## Supplemental Material

**Table S1. Etest and VITEK 2 antibiotic susceptibility testing results**

| | Ampicillin | | | Cefotaxime | | | Ciprofloxacin | | |
|---|---|---|---|---|---|---|---|---|---|
| | Etest[1] | | VITEK 2[2] | Etest | | VITEK 2 | Etest | | VITEK 2 |
| Strains | µg/ml | Classification | Classification | µg/ml | Classification | Classification | µg/ml | Classification | Classification |
| ARD144 | > 256 | R | R | 0.5 | S | S | > 32 | R | R |
| ARD145 | > 256 | R | R | > 32 | R | R | 0.19 | S | S/I |
| ARD146 | > 256 | R | R | 0.125 | S | S | 0.19 | S | S |
| ATCC 25922 | 8 | NA[3] | NA | 0.19 | NA | NA | 0.012 | NA | NA |

[1]The MIC detected is shown in µg/ml and interpreted to classify a strain as susceptible (S), intermediate (I), or resistant (R) according to the CLSI guidelines.

[2]VITEK 2 results classify a strain as susceptible (S), intermediate (I), or resistant (R)

[3]Not applicable. S, I, or R classification does not apply for the Quality Control strains

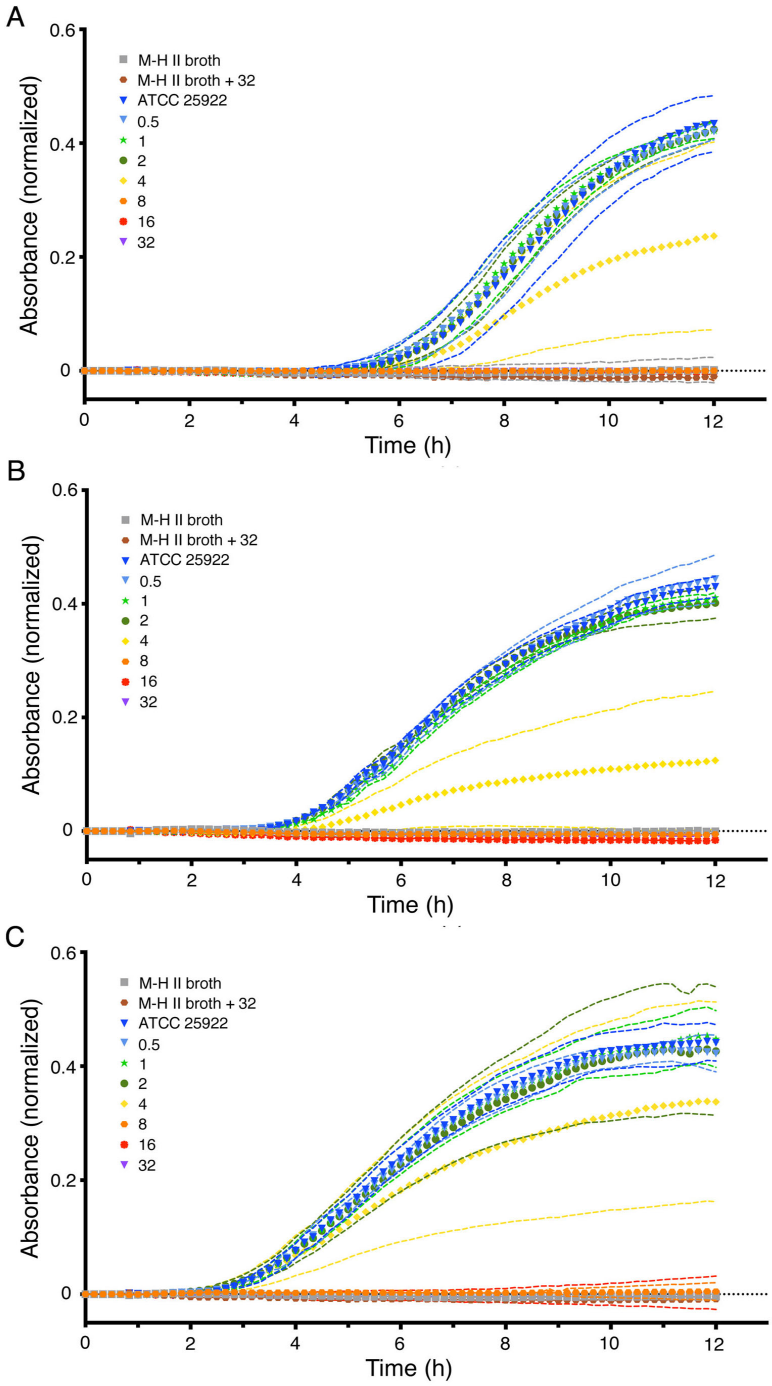


**FIG. S1** Susceptibility of ATCC 25922 to ampicillin using the nanowell AST device. Growth curves of the reference strain ATCC 25922 exposed to 0-32 µg/ml of ampicillin, using an inoculum of (A) 5 cfu/well, (B) 50 cfu/well and (C) 500 cfu/well. Each curve represents the average normalized $OD_{600}$ from 12 wells of a single experiment with standard deviations shown as two dashed lines (---) of the same color. Positive control (ATCC 25922) representing bacteria in M-H II broth, and negative controls (M-H II broth, M-H II broth +32) representing medium with and without ampicillin were included.

