

SUPPLEMENTARY INFORMATION for Antimicrobial susceptibility assays in paper-based portable culture devices

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Details about the bacteria used in this report:

Escherichia coli K12 ER 2738 (New England Biolabs)

F' *proA*⁺ *B*⁺ *lacI*^q Δ (*lacZ*)M15 *zzf::Tn10*(Tet^R)/*fhuA2 glnV* Δ (*lac-proAB*) *thi-1* Δ (*hsdS-mcrB*)5.

This strain is an *fhuA2* version of *E. coli* NM522 in which the F' can be selected for using tetracycline (Tet). The strain was maintained on 1.5 % agar solid media supplemented with Tet (20 μ g/mL); the plates with colonies were maintained in the dark at 4 °C for up to two weeks. For individual experiments, one colony was used to inoculate the antibiotic-free LB medium and cultured at 37 °C in 25 mL baffled flasks shaken at 200 r.p.m. for 18 hours.

Pathogenic bacteria *E. coli* O157:H7 (Félix D'Hérelle Reference Center for Bacterial Viruses, Université Laval) and *Salmonella Typhumurium* LT2 (ATCC® 19585) were generously provided by Szymanski lab (Department of Microbiology, University of Alberta). The maintenance and handling of these bacteria was performed in accordance with protocols for biosafety level 2 (BL2) pathogens.

Ampicillin-resistant strains R, I1, I2 were evolved from *E. coli* K12 ER 2738 and characterized by micro-broth dilution to give the following minimum inhibitory concentrations (MIC): MIC(R)= 32 μ g/mL, MIC(I1)= 16 μ g/mL, MIC(I2)= 16 μ g/mL and MIC(*E. coli* K12 ER 2738)=8 μ g/mL. The strains were expanded on 1.5 % agar solid media supplemented with Amp (20 μ g/mL); the plates with colonies were maintained at 4 °C for up to two weeks. For individual experiments, one colony was used to inoculate the antibiotic-free LB medium and cultured at 37 °C in 25 mL baffled flasks shaken at 200 r.p.m. for 18 hours.

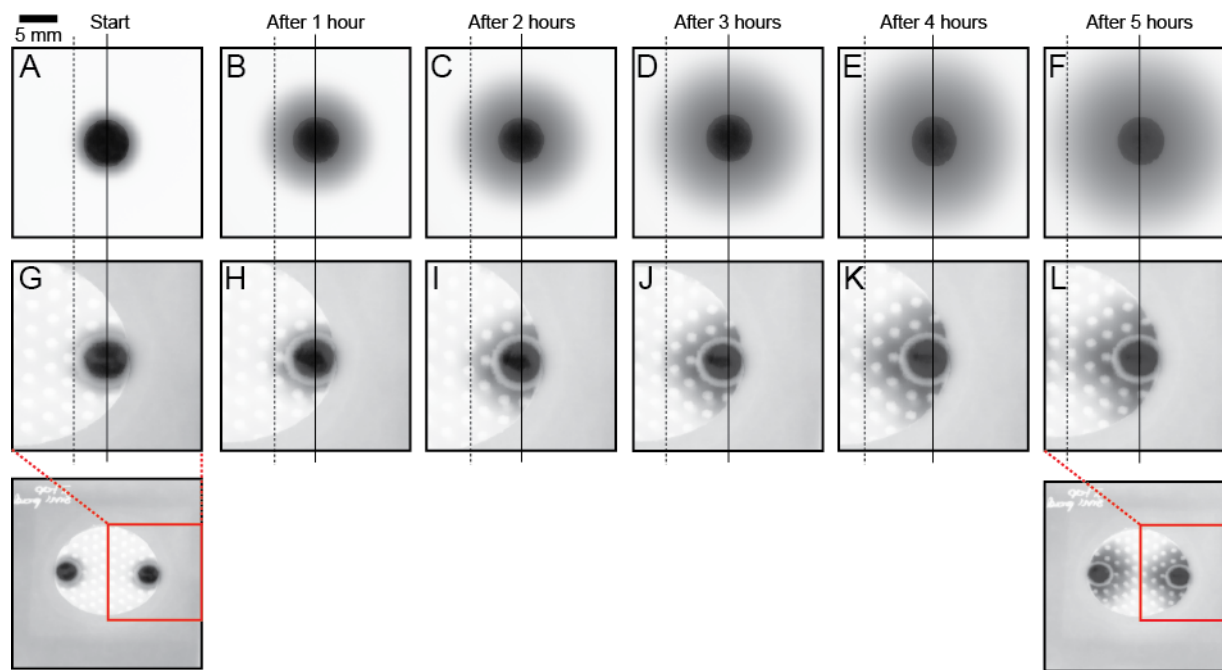
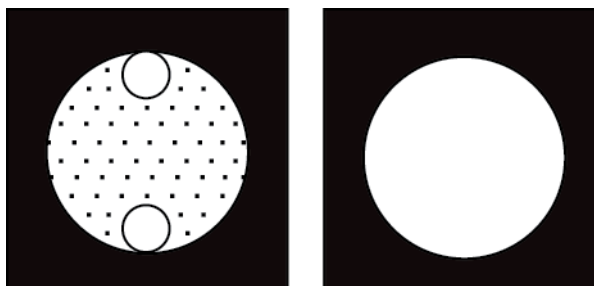


