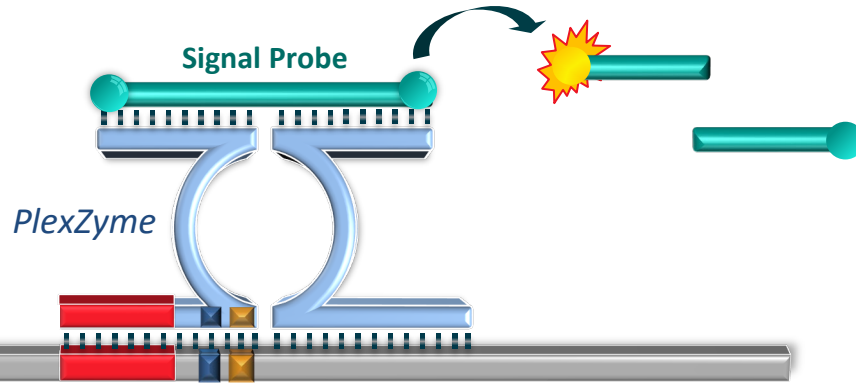- UCLA Health System has implemented a gyrA molecular assay to identify patients for ciprofloxacin treatment

  Allan-Blitz et al. Sex Transm Dis. 2018 Apr;45(4):e18.
  - 100% Ciprofloxacin cure rate for gyrA WT (n=25; 7 urethral, 7 pharyngeal, 7 rectal, 4 genital)

*Plex*PCR® *Technical Advantages*

- **Specific amplification and detection**

- **Universal probe for easy multiplexing**

- **Maintains sensitivity**

PlexPrimer

Insert
xxxxx

Signal Probe

PlexZyme

Tan et al PLoS ONE (2017) and Patent: WO 2013/123552

Ideal molecular diagnostic technology for multiplexed detection of resistance/susceptibility markers

ResistancePlus GC

🧬 **GC detection and ciprofloxacin resistance/susceptibility information**
Rapid qPCR format (<1.5 hours)

Direct from clinical specimens

🧬 **Specimen Types**
Urine, Swabs

	Channel	Target
1 Well	1	*N. gonorrhoeae* (opa)
	2	*N. gonorrhoeae* (porA)
	3	gyrA S91 wild type
	4	gyrA S91F mutation
	5	Internal Control

ResistancePlus GC is a molecular diagnostic test for ciprofloxacin resistance/susceptibility

Not available for sale in the USA

ResistancePlus GC (beta) Analytical Performance SpeeDx

- **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*.

Not available for sale in the USA

ResistancePlus GC (beta) on GC Clinical Isolates

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

SpeeDx		WGS	
		S91F mut	**S91 WT**
	S91F mut	28	0
	S91 WT	0	42
	Total	**28**	**42**
	Sensitivity	**100.0%** (95% CI 87.7-100.0%)	
	Specificity	**100.0%** (95% CI 91.4-100.0%)	

SpeeDx		Ciprofloxacin AST	
		Resistant (R)	**Susceptible (S)**
	S91F mut	27	1*
	S91 WT	0	42
	Total	**27**	**43**
	Sensitivity	**100.0%** (95% CI 87.7-100.0%)	
	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

Not available for sale in the USA

ResistancePlus GC (beta) Clinical Performance

- University of Queensland Centre for Clinical Research (Brisbane, Australia)
- Cobas specimens from 2014-2017
  - 361 genital swabs
  - 191 pharyngeal swabs

	Cervical	Vaginal	Penile	Urethral	Genital ns	Pharyngeal	Total
Male			27	49		27	312
Female	166	106			13	163	239
Unknown						1	1
Total	166	106	27	49	13	191	552

ns – not specified

Not available for sale in the USA

ResistancePlus GC (beta) Clinical Performance
GC detection

Genital Swabs		GC Clinical Results	
		+	**-**
SpeeDx	GC gyrA S91F Mut	35	0
	GC gyrA S91 WT	117	0
	GC Indeterminate	20	0
	GC Negative	7	180
	Total	**179**	**180**
	Sensitivity	**96.1%** (95% CI 92.1-98.4%)	
	Specificity	**100.0%** (95% CI 97.9-100.0%)	

Pharyngeal Swabs		GC Clinical Results	
		+	**-**
SpeeDx	GC gyrA Mut	20	0
	GC gyrA WT	49	0
	GC Indeterminate	11	1
	GC Negative	1	109
	Total	**81**	**191**
	Sensitivity	**98.8%** (95% CI 93.3-100.0%)	
	Specificity	**99.1%** (95% CI 95.0-100.0%)	

§ SpeeDx GC indeterminate rate: ~11% for genital and ~14% for pharyngeal swabs

Not available for sale in the USA

ResistancePlus GC (beta) Clinical Performance
GC gyrA detection

Genital Swabs		In-house gyrA qPCR	
		Mutant	WT
SpeeDx	GC gyrA S91F Mut	33	0
	GC gyrA S91 WT	0	110
	GC Indeterminate	0	13
	GC Negative	0	0
	Total	**33**	**123**
Sensitivity		**100.0%** (95% CI 89.4-100.0%)	
Specificity		**100.0%** (95% CI 96.7-100.0%)	

Pharyngeal Swabs		In-house gyrA qPCR	
		Mutant	WT
SpeeDx	GC gyrA S91F Mut	12	0
	GC gyrA S91 WT	0	39
	GC Indeterminate	0	0
	GC Negative	0	0
	Total	**12**	**39**
Sensitivity		**100.0%** (95% CI 73.5-100.0%)	
Specificity		**100.0%** (95% CI 91.0-100.0%)	

§ Prevalence GC gyrA mutation: ~21% in female and ~22.5% in male patients

Not available for sale in the USA

ResistancePlus GC (beta) Clinical Performance
Additional specimen types

- 45 GC positive urine (18 female, 27 male)
  - GC detection: 100.0% sensitivity
  - gyrA detection: 100.0%/97.1% sensitivity/specificity (n=5+34)

- 16 GC positive rectal swabs (1 female, 15 male)
  - GC detection: 93.8% sensitivity
  - gyrA detection: 100.0%/100.0 % sensitivity/specificity (n=4+10)

Not available for sale in the USA

ResistancePlus GC – Future work

- Expected date for CE-IVD (Sept 2018)
  - Cobas extracts, Aptima Sample, BD Viper sample
  - Not available for sale in the USA

- GRAND2 – GC clinical study

Not available for sale in the USA

Potential Implementation Pathways

Screening or postal service

```
Asymptomatic
Test CT/GC
      ↓
Confirm GC
ResistancePlus GC
```

GC + gyrA wild type Treat with cipro	GC + gyrA mutant Treat with ceft+azo

Partner testing

```
Partner test
ResistancePlus GC
```

GC + gyrA wild type Treat with cipro	GC + gyrA mutant Treat with ceft+azo

Not available for sale in the USA

Acknowledgements

UQCCR, Australia

- David Whiley
- Lebogang Mhango
- Amanda Bordin
- Ella Trembizki

SpeeDx, Australia

- Samantha Walker
- Madeline Windsor
- Simon Erskine
- Elisa Mokany
- Colin Denver