RAPID ENUMERATION OF BACTERIA IN SIMULATED URINE

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BACKGROUND AND AIM

- Urinary Tract Infections (UTIs) are one of the most common infections, with a 50% lifetime incidence in adult women[1].
- Diagnosis of UTIs is slow leading to empirical treatment frequently being used.
- Ineffective or overly-broad antimicrobial treatment can lead to poor patient outcomes and exacerbate the spread of antimicrobial resistance.
- The ability to rapidly diagnose UTIs could allow for the swift use of specific, targeted treatments, reducing the use of broad-spectrum treatments.

Aim: to assess the CyteCount for rapid enumeration of live bacteria in stationary and exponential growth phase in simulated urine.

THE CYTECOUNT

- The CyteCount system harnesses bacterial electrophysiology to rapidly distinguish and count proliferative bacteria in just 45 seconds.
- This is due to the distinctive dynamic membrane potential responses of proliferative and inhibited bacteria to electrical stimulation.
- Proliferative cells show a characteristic hyperpolarisation response (measured as increase in fluorescence), whilst inhibited cells show a depolarisation response (decrease in fluorescence) to an external electrical stimulation (Fig 1).[2]

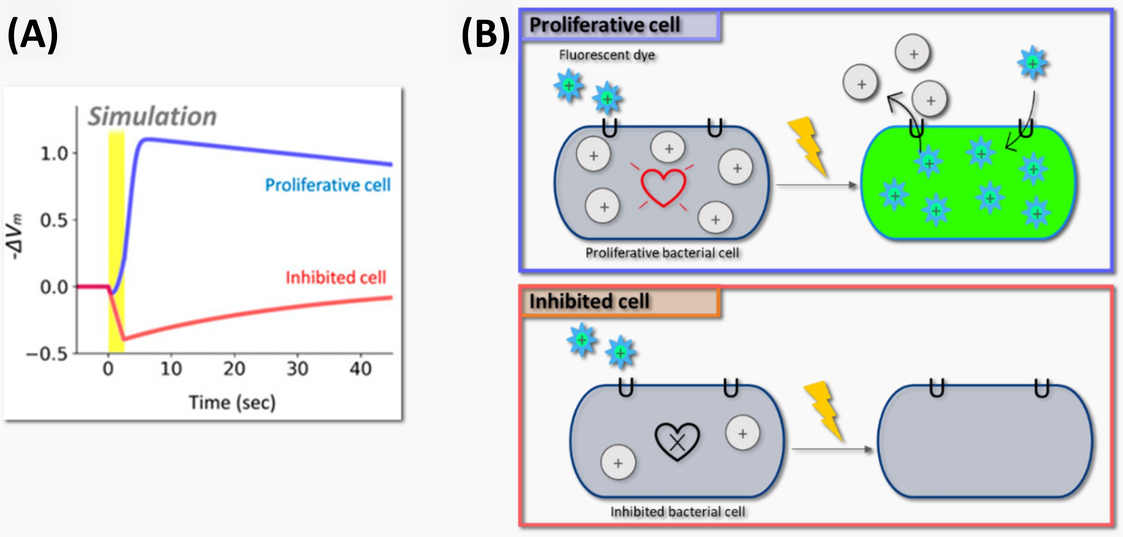


Figure 1. *Shift in resting membrane potential is sufficient to describe the distinct responses between proliferative and inhibited cell. (A) Numerical simulation of the FitzHugh-Nagumo bacteria model. Despite being simulated by an identical electrical stimulus, proliferative cells (blue) hyperpolarise and inhibited cells (red) depolarise. (B) Illustration of the biological mechanism of the response differentiation between proliferative and inhibited cells. Adapted from Stratford et al., 2019.*[2]

MATERIALS AND METHODS

Culture Preparation

Dilute culture in fresh TSB and incubate for 3-4 hours at 37°C and 150RPM. Skip this step for stationary phase culture.

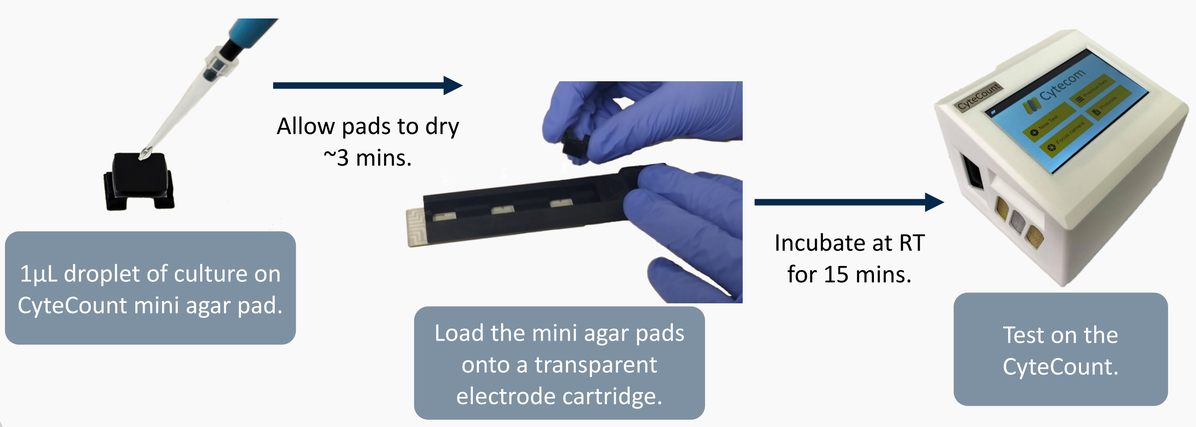
→ Centrifuge at 3000x*g* for 5min.

Resuspend cells in TSB (exponential phase) or PBS (stationary phase) and OD standardise to 1-3x10^8 CFU/mL.