Fig. S1. Diffusion of small molecules in patterned paper and in agar. To validate the use of patterned paper for AST by diffusion, we monitored the diffusion of fluorescein (1 mg/mL solution in PBS, 2 μ L per area) within the culture device and from the impregnated disk on the agar-filled Petri dish using a Typhoon FLA9500 gel scanner (GE Healthcare; 473 nm laser, PMT=300 V, BPB1 filter). Black color represented high intensity of fluorescence while white represented no fluorescence. In (A) through (F), images were acquired 4 minutes after the addition of the fluorescein-impregnated disk to agar and every hour thereafter. Images (G) through (L) were acquired simultaneously with (A-F). The devices were fabricated from standard components (paper, pad, tape, PDMS, LB media). This figure illustrates that the diffusion of fluorescein exhibits a similarly uniform and radial profile in both the agar and the paper-based portable culture device.

Protocol established by and for minimally-trained personnel (high-school students)

I. Fabrication of the device:

- 1) Fabrication of antibiotic culture device from paper, tape, and PDMS as reported previously¹
A supporting video [movie S1 in ESI] is also available.
 - a. Wax-pattern the culture zone with hydrophobic grey spots and two side-zones for antibiotics, and the media zone.



- b. Prepare the pad by cutting a 24-mm circle in Western Blotting thick filter paper (cut in half along the thickness).
 - c. Cut a 26-cm-long, double-layer band of packaging tape (48 mm wide).
 - d. Punch a 24.5 mm (1 inch) hole, centered in one half of the band.
 - e. Fold both short edges to create a 5-mm wide border.
 - f. Place a PDMS membrane on the hole, against the sticky side of the tape, and trim the excess at 2 mm around the hole.
 - g. Place the culture zone paper against the PDMS window and press to seal it against the tape.
 - h. Align the pad and media zone paper with the culture zone and press them onto the other side of the tape to seal the media reservoir.
 - i. Fold the device in half to align the culture zone with the media zone.
- 2) Prepare a protective layer by cutting a 26-cm-long band out of the packaging tape roll. Then, fold the tape in half to create a 13-cm-long non-sticky protective band.

II. Preparation of the device:

Addition of the LB media and sterilization

- 1) Position the device patterned papers facing up on a flat surface. Add LB media in the media zone (paper against the pad) with an eyedropper until no more liquid can be absorbed (~400 μL).
- 2) Close the device for few seconds to put both zones in contact, and thus, wet the culture zone (the zone with grey hydrophobic spots and two circles, on the PDMS window side).
- 3) Open the device and seal it onto the protective band. Proceed with autoclaving.

