# CRISPR-diagnostics for sexually transmitted infections

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THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

# Disclosures

No disclosures of interest to declare

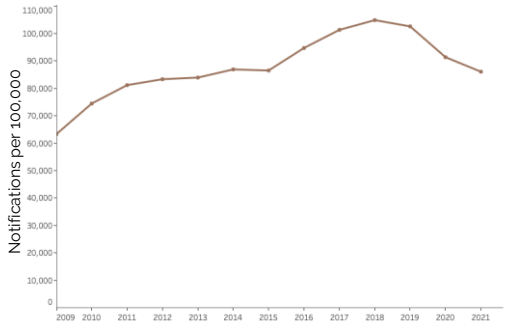
# Acknowledgements

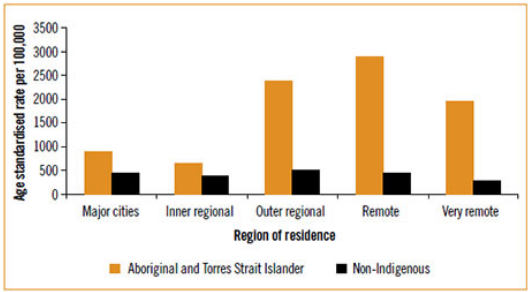
Acknowledge the traditional owners of the lands on which we meet today - the Kaurna people. As well as the Wurundjeri people of the Kulin Nation where I call home.

I recognise Aboriginal and Torres Strait Islander peoples' continuing connection to land, water, and community and pay my respects to Elders past and present.

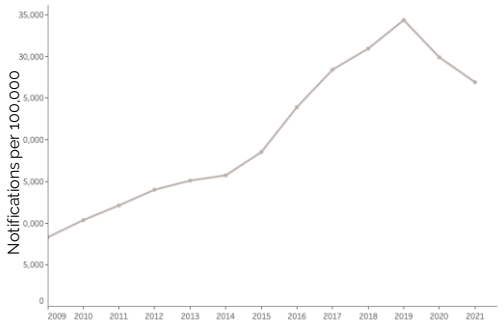
# Bacterial STI incidence higher in remote Australia

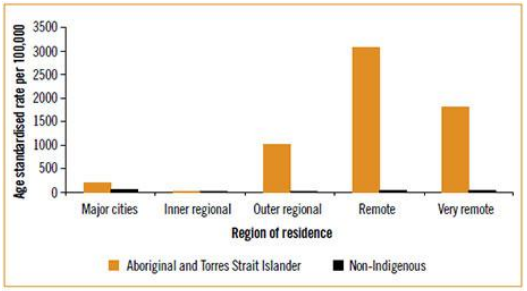
Chlamydia



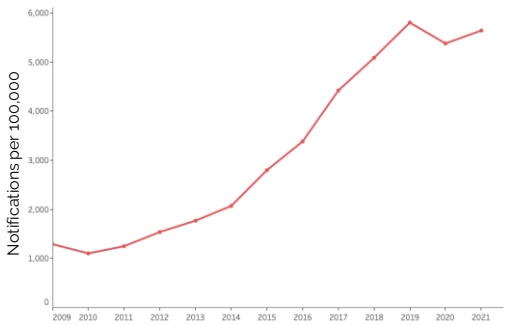



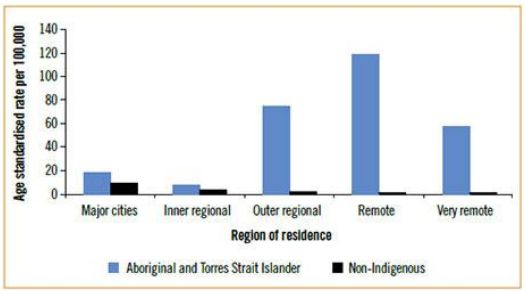

Gonorrhea




Syphilis <2 years duration




*Source: National Notifiable Diseases Surveillance System, Australian Government*

# Reliance on lab testing reduces access

## Global Problem

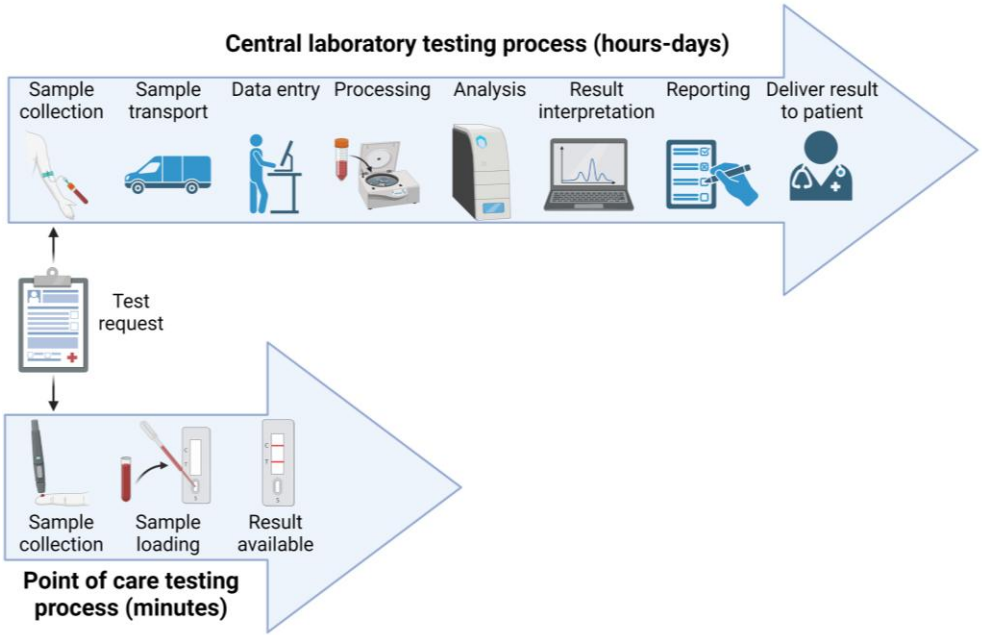
Accurate diagnosis relies on expensive laboratory tests

## Current challenges in Australia

Access & Duration - over two weeks for clinical samples to be processed from remote regions

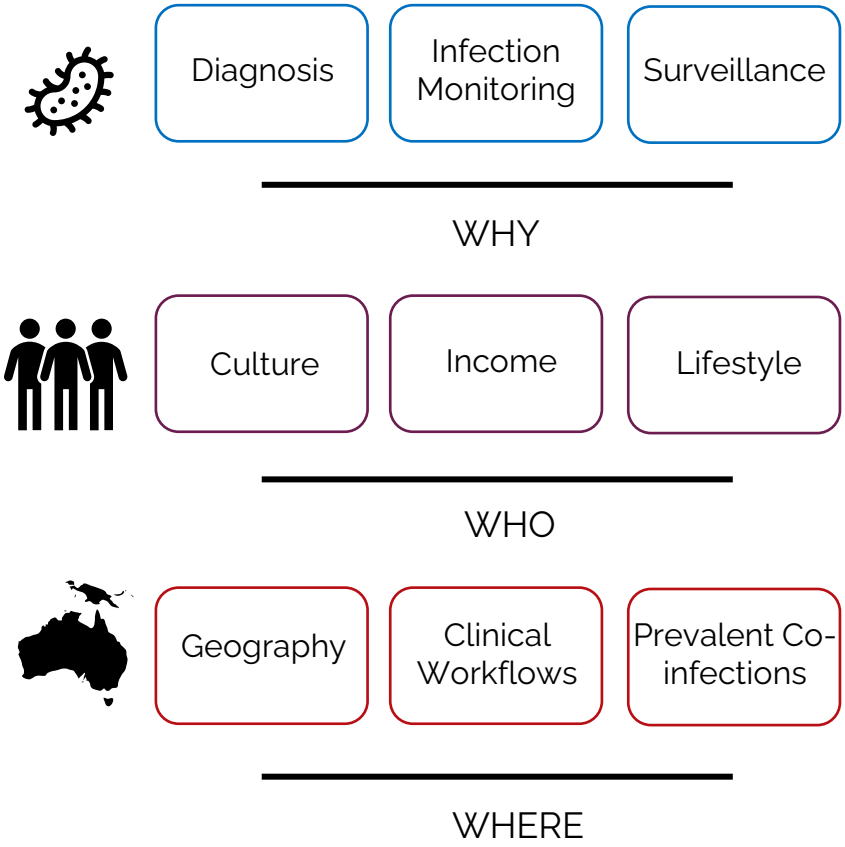
Testing Platforms - small number available

