Dead or alive? New precision tools for determining microbial viability in *Mycoplasma genitalium* and *Neisseria gonorrhoeae*

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Disclosure of Interest

The investigators report there are no competing interests to declare.

Acknowledgement of Country

I would like to acknowledges the Traditional Owners and their custodianship of the lands on which we meet today, the Kaurna People.

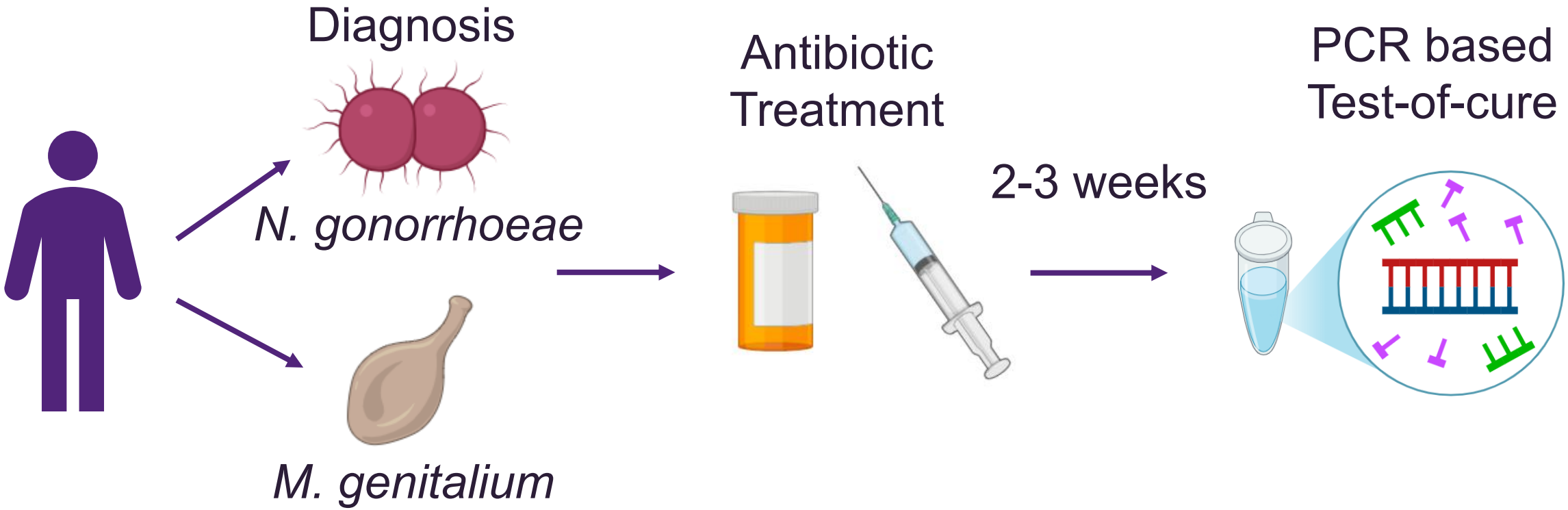
I would like to pay my respects to their Ancestors and their descendants, who continue cultural and spiritual connections to Country.

