Miniaturised agar plates: microfluidic tools to determine and study antibiotic resistance in bacteria

Petra Jusková[a], A. Kling[a], S. Schmitt[b], A. Egli[c] and P. S. Dittrich[a]

a Department of Biosystems Science and Engineering, ETH Zurich, Switzerland
b, Myria Biosciences AG, Department of Biosystems Science and Engineering, ETH Zurich, Switzerland
c, Clinical microbiology, University Hospital Basel, Switzerland

Microfluidic technologies offer extensive control over microliter sample volumes and are compatible with a broad range of supplementing systems for downstream analysis. The possibility of performing antibiotic susceptibility testing (AST) in the miniaturised cultivation systems holds great potential to significantly decrease required pathogen biomass and shorten the overall analysis time.
Here, we present a simple microfluidic platform consisting of four sets of chamber arrays made in air-tight cyclic olefin copolymer (COC). The system was designed to require minimal equipment and user manipulation for potential clinical use. The principle of the proposed platform is presented in Figure 1. The system contains four sets of chambers, pre-filled with agarose gel, containing oxygen sensing nanoprobes and different concentrations of the studied antibiotics. Oxygen consumption during bacterial growth is monitored as an increase of the luminescence signal collected from the oxygen sensing nanoprobes inside the individual chambers.
The system performance was characterised using *Escherichia coli* strains (ATCC 25922, ATCC 35218) and clinical isolate obtained from a patient blood culture. Further, we applied the platform for antibiotic susceptibility measurement of a fast-growing nontuberculous *Mycobacterium smegmatis* (ATCC 19420). We performed AST of these strains in response to several different antibiotic compounds and concentrations, including bacteriostatic and bactericidal antibiotics. The obtained results were in good agreement with values given in reference guidelines and independent measurements using a clinical AST protocol.
The resistant profiles of the tested *E. coli* strains were obtained within 2.5 hours of incubation, while for *M. smegmatis,* we obtained AST results in approximately 15 hours of incubation. For both strains, this represents a significant improvement compared to the current state-of-an-art technologies or cultivation on agar plates which require 16 hours to several weeks to determine the AST results.

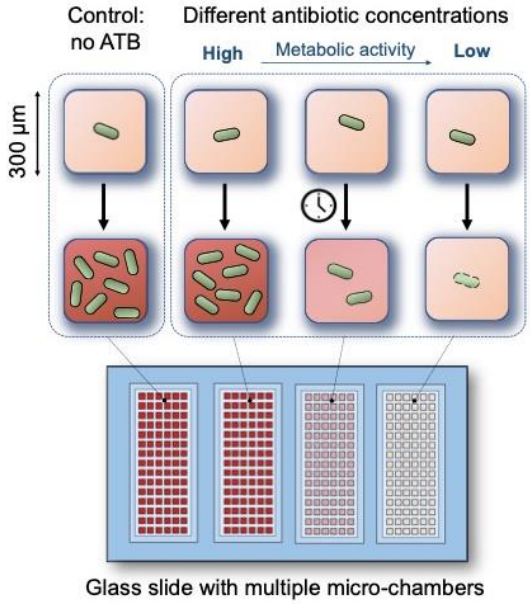

Figure 1: Conceptual sketch of the microfluidic system for antibiotic susceptibility testing (AST). The platform consists of hundreds of growth chambers (300 x 300 x 75 µm), each pre-filled with oxygen sensing nanoprobes and different concentrations of the studied antibiotics (ATBs) embedded in the agarose gel. The metabolic activity of bacteria after exposure to antibiotics is determined based on oxygen consumption.