Addition of the reagents (antibiotics and PrestoBlue™)

- 4) After autoclaving, put the device on a flat, clean surface with the protective band facing up. Remove the protective band on the culture zone side. Using a pipette, add 2 μL of antibiotic antibiotic zones (two small circles).
- 5) Add 50 μL of Presto Blue, using a pipette, in the central part of the culture zone, avoiding the two antibiotic reservoirs.
- 6) Seal the protective band back onto the device.

III. Performing Antimicrobial susceptibility test:
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When working with bacteria, it is preferable to wear gloves.

- 1) Place the device on a flat, clean surface with the protective band facing up.
- 2) Remove the protective layer. Mix your bacterial sample. Add 50 μL of the bacterial sample in the centre of the zone with grey spots using the eyedropper (50- μL marked on the eyedropper).
- 3) Close the device by folding it in half along the central crease. Press along all four sides to seal it. Place the device in a container, and keep it protected from light.
- 4) Incubate the device at 37 °C for 18 hours (overnight).
- 5) After incubation, take a picture of the culture zone through the PDMS window.
- 6) Count the number of grey spots in the blue area around each antibiotic zone, and report it for each antibiotic.

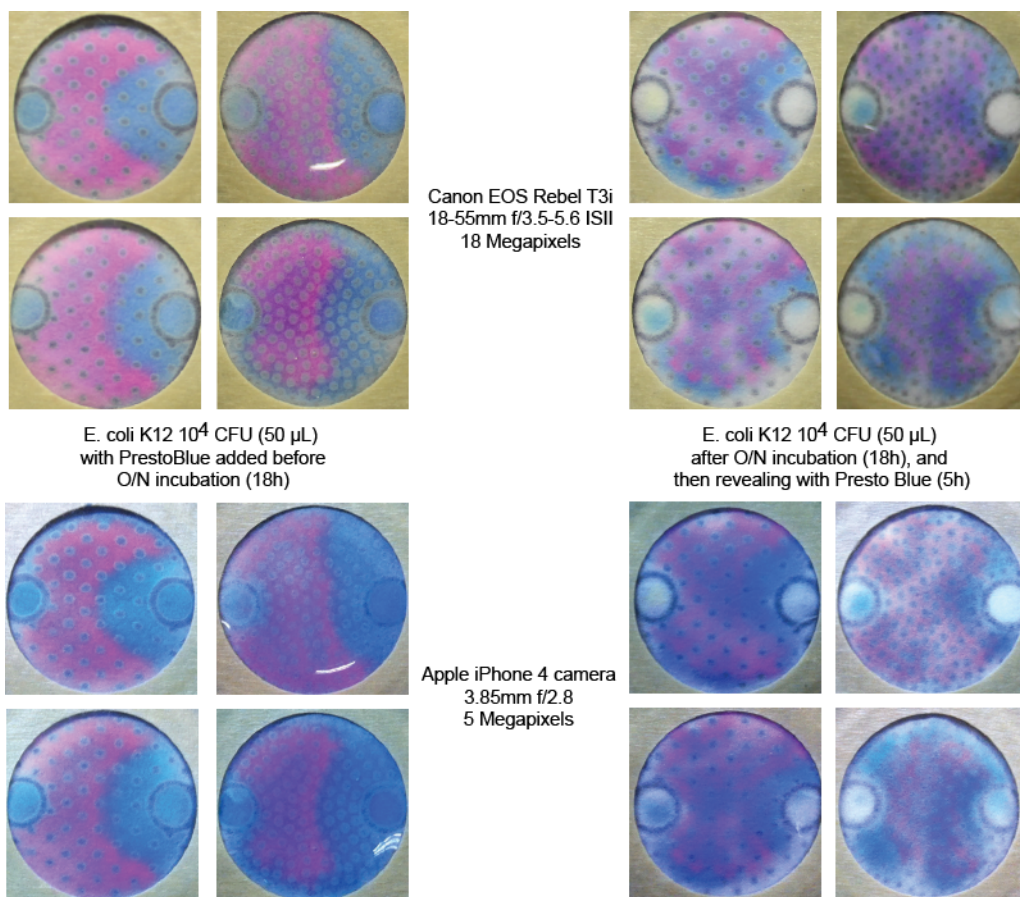


Fig. S2. Optimizing the protocol for visualization of viable cells in devices that contained two types of antibiotics (tetracycline in the left reservoir, ampicillin in the right reservoir). In left group of eight samples, the devices were inoculated with *E. coli* K12 ER 2738 and incubated for 18 hours in the presence of PrestoBlueTM. In the right group of eight samples, the devices were sprayed with PrestoBlueTM after culture. Adding PrestoBlueTM before the culture yields images that are more suitable for analysis either by high-resolution camera (top eight images) or lower-resolution iPhone-4 camera (bottom 8 images). Each 2x2 group of pictures contains two patterns of hydrophobic dots that enhance distribution of cells inside the culture and simplify quantification of the size of the zone of inhibition of growth.

