Electronic Supplementary Material (ESI) for Lab on a Chip.


# Supplementary Information

## A rapid antimicrobial susceptibility testing for low bacterial concentrations integrating centrifuge based bacterial cell concentrator

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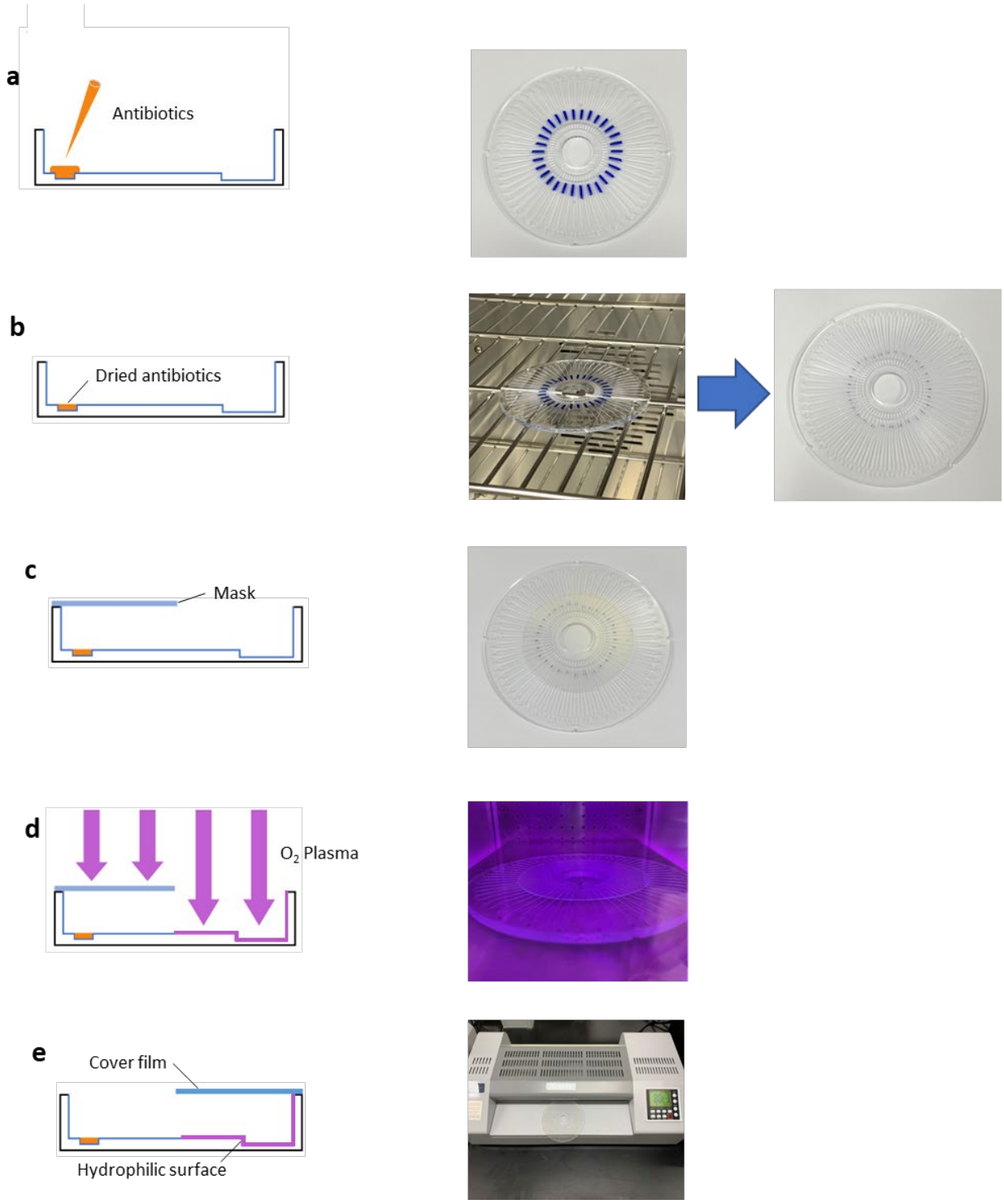


**Figure S1. Process of preparing the AST chip (a-e);** (a) Antibiotics are loaded into the reservoir in the chip. (b) Antibiotics are dehydrated by heat in the oven. (c) Chip is covered with mask to protect antibiotics. (d) Oxygen plasma treatment to make channels partially hydrophilic. (e) Chip is sealed with film to make the centrifuge chip.

