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Rapid antibiotic susceptibility testing using resazurin bulk modified screen-printed electrochemical sensing platforms

Supporting Information

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To determine the length of time taken for *E. coli* to reach the exponential phase of growth after the inoculum is added to artificial urine, a bacterial growth curve was conducted. An overnight culture of *E. coli* was standardised to an optical density of 0.1804 at 600nm. A series of wells on a 96 well plate were then filled with 180 of artificial urine, a 20 µL of the standardised bacterial overnight culture was then added to each of the wells to produce an initial bacterial concentration of 1.76E7 colony forming units per mL (CFU/mL). The plate was read at 600 nm using a Thermo Scientific Multiscan GO 96 well plate reader using SkanIt RE for Multiskcan GO 3.2 software straight after the inoculation step and then read every 20 m for six hours. Whilst not being read, the plate was kept incubated at 37°C on a shake plate.

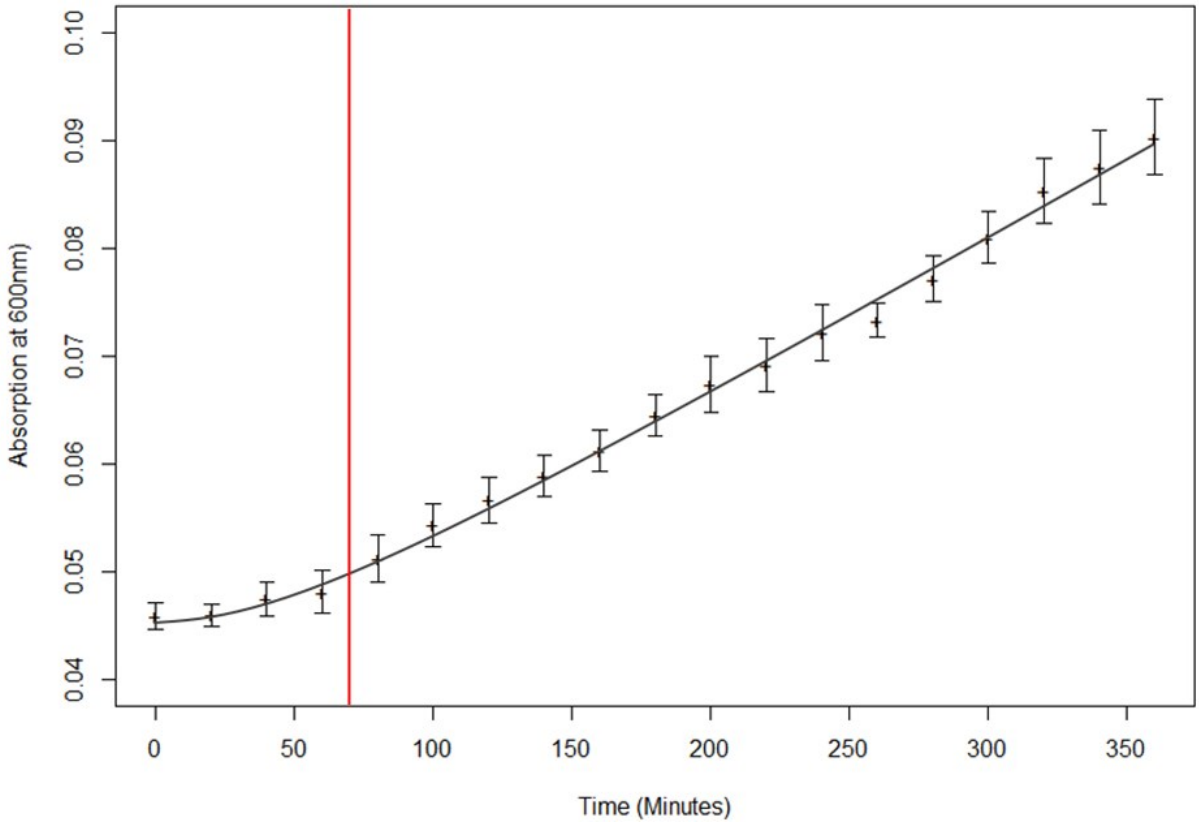

Figure S1. Change in optical density cause by the growth of *E. coli* in pH 6 artificial urine. The red line marks 70 minutes where the exponential phase of the *E. coli* growth starts, and the bacterial concentration was approximately 1.75E8 CFU/mL. Each datapoint is the average of 30 measurements with associate standard deviation.

Plotting resazurin reduction peak height as a function of pH yielded a nonlinear trend. As such, the peak height was plotted as a function of the reduction peak position (E_p) to determine how the signal height changed as the concentration of resazurin decreased. The resulting plot showed an overlap in signal height standard deviation between concentrations of 62.5 and 31.25 µM. However, when using R – SPEs to conduct electrochemical AST using a concentration of 1.55 µM (0.8 µg/mL) gentamicin sulphate, the R – SPEs could detect, and differentiate resazurin concentrations even when the concentration of resazurin had decreased, due to electrochemical and biological reduction, into the range to yield signal heights in the range of -1 to approximately -1.5 where the overlap occurred using bare/unmodified SPEs. As such, the R – SPEs demonstrate a high degree of resolution over their unmodified counterparts.

