Supporting Information for

Portable cultures for phage and bacteria made of paper and tape

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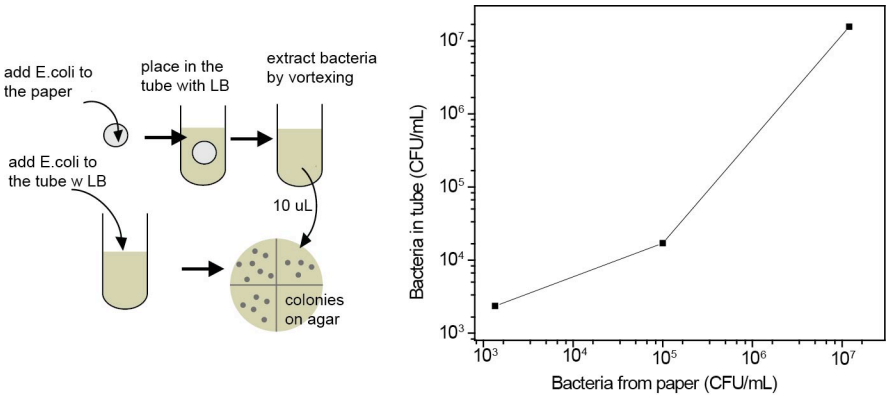

Figure S1. Extraction of bacteria from paper (calibration). The paper was inoculated with 10 µL E. coli 10^3, 10^5 or 10^7 CFU/mL. The paper was then placed in a tube containing 5 mL LB, and vortexed for 30 seconds. In a control experiment, the same suspension of E.coli was added directly to 5 mL of LB. The number of viable bacteria in both tubes was compared using colony-forming assay. We observed that bacteria can be extracted with good yield from the paper.

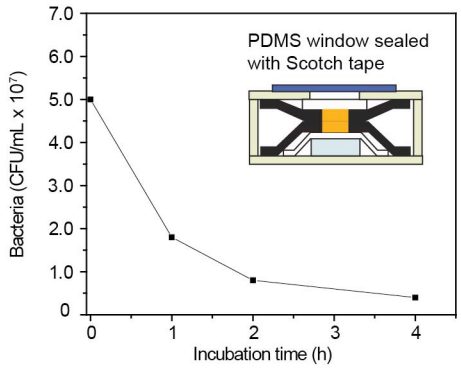

Figure S2 Sealing the window with scotch ablates bacterial growth. $5x10^7$ CFU of bacteria were seeded into the device; at the specific time, bacteria were extracted from the device and quantified using colony forming assay.

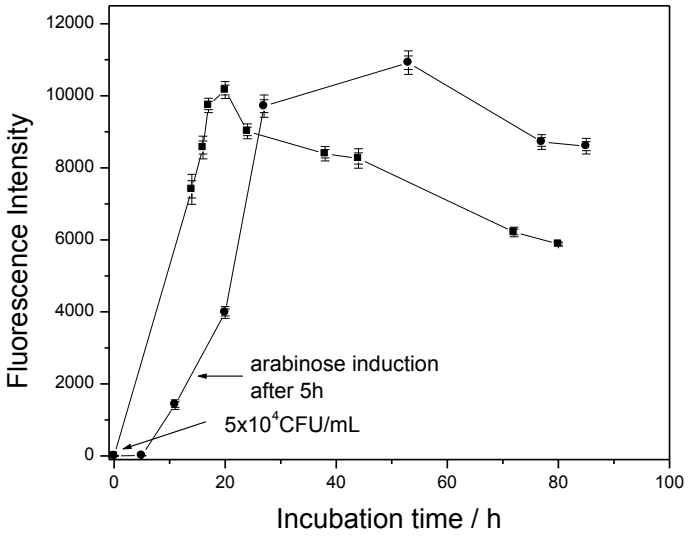

Figure S3: Development of fluorescence during growth of mCherry *E.coli* in shaking culture. Bacteria ($5x10^4$ CFU) were inoculated in LB+ampicilin media or in LB+ampicilin+arabinose media. After 5h of culture, arabinose was added to LB+ampicillin medium to a total concentration of 0.02% (marked as "arabinose induction"). Culture without induction produced no fluorescent signal (not shown). Fluorescence was measured using plate reader (Spectra Max M2, Molecular Devices) 584 nm excitation, 612 nm emission and 610 nm filter.