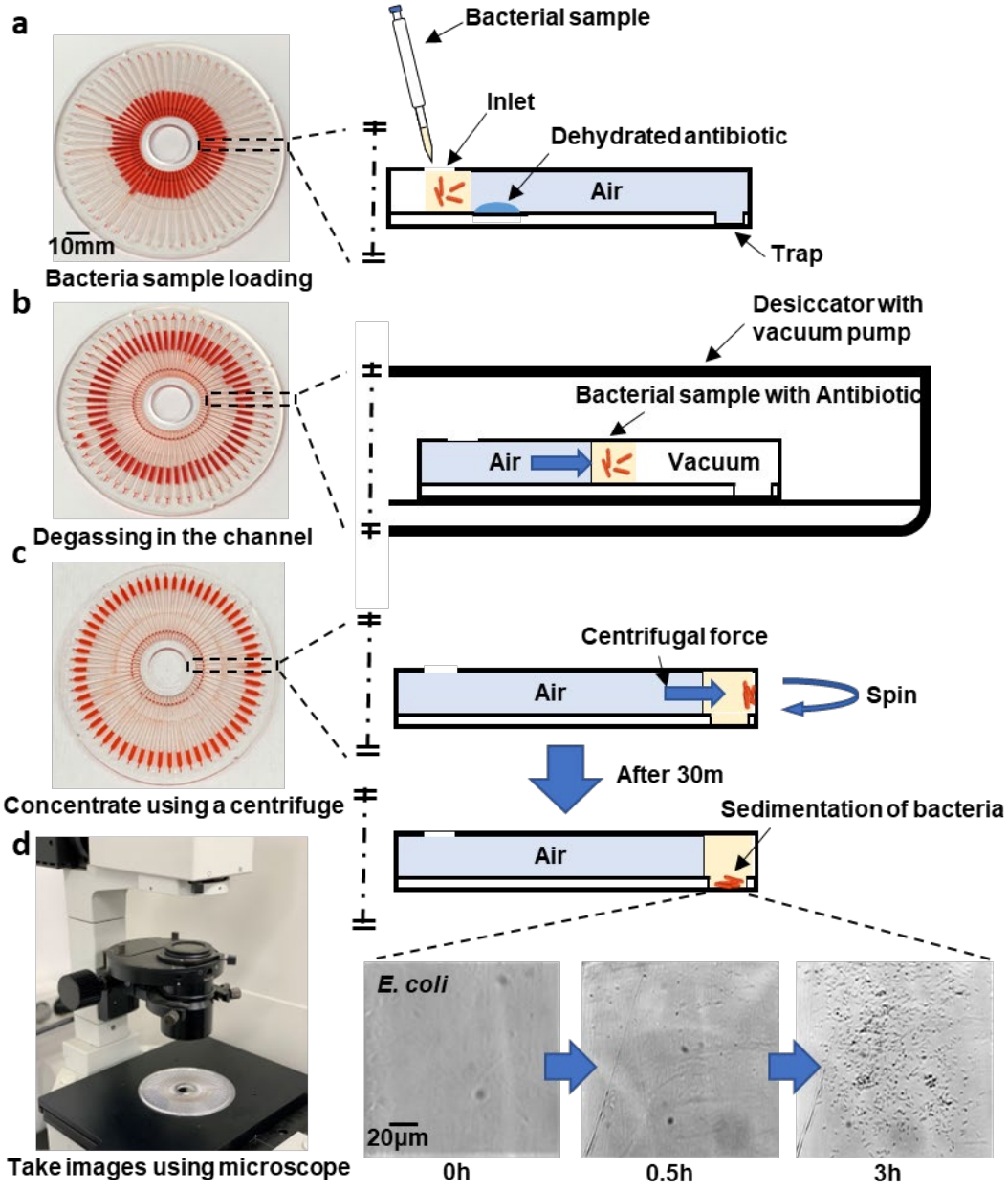


**Figure S2. AST process of the Microfluidic AST chip (a-e);** (a) Bacterial samples are injected on the channel. (b) Discharging the air inside the channel. (c) The chip is rotated by using centrifuge to concentrate the bacteria. (d) After the bacteria has completely sunk on the bacterial trap, bacterial growth is tracked by using a microscope in a time lapse image method.

