Rapid selection of an appropriate antibiotic

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Faster diagnostics can better inform antibiotic prescription

Prescription of ineffective antibiotics due to urgency in treatment combined with slow, culture-based diagnostics can result in treatment failure and promote development of antimicrobial resistance.

A rapid alternative is application of the LIVE/DEAD® BacLight™ Bacterial Viability Kit to determine antibiotic susceptibility to better inform antibiotic choice.

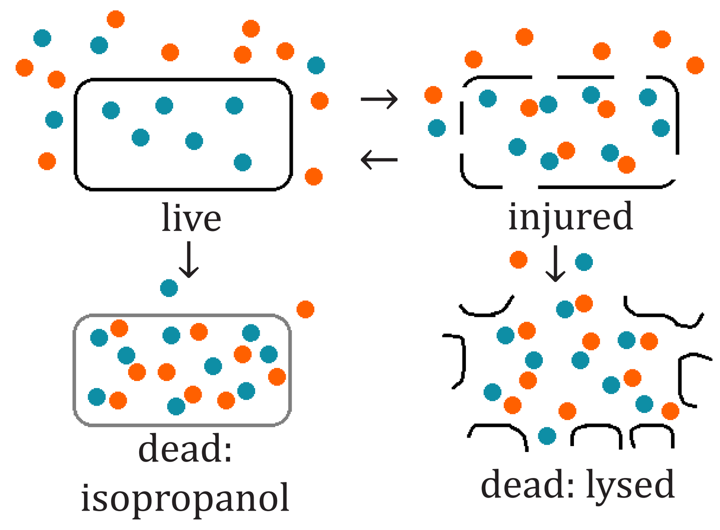

Fig 1. SYTO 9 and PI staining of live, injured, and dead bacterial cells. SYTO 9 (teal) can enter all cells while PI (orange) can only enter cells with compromised membranes.

LIVE/DEAD® BacLight™ Bacterial Viability Kit

SYTO 9: membrane permeable → enters all cells → green emissions

Propidium iodide (PI): membrane impermeable → only enters cells with compromised membranes → red emissions

Live and dead cell differentiation: relative green and red fluorescence

SYTO 9 and PI bind nucleic acid → increase in fluorescence emissions

Excitation/emission maxima:
480/500 nm (SYTO 9) 490/635 nm (PI)

Optimised live/dead spectrometry

Measure fluorescence spectra (10 s) of stained samples using the Optrode → Normalise spectra to integration time (20 ms) and illumination power → Integrate spectra at 505 - 515 nm (green) and 600 - 610 nm (red) → Calculate the proportion of live cells using the adjusted dye ratio (ADR) → Adjusted dye ratio:

$$\text{Proportion of live} \propto \frac{(100\times\text{SYTO 9/PI})}{(1+\text{SYTO 9/PI})}$$

Characterisation of *Escherichia coli* live/dead staining: interaction between SYTO 9 & PI

The nature of the SYTO 9 and PI interaction is indicative of the live/dead status of the cells

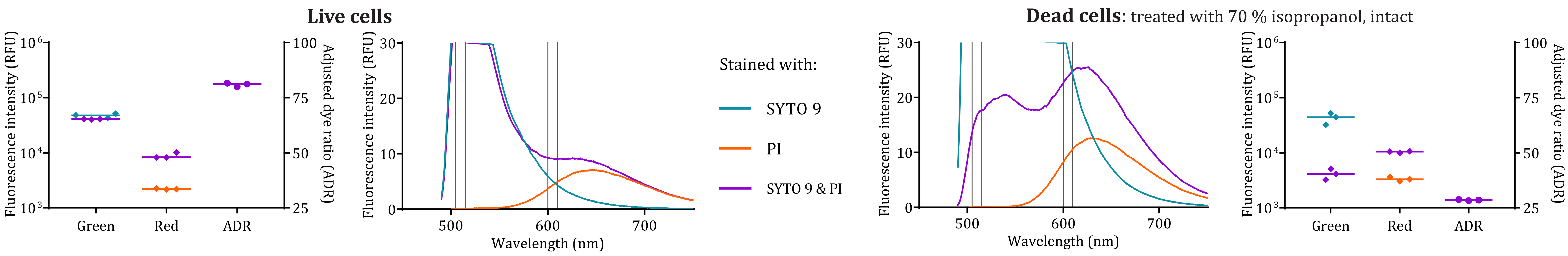

Fig 2. Live/dead spectrometry of *E. coli*. Live and isopropanol killed *E. coli* stained with SYTO 9, PI, and SYTO 9 & PI. Data is presented as integrated fluorescence intensities with the proportion of live cells (outer graphs) and fluorescence spectra (inner graphs).

Crosstalk

Green emissions from SYTO 9 = SYTO 9 & PI

Red emissions from PI < SYTO 9 & PI

Red emissions are from bound SYTO 9 and unbound PI

Indicative of PI unable to enter cells

Quenching/enhancement

Quenched bound SYTO 9 emissions with enhanced bound PI emissions

Dyes are bound to DNA in close proximity → indicative of PI entry into cells

Green emissions from SYTO 9 > SYTO 9 & PI

Red emissions from PI < SYTO 9 & PI

Determination of viability of antibiotic challenged *E. coli*: live/dead spectrometry vs culture-based enumeration

Antibiotic treated and untreated: sample at 0 h, 0.5 h, 2 h, and/or 5 h → Live/dead spectrometry: SYTO 9, PI, and SYTO 9 & PI → Interpretation of spectrometric data →

Live and dead cell differentiation criteria		Deduction
Green emissions	SYTO 9 only vs SYTO 9 & PI	Interaction type
Red emissions	PI only: treated vs untreated	Membrane integrity status
ADR	Treated vs untreated	Estimated proportion of live

Antibiotic treated and untreated → Viable cell plate counts: spread 20 µl on TSA plates from serial dilution → Incubate plates for 1 - 2 days. Count colonies and calculate CFU/ml.