→ Centrifuge at 3000x*g* for 5min.

Resuspend cells in artificial urine.

Perform serial dilution and standard plating methods alongside the CyteCount protocol



RESULTS AND FINDINGS

- The viable bacterial counts obtained with the CyteCount assay were comparable to the traditional plate counting methods with results available ~50x faster (Fig 2A).
- The CyteCount membrane potential signal analysis distinguished between bacteria in stationary and exponential growth phases, which could be of clinical importance for Antimicrobial Susceptibility Testing (AST) (Fig 2B).

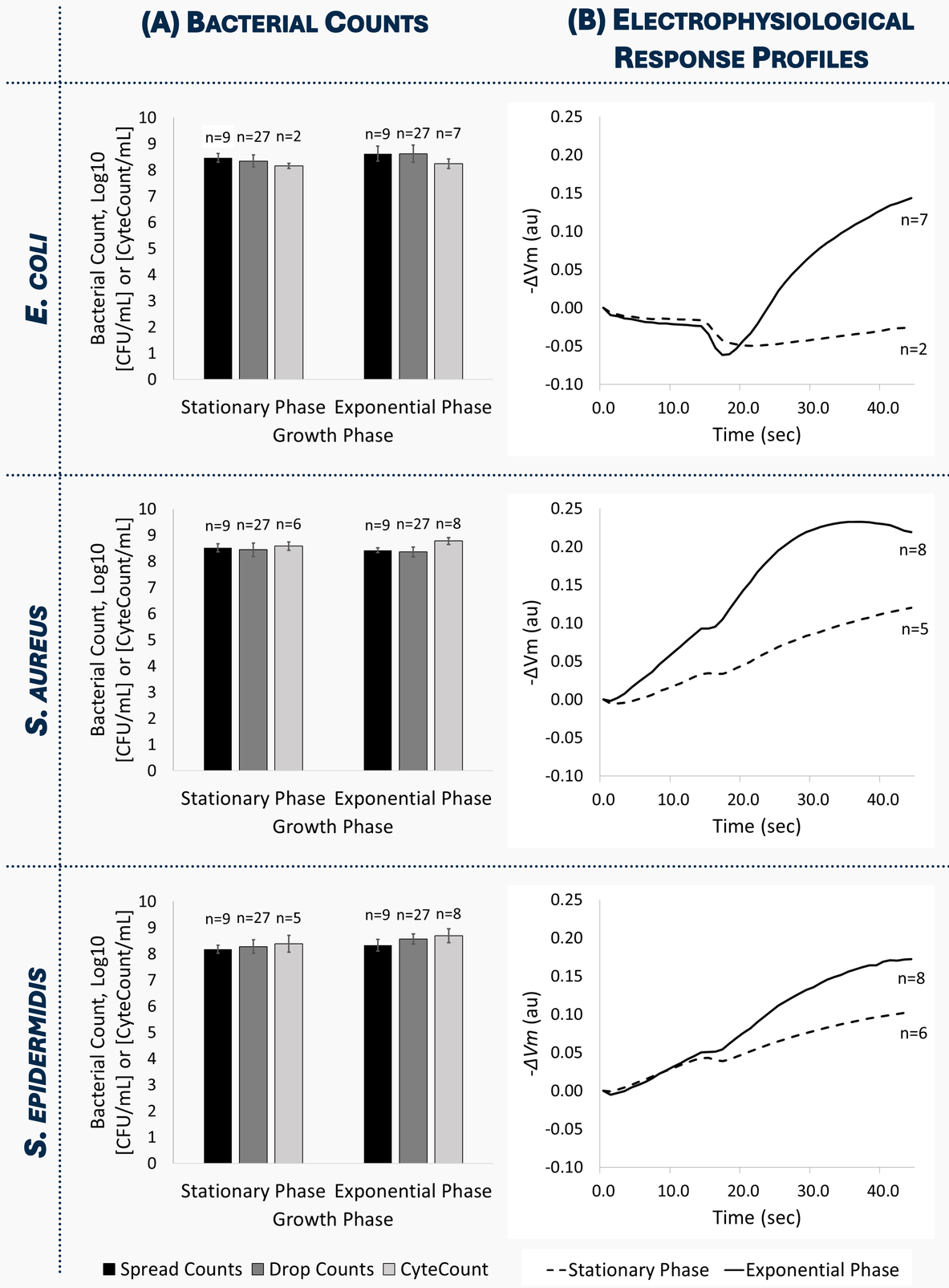


Figure 2. *(A) Bacterial counts of stationary and exponential phase E. coli, S. aureus & S. epidermidis cultures obtained using traditional plating methods and the CyteCount. The number of replicates are shown, error bars are ± standard deviation. (B) Time trace of mean membrane potential change (−ΔVm) of stationary (dashed line) and exponential (solid line) phase E. coli, S. aureus & S. epidermidis cultures with number of replicates shown. The mean −ΔVm was calculated based on the mask identifying all viable and inhibited cells.*

CONCLUSION

- Results showed proof of concept data that the CyteCount can rapidly count bacteria and differentiate between bacterial growth states in artificial urine.
- Further work should assess the assay for enumeration in clinical samples as well as evaluation of the membrane signal response analysis for rapid AST.

REFERENCES

1. Fu, Z., et al., Cranberry Reduces the Risk of Urinary Tract Infection Recurrence in Otherwise Healthy Women: A Systematic Review and Meta-Analysis. J Nutr, 2017.147(12):p. 2282-2288.
2. Stratford, J.P., et al., Electrically induced bacterial membrane-potential dynamics correspond to cellular proliferation capacity. Proceedings of the National Academy of Sciences, 2019. 116(19): p. 9552-9557.