A novel rapid molecular-phenotypic assay for viability and antibiotic susceptibility assessment of *Neisseria gonorrhoeae* (NG) direct from patient samples

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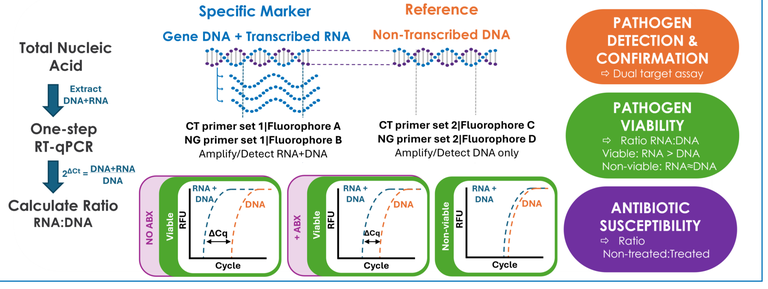
INTRODUCTION

- Current Nucleic Acid Amplification Test (NAATs) rely on detection of DNA or RNA only, which limits their ability to distinguish between alive and dead cells or to perform phenotypic measures of antibiotic susceptibility (AST). This limitation highlights the need for a simple, fast and accurate method for determining viability of pathogens, and performing rapid AST, **which is vital for:**
  - supporting antimicrobial stewardship efforts,
  - continuing effective screening programs, and
  - providing accurate measures of cure post-treatment
- This is specifically true for sexually transmitted infections caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG); the latter being listed as a priority pathogen due to increases in antimicrobial resistance and its current reliance on last-line antibiotics for treatment
- To address this gap, ***In**Signia*®, an innovative molecular-phenotypic NAAT technology, co-amplifies and determines the ratio of RNA to DNA from pathogens enabling detection, viability assessment and AST in a single RT-qPCR
  - **Viability** status is measured as the ratio of RNA to DNA providing an Index, reflective of the level of active transcription
  - **AST** is enabled by exposing the sample to antibiotic and analysing the Index change compared to the untreated control
- Feasibility of the SpeeDx ***In**Signia*® CT/NG workflow and assay was tested in a cross-sectional cohort study to assess performance in determining **viability and AST of NG** rapidly and directly from clinical specimens



PATHOGEN DETECTION & CONFIRMATION
⇨ Dual target assay

PATHOGEN VIABILITY
⇨ Ratio RNA:DNA
Viable: RNA > DNA
Non-viable: RNA=DNA

ANTIBIOTIC SUSCEPTIBILITY
⇨ Ratio
Non-treated:Treated

An innovative NAAT which, co-amplifies and then determines the ratio of RNA to DNA from the intended organism to enable **pathogen detection**, **viability assessment** and **antibiotic susceptibility** in a **single RT-qPCR reaction**.

METHOD

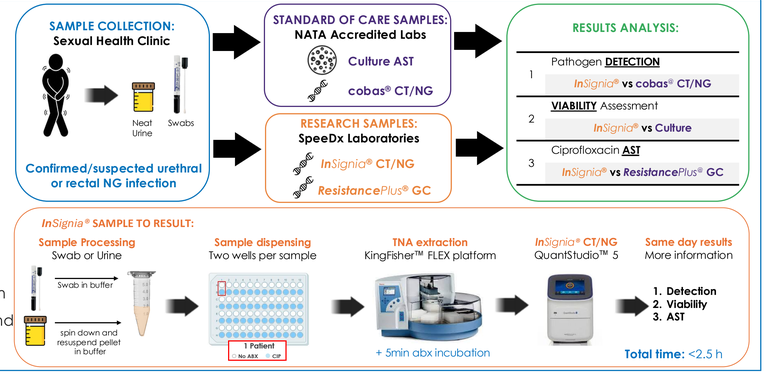
- Cases of confirmed or suspected gonorrhoea infection in cisgender men were recruited; paired urine and urethral swabs or rectal swabs were collected from each participant

Standard of care samples:

- Urethral or rectal swab for culture
- Urine and rectal for NAAT with cobas® 4800 CT/NG test

Research samples:

- Rectal swab, urethral swab and neat urine for NAAT with ***In**Signia*® CT/NG prototype and ***Resistance**Plus*® GC which detects NG and assesses ciprofloxacin sensitivity by determining the gyrA genotype
- Samples were tested with ***In**Signia*® CT/NG within 6 hours of collection
- ***In**Signia*® CT/NG results were compared to cobas® CT/NG, culture and ***Resistance**Plus*® GC for detection, viability and AST, respectively



RESULTS

A total of 81 samples were obtained during recruitment

- 24 urethral swabs, 23 urine, 34 rectal swabs

Detection

- ***In**Signia*® CT/NG compared to cobas® CT/NG demonstrated 92.59% concordance for NG for all sample types
- Discrepant results in 6 asymptomatic patients arose from the time difference between sample collection for screening and ***In**Signia*® test

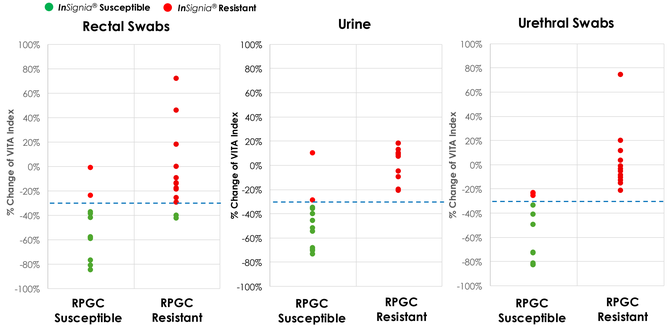
Viability

- ***In**Signia*® CT/NG compared to culture had 100% concordance for neat urine and urethral swabs and 96.90% concordance for rectal swabs

AST

- AST with ciprofloxacin was assessed for the viable samples only (25 rectal, 23 urine and 24 urethral)

***In**Signia*® CT/NG AST results by sample type



- ***In**Signia*® CT/NG compared to ***Resistance**Plus*® GC demonstrated 91.30% concordance for urine, 87.50% for urethral and 84% concordance for rectal swabs

CONCLUSIONS

- ***In**Signia*® CT/NG has shown to be feasible for direct use on clinical samples with a turnaround time of less than 2.5 hours
- With one simple-to-use test, results for detection, viability and AST can be obtained for NG
- ***In**Signia*® CT/NG demonstrated high concordance with gold standard culture and genotypic NAAT for viability and AST respectively
- ***In**Signia*® CT/NG offers a pathway to minimise over-treatment of patients with non-viable NG AST capabilities will be expanded to clinically relevant antibiotics ceftriaxone and cefixime in the future

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