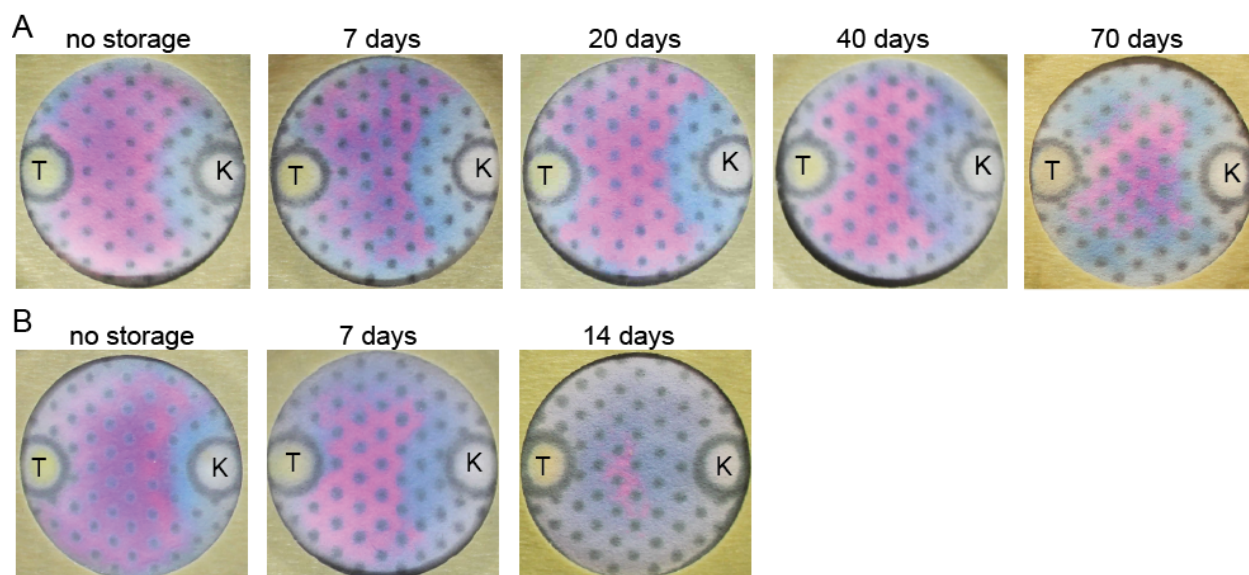


Fig. S3. Assessment of shelf life of portable culture devices (A) We autoclaved culture devices with 400 μL of LB media, added a solution of tetracycline 'T' and kanamycin 'K' (10 μg in 2 μL), onto each reservoir, and stored the devices in heat-sealed moisture- and puncture-resistant bags from 0 to 70 days at room temperature. After the indicated number of days in storage, we opened the storage bag, added PrestoBlueTM (50 μL) to the devices, and the inoculum (50 μL containing 10^4 CFU *E. coli* K12 ER 2738). We imaged the devices after overnight incubation at 37 $^{\circ}\text{C}$. After prolonged storage (70 days), we observed a diminished growth of bacteria in devices, presumably due to significant evaporation of the media (as verified by the reduced weight of the devices after storage). (B) Assessment of shelf-life when PrestoBlueTM was added before storage. We autoclaved culture devices with 400 μL of LB media, added a solution of tetracycline 'T' and kanamycin 'K' (10 μg in 2 μL) onto each reservoir and 50 μL PrestoBlueTM onto the culture zone. We stored and tested devices as described in (A)

