

Title: The MilliDrop Analyzer : an enabling technology for antimicrobial drugs development

Abstract:

We present the MilliDrop Analyzer (MDA), a droplet-based millifluidic system for antimicrobial susceptibility testing. We demonstrate its application to determine high precision minimum inhibitory concentrations (MICs) either for traditional antibiotics or innovative antimicrobial candidates such as bacteriophages or small RNAs.

The MilliDrop technology was combined with a metabolic (cell respiration) fluorescent reporter, for microbial growth detection. For comparison with standard methodology, and for conducting reproducibility studies, the susceptibility of a reference Gram negative strain *Escherichia coli* ATCC 25922 to gentamicin, chloramphenicol, and nalidixic acid were tested by the MDA, VITEK®2, and broth microdilution as a reference standard. These experiments validated the technology for measuring precise MICs. Additionally, we tested the susceptibility to ampicillin, cefotaxime and gentamicin of a clinical strain isolated from a patient suffering from chronic Urinary Tract Infection. The results were in agreement with microdilution standard tests.

Finally, alternatives to antibiotics were also tested. We screened a small RNA library against a known pathogen and different phages concentration against *E. coli*. These studies revealed the two advantages of the MDA compared to conventional techniques. First, the large number of droplets provides a unique tool for rapid MIC results can be obtained in less than 2 h when we test fast-growing strains such as *E. coli* and precise phenotypic bacterial antibiotic screening. Second, the fine control of inoculum sizes in droplets down to individual bacterial cell allows the measurement of growth heterogeneities within bacterial population which enabled the discrimination of resistant strains from intermediate variants.

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