Electronic Supplementary Material (ESI) for Analyst.


**Versatile Printed Microheaters to Enable Low-Power Thermal Control in Paper**

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## SUPPLEMENTAL INFORMATION

**Table S1:** Nucleotide sequences of LAMP primers that target the *V. cholerae ctxA* gene.

| Primer | Sequence (5' – 3') |
|---|---|
| B3 | GTGGGCACTTCTCAAACT |
| F3 | TCGGGCAGATTCTAGACC |
| BIP | TCAACCTTTATGATCATGCAAGAGGGGAAACATATCCATCATCGTG |
| FIP | TTGAGTACCTCGGTCAAAGTACTTCCTGATGAAATAAAGCAGTCA |
| LB-FITC | /56-FAM/AACTCAGACGGGATTTGTTAGG |
| LF-Biotin | /5-Biosg/CCTCTTGGCATAAGACCACC |

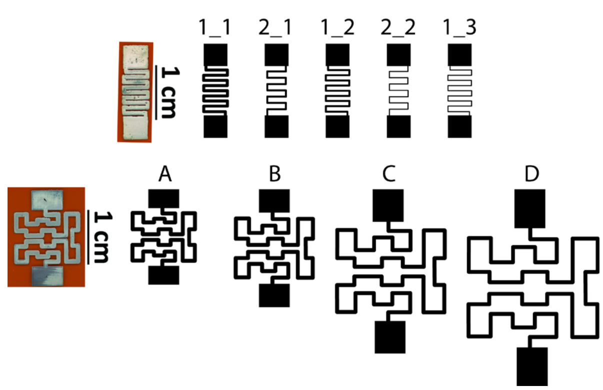



**Figure S1:** Microheaters designs summary. The top set of designs is useful for heating short regions of lateral flow assays while the lower set of designs is useful for chips or 2-Dimensional Paper Network devices with wide heating regions.

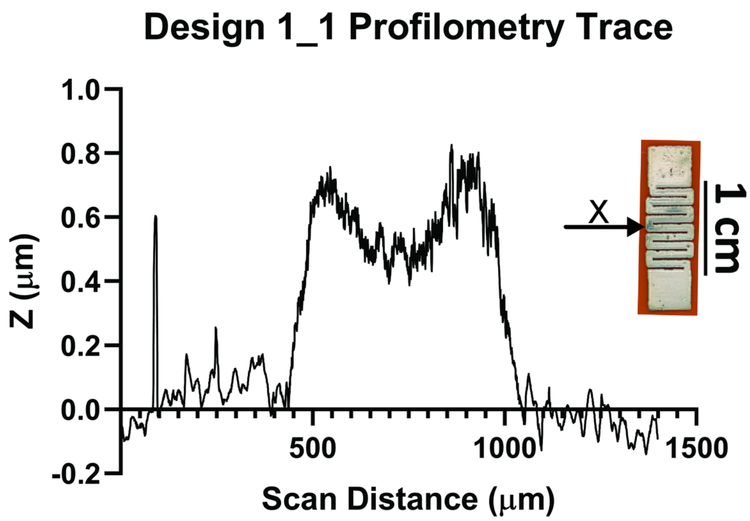
**Table S2:** Microheaters design details and resistance predictions given ink resistivity of 4.3E-5 Ohms*mm and printed trace height of 700 nm.

| **Design** | **1_1** | **2_1** | **1_2** | **2_2** | **1_3** | **A** | **B** | **C** | **D** |
|---|---|---|---|---|---|---|---|---|---|
| **Path length (mm)** | 46.14 | 32.45 | 46.14 | 32.45 | 46.14 | 82.03 | 105.19 | 147.82 | 177.38 |
| **Stroke/width (mm)** | 0.53 | 0.35 | 0.35 | 0.18 | 0.18 | 0.70 | 0.70 | 0.70 | 0.70 |
| **Heating Area (mm x mm)** | 4.8 x 8.75 | 3.8 x 8.75 | 4.6 x 8.75 | 3.6 x 8.75 | 4.4 x 8.75 | 11.9 x 9.2 | 14.5 x 11.3 | 20.1 x 15.4 | 23.7 x 18.3 |
| **Calculated Resistance** | **5.4** | **5.7** | **8.0** | **11.3** | **16.1** | **7.2** | **9.2** | **13.0** | **15.6** |


**Figure S2:** Setup for incubations powered by AA battery. Box was closed after initiation.

**Table S3: COMSOL Model Inputs & Outputs**

| Model Input | Source | Design 1_1 | Design A |
|---|---|---|---|
| Simulation Time | - | 30 min | 5 hours |
| Terminal voltage (V) | - | 1.2 | 1.2 |
| Ambient Heat Flux W/($m^2$K) | Manