

PrestoBlue Colorimetric Viability Assay for the Identification of Nitrofurantoin Resistant *Escherichia coli*

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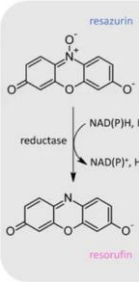
OVERVIEW

Uropathogenic *Escherichia coli* (UPEC) accounts for 80% of uncomplicated Urinary Tract Infection (UTI) diagnoses (Asadi Karam et al., 2019). In the UK, nitrofurantoin (NIT) has been increasingly prescribed as the front-line antibiotic for uncomplicated UTIs (Kashouris et al., 2023), overcoming carbapenem resistant bacterial strains. Novel diagnostic tests for NIT resistant *Escherichia coli* (*E. coli*) are essential to prolong the efficacy of this broad-spectrum antibiotic.

Here, we propose the application of PrestoBlue (PB) as a Colorimetric Viability Assay for the Identification of Nitrofurantoin Resistance in *E. coli*.

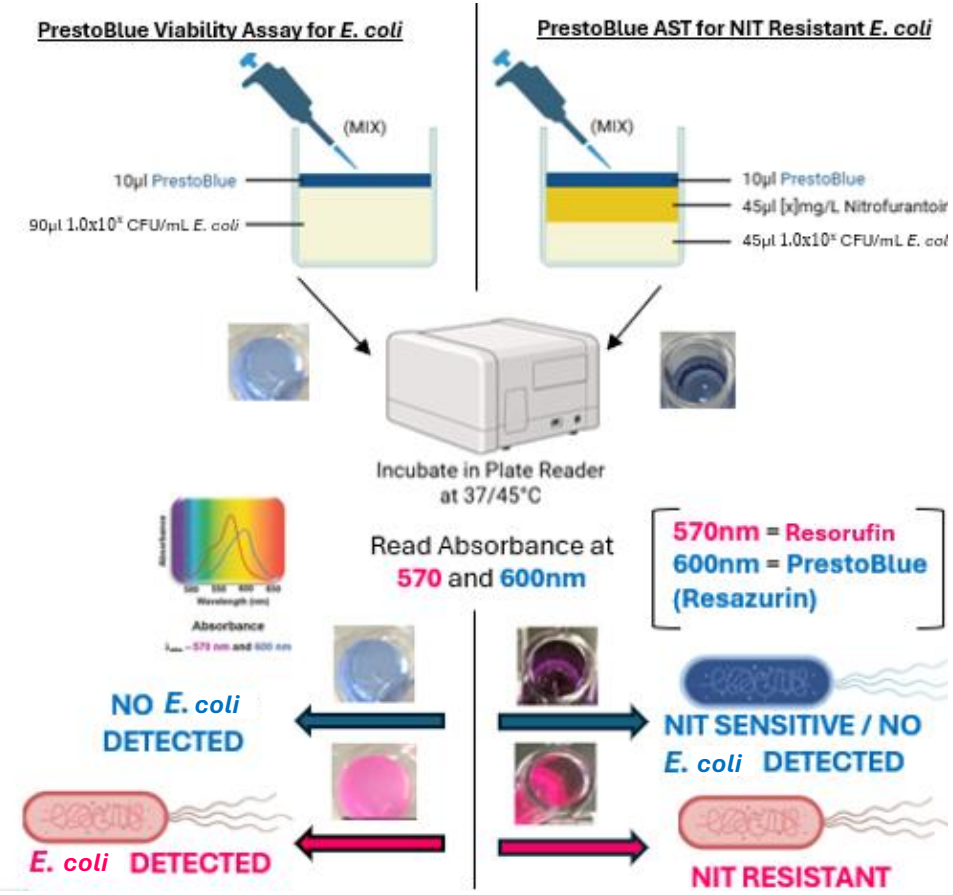
MECHANISM: In live bacteria, NADPH dehydrogenase reduces **resazurin** within PrestoBlue into **resorufin**. If PrestoBlue is added to *E. coli* exposed to nitrofurantoin, we can discriminate resistant and sensitive strains

- NIT Sensitive:** Sensitive *E. coli* are unable to overcome NIT challenge, no PrestoBlue reduction -> no colour change
- NIT Resistant:** Resistant *E. coli* survive despite NIT challenge, reduce PrestoBlue into resorufin -> colour change from blue to pink



- AIM 1: Establish a PrestoBlue Cell Viability Assay for *E. coli*
- AIM 2: Adapt PB Viability Assay as a NIT Susceptibility Test, with discriminative colorimetric and kinetic results between Resistant and Sensitive *E. coli*

METHODOLOGY



CONCLUSIONS

- AIM 1: PrestoBlue Cell Viability Assay for *E. coli***
- PrestoBlue yields colorimetric results for *E. coli* viability at a range of PB concentrations (1.0 – 0.1x)
 - Reduction accelerated when temperature increased to 45°C
 - Sensitivity increased by extending incubation and decreasing PrestoBlue concentration

