Identifying viable *Neisseria gonorrhoeae* through validation and application of viability RT-PCR

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KEY MESSAGE

- Viability PCR **effectively discriminates viable *Neisseria gonorrhoeae*** from non-viable cells and performs well across varying bacterial concentrations
- Viability PCR reliably assesses viability in clinical specimens, with **higher absolute and relative viability shown in culture-positive samples** compared to culture-negative specimens

INTRODUCTION

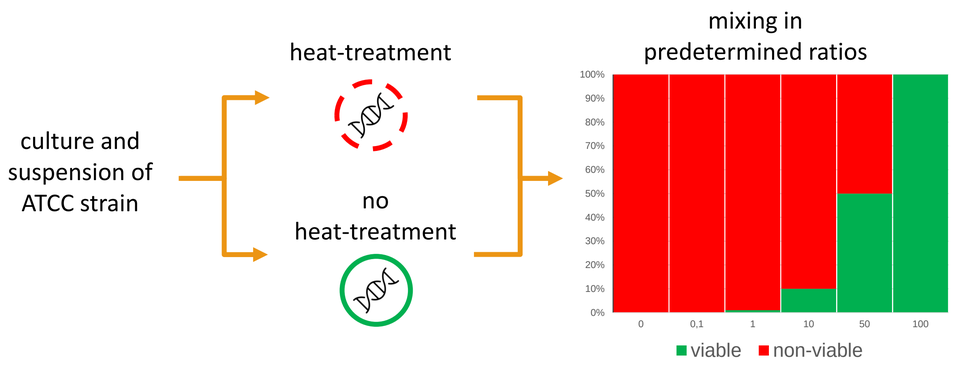
- The incidence of gonorrhea is rising worldwide, including antimicrobial resistance to first line treatment
- Current molecular diagnostic methods, such as nucleic acid amplification tests, cannot differentiate between viable and non-viable bacteria
- Knowledge of bacterial viability may have implications for research, diagnostic, and treatment purposes
- DNA-binding dyes like propidium monoazide (PMA) cannot penetrate intact cell membranes and inactivate bound DNA for PCR, enabling selective detection of DNA in intact cells only[1,2]

OBJECTIVE

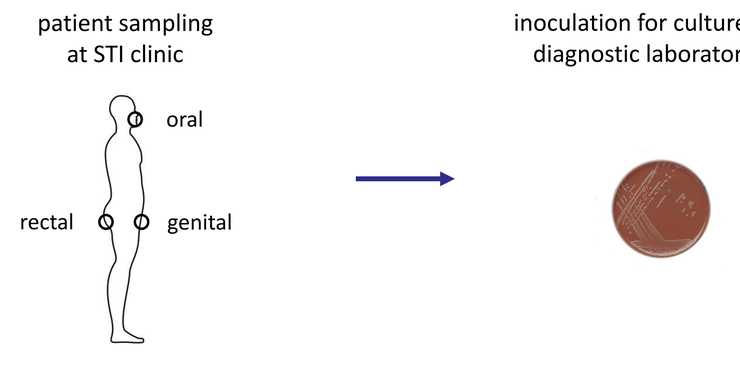
To validate and apply a viability PCR method for *Neisseria gonorrhoeae*, while correlating the results to successful culture recovery in clinical samples