Outbreak Response – critical shortages of reagents lead to extreme delays

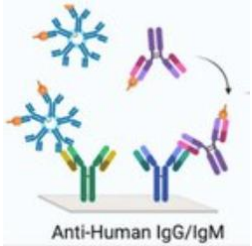
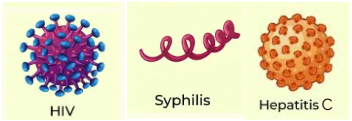
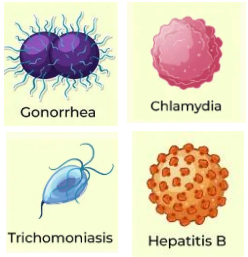


# In 2004 the WHO declares urgent need for novel point-of-care (Poc) tests for bacterial STIs

"One size does not fit all"
Solutions need to be tailored



# We need more options at the point of care

| | INDIRECT | DIRECT |
|---|---|---|
| **Technology** | Detects antigens/proteins made by a pathogen<br><br>Detects antibodies produced by humans in response to infection<br><br>  | Detects pathogen DNA/RNA and makes copies/amplifies<br><br>Complex temperature dependent molecular genetic cycles<br><br>  |
| **Approved STI PoCTs** |  <br>**Lateral flow immunoassay**<br>Bioline 3.0 HIV, TP<br>INSTi – Biolytics HIV, HepC, TP<br>Determine –Abbott - TP<br>PanBio HIV<br>Atomo HIV<br>  <br>Fast & Cheap<br>Can have lower sensitivity<br>Cannot distinguish past versus active infection for syphilis |  <br>**RT-PCR**<br>GeneXpert<br>  <br>Highly-sensitive<br>Non-quantitative<br>Expensive |

# Next-generation platform technologies

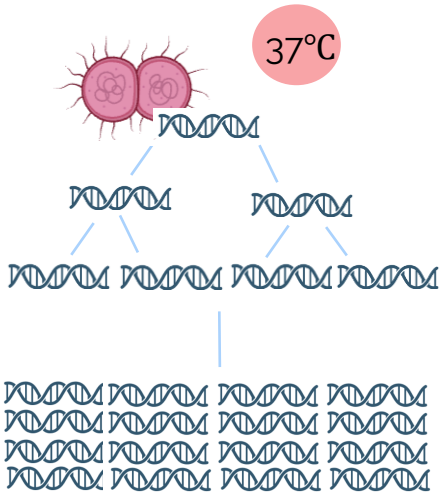
## Adding Diversity to diagnostics


Image source: Chacon GP et al. Micro Aus 2024

# Next-generation platform technologies

## Adding Diversity to diagnostics

| Innovative diagnostic method | Affordable | Sensitive | Specific | User-friendly | Rapid & Robust | Equip free | Deliverable |
|---|---|---|---|---|---|---|---|
| Lateral flow devices (antigen or antibody) | 🟢 | 🔴 | 🟡 | 🟢 | 🟢 | 🟢 | 🟢 |
| Miniatured PCR devices | 🔴 | 🟢 | 🟢 | 🔴 | 🟡 | 🔴 | 🟡 |
| Isothermal nucleic acid amplification devices | 🟡 | 🟢 | 🟡 | 🟡 | 🟢 | 🟡 | 🟡 |
| Metagenomics (e.g MinION) | 🔴 | 🟡 | 🟡 | 🟡 | 🟡 | 🟡 | 🟢 |
| Lab-on-a-chip devices (microfluidics) | 🔴 | 🟢 | 🟢 | 🟡 | 🟢 | 🔴 | 🟢 |