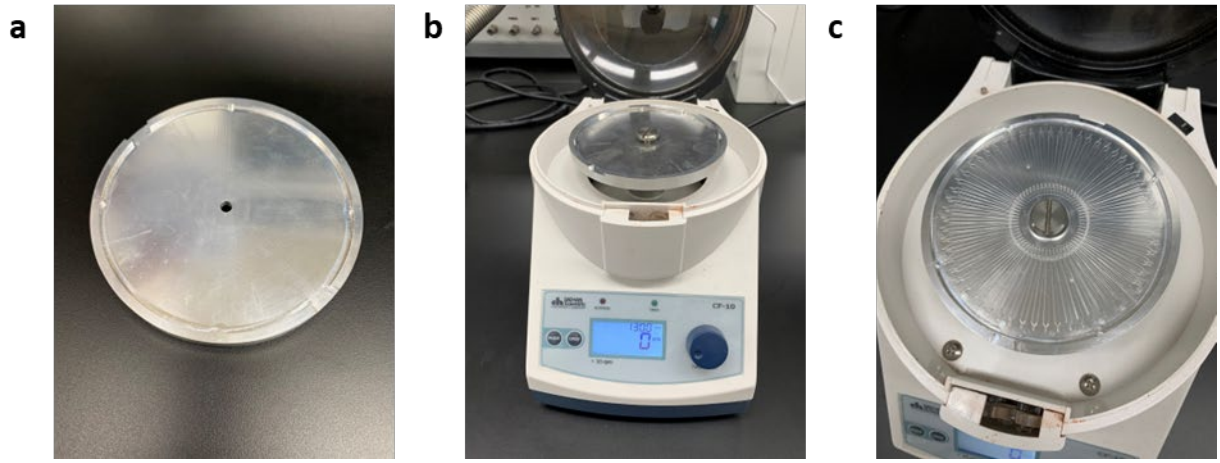


**Figure. S3.** (a) The aluminum chuck with the four pins at the corners. (b) The e-tube centrifugation. (c) The chuck is assembled to the e-tube centrifugation.