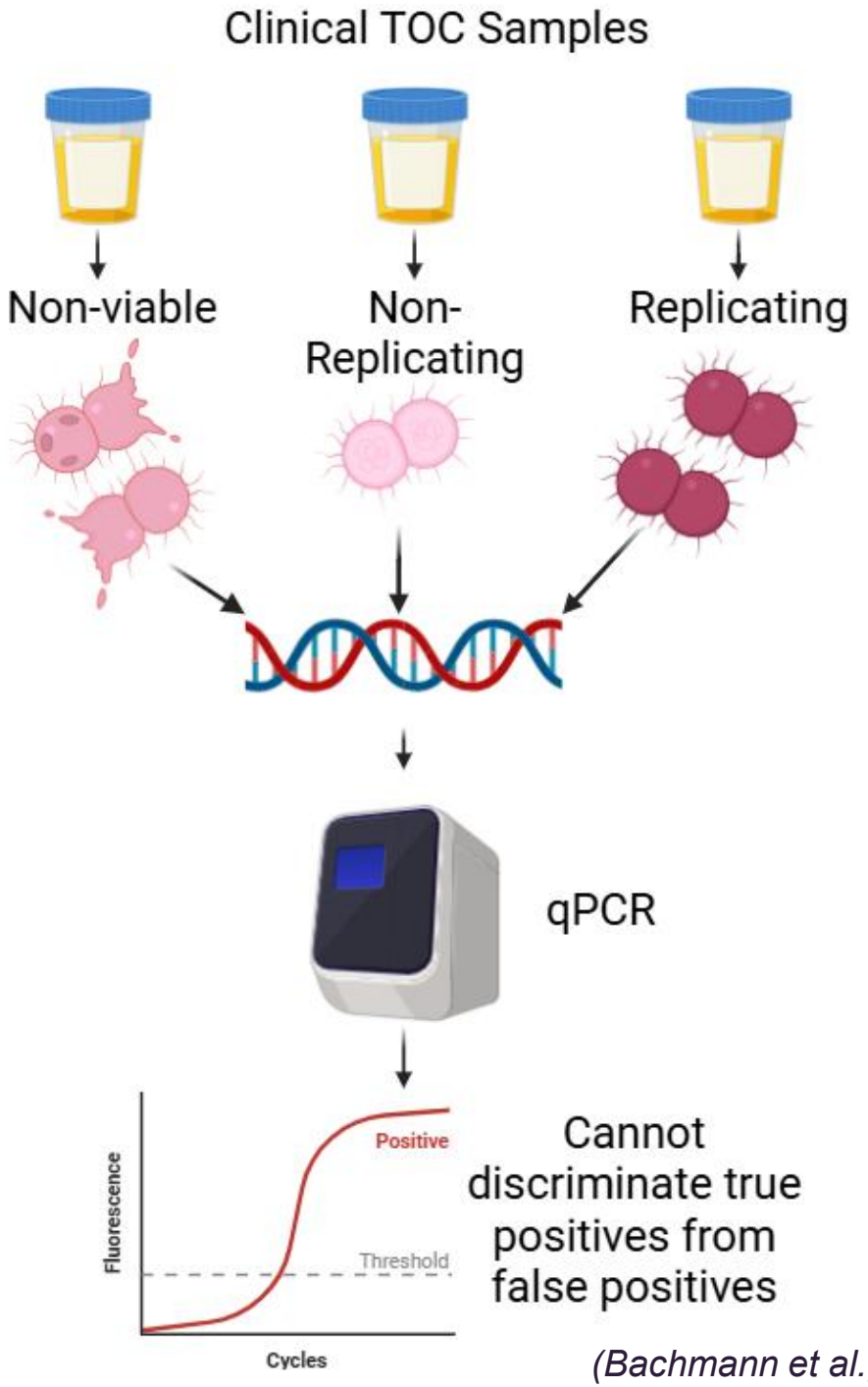
We recognise their valuable contributions to Australian and global society and the work we do at UQCCR.

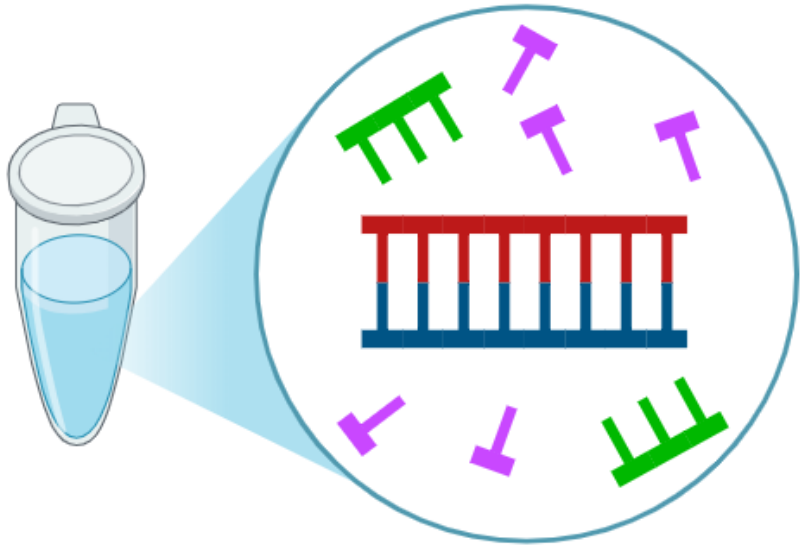
The Brisbane River pattern from *A Guidance Through Time*
by Casey Coolwell and Kyra Mancktelow.