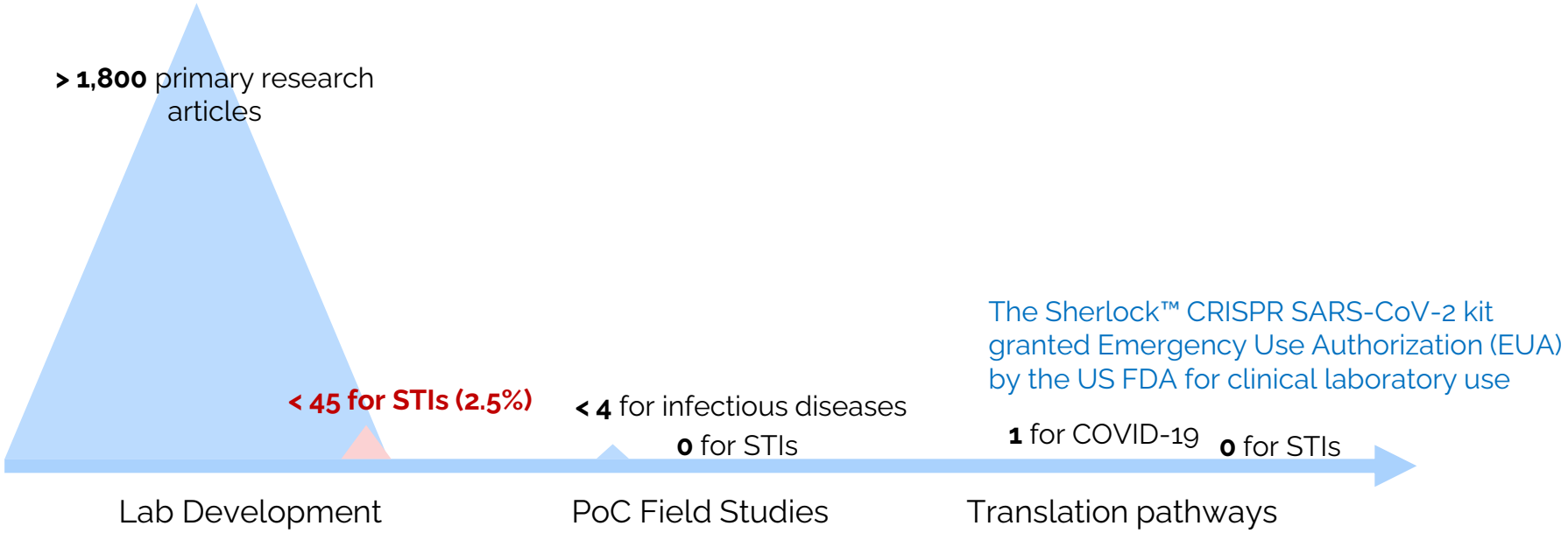


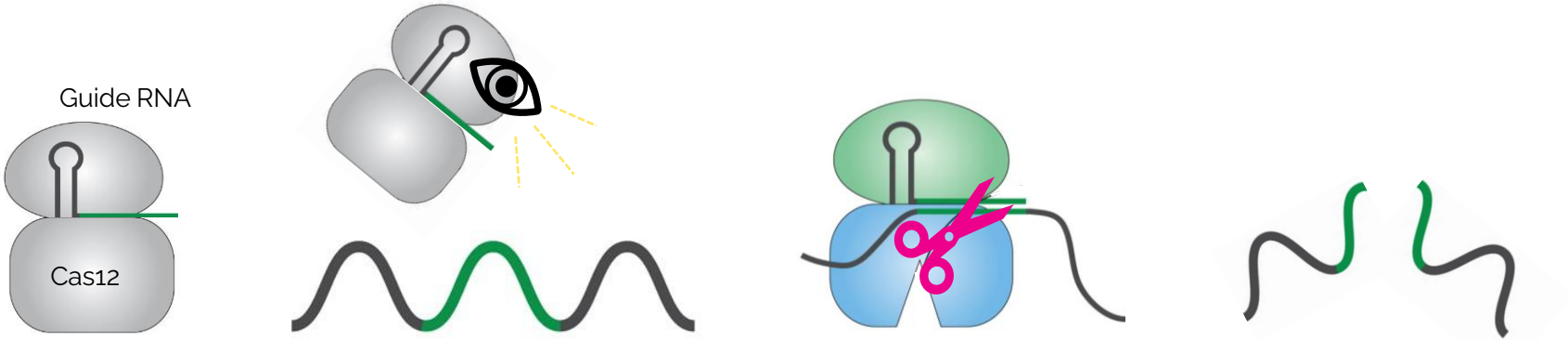
# CRISPR biosensors

# From the bench to the point of care

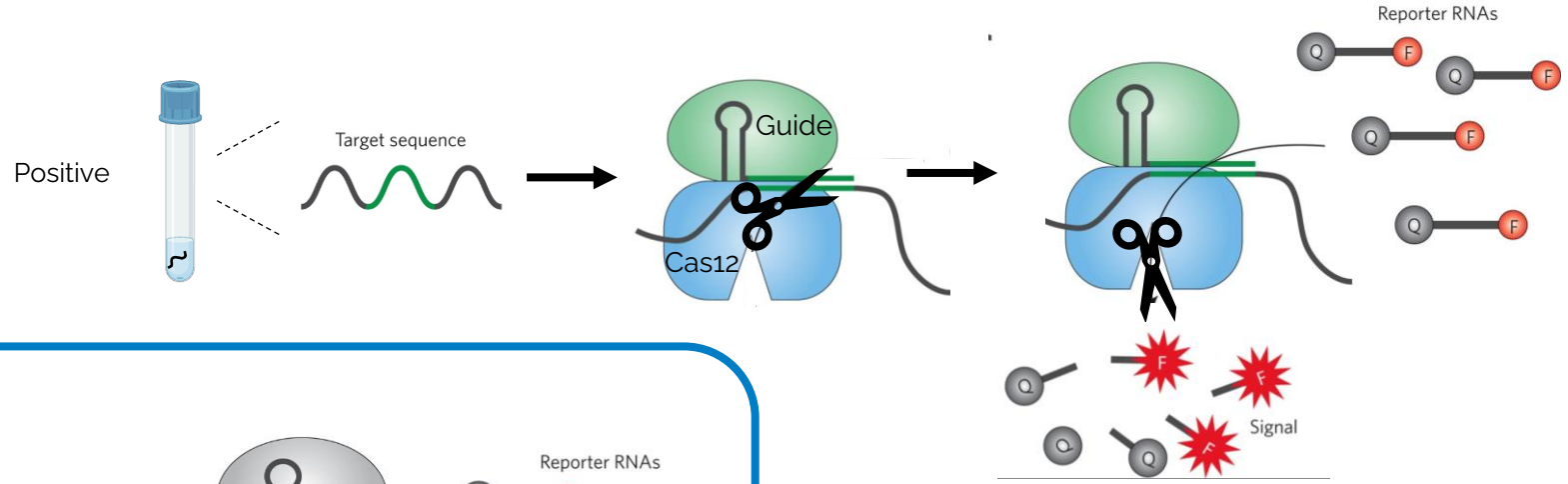
# CRISPR-diagnostics for STIs

## The Landscape




Guide RNA
Cas12

# Exploiting CRISPR technology for diagnostics



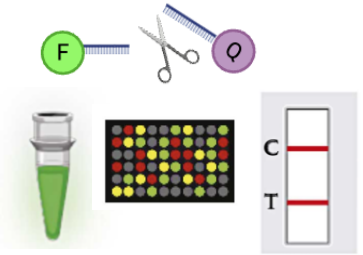

Adapted from Zuo et al. (2017) Nature biomedical engineering

# **A Versatile Platform:**
## Ideal for the point of care

FAST
Enzymatic reaction
Minutes

Single Temperature
37C

High Sensitivity
Coupled with Isothermal
amplification

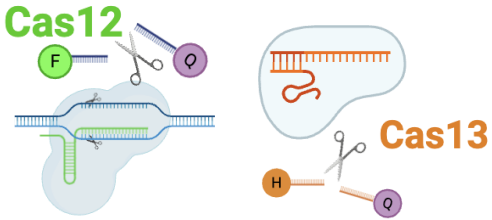



Diverse **readouts**/devices

# A Versatile Platform:
## Amendable to use-case needs

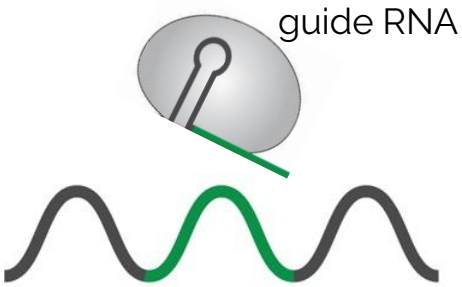
**Avoid diagnostic escape**

Adding Guide RNAs




Detect **multiple pathogens** and distinct reporters

Using different Cas enzymes

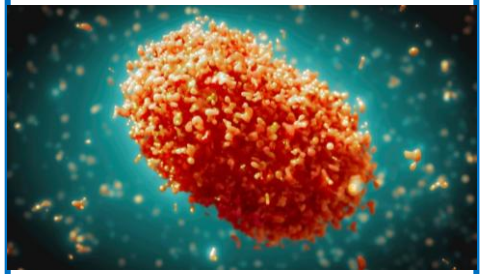



Detect nucleotide **mutations**

Inherent specificity of CRISPR

# CRISPR solutions to overcoming barriers to PoC STI diagnosis

Fast Development
Versatile Readouts

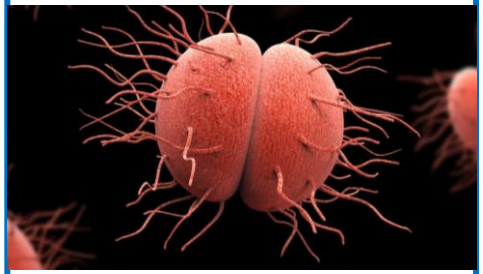


Emerging Pathogens
Outbreak Response

Multiplex Pathogen
Detection

Misdiagnosis/Co
-infections

High specificity to
detect gene mutations



Antimicrobial
Resistance

# 2022 mpox outbreak – WHO declares Health Emergency

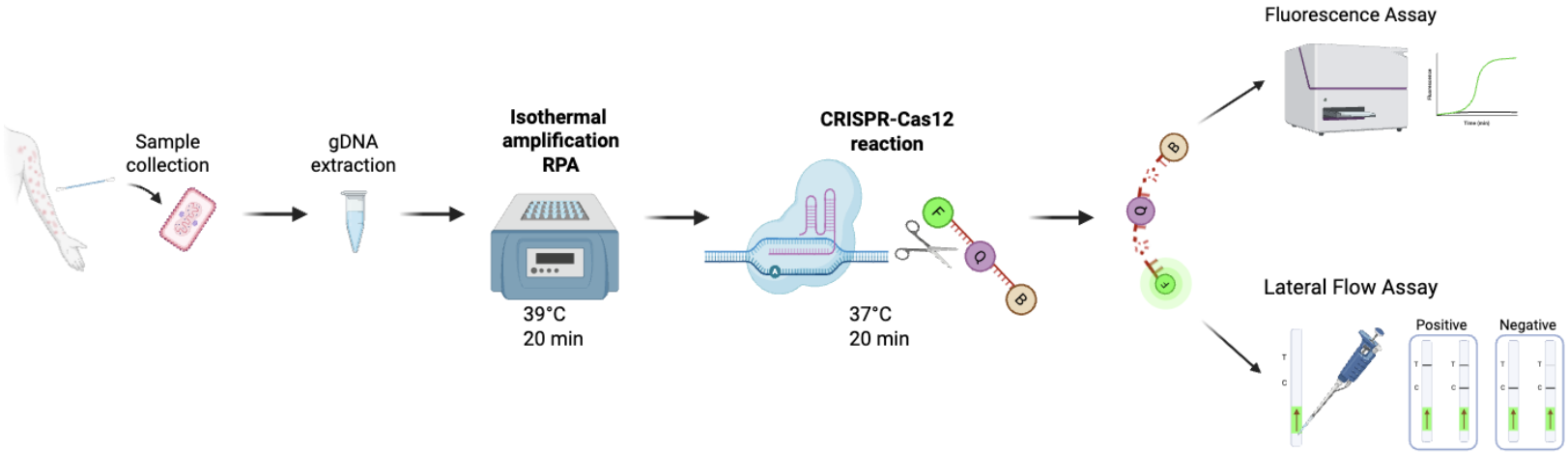
**Outbreak**: May - Nov 2022 – over 80,000 new cases of mpox in 110 countries

**Cases**: Predominately reported in men who have sex with men (MSM), with cases also described in women and infants

**Monkeypox virus**: Orthopoxvirus with **dsDNA** (~197 kb)

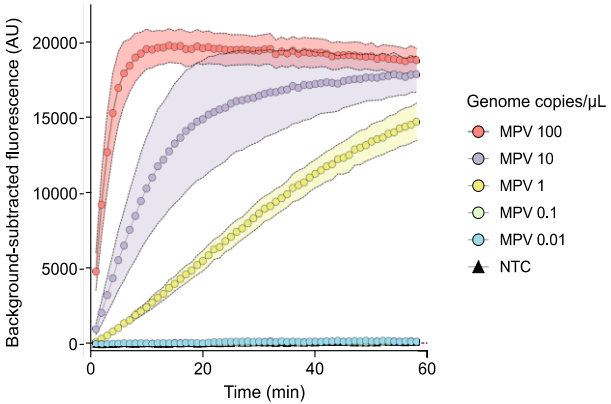