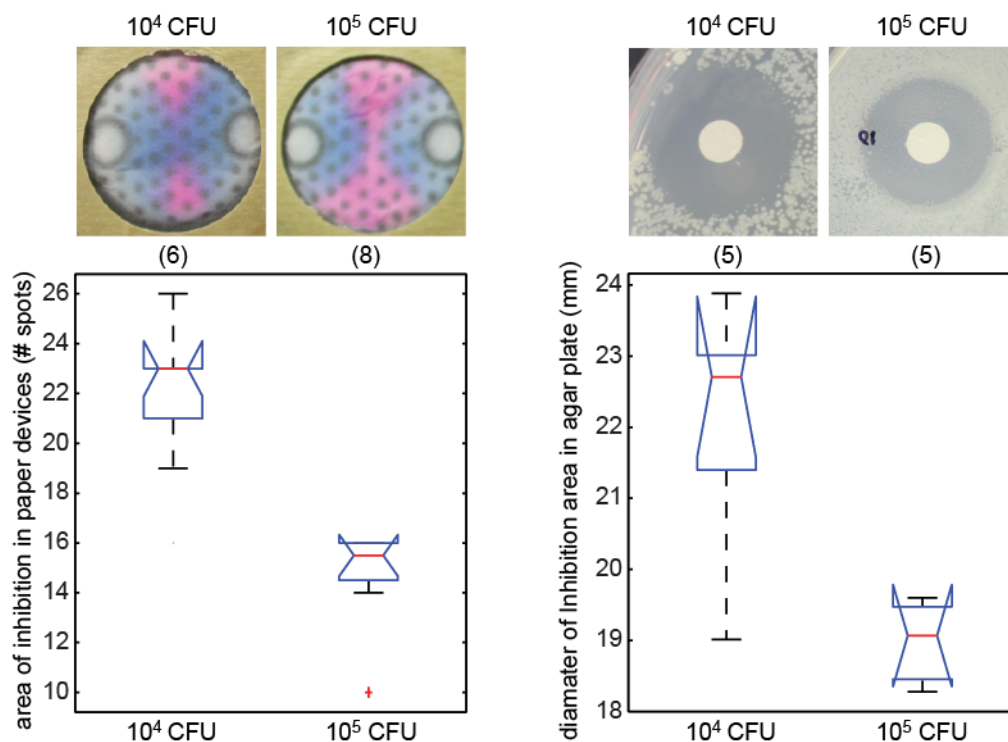


Fig. S4. Effect of concentration of the inoculum on the zone of inhibition of growth.

After autoclaving devices with 400 μ L LB media, we added 10 μ g of ampicillin to each antibiotic zone and 50 μ L of PrestoBlueTM. We then inoculated them with the evolved strain 'I1' of *E. coli* K12 ER2738 at two different concentrations, achieving 4.1×10^4 CFU or 3.3×10^5 CFU per device. We inoculated agar-filled Petri dishes with the same amount of bacteria, and placed paper disks impregnated with 10 μ g of ampicillin on top of the agar. The images are representative of the results after 18 hours of incubation at 37 $^{\circ}$ C for agar plates and paper devices. The notched box plots were generated by Anova1 function (MATLABTM). Concentration of the inoculum influenced the size of the zone of inhibition in both platforms. It is, thus, important to perform AST with a constant concentration of inoculum.