Introduction

- *M. genitalium* and *N. gonorrhoeae* infect more than 150 million people each year worldwide.
- Both microbes are rapidly acquiring antimicrobial resistance (AMR), to all available treatments.
- This necessitates the use of test-of-cures to ensure infection clearance.







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The issue with PCR-based tests-of-cure (TOC):

- cannot distinguish actively replicating bacteria, indicating treatment failure, from non-viable and non-replicating bacteria

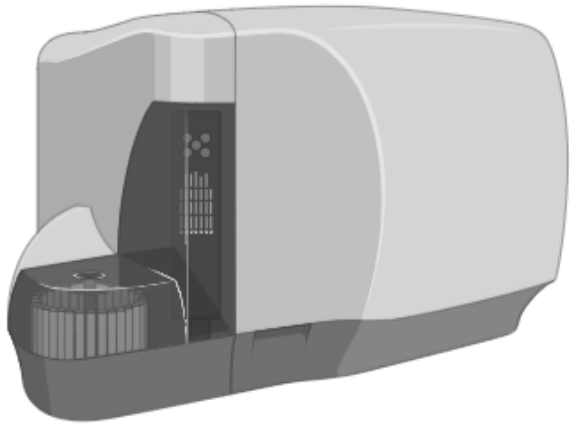
= **high rate of false-positivity**.

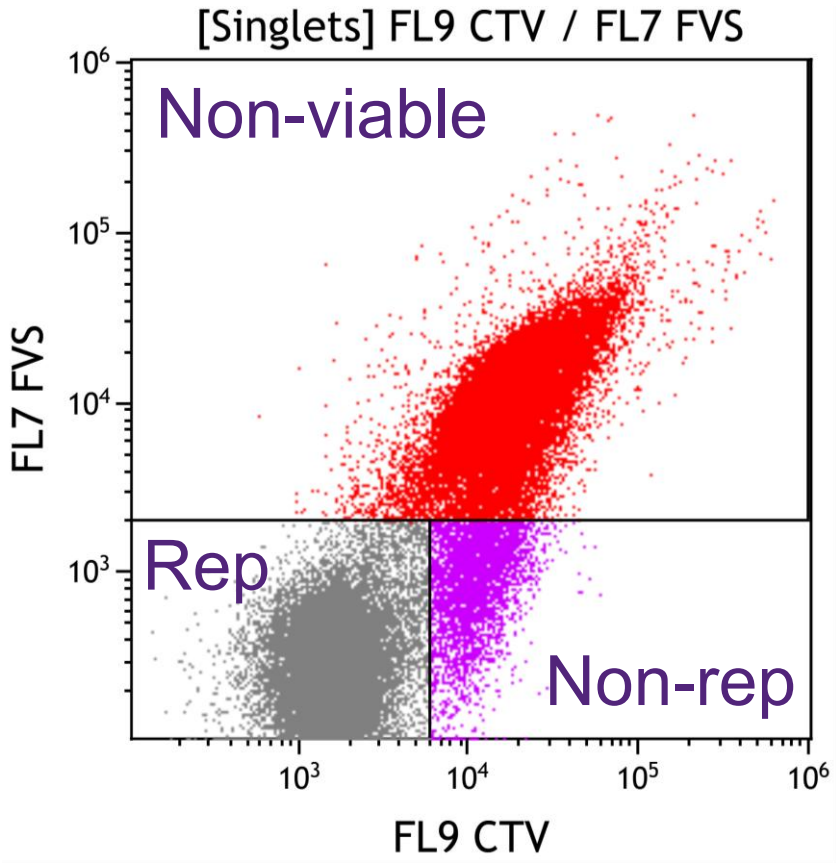
(Bachmann et al., 2002; Sultan et al., 2020; Williams et al., 2014; Falk et al., 2015; Schlanger et al., 2021*)*

Methods

We need tools that can identify replicating, non-replicating, and dead populations to better understand treatment failure and use this understanding to create and optimise precise molecular diagnostics.