**Diagnostics** – predominately PCR, serologically cross-reactive with other orthopoxviruses - antigen and antibody detection methods lack specificity

# Monkeypox virus detection using the MPXV-CRISPR assay

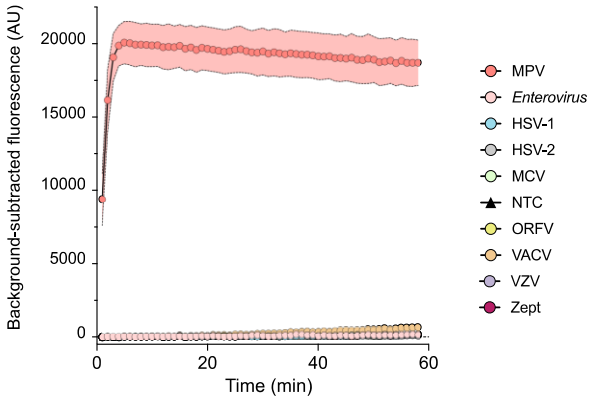


# Analytical sensitivity and specificity

Detects single genome copies/ µL with no cross-reactivity



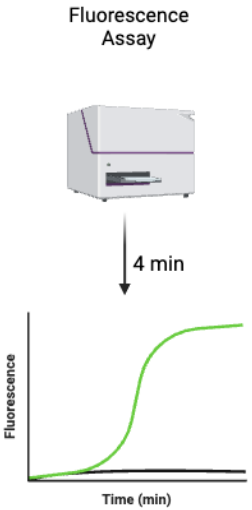

**Sensitivity** - Assay can detect single 1 copy/µL of spiked monkeypox virus gDNA



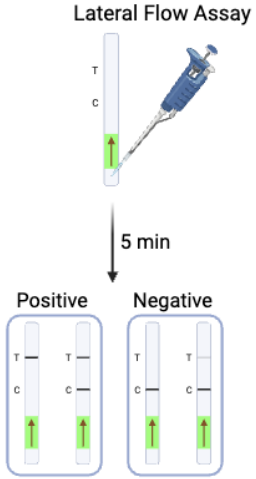

**Specificity** - No cross-reactivity against a panel of viruses

# Validated on 185 oral, anal and skin lesion clinical specimens

| | Concordance |
|---|---|
| Positive by qPCR | |
| True positive | 40 |
| False negative | 0 |
| **Sensitivity**, % (95% CI) | **100** (89-100) |
| Negative by qPCR | |
| True negative | 144 |
| False positive | 1 |
| **Specificity,** % (95% CI) | **99.3** (96-100) |

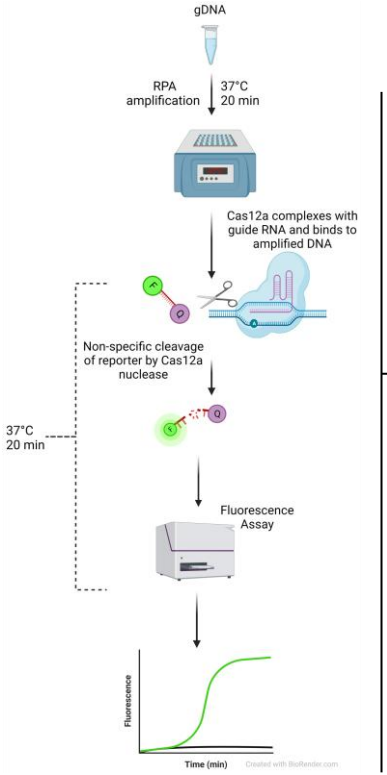



| Concordance |
|---|
| |
| 40 |
| 0 |
| **100** (89-100) |
| |
| 143 |
| 2 |
| **98.6** (96-100) |




Soo Jen Low et al, Lancet Microbe, 2023

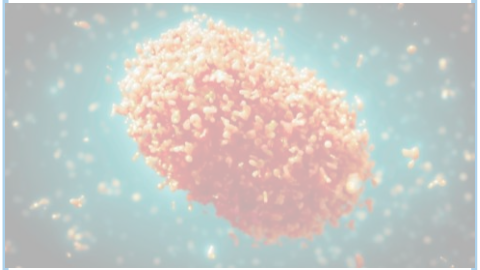
# In response to the 2024 outbreak - updated assay and transferred to portable device




- Direct from clinical sample
- Picks up Clade 1 & 2
- Dual targeting
- Submitting for IVD Accreditation by Victorian Infectious Disease Reference Laboratory (VIDRL)

