Supporting Information

Cell-on-Hydrogel Platform Made of Agar and Alginate for Rapid, Low-cost, Multidimensional Test of Antimicrobial Susceptibility

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(1) Evaluation of device performance

Different tests were carried out to evaluate the performance of the device in fluid manipulation. Fig. S1 shows the thickness and maximum supported flow rate of the device. Fig. S2 and Fig. S3 show the results of one-dimensional and two-dimensional diffusion tests, respectively.

![Side view of the device and flow rate test result](image1)

Fig. S1 (A) Side view of the completed device. The thickness could be as low as 1 mm, as shown in the image. (B) Result of a flow rate test. The device was broken at maximum linear flow rate of 11.1 cm/s, which is about 100-fold of the operation condition used in our work.

![Diffusion test results](image2)

Fig. S2 The vertical distribution of gradients (120 min) in diffusion test. (A) The inspected area (operation zone) was divided into five equal zones (B) The functions of fluorescence intensity over distance in these five zones were plotted. The closely-packed lines suggest even distribution of gradient over the width of the operation zone.
**Fig. S3** (A) Two-dimensional diffusion test using Rhodamine B (B) and fluorescein (C) as samples. The two gradients were in perpendicular positions. (D) The fluorescence intensity over distance of two dyes was plotted, and both gradients achieved good linearity.

**2) Two-dimensional AST**

**Fig. S4** Schematics of the device used for Two-dimensional AST. (A) The tested gradient zone was located in the center of the diffusion region. (B) Grids were fabricated inside the device to help locate the sample and capture images for analysis.

**3) Two-dimensional AST on wild type bacteria**

Experiments were carried out to evaluate the feasibility of using our device to examine normal bacterial cells without fluorescence mode. Fig. S5 and Fig. S6 show the results of
trials with wild *E. coli* and *S. aureus*. Changes in bacterial growth could be observed in the captured images.

**Fig. S5** Two-dimensional AST of Wild *E. coli* (ATCC 25922). (A) Ampicillin gradient ran from right to left, and gentamicin from top to bottom. Morphologies of *E. coli* at different concentrations of the two antibiotics are shown. At high concentration of ampicillin, cells showed elongation (B); at high concentration of gentamicin, cells ceased growth (C); and at low concentration of both drugs, bacteria grew normally (D).
Fig. S6 Two-dimensional AST of *S. aureus* (ATCC 29213). (A) Ampicillin gradient ran from right to left, and gentamicin from top to bottom. The morphologies of *S. aureus* at different concentrations of two antibiotics are shown, as inhibition (B), partial inhibition (C) and normal growth (D), at decreasing drug concentrations.