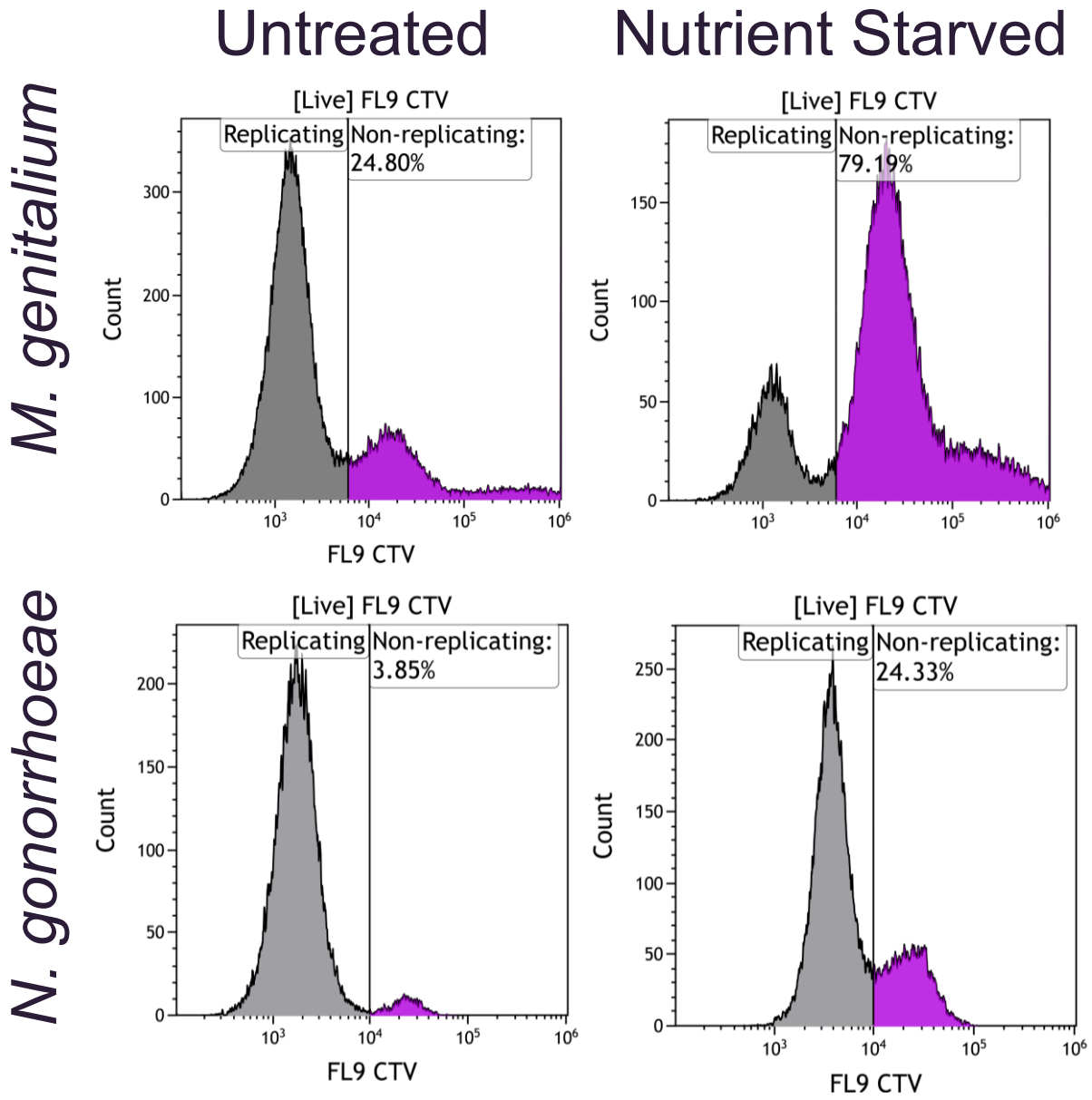
Flow Cytometry





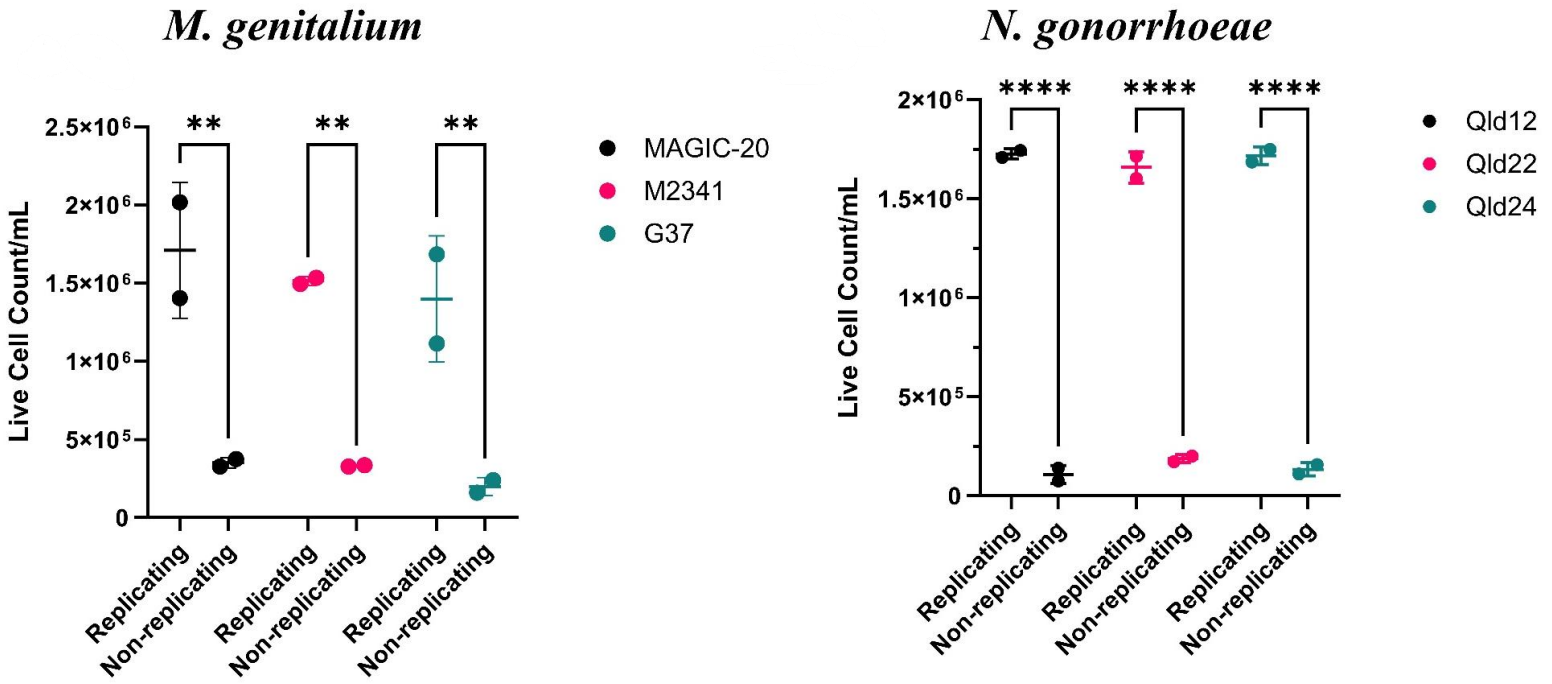
Determining Flow Assay Efficacy

- Tested assay using three strains of *M. genitalium* and *N. gonorrhoeae*
- Used nutrient starvation to induce stasis
- Larger proportion of nutrient starved samples were non-replicating compared to untreated samples



