

Supplemental Information

A multiplexed nanoliter array-based microfluidic platform for quick, automatic antimicrobial susceptibility testing

Mohammad Osaid^a, Yi-Sin Chen^a, Chih-Hung Wang^a, Anirban Sinha^b, Wen-Bin Lee^a, Priya Gopinathan^a,

Hung-Bin Wu and Gwo-Bin Lee^{a,b,c*}

^aDepartment of Power Mechanical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan

^bInstitute of NanoEngineering and Microsystems, National Tsing Hua University, Hsinchu 30013, Taiwan

^cInstitute of Biomedical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan

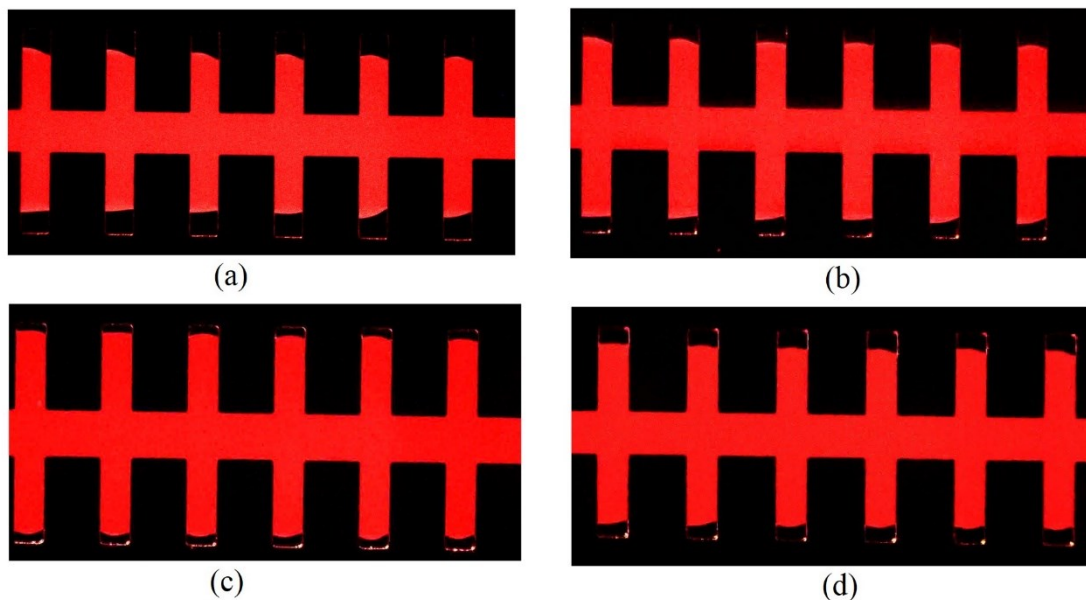
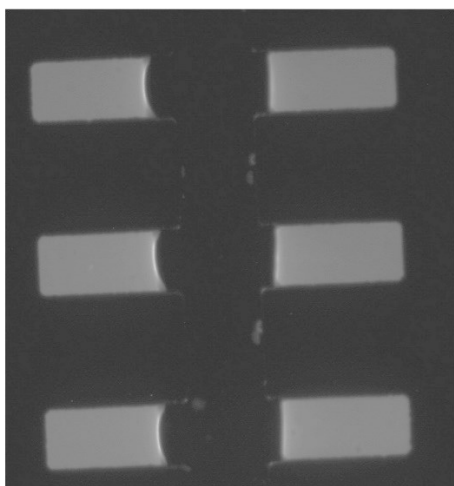
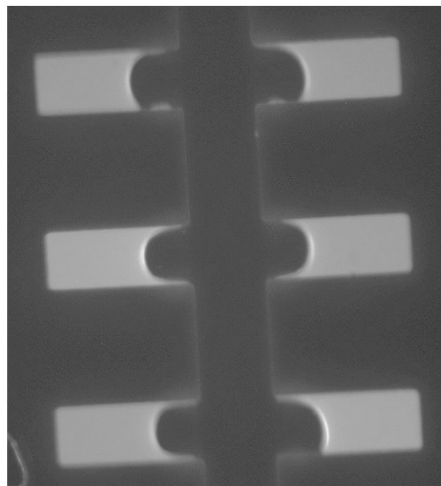