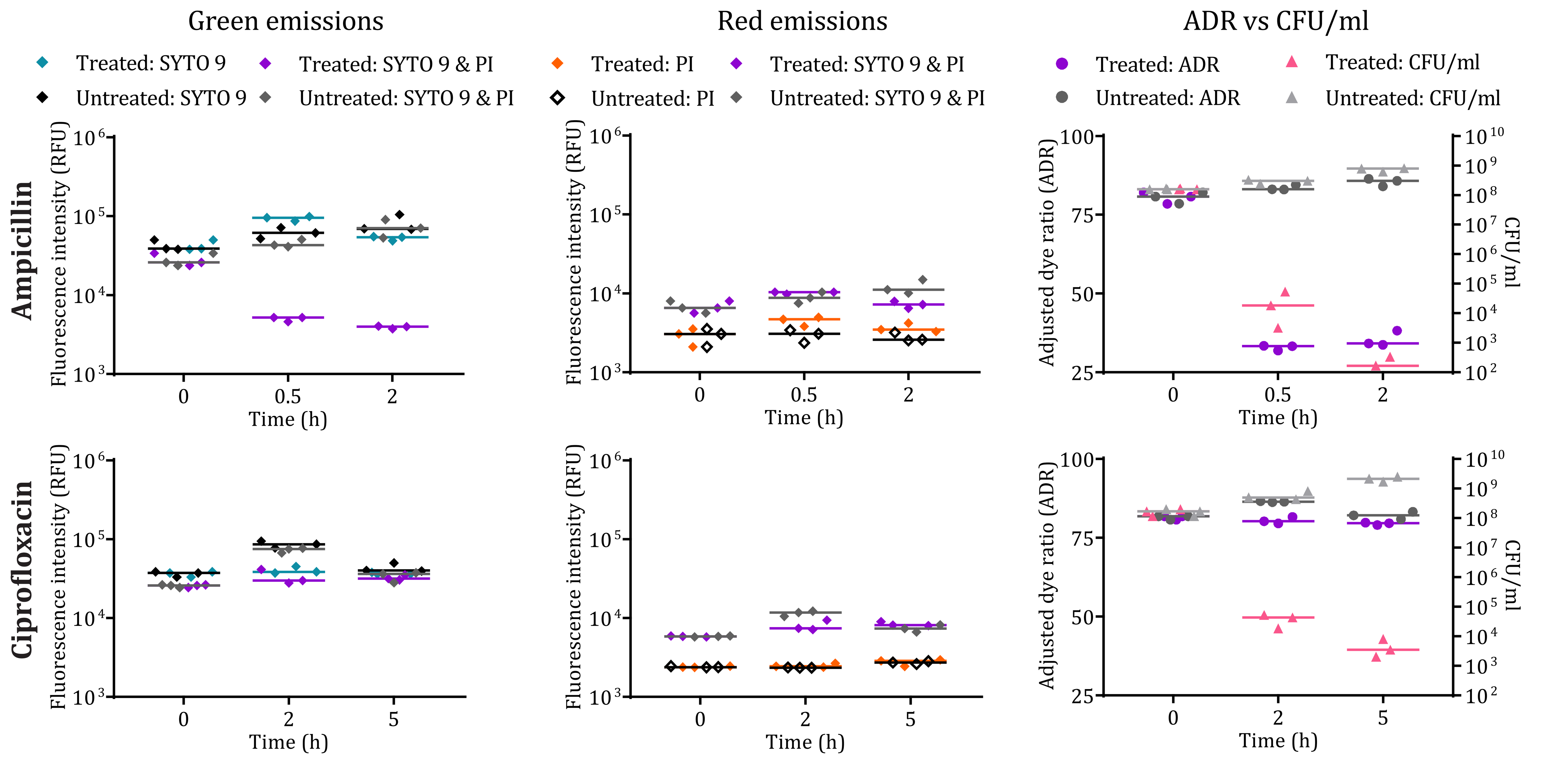

Fig 3. Live/dead spectrometry and culture-based enumeration of antibiotic challenged *E. coli*. Viability of *E. coli* challenged with ampicillin, ciprofloxacin, and untreated was determined by measuring fluorescence of SYTO 9, PI, and SYTO 9 & PI stained samples, and viable cell plate counts. The adjusted dye ratio was used to determine the proportion of live cells in a sample. Data presented is from three biological replicates with a line at the median.

Ampicillin
Dead: lysed cells
Quenching/enhancement
↑ red emissions (PI only)
ADR matches CFU/ml

Untreated
Live cells
No ↑ red emissions (PI only)
Crosstalk
ADR matches plate counts

Ciprofloxacin
Dead: intact membrane
Crosstalk
No ↑ red emissions (PI only)
ADR does not match CFU/ml

Detection of antibiotic susceptibility is influenced by the bactericidal mechanism

We could detect the lytic activity of ampicillin using live/dead spectrometry after a 2 h treatment while the non-lytic activity of ciprofloxacin could not be detected after a 5 h treatment. Determination of cell viability requires staining with SYTO 9 and PI, separately and in combination.

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