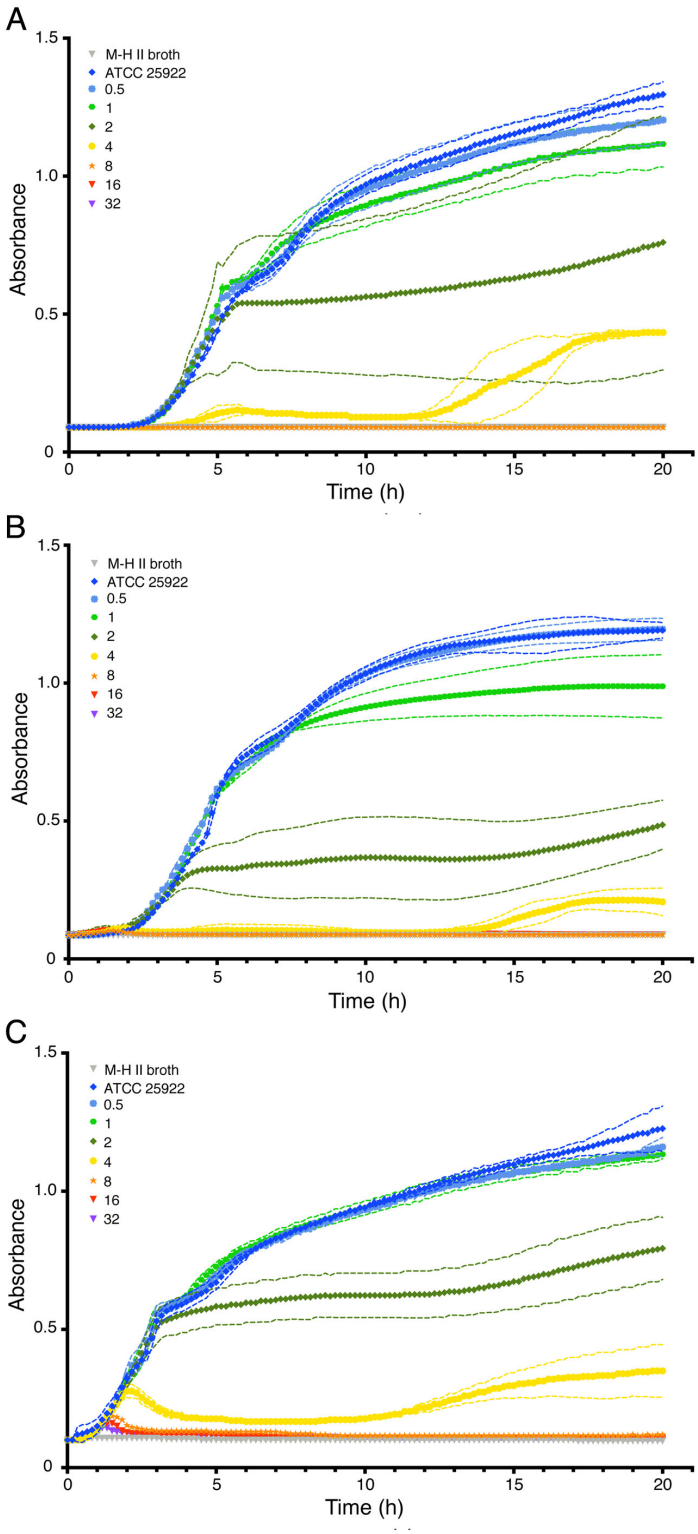


**FIG. S2** Ampicillin susceptibility of ATCC 25922 in a microbroth dilution assay. Growth of the reference strain ATCC 25922 subjected to 0-32 µg/ml of ampicillin, using an inoculum of (A) $10^4$ cfu/ml (B) $10^5$ cfu/ml and (C) $10^6$ cfu/ml. ATCC 25922 = M-H II broth + bacteria; M-H II broth = medium; 0.5-32 = M-H II broth + bacteria + indicated ampicillin concentration (µg/ml). Each curve represents the average $OD_{600}$ from triplicate wells from a single experiment. Standard deviation is illustrated as two dashed lines (---) of the same color as the growth curve.