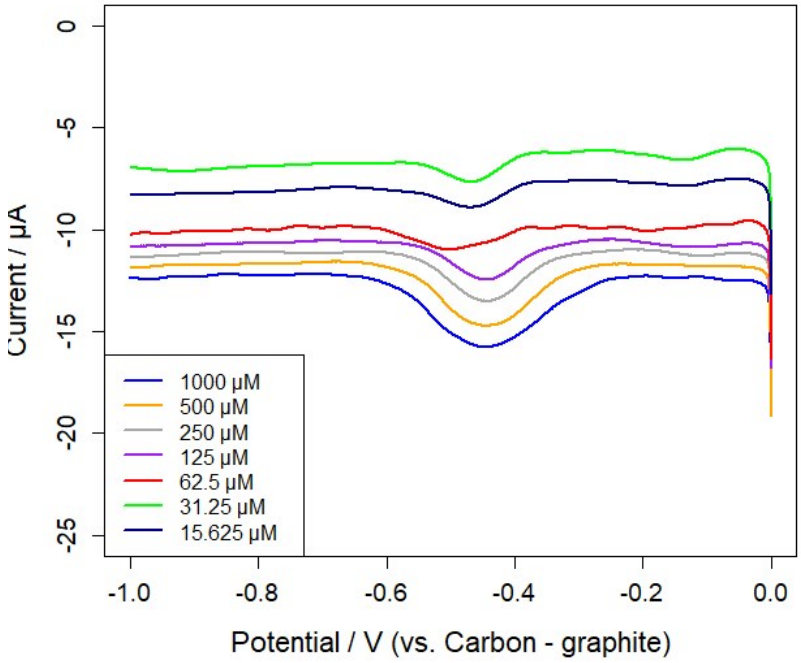

Figure S2. Differential pulse voltammogram current response versus potential for resazurin concentrations of 1000 to 15.625 µM. using bare SPEs.

The significant differences in the resazurin reduction peak height between the antibiotic treated, and the antibiotic free resazurin reduction was taken to indicate that the antibiotic was effective. To validate the antibiotic concentration used, culture based antibiotic susceptibility testing was done in parallel. The method used was the same as was discussed in the experimental section pertaining to detecting bacterial growth in response to different antibiotic concentrations. However, instead of µDrop the artificial urine was loaded into 96 well plates. At each incubation point, a 10 µL sample was extracted, and then serially diluted to a factor of 10^{-5} before being plated on nutrient agar plates that were then incubated for 24 hours at 37°C. Extractions were performed at time zero, and then either every hour for a total of four hours, or as every 20 minutes for a total of 80 minutes.

The colony forming units per mL (CFU / mL) were then calculated, and then statistically compared to determine if the inhibition of bacterial growth was significant. The MIC50 of gentamicin sulphate versus E. coli ATCC 25922 is quoted as 0.968 µM (0.5 µg/mL).[1, 2] Concentrations of 0.968 µM (0.5 µg/mL) and 1.55 µM (0.8 µg/mL) inhibited bacteria growth by 50% with 1.55 µM showing slightly more inhibition of bacterial growth than 0.968 µM over time. As such the concentrations used showed the degree of bacterial growth inhibition that was expected.

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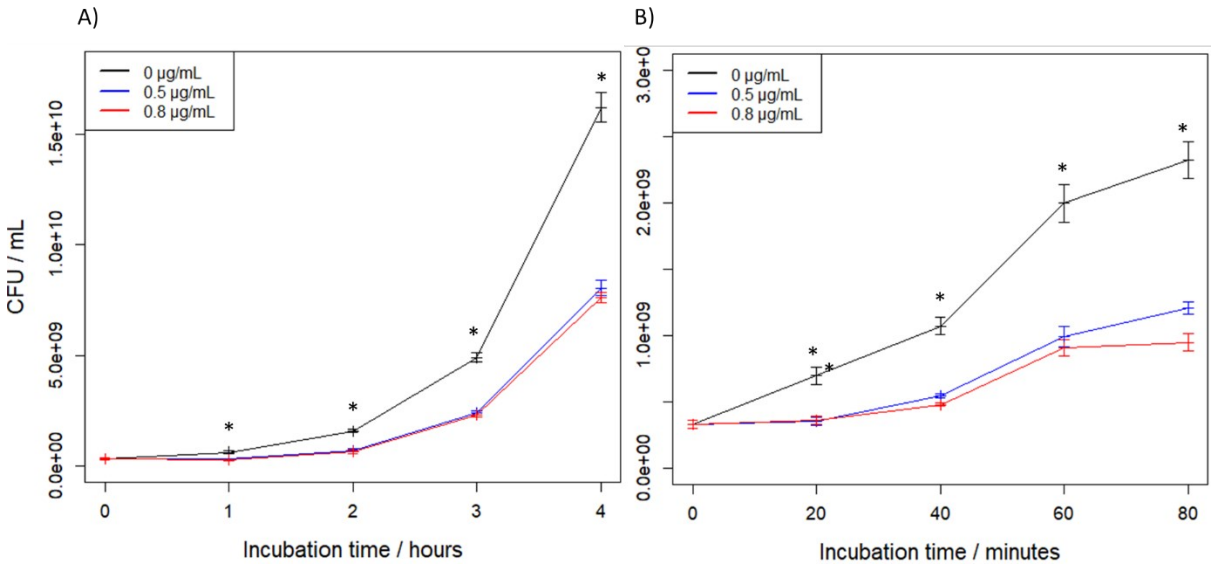

Figure S3. CFU / mL counts derived using concentrations of 0, 0.5, and 0.8 µg / mL gentamicin sulphate versus *E. coli* ATCC 259222. Each data point is the average of 5 measurements with associated standard deviation. Statistical significance between the antibiotic treated CFU / mL values and the antibiotic free control are marked with a * symbol.

References

1. J. M. Andrews, *Journal of Antimicrobial Chemotherapy*, 2001, **48**, 5 - 16.
2. H. Sun, C.-W. Chan, Y. Wang, X. Yao, X. Mu, X. Lu, J. Zhou, Z. Cai and K. Ren, *Lab on a Chip*, 2019, **19**, 2915-2924.