# CRISPR solutions to overcoming barriers to PoC STI diagnosis

Fast Development
Versatile Readouts



Emerging Pathogens
Outbreak Response

Multiplex Pathogen
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Misdiagnosis/Co
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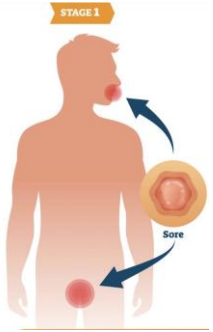
High specificity to
detect gene mutations

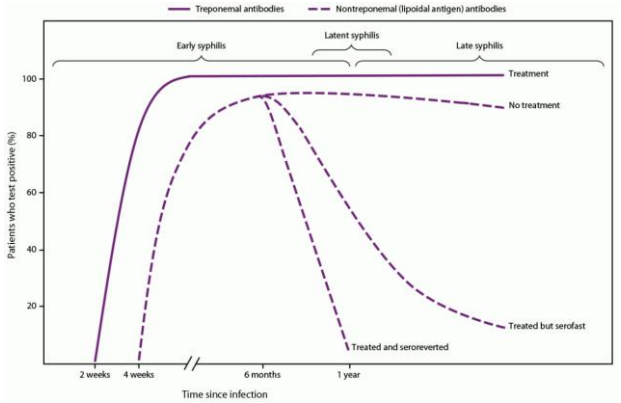
Antimicrobial
Resistance

# Barriers to early syphilis diagnosis



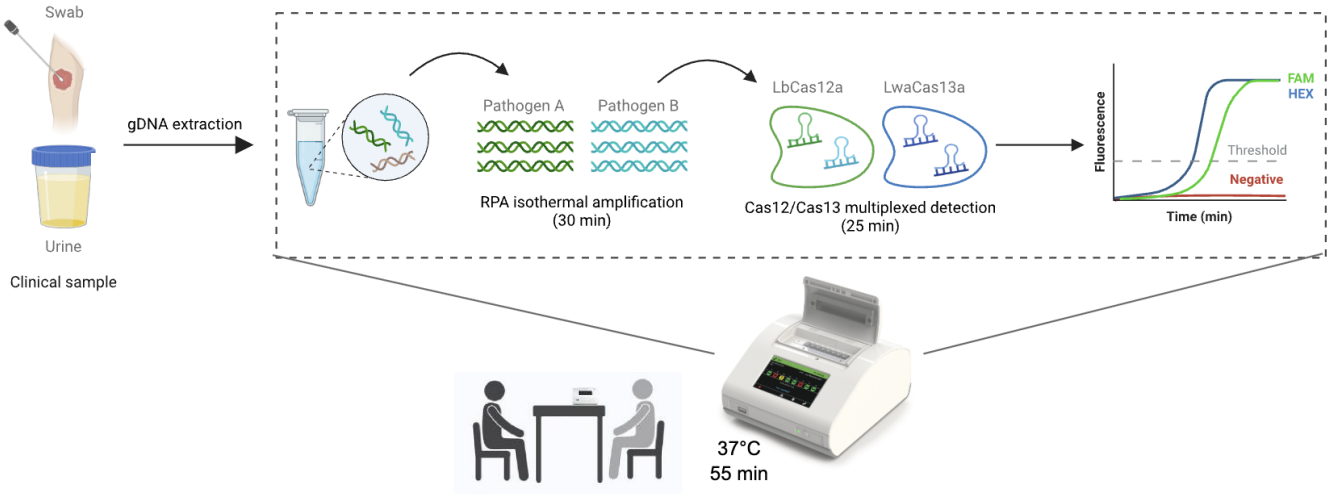

**Clinical presentation** (misdiagnosis)
- Symptomatic diagnosis
- Occult lesions (oropharynx, anus, vagina)
- Hard to distinguish between primary syphilis and herpes

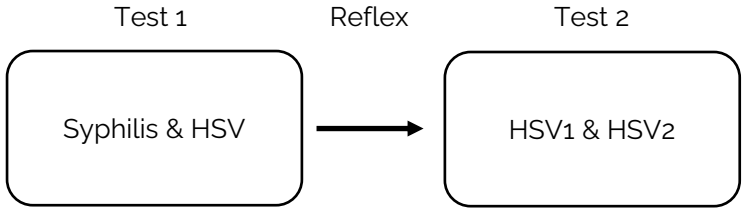
*Rebecca Wigan et al. 2024 ASHRM Abstract; Multiplex PCR testing of anogenital lesions for TP and HSV in primary care improves detection of primary syphilis*




**Diagnostic tests**
- Invasive intravenous blood collection
- Relies on serology which shows reduced performance in very early infection
- Limited access to *T. pallidum* PCR

# Multiplexed detection of *Treponema pallidum* and Herpes simplex virus




Preprints with THE LANCET

CRISPR-Cas-b-Based Diagnostics for Point-of-Care Detection of Sexually Transmitted Infections: A Laboratory Development and Evaluation Study

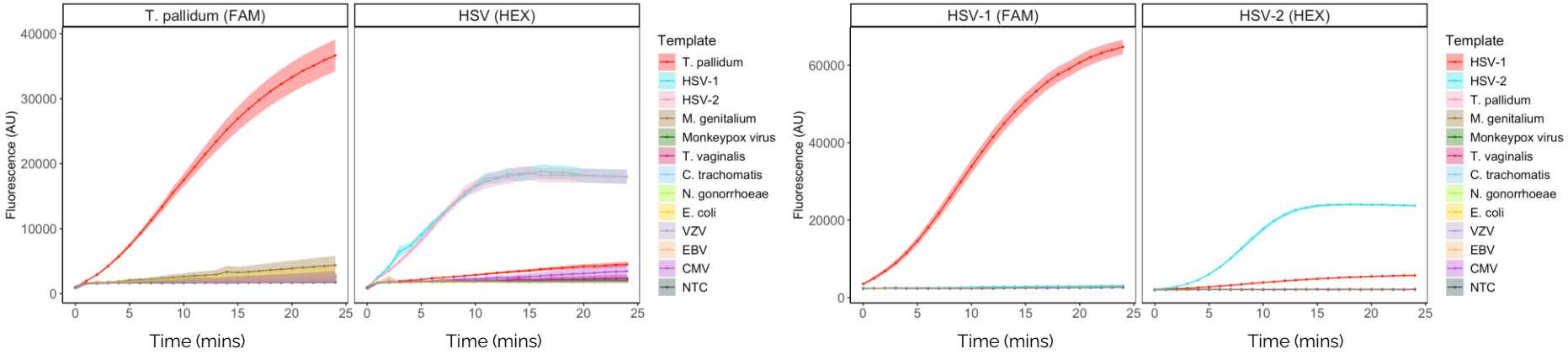
Low SJ, O'Neill M, Fernando JA, Kerry WJ, Prestedge J, Wild N, Chahal S, Pollock GL, Papadakis G, Krysiak M, Williams E, Azzato F, Tran T, Fairley CK, Lim CK, Bradshaw C, Chen MY, Williamson DA, Pasricha, S. Available at SSRN: https://ssrn.com/abstract=5230642