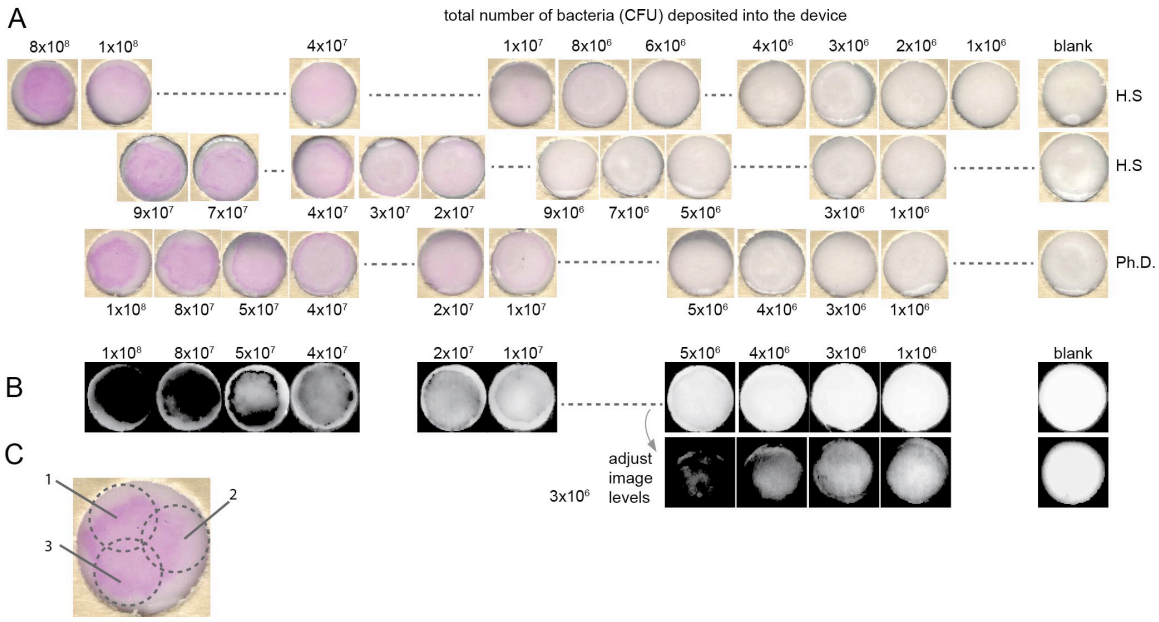

Figure S4. (A) Representative images of the devices inoculated with a pre-calculated number of mCherry E. coli. Each row represents a separate experiment. HS – data acquired by high-school students. Ph.D. – data acquired by the fisrt author. (B) Fluorescent images of the devices from the third row in (A), acquired using Fluoro Image Analyzer (FLA-5000). Black color is proportional to red fluorescence. Second row represents the same images with adjusted grey scale levels. Fluorescence generated by low number of bacteria can be distinguished from autofluorescence of the paper. The lower limit of detection is 1 million bacteria per 6x6 mm culture window of the device. (C) To quantify fluorescence or color intensity, each zone was measured 3 times using weakly-overlapping regions of interest (ROI). Integrated cyan channel intensity (color) or grey scale intensity (fluorescence) was measured within each ROI. These values, or their average were used in Figures 4 and 5 (main text).