Figure S1. Simultaneous loading of microarrays of different lines of the ASTM. (a), (b), (c) and (d) represent the loading process of microarrays of line 1, 3, 5 and 7, respectively. Bacterial solution mixed with resazurin, emitting fluorescence, was used to analyze the process. It should be noted that microarrays of different lines fill simultaneously which ensures uniform distribution of bacteria in different lines.



(a)



(b)

Figure S2. Testing evaporation of the liquid in nanoliter microarrays of the ASTM. The microarrays generated by shearing the main-channel with oil were incubated at 37°C for testing the evaporation of liquid on the developed platform. (a) Microarrays at $t = 0$, i.e. just after generation. (b) Microarray after 7 hrs of incubation; no substantial evaporation was observed.

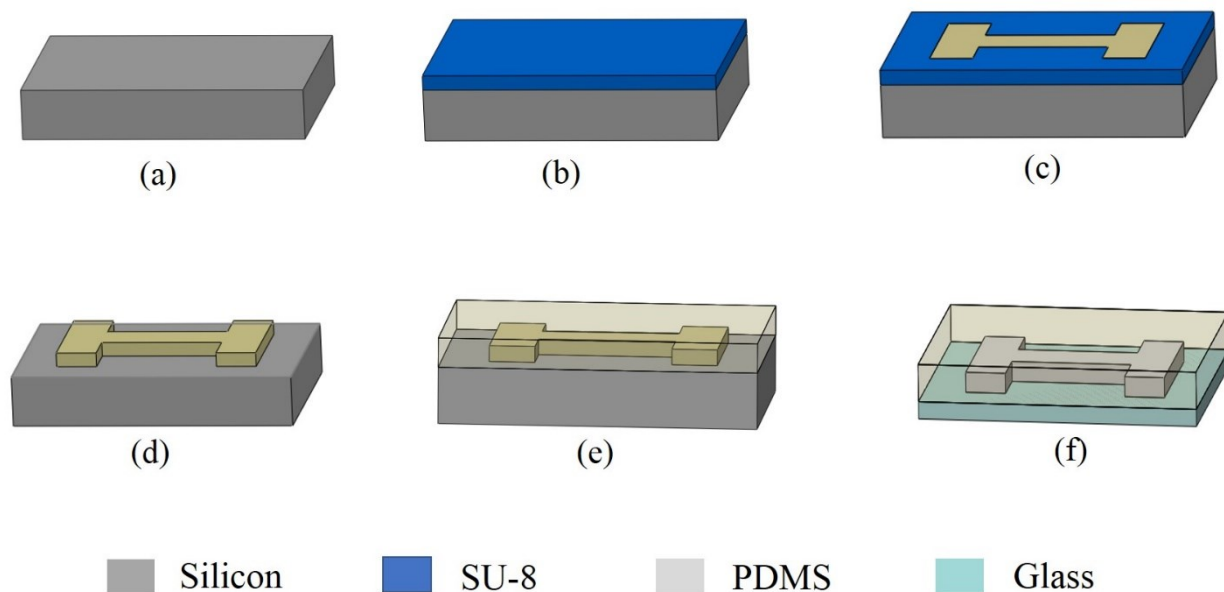


Figure S3. Schematic of PDMS microfabrication procedure. (a) The silicon surface was cleaned prior to photoresist spin-coating. (b) SU-8 was spin-coated on the surface and soft-baked at 90°C. (c) The silicon surface was exposed with UV to form structures on the silicon. (d) The silicon mold was obtained after removing unexposed SU-8. (e) PDMS was poured on the silicon mold followed by curing. (f) The PDMS layer was bonded to glass using oxygen plasma treatment.