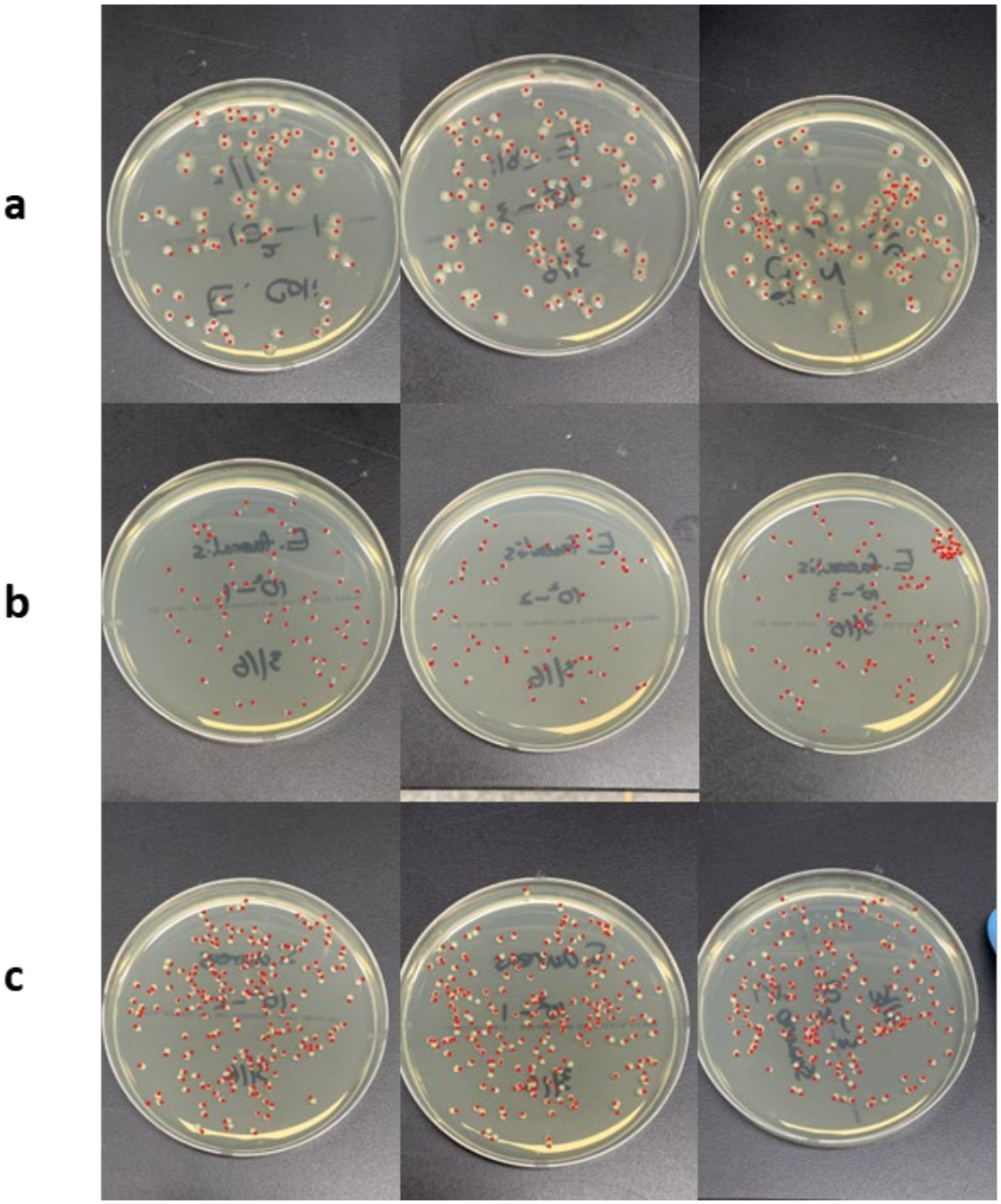


**Figure. S4. Colony counting of three strains.** (a) *Escherichia coli*. (b) *Enterococcus faecalis*. (c) *Staphylococcus aureus.*

**Table S1. Colony counting of three strains.** (A) *Escherichia coli*. (B) *Enterococcus faecalis*. (C) *Staphylococcus aureus.*

| Bacteria | Plate 1 | Plate 2 | Plate 3 | Average |
|---|---|---|---|---|
| *E. coli* (ATCC 25922) | 88 | 69 | 99 | 85 |
| *E. faecalis* (ATCC 29212) | 59 | 57 | 93 | 70 |
| *S. aureus* (ATCC 29213) | 168 | 189 | 190 | 182 |