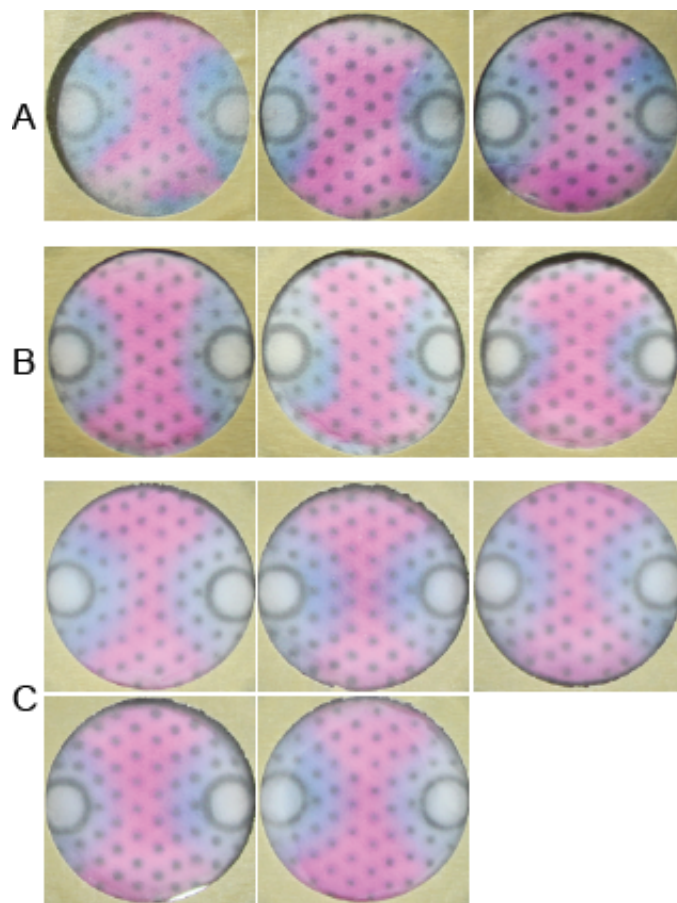


Fig. S5. Reproducibility of antimicrobial susceptibility assays in portable culture devices.

Examples of replicates collected for the strain ‘S’. The devices have been made with patterned paper Whatman 114 (set A) and Whatman 1 (sets B and C). After adding 400 μL LB media and sterilizing the devices, we added 10 μg of ampicillin to each antibiotic zone and 50 μL of PrestoBlueTM to each culture zone. We then inoculated them with the strain ‘S’ of *E. coli* K12 ER2738 achieving $\sim 5 \times 10^5$ CFU per device by adding 50 μL of bacterial sample (sets A and B; these devices are the 12 replicates used for Fig. 4C) and $\sim 1.8 \times 10^5$ CFU per device by adding 45 μL of bacterial sample (set C). These devices are the results of three distinct experiments.

MATLAB script used to generate Fig.4

```
clear all
close all

[agarN, agarID, ~]=xlsread ('amp_agar', 1, '', 'basic');

%extract ID, diameter for agar plates
agarID=agarID(2:end,1);
agarD = agarN(:,2);

%extract ID, S number of spots for PCD
[pcdN, pcdID, ~]=xlsread ('amp_pcd', 1, '', 'basic');
pcdID= pcdID(2:end,1);
pcdS = pcdN(:,1);

%Anova and multiple comparison test for spots in inhibition area in pcd
[~,~,st] = anova1(pcdS,pcdID);
figure(6)
multcompare(st);

%Anova and multiple comparison test for diameter of inhibition area in agar
[~,~,st] = anova1(agarD,agarID);
figure(12)
multcompare(st);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

Examples of excel sheets for devices ('amp_pcd.xls') and agar plates ('amp_agar.xls')

ID	spots (#)
R	8
R	8
R	9
R	3
R	8
I1	9
I1	10
I1	12
I1	10
I1	9
I3	9
I3	8
I3	7
I3	9
S	13
S	12
S	9
S	11
S	9

ID	diam (mm)
R	9.3
R	9.3
R	9.5
R	10.4
I1	9.6
I1	11.2
I1	13.4
I1	9.6
I2	11.4
I2	11.0
I2	10.9
I2	10.5
S	13.4
S	12.6
S	11.4
S	11.9

MATLAB script used to generate Fig.5