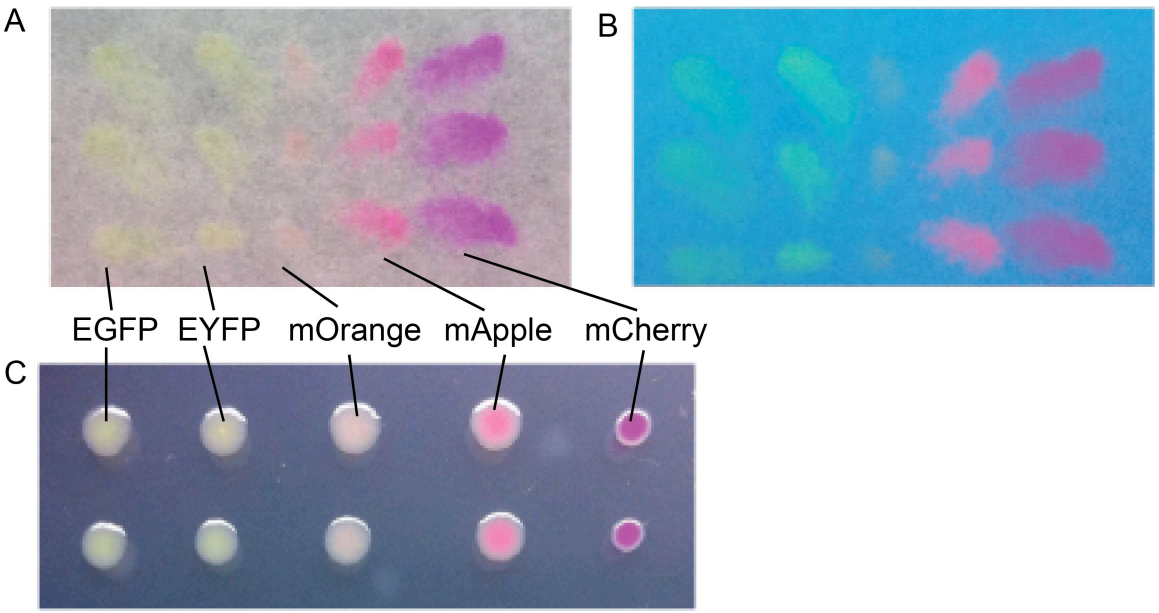

Figure S5. Visualization of bacteria expressing EGFP, EYFP, mApple, mOrgange and mCherry constructs after culture in paper (A, B) or as colonies on agar (C).[1] Image in (A) was acquired using illumination with white light. Image in (B) depicts samples on a UV trans-illuminator (both A and B were acquired by ordinary digital camera). mCherry has the deepest and brightest color, but mApple and mOrange can be also detected under white-light illumination. Their color is distinctly different from that of mCherry. EYFP- and EGFP-expressing bacteria are nearly invisible under white light illumination when cultured in paper, but their fluorescence is clearly visible.

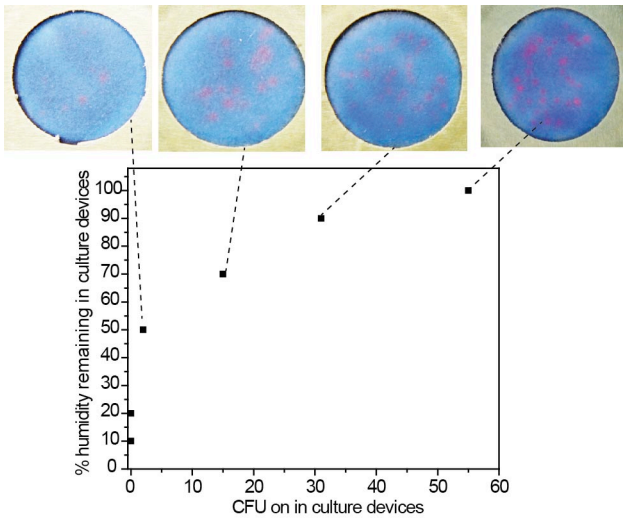

Figure S6. Performance of the device after partial evaporation of media. We generated a series of identical devices, loaded them with LB media, sterilized them and allowed some liquid to evaporate from the device. We determined % humidity remaining in the devices (Y-axis) by weighing the devices before and after evaporation. We then inoculated the devices with 100 μL of the solution that contained low concentration of *E. coli* (~100 CFU in 100 μL), allowed the bacteria to growth for 24 hours, and visualized the number of colonies using PrestoBlue. Clonal growth in devices that lost >10% of humidity was significantly worse than growth in devices that were not exposed to any evaporation. No growth was detected in devices that lost over 50% of humidity.

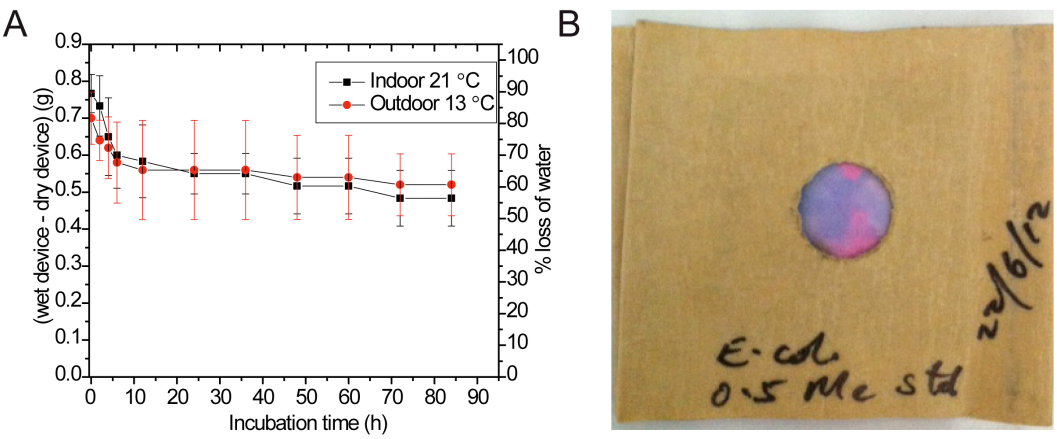

Figure S7. Preliminary evaluation of the performance of the culture devices assembled at the Institute of Primate Research (IPR) in Nairobi, Kenya. (A) Quality of the devices could be assessed by their ability to retain humidity over time. Due to high humidity both indoors and outdoors, the evaporation rate from these devices is considerably slower than that observed in the lab in Canada (main text Figure 2). (B) Example of the device that contains colonies of microorganisms visualized by PrestoBlue. Device was sterilized in IRP labs, cultured and visualized on site during the International Diagnostic Workshop.