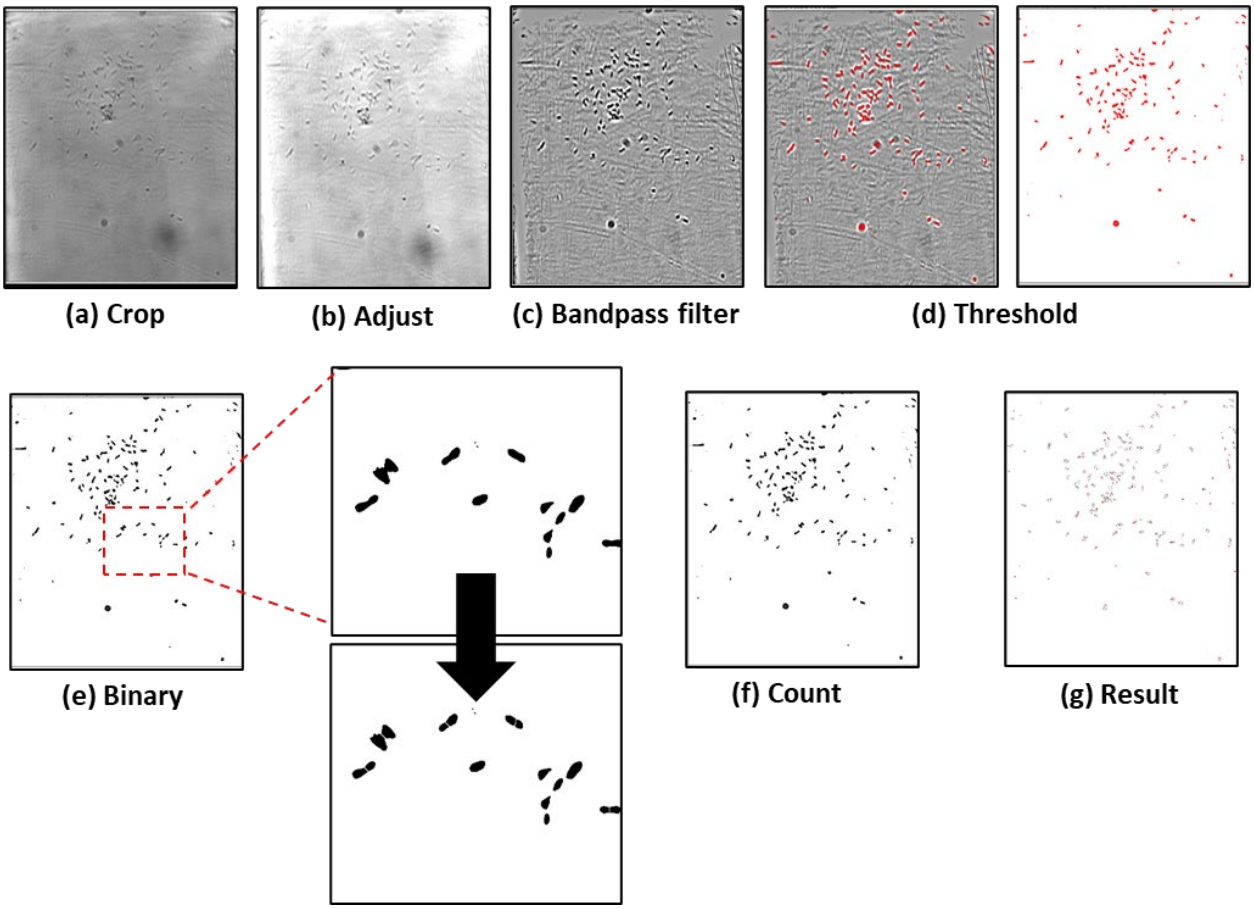


**Figure. S5. Image process of microscopic image of bacteria** (a) Crop the raw image to optimal size for image processing. (b) Adjust the brightness and contrast of the image to easily identify bacteria. (c) Reduce the noise in the image using the bandpass filter tool. (d) Extracting bacterial area from the background by adjusting thresholds. (e) Divide the overlapping area of bacteria using a watershed tool. (f) Count the number of bacteria using an analysis tool. Particles with an area in the range 0-600 pixels are counted as one bacterial cell. (g) The counted total number and the area of each particle are displayed as a pop-up.