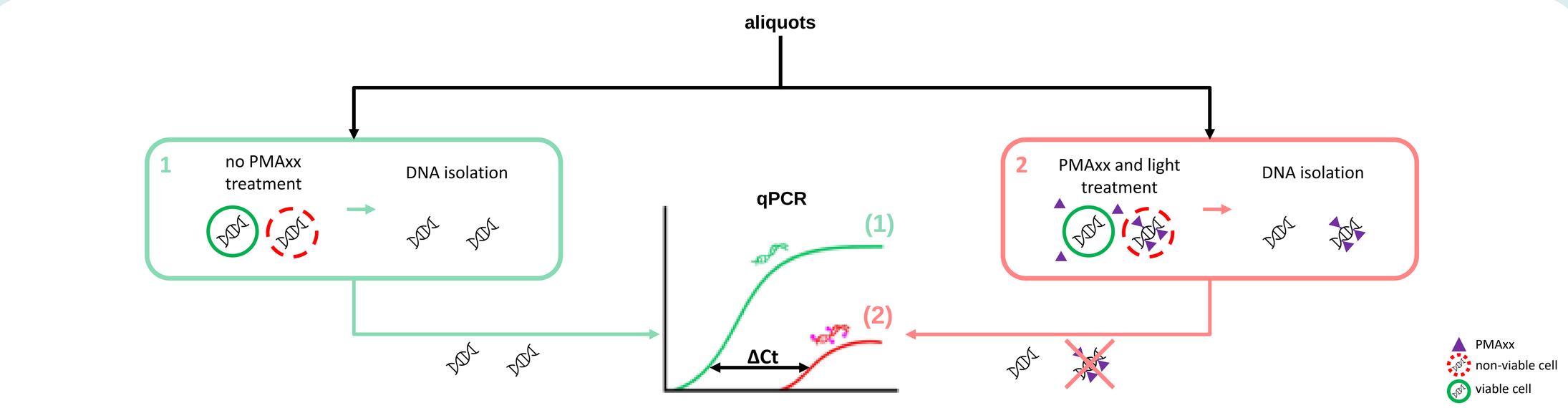
METHODS

TECHNICAL VALIDATION

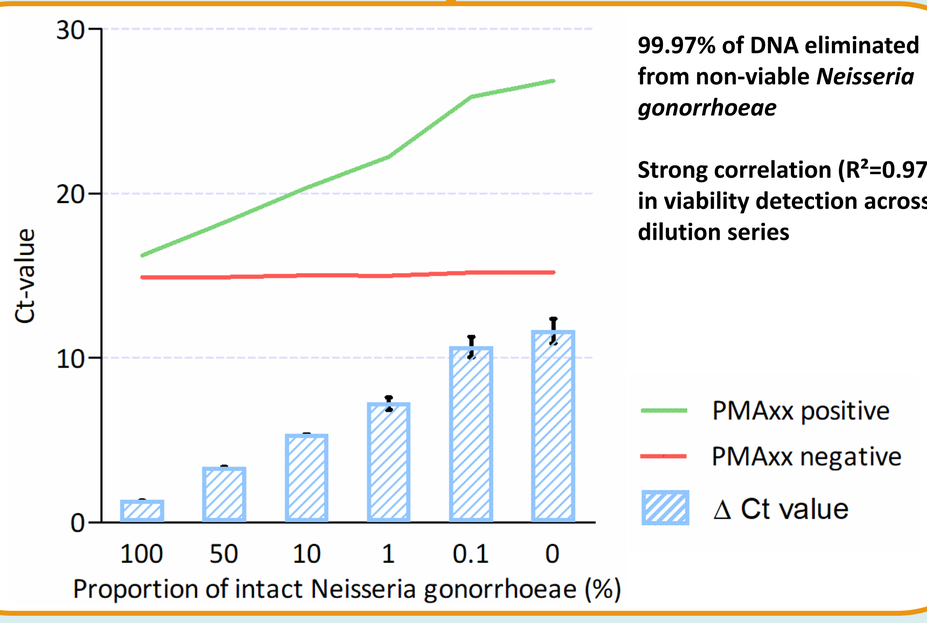


CLINICAL VALIDATION





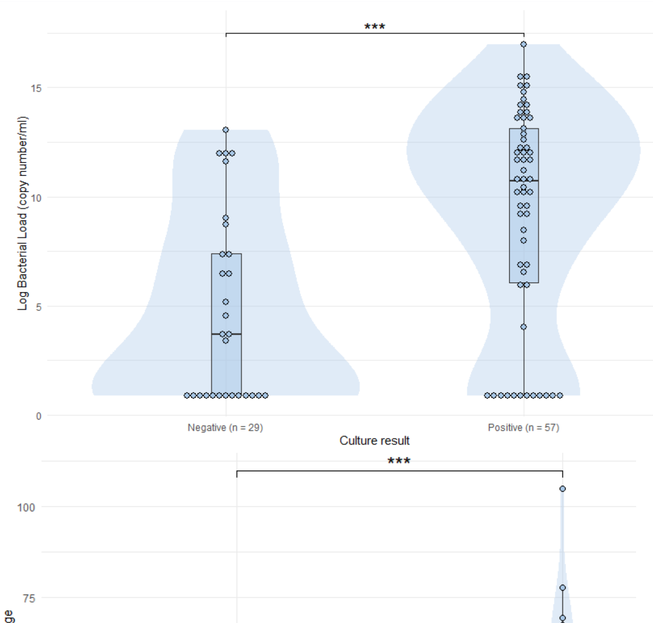
RESULTS



99.97% of DNA eliminated from non-viable *Neisseria gonorrhoeae*

Strong correlation (R^2=0.973) in viability detection across dilution series

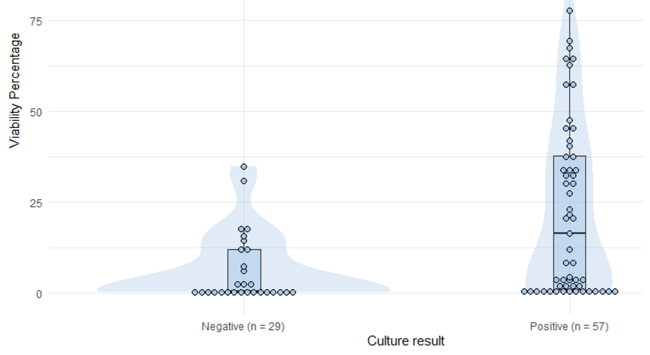
Absolute viability



Median log viable load was significantly higher in culture-positive samples than culture-negative samples (p=0.0003).

3.69 log_{10} copies/mL (IQR: 0.872–7.39) versus 10.7 log_{10} copies/mL (IQR: 6.05–13.1)

Relative viability



Median viability percentage was significantly higher in culture-positive samples (p=0.0003) compared to culture-negative samples.

16.4%; IQR: 0.887–37.7 versus 0.31%; IQR: 0.01–11.9

CONCLUSION

Viability PCR:

- effectively discriminates viable NG from non-viable cells and performs well across varying bacterial concentrations.
- reliably assesses viability in clinical specimens, with higher absolute and relative viability shown in culture-positive samples.
- holds potential for improving diagnostic stewardship and provides insights into factors affecting culture recovery and infection dynamics.

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References

1. Veugen, J. M. J., et al. (2024). "Advancing COVID-19 diagnostics: rapid detection of intact SARS-CoV-2 using viability RT-PCR assay." Microbiol Spectr 12(9): e0016024
2. Janssen, K. J., et al. (2016). "Viability-PCR Shows That NAAT Detects a High Proportion of DNA from Non-Viable Chlamydia trachomatis." PLoS One 11(11): e0165920.

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