Quantifying **Replicating** and **Non-replicating** Microbes

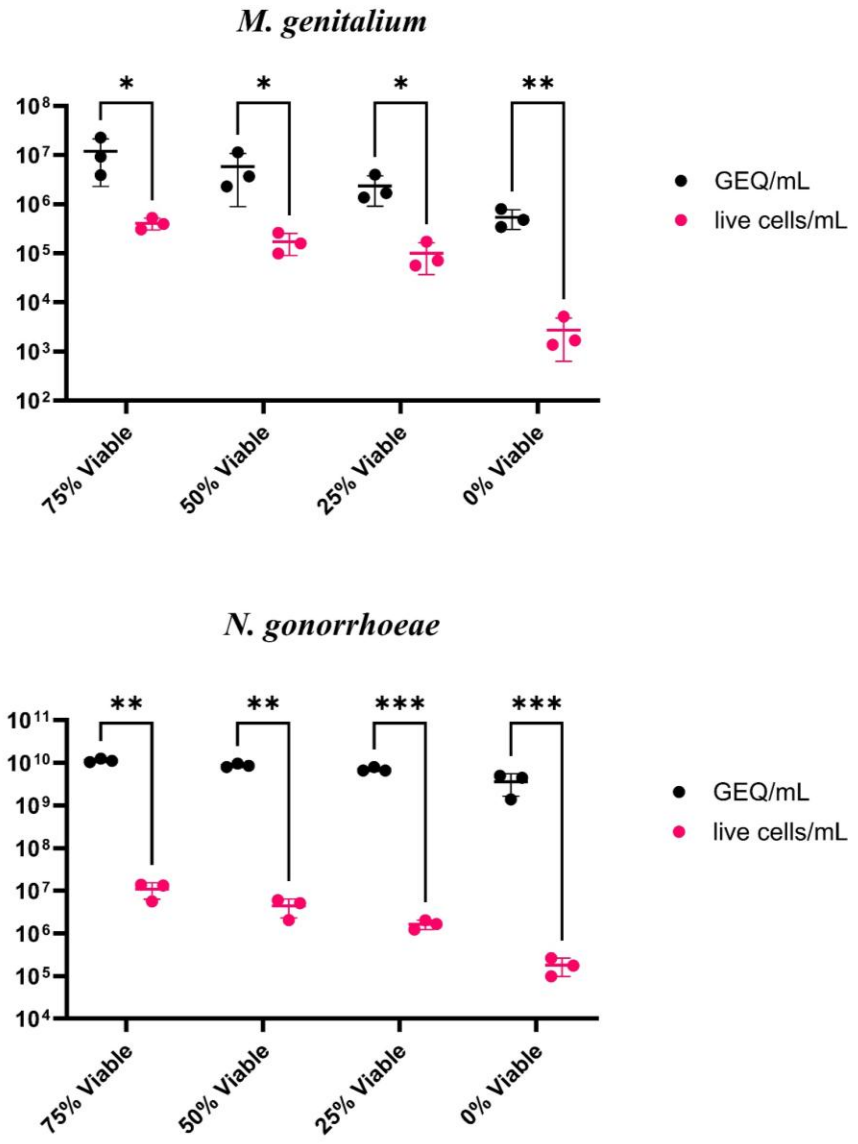
- Flow cytometry: significantly fewer (*P* <0.01) live cells in the non-replicating samples than in the replicating samples



*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Quantifying **Viable** and **Non-viable** Microbes