# Analytical specificity

Displays no significant cross-reactivity with other pathogens

*T. pallidum* & pan*HSV*

HSV1 & HSV2

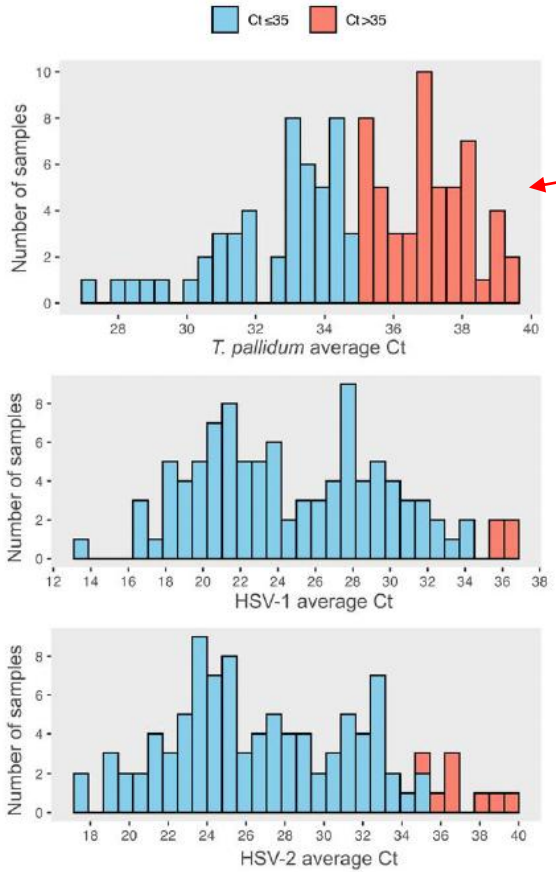


# Validation on 400 clinical samples including vaginal, anal, urine and skin lesion specimens

Syphilis & HSV

| Concordance | Primary | |
|---|---|---|
| | *T. pallidum* | panHSV |
| Positive by qPCR | | |
| True positive | 85 | 187 |
| False negative | 18 | 11 |
| **Sensitivity %** | **82.5** (74–88.7) | **94.4** (90.2–97.0) |
| | | |
| Negative by qPCR | | |
| True negative | 291 | 198 |
| False positive | 6 | 4 |
| **Specificity %** | **98.0** (95.6–99.2) | **98.0** (94.8–99.4) |

# Lower Pathogen Load in *T. pallidum* – positive cohort




17 of the 18 missed by the CRISPR assay

**All 17 were detected upon repeat testing**

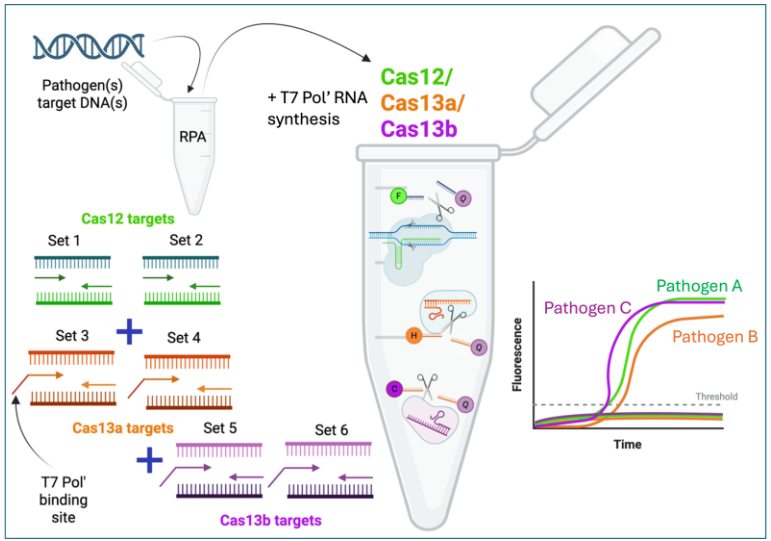
**Context**

Lab Testing includes at least 2 serological assays and a PCR test

# Towards Triplex detection

## Can we now identify cases of syphilis and mpox that would have otherwise been missed?

### Triplex detection of syphilis, HSV, mpox




Evaluate diagnostic precision of triplex CRISPR-testing compared to symptomatic diagnosis by clinicians.

In collaboration with Prof Marcus Chen and Ms Rebecca Wigan
Melbourne Sexual Health Centre

VICTORIA State Government

# CRISPR solutions to overcoming barriers to PoC STI diagnosis

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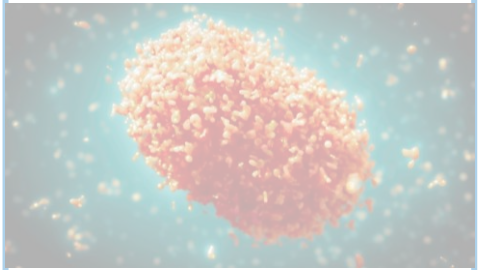

Emerging Pathogens
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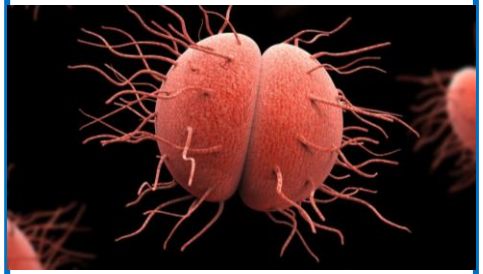


Antimicrobial
Resistance

# Can a CRISPR-PoC test support resistance-guided therapy and reduce broad spectrum antibiotic use?

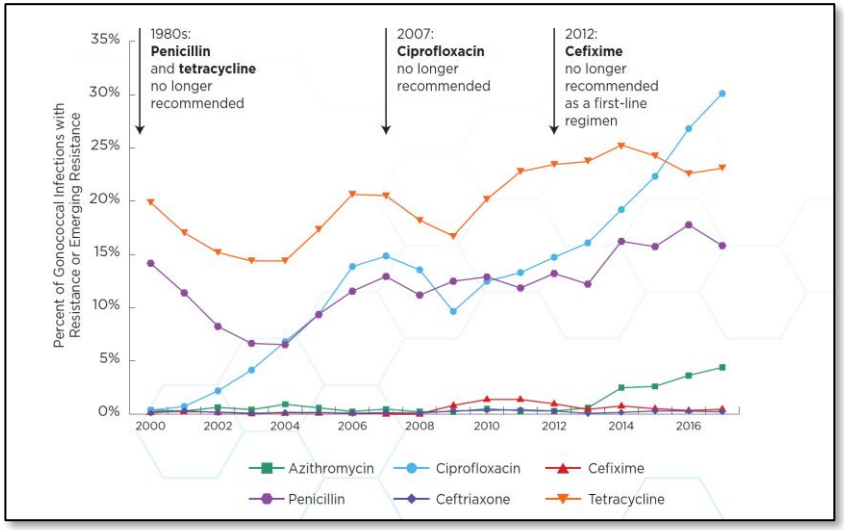
Gonorrhea & Chlamydia

Coinfection of CT and NG is between 10-50%

Gonorrhea & ciprofloxacin resistance (*gyrA* S91F mutation)