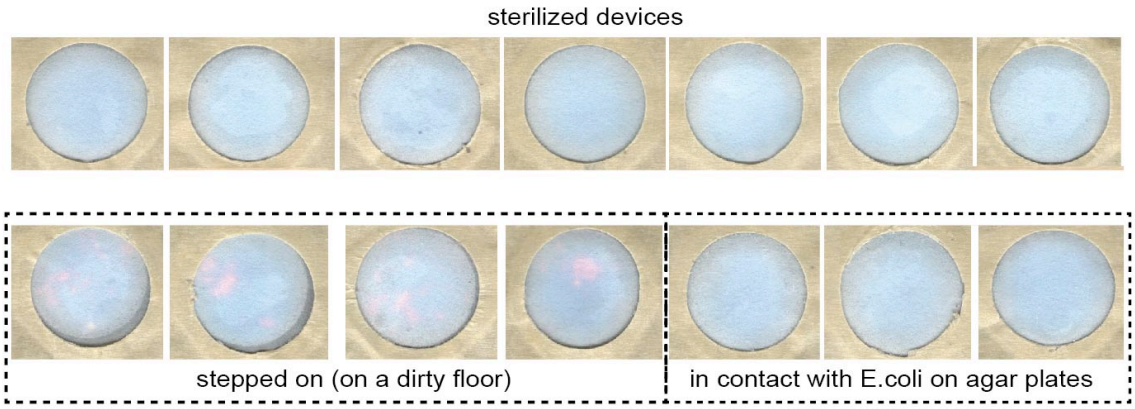

Figure S8. Sterility of the devices in different conditions. Top: devices that were not inoculated with any bacteria remain sterile. Autoclaved devices with LB media were loaded with sterile PrestoBlue (PB) reagent and cultured for 48 hours. Some devices were loaded with PB first and then dropped on the dirty floor and stepped on. Some were placed inside E. coli-filled petri dish. After each "mistreatment", the devices were cultured for 48 hours to reveal contaminants. Contamination happens only after harsh mechanical impact, which would normally shatter all known glass, and plastic culture devices.

Cost Analysis of Culture Devices (based on retail prices):

1) PDMS sheet used per device weighs ~150 mg, a bucket of PDMS costs $355 for 4 Kg. One bucket could yield ~26,000 devices.

   The cost of one sheet is $355 x 0.15 g / 4000 g = $0.013

2) Dialysis membrane used per device ~4 cm^2 , pack of dialysis membrane costs $131 and it has 0.64 m^2 (6400 cm^2). One pack could yield 1600 devices.

   Cost of one dialysis membrane: $131 x 4 cm^2 / 6400 cm^2 = $0.08

3) Masking tape: we make each device using two layers of 12 cm (total 0.24 m) , the pack of tape is $4 for 55 m. One roll could yield 230 devices.

   The cost of tape for one device is: $4 x 0.24 m / 55 m = $ 0.017

4) Blotting filter paper: we used one pad of 1.75 cm^2, pack of filter membrane is $93 for 0.58 m^2 (5800 cm^2). One pack could yield 3300 devices.

   The cost of one filter pad is $93 x 1.76 cm^2 / 5800 m^2 = $0.028

5) Chromatography paper W114: A pack of paper is ~$400 for 400 letter-size sheets of paper; each sheet could accomodate 35 patterned papers (3.5 cm x 3.5 cm).

   The cost of each patterned paper is: $400/ (400 sheets x 35 pieces) = $0.028
   For each device we used 2 pieces of patterned paper= $0.028 x 2 = $ 0.057

Price for each device:

PDMS	$0.013
Dialysis membrane	$0.08
Masking tape	$ 0.017
Blotting paper	$0.028
Patterned paper	$ 0.057
Total	$0.195

The cost of items highlighted red could be decreased significantly. For example, expensive controlled-pore size dialysis membrane could be replaced by cellophane. High-quality wet-strengthened filter paper and blotting paper could be potentially replaced by low-grade thin and thick paper.

1. Shaner, N. C.; Campbell, R. E.; Steinbach, P. A.; Giepmans, B. N. G.; Palmer, A. E.; Tsien, R. Y., Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp red fluorescent protein. *Nat. Biotechnol.* **2004,** *22* (12), 1567-1572.