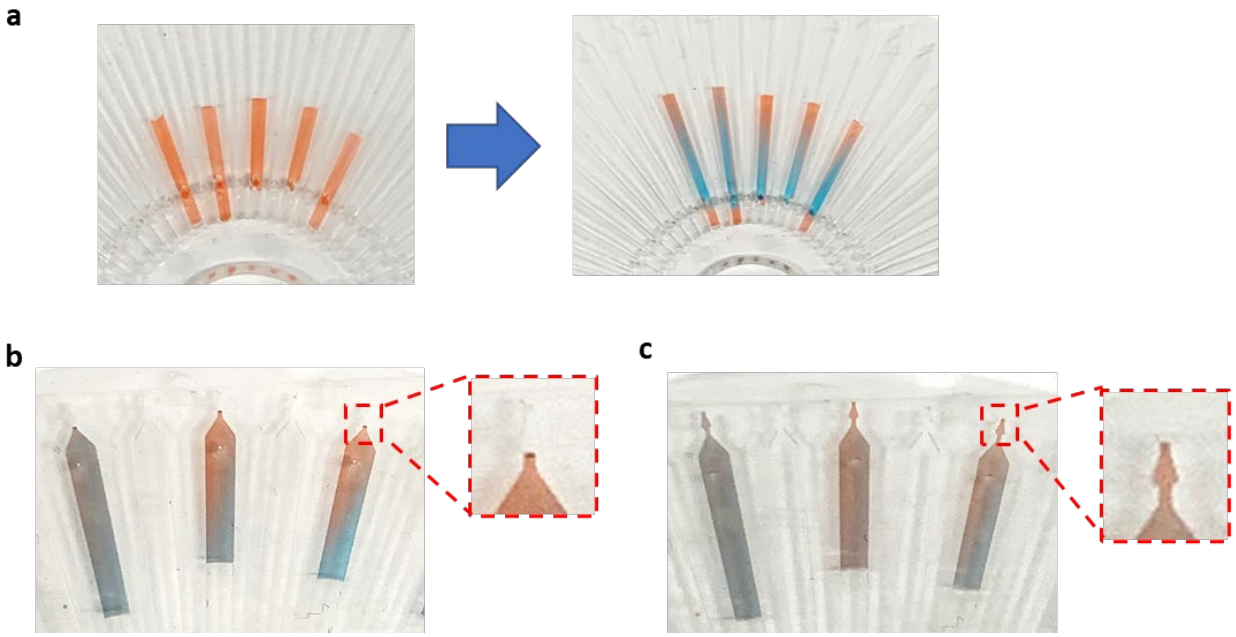


**Figure. S6. Validation of the mixing performance of the chip and the minimum RCF to concentrate;** (a) Antibiotics (Blue color) were preloaded on the reservoir of the chip. When bacterial samples (Orange color) were injected into the channel, it moved into the channel and mixed with antibiotics. The color of the bacteria samples gradually changed to blue. This diffusion phenomenon confirmed that the two samples were properly mixed. (b) The chip was rotated below 450 RCF. The sample did not reach the end of the channel well. (c) The chip was rotated over 450 RCF. The sample reached the end of the channel. The minimum RCF needed for the sample to reach the end of the channel was confirmed to be 450 RCF.

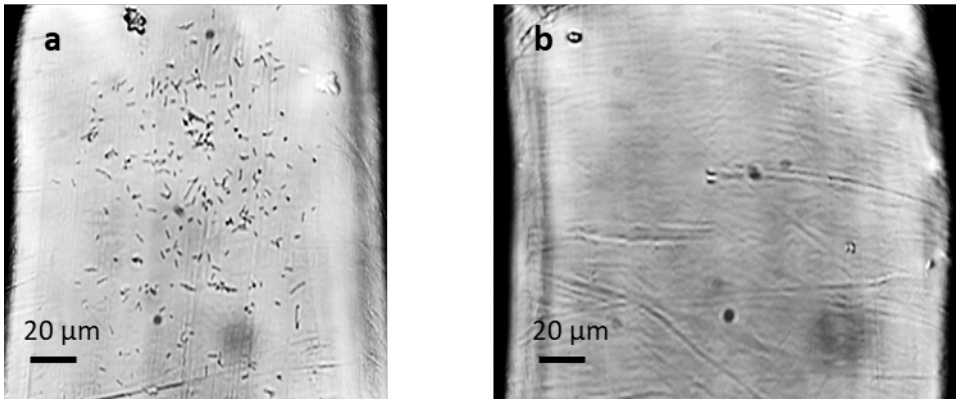


**Figure. S7. Images of the concentrated bacteria at 6000 RCF;** (a) *Escherichia coli* and (b) *P. aeruginosa;* there are no bacteria in this image. The motility of the bacteria was high. Even after concentration, *P. aeruginosa* easily escaped the bacterial traps. Scale bars represent 20 µm.

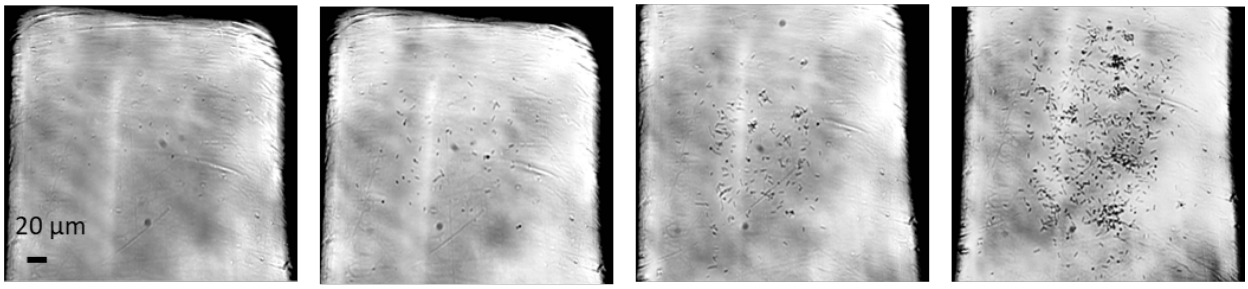


**Figure. S8. Time-lapse images of the concentrated bacteria (*E. coli*) in the trap.** scale bars represent 20 µm.