Treatment guidelines keep changing due to AMR

Culture rates to confirm susceptibility are declining given molecular testing

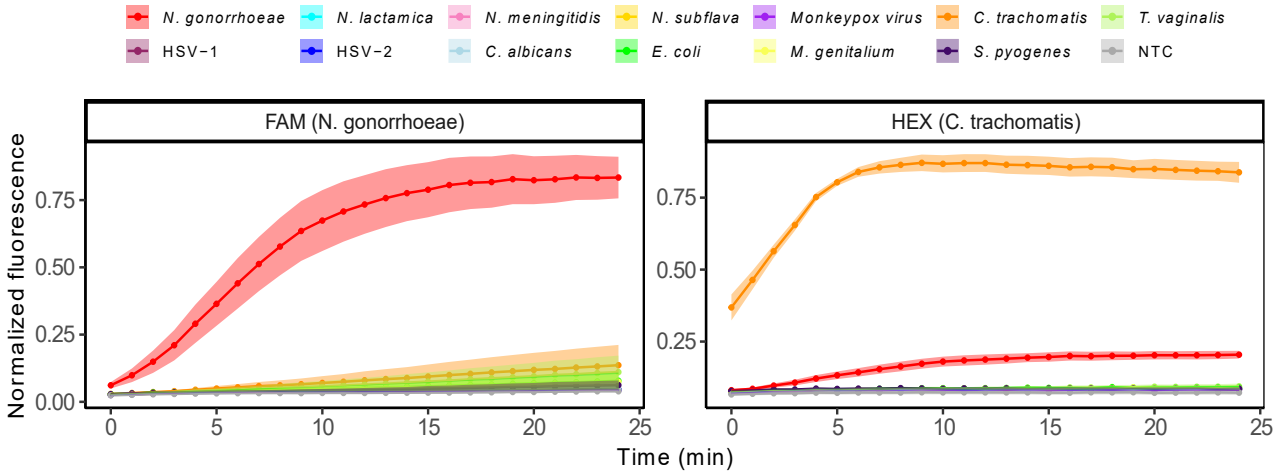



*Source: CDC*

# Analytical specificity

Displays no significant cross-reactivity with other pathogens

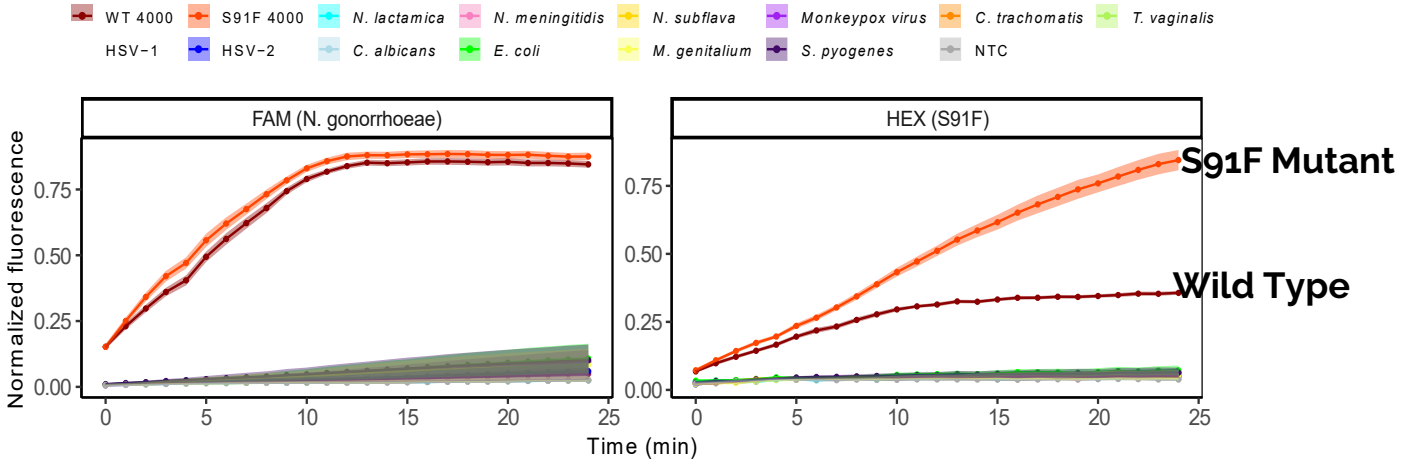
Gonorrhea &
Chlamydia



# Analytical specificity

## CRISPR- Cas enzyme preferentially cuts the mutant strain

Gonorrhea & *gyrA* S91F mutation



# Validation using clinical samples

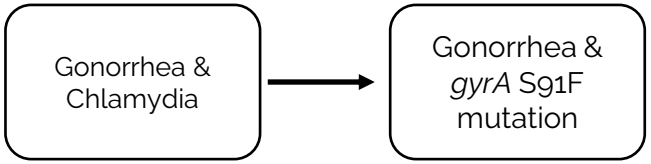
Blind test of 500 clinical samples including vaginal, anal, urine and throat specimens

Gonorrhea & Chlamydia

| Concordance | Primary | |
|---|---|---|
| | *N. gonorrhoeae* | *C. trachomatis* |
| Positive by qPCR | | |
| True positive | 168 | 116 |
| False negative | 42 | 43 |
| **Sensitivity %** | **80.0** (74–84.9) | **73.0** (65.5–79.3) |
| | | |
| Negative by qPCR | | |
| True negative | 281 | 335 |
| False positive | 9 | 6 |
| **Specificity %** | **96.9** (94.1–98.4) | **98.2** (96.1–99.3) |

# Validation using clinical samples

Blind test of 500 clinical samples including vaginal, anal, urine and throat specimens




| Concordance | Primary | | Reflex | |
|---|---|---|---|---|
| | *N. gonorrhoeae* | *C. trachomatis* | *N. gonorrhoeae* | *gyrA* S91F |
| Positive by qPCR | | | | |
| True positive | 168 | 116 | 197 | 94 |
| False negative | 42 | 43 | 1 | 55 |
| **Sensitivity %** | **80.0** (74–84.9) | **73.0** (65.5–79.3) | **99.4** (96.9-100) | **63.1** (55.1-70.4) |
| Genital Specimens | **93.3%** | **72.9%** | **99.4%** | **85.7%** |
| Negative by qPCR | | | | |
| True negative | 281 | 335 | 99 | 41 |
| False positive | 9 | 6 | 0 | 8 |
| **Specificity %** | **96.9** (94.1–98.4) | **98.2** (96.1=99.3) | **100.0** (95.5–100) | **83.7** (70.7=91.8) |

# Next step: multi-centre pilot study of CT/NG+GyrA PoC test

## Darwin, NT

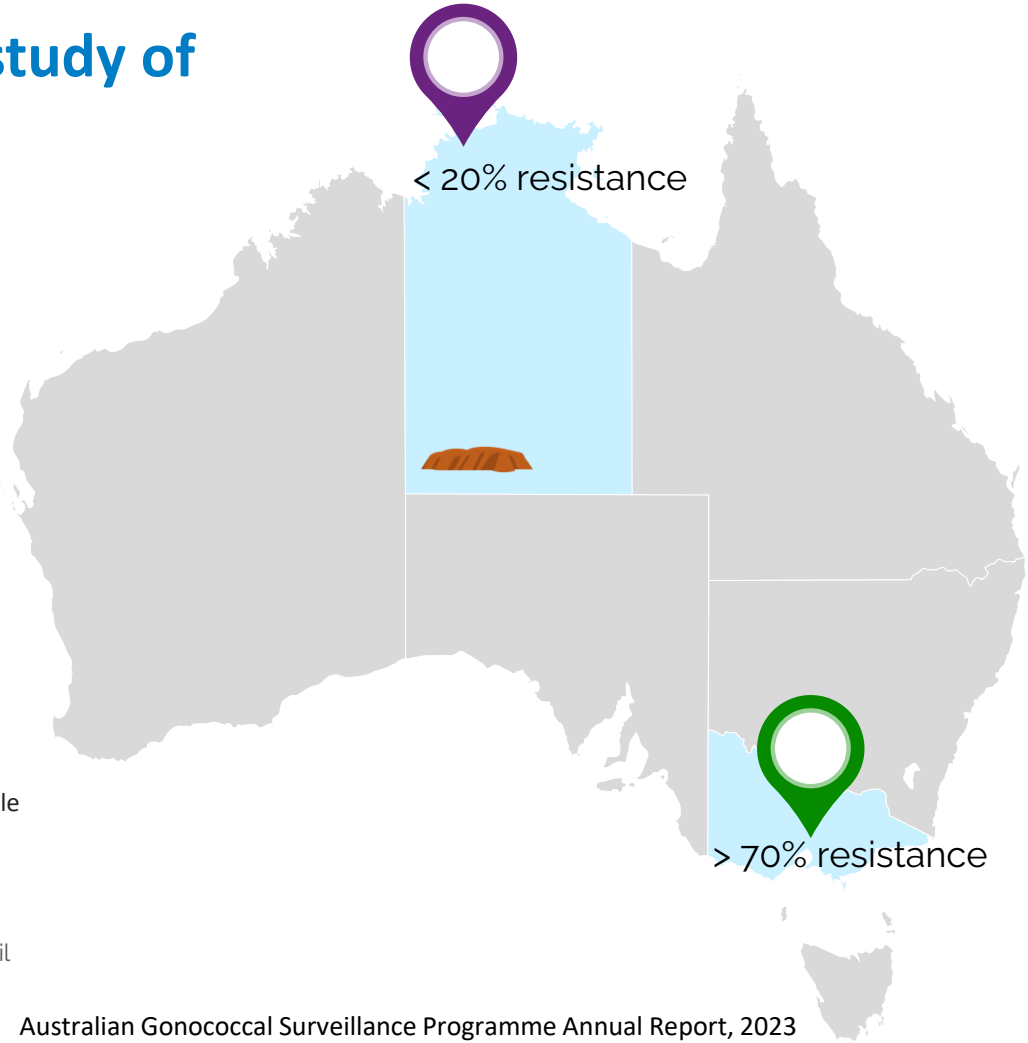
- NT is the least populated jurisdiction, 1% of Australia's population with 230k people
- 27% of NT population is Indigenous
- Clinic 34 sees predominantly heterosexual clients