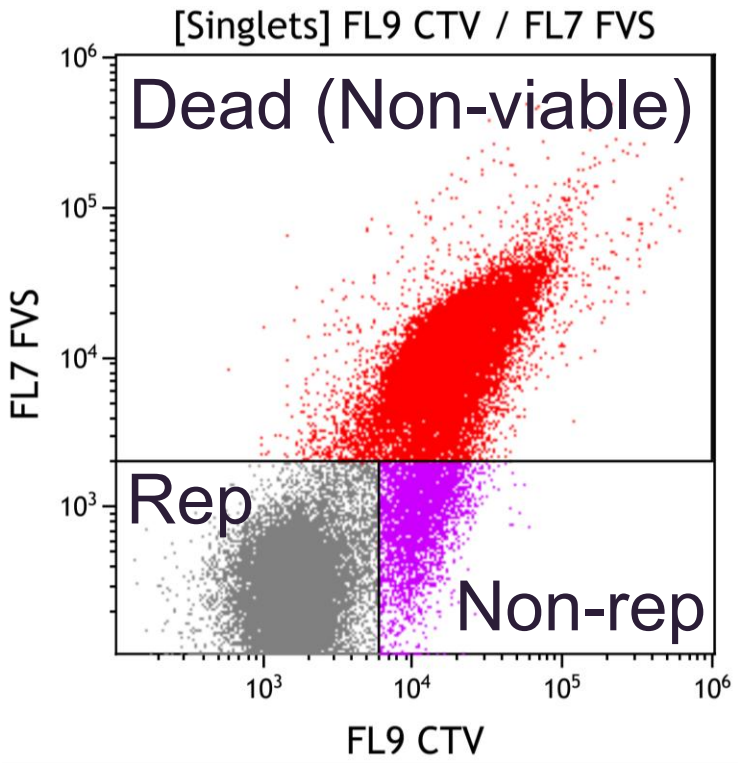
- qPCR overestimated viable cell counts by:
  - 1-2 orders of magnitude for *M. genitalium*
  - 2-4 orders of magnitude for *N. gonorrhoeae*
- This difference was most pronounced at 0% viable
- qPCR amplifying DNA from non-viable cells



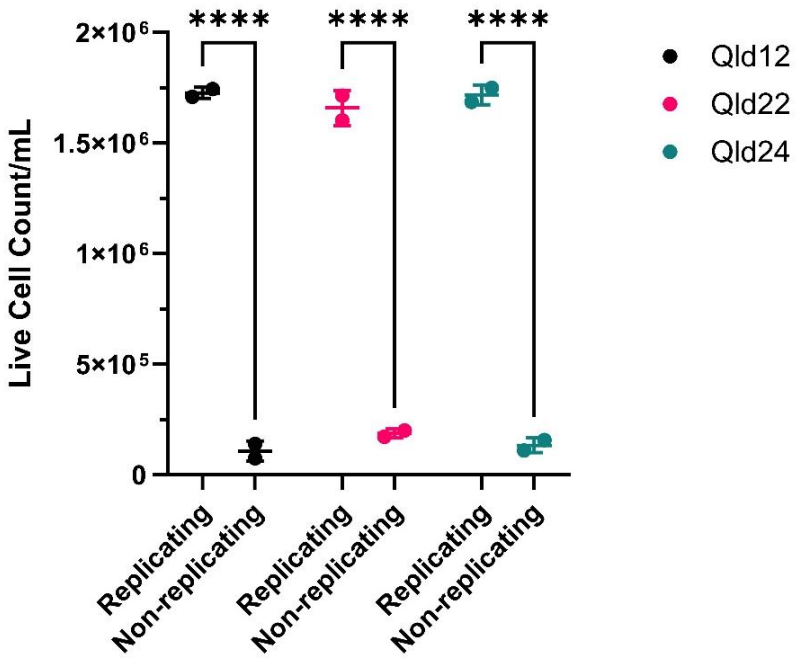
*, $P < 0.0332$; **, $P < 0.0021$; ***, $P < 0.0002$.