- AIM 2: PrestoBlue NIT Susceptibility Test**
- NIT concentration must exceed 32mg/L to avoid false positives
 - Kinetics of both NIT (R) and (S) *E. coli* strains slowed by NIT; this can be overcome by extending incubations, optimising NIT concentration and increasing incubation temperature to 45°C

AIM 1: PrestoBlue Cell Viability Assay for *E. coli*

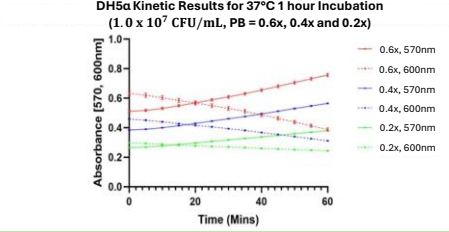
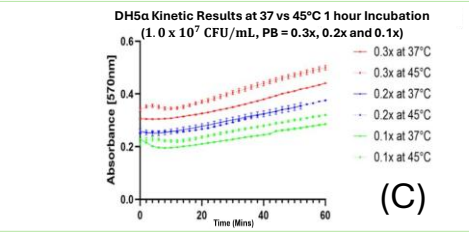
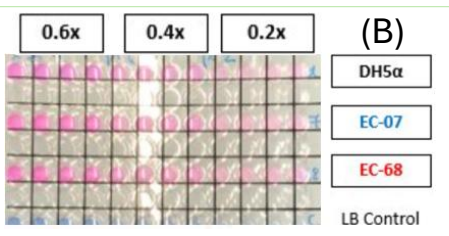
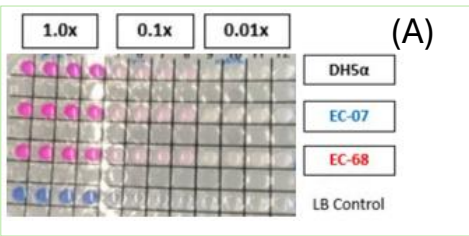


Figure 1:

- Colorimetric results for PB conc. 1.0x, 0.1x, 0.001x. 1 hr incubation at 37°C. 1.0×10^7 CFU/mL *E. coli*
- Colorimetric and kinetic results for PB conc. 0.6x, 0.4x and 0.2x. 1 hr incubation at 37°C. 1.0×10^7 CFU/mL *E. coli*
- Kinetic results for PB conc. 0.3x, 0.2x and 0.1x. 1.0×10^7 CFU/mL *E. coli*, 1 hr incubation at 37 vs 45°C
- Colorimetric results for 0.1x PB sensitivity test. 1 hr incubation at 37°C. $1.0 \times 10^7 - 1.0 \times 10^4$ CFU/mL *E. coli*

AIM 2: PrestoBlue NIT Susceptibility Test

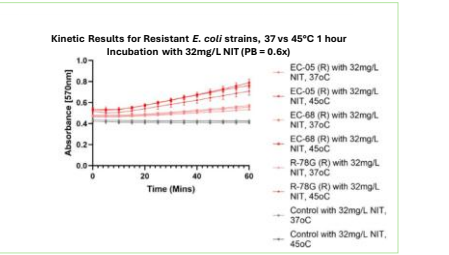
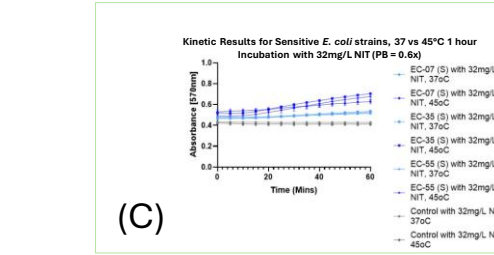
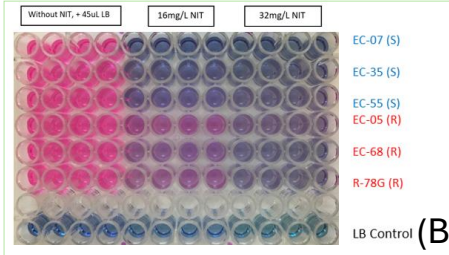
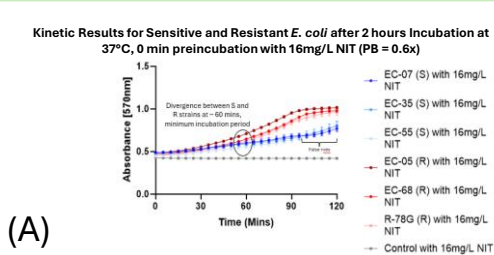


Figure 2:

- Kinetic results for NIT Sensitive (S) and Resistant (R) *E. coli* over 2 hr, 37°C incubation with 16mg/L NIT. PB = 0.6x, 1.0×10^7 CFU/mL *E. coli*
- Colorimetric results for NIT (S) and (R) incubated with 16mg/L and 32mg/L NIT. 20 min preincubation and 90 min incubation at 37°C. PB = 0.6x, 1.0×10^7 CFU/mL *E. coli*
- Kinetic results for NIT (S) and (R) *E. coli*, 1 hr incubation with 32mg/L NIT at 37 vs 45°C. PB = 0.6x, 1.0×10^7 CFU/mL *E. coli*

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