In collaboration with Dr Ella Meumann & Dr Manoji Gunathilake Menzies

## Melbourne, VIC

- Victoria is the most populated jurisdiction, 7 million people (5.3m in Melbourne)
- 1% of Victoria's population is Indigenous
- MSHC sees predominantly GBMSM and oversees born clients

In collaboration with Prof Catriona Bradshaw & Dr Lenka Vodstrcil Melbourne Sexual Health Centre

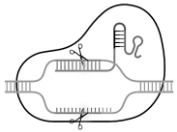


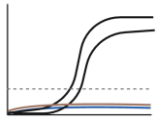

Australian Gonococcal Surveillance Programme Annual Report, 2023

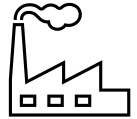
# CRISPR-technology– A versatile diagnostic platform

- Emerging Pathogens
- Resistance Guided Therapy
- Symptomatic Misdiagnosis
- Remote Settings
- Critical Care, Informing Comorbidity Treatment
- Viral Load monitoring
- Host Biomarkers
- Surveillance

# Breaking the innovation to translation barrier







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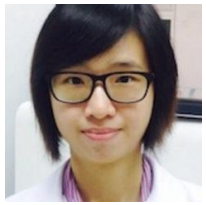


Soo Jen Low

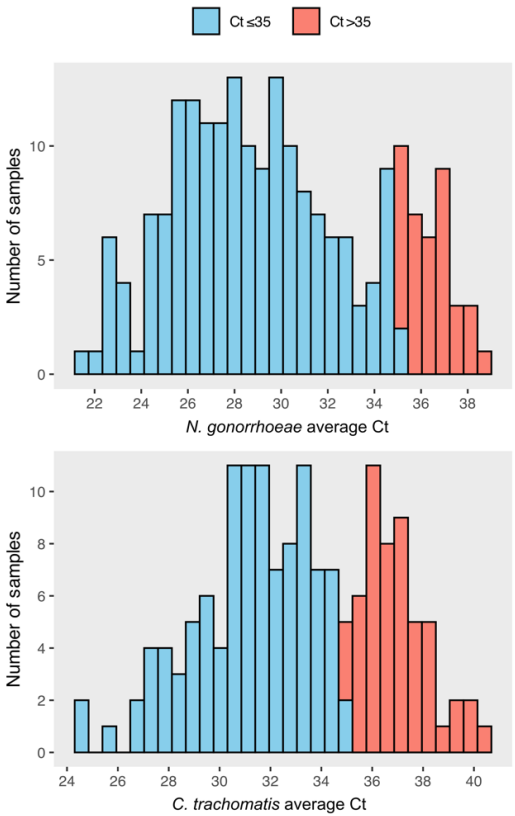
Australian Government

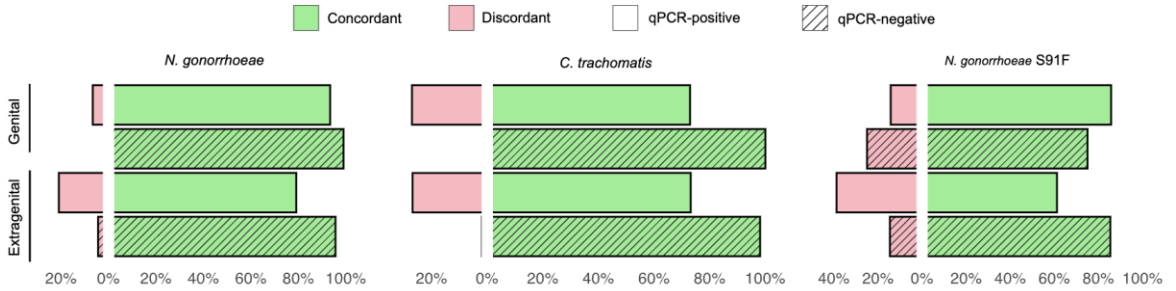
Department of Health

VICTORIA State Government

AMR Hub

# Assay sensitivity associated with sample type




*N. gonorrhoeae*
genital 93.3% (14/15)
extragenital 79.1% (151/191)

*C. trachomatis*
genital 72.9%
extragenital 73.1%

*gyrA mutation*
genital 85.7% (6/7)
extragenital 61.2% (85/139)

# There are geographical differences in *N. gonorrhoeae* susceptibility patterns

**Table 1: Gonococcal isolates resistant to azithromycin, ciprofloxacin, and penicillin, Australia, 1 July to 30 September 2023, by state or territory**

| Jurisdiction | Number of isolates tested | Resistance[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Azithromycin | | Ciprofloxacin | | Penicillin | |
| | Q3, 2023 | n | % | n | % | n | % |
| Australian Capital Territory | 71 | 0 | 0 | 35 | 49.3 | 21 | 29.6 |
| New South Wales | 896 | 44 | 4.9 | 589 | 65.7 | 231 | 25.8 |
| Queensland | 373 | 10 | 2.7 | 199 | 53.4 | 104 | 27.9 |
| South Australia | 150 | 3 | 2.0 | 74 | 49.3 | 55 | 36.7 |
| Tasmania | 37 | 2 | 5.4 | 22 | 59.5 | 12 | 32.4 |
| Victoria | 671 | 50 | 7.5 | 469 | 69.9 | 236 | 35.2 |
| Northern Territory non-remote | 24 | 0 | 0 | 5 | 20.8 | 2 | 8.3 |
| Northern Territory remote | 17 | 0 | 0 | 3 | 17.6 | 0 | 0 |
| Western Australia non-remote | 262 | 15 | 5.7 | 151 | 57.6 | 85 | 32.4 |
| Western Australia remote | 19 | 1 | 5.3 | 3 | 15.8 | 3 | 15.8 |
| **Australia** | **2,520** | **125** | **5.0** | **1,550** | **61.5** | **749** | **29.7** |

a Resistance as defined by jurisdictional reporting criteria.

Australian Gonococcal Surveillance Programme Annual Report, 2023