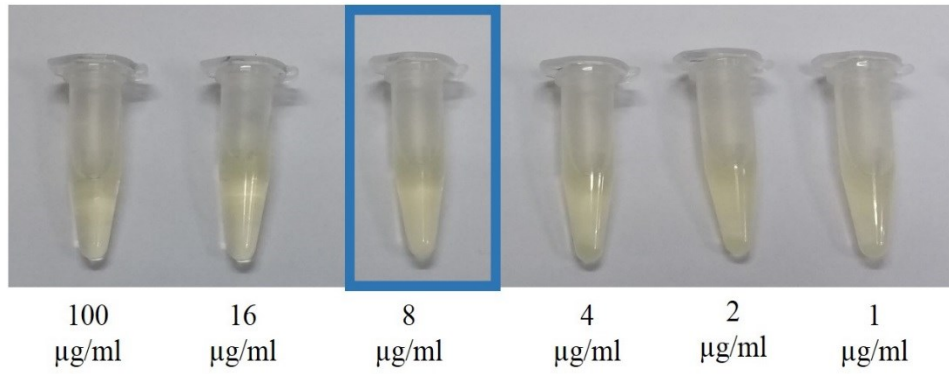


Figure S4. On-bench broth dilution assay for MIC determination of *E. coli ATCC 25922* with ampicillin. *E. coli ATCC 25922* (10^6 CFU/ml) with ampicillin was grown in LB broth with a total volume of 1 ml for 24 hrs. Visible growth of bacteria was observed at lower concentrations, 2 and 4 µg/ml. 8 µg/ml was deduced as the MIC value following the CLSI guideline.