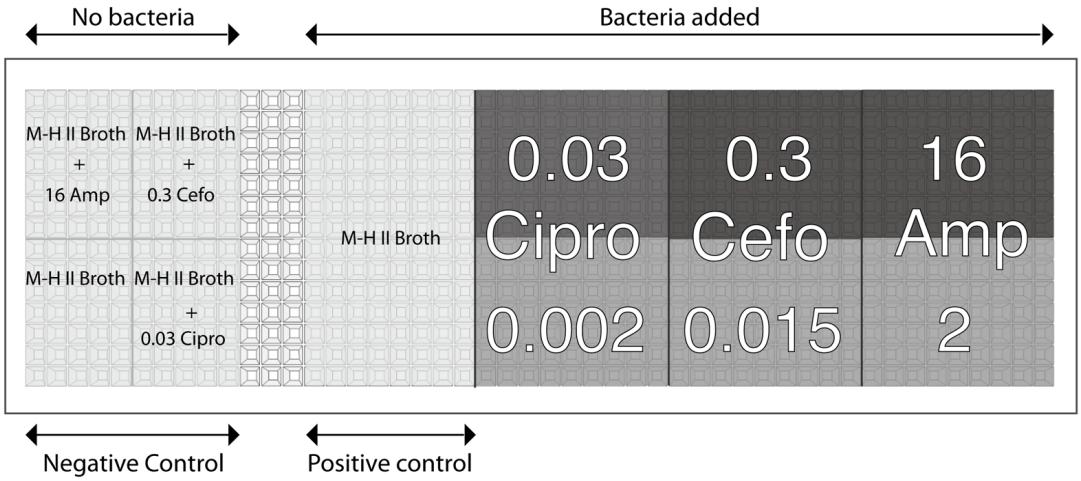


**FIG. S3** Schematic of the multiplex nanowell AST device. Two concentrations of ampicillin (2 and 16 $\mu$g/ml), ciprofloxacin (0.002 and 0.030 $\mu$g/ml) and cefotaxime (0.015 and 0.3 $\mu$g/ml), corresponding to values below (light grey) and above (dark grey) respective MICs against strain ATCC 25922, were used. Positive (ATCC 25922 in M-H II broth) and negative controls (M-H II broth, M-H II broth + 16 Amp, M-H II broth + 0.3 Cefo, M-H II broth + 0.03 Cipro) were included.