Electronic Supplementary Information for

**Time Lapse Investigation of Antibiotic Susceptibility**

**using a Microfluidic Linear Gradient 3D Culture Device**

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Thermostat

A thermostat was used to heat the chip and maintain the temperature. Generally, we used a digital thermometer (DS18b20, Dallas company), a chromium alloy resistance wire heater and a single-chip microcomputer (Arduino UNO R3, Arduino) to build a proportional-integral-derivative (PID) feedback controller system. The PID controlling is described by the following function.

\[ P = K_p \left( T_s - T_c \right) / T_s + K_i \int_0^t \left( T_s - T_c \right) dt + K_d dT_c / dt + M \]  

(Eq. S 1)

where \( P \) is the power output of the heater; \( T_s \) is the temperature set point value; \( T_c \) is the current temperature value; \( t \) is time; \( dT \) is the derivative of the temperature with respect to time; \( K_p, K_i, K_d \) are the parameters of the Proportional, Integral and Derivative term; \( M \) is the constant term.

The PID control was programmed into the Arduino Board, when \( T_C > T_S \), the \( P \) would be set down to zero. Room temperature was 26°C ± 1°C and the chip was cooled by dissipation. The thermometer DS18b20 can provide a 12-bits serial signal as the temperature through the bus connect to the Arduino UNO. Temperature resolution is 0.06°C. The heater was made from a 25 cm chromium alloy resistance wire which resistance per unit length is 0.01 \( \Omega \cdot m^{-1} \). That makes the resistance of the heater is 25 \( \Omega \). Aluminum foils was attached to both sides of the heater to make the heating evenly, Fig. S2.

Gradient formation

In order to use a specific length of the width to represent specific concentration of the antibiotics, it is necessary to obtain a linear and stable distribution of the antibiotics before the cells grow into the logarithmic phage. As both the particle size of the antibiotics and the glutinousness of the agarose (we used it to trap the cells) contribute
to the distribution of the antibiotic in the agarose, it needs to determine the time lag of the distribution becoming linear. A first approximation is given by,

\[ T_{\text{lag}} = \frac{d^2}{2D} \]  

(Eq. S2)

where \( D \) is the diffusion constant of the molecule in the specific substance and \( d \) is the distance of the distribution.

Figure S5 is a result from a simulation in Comsol Multiphysics, showing how with time the gradient changes towards linearity. At \( T_{\text{lag}} \) it is very close to linearity. At \( \frac{2}{3} T_{\text{lag}} \) the maximal error is -2 % at the middle of the chamber. At \( \frac{1}{3} T_{\text{lag}} \) the maximal error is -12 %.

The question is what value for \( D \) should be used. We have not been able to find a value for any of the antibiotics for this agarose gel and buffer loaded with bacteria. Hence, we need to make some estimates to get an approximate value. According to Stokes-Einstein approximation,

\[ D = \frac{kT}{6\pi r \eta} \]  

(Eq. S3)

where \( k \) is the Boltzmann constant, \( T \) is temperature, \( r \) is the hydrodynamic radius of a particle, and \( \eta \) is viscosity. Hence, the product \( D\eta/T \) is constant. For ampicillin in 2% agarose, \( D \) is 0.016 cm•h\(^{-1}\) [Ref. S1]. The viscosity for 0.5% agarose with a similar buffer and at 37°C is 26% of that of 2% agarose [Ref. S2, S3]. Hence, approximately, for ampicillin in 0.5% agarose, \( D \) is 0.061 cm•h\(^{-1}\). In our device, the length of the chamber is 4 mm. According to the Eq. S2, the time lag of ampicillin distribution before getting linear should be 79 min. For the other antibiotic molecules we used as in Tab. S1, according to Eq. S3 a first approximation is that their diffusivity will be proportional to their hydrodynamic radius, which approximately follows the cubic root of their molecular mass. With this reasoning, the heaviest antibiotic used –
vancomycin, will have a time lag of 128 min. But after less than 45 min the maximum error is not more than -10% from the linear estimation of this the largest antibiotic in the study. From this we will assume linearity from 45 min after measuring. This is less than two generation times for the bacteria in this set-up. That is, we cannot say what the concentration distribution was initially but that it is linear during the logarithmic and stationary phases of growth. A specific system should be measured for more exact determination of when different chemicals reach a linear gradient. One way to meet this would be to have much shorter gradient chambers when studying the effect on cell growth of a very large molecule.

Our evaluation may be compared with results from Fluoroscein (332 g/mol) gradient formation in a similar set-up, where the diffusion creates an approximately linear gradient within 20 min in a 2% agarose solution over 1 mm length, Ref S5. Following the arguments above, in our system it would reach the same state within $16 \times 0.26 \times 20 = 83$ min, which is similar to our time lags.

**Agarose gel strength**

The capability of the gel to immobilize motile bacterial cells is related to the gel strength, and the gel strength is highly related to the gel concentration. We initially tested both 1% and 0.5% agarose and reached the same result for the bacterial growth curves. For convenience a 0.5% agarose is easier to work with. Many studies have used agar for immobilization of bacteria. The difference between agar and agarose is that agar is the combination of agarose and agaropectin. Agaropectin is a kind of smaller molecule heavily modified with acidic side-groups, such as sulfate and pyruvate, Ref S6. The acidic side-groups can weaken the gel strength in agar, Ref S7. In conclusion, when 0.5% agar can immobilize the motile bacteria cells, 0.5% agarose should be more capable in achieving this objective.
Reference


Figure S1 Experimental setup.
Figure S2 The circuit diagram of the thermostat
Figure S3 Optical images of the device set up on the microscope and the set up of the single-chip microcomputer.
Figure S4 Reference growth curves. (A) Growth curve of *Escherichia coli* K12 MG1655 measured in the gradient generator by using Muller-Hinton medium. (B) Growth curve of *Salmonella* Typhimurium LT2 measured in the gradient generator by using Muller-Hinton medium.
Figure S5 Gradient distribution in relation to the time lag before it reaches linearity.
<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>349</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>495</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>728</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>481</td>
</tr>
<tr>
<td>Vancomycin</td>
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