```
clear all
close all

%salmonella typhimurium LT2

%extract ID, diameter and A area for agar plates
[SagarN,SagarID,~]=xlsread ('pathoSagar', 1, '', 'basic');
SagarID=SagarID(2:end,2);
SagarD = SagarN(:,3);

%Anova and multiple comparison test for diameter of inhibition area in agar
[~,~,st] = anova1(SagarD,SagarID);
figure(3)
multcompare(st);

%extract ID, S number of spots in blue area for paper device
[SpcdN, SpcdID ,~]=xlsread ('pathoSdevice', 1, '', 'basic');
SpcdID= SpcdID(2:end,2);
SpcdS = SpcdN(:,1);

%Anova and multiple comparison test for spots in blue area for paper device
[~,~,st] = anova1(SpcdS,SpcdID);
figure(6)
multcompare(st);

%%%%%%%%%%%%%%

%Escherichia coli O157:H7

%extract ID, diameter and A area for agar plates
[EagarN,EagarID,~]=xlsread ('pathoEagar', 1, '', 'basic');
EagarID=EagarID(2:end,2);
EagarD = EagarN(:,3);

%Anova and multiple comparison test for diameter of inhibition area in agar
[~,~,st] = anova1(EagarD,EagarID);
figure(9)
multcompare(st);

%extract ID, S number of spots in blue area for paper device
[EpcdN, EpcdID ,~]=xlsread ('pathoEdevice', 1, '', 'basic');
EpcdID= EpcdID(2:end,2);
EpcdS = EpcdN(:,1);

%Anova and multiple comparison test for spots in blue area for paper device
[~,~,st] = anova1(EpcdS,EpcdID);
figure(12)
multcompare(st);
```

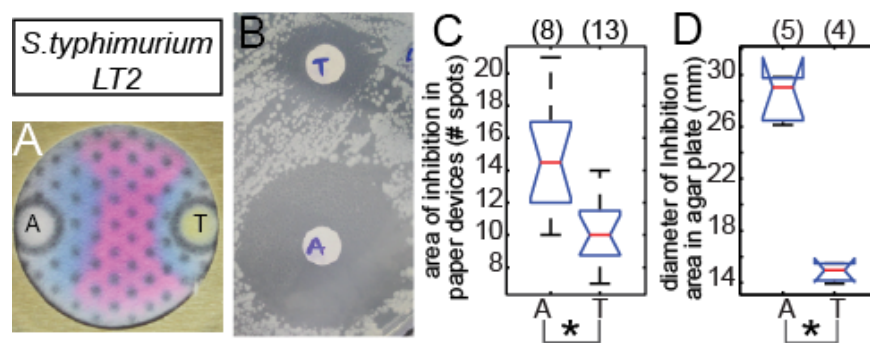


Fig. S6. Antimicrobial susceptibility tests with the pathogenic bacteria *S. typhimurium* LT2. Representative images, after 18 hours of culture at 37 °C, of culture device (A) and Petri dish (B), inoculated with 10^4 CFU of *S. typhimurium* LT2 and exposed to ampicillin 'A' or tetracycline 'T' (10 μ g/zone or disk). The notched box plots display the number of spots in the blue-colored area of inhibited growth in paper culture devices(C) and the diameters of the zone of inhibited growth on agar plate (D). See Fig. 4 for the description of the notched box plots.

References:

1. M. Funes-Huacca, A. Wu, E. Szepesvari, P. Rajendran, N. Kwan-Wong, A. Razgulin, Y. Shen, J. Kagira, R. Campbell and R. Derda, *Lab on a Chip*, 2012, **12**, 4269-4278.