Key Findings

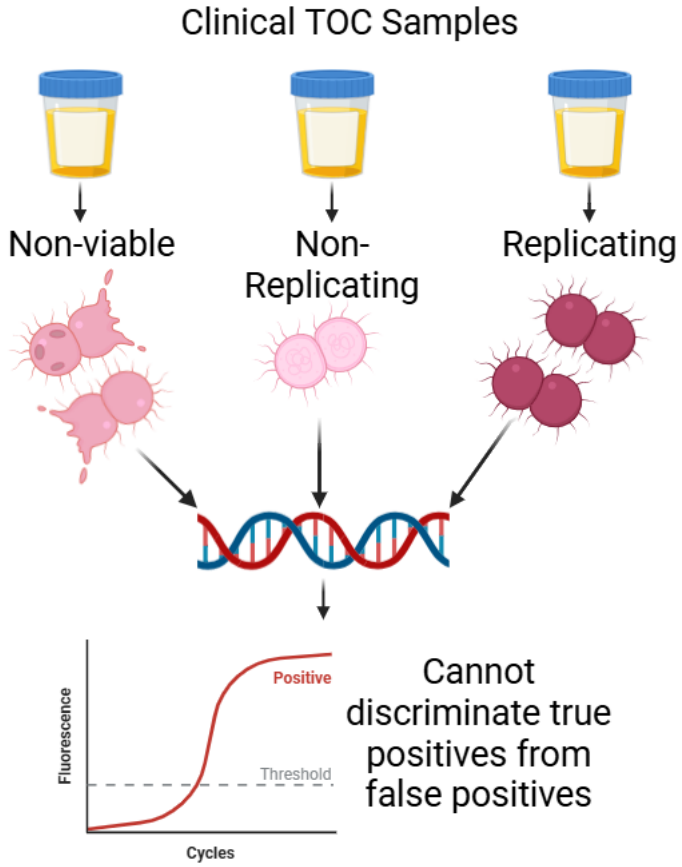
1. Characterise



2. Quantify



3. Support

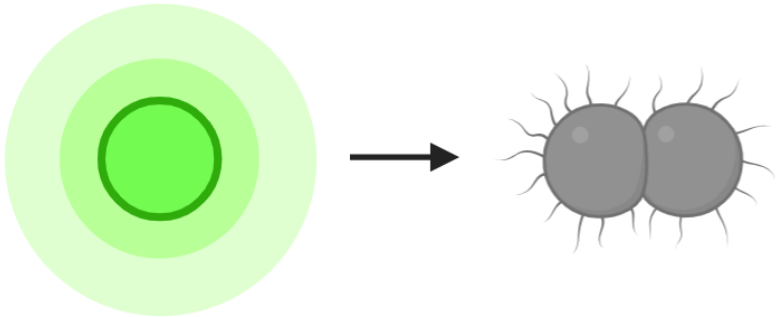


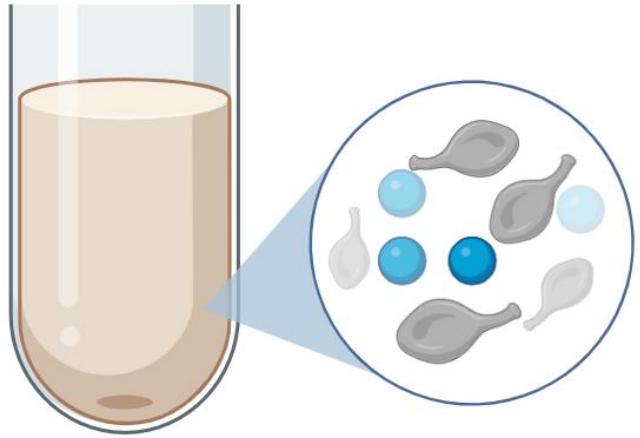
Discussion

Some limitations of the flow cytometric assay include:

- Not suitable for use in a pathology lab setting
- Can only track 4-5 replication cycles until CTV levels diminish to background levels
- Variability in cells/mL quantification capabilities between different flow cytometers

The Way Forward





Acknowledgments

Herring EO[1], Maidment TI[1], Andersen TK[1], Gough K[1], Zacchi L[2], Murray G[3–5], Jensen JS[6], Tickner JA[1,7], Whiley DM[1,8], Sweeney EL[1] and Bryan ER[1]

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