Rapid and high-throughput phenotypic AST directly from positive blood cultures with very high precision MIC values

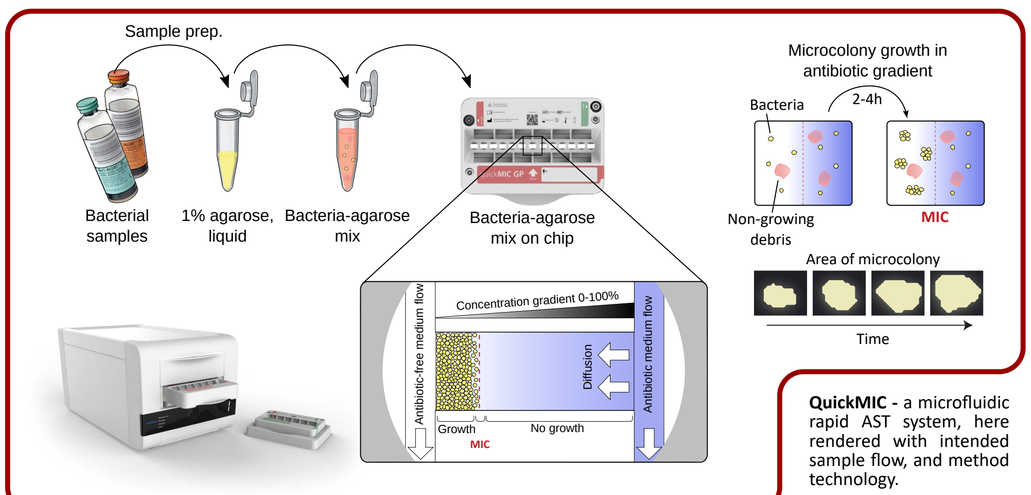
Pikkei Wistrand-Yuen[3], Christer Malmberg[1,3], Thomas Läräng[3], Thomas Tängdén[2], Johan Kreuger[1]

1: Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden 2: Department of Medical Sciences, Uppsala University, Uppsala, Sweden 3: Gradientech AB, Uppsala, Sweden

1 AMR is a challenge for empirical therapy

The changing landscape of antimicrobial resistance poses a challenge for empirical therapy, increasing the need for antimicrobial susceptibility testing (AST) to guide treatment. However, traditional methods such as broth microdilution (BMD) are comparatively slow and with high variance, typically allowing +-1 log2 dilution steps of variation. New rapid AST methods usually show low precision or narrow range, sometimes only measuring one single concentration point. We have developed a lab-on-a-chip gradient method for rapid AST directly from positive blood cultures with high precision and resolution.

Here we evaluate the gradient linearity and speed, precision and accuracy in comparison to BMD.

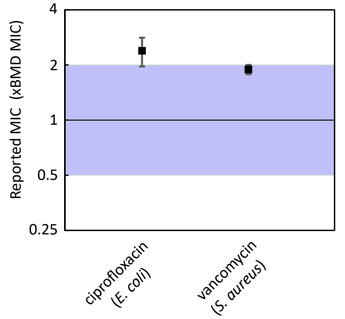


QuickMIC - a microfluidic rapid AST system, here rendered with intended sample flow, and method technology.

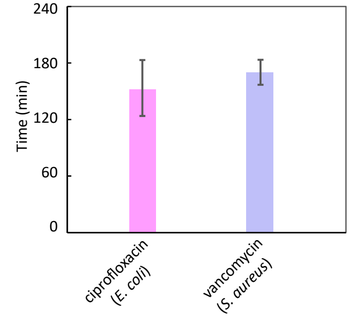
2 QuickMIC: Precise MIC results in 2-4 h

The fluorescent gradient was linear (R^2 0.98) and with a high range (90.4%). The MIC results were 0.019 mg/L (SD: 0.0033) and 1.9 mg/L (SD: 0.11) for *E. coli* and *S. aureus*, respectively (BMD: 0.008 and 1 mg/L), with a low variation compared to the acceptable standard of +-1 log2 unit (-50 to +100%) in BMD. Times to result were on average 155 min (SD: 30 min) for *E. coli* and 173 min (SD :13 min) for *S. aureus*.

Comparison of method variability



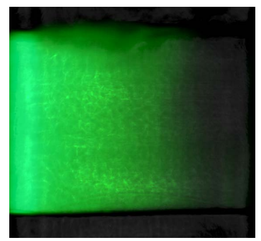
Time-to-answer (MIC)



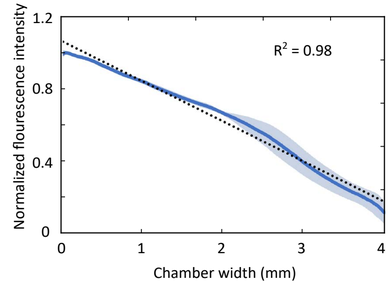
3 Materials and methods

Fluorescein was used to quantify gradient linearity in 12 chambers. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29213) were grown in blood culture bottles, extracted by centrifugation (150 rcf, 5 min), and measured a total of 30 times each to quantify the method variability against ciprofloxacin (*E.coli*) and vancomycin (*S. aureus*). An automatic read-out instrument using quantitative light-scatter microscopy tracked microcolony growth rates through the linear antibiotic gradient. Automatic MIC read-out result was compared to BMD.

Fluorescein gradient (2h)



Gradient linearity (n=12)



4 We conclude:

The presented method can provide rapid and precise antibiotic susceptibility data directly from positive blood cultures. Times-to-result and interexperiment variability is low compared to BMD. The new method could be valuable in AST where wildtype and resistant distributions are close or overlapping; to accurately quantify small changes in evolution of resistance, or to rapidly screen candidate drugs in drug development.

Acknowledgements:
The authors wish to thank Vinnova, Uppsala Antibiotic Center and the European Union's Horizon 2010 research and innovation program (grant agreement No. 642866) for funding this research.

Contact:
christer.malmberg@mcb.uu.se