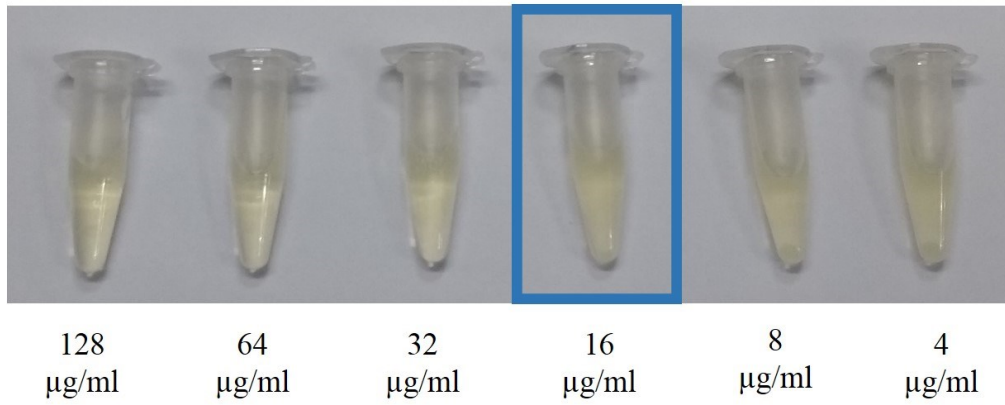
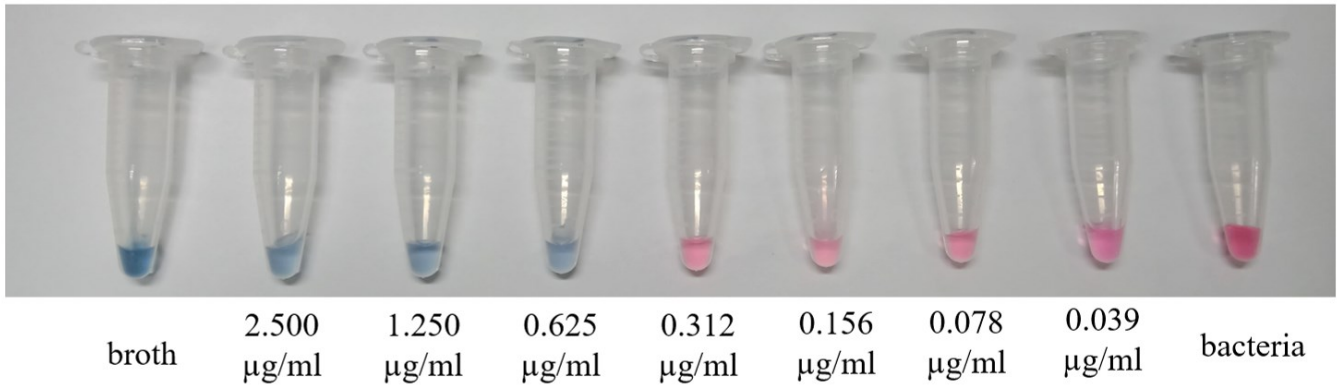
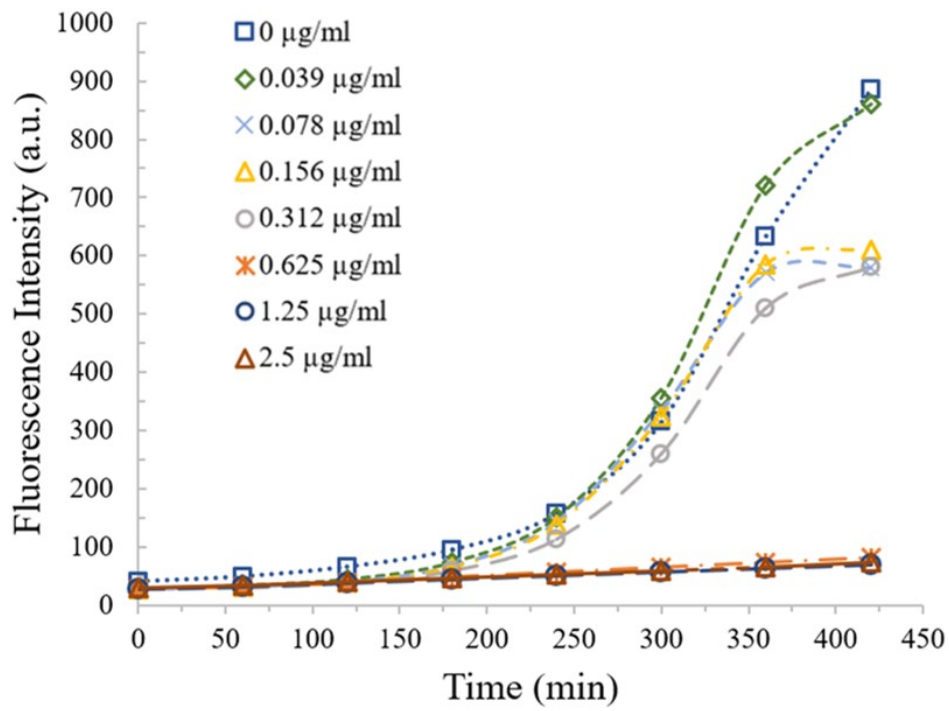


Figure S5. On-bench broth dilution assay for MIC determination of *E. coli* ATCC 25922 with streptomycin. *E. coli* ATCC 25922 (10^6 CFU/ml) with streptomycin was grown in LB broth with a total volume of 1 ml for 24 hrs. Visible growth were observed at lower concentrations, 4 and 8 µg/ml. 16 µg/ml was evaluated as the MIC value following the CLSI guideline.



(a)



(b)

Figure S6. (a) On-bench AST results using MRSA (S ATCC 43300), a gram-positive bacteria and vancomycin as an antibiotic. (b) Variation in fluorescence intensity over time for using vancomycin as antibiotics. The MIC value against vancomycin was measured to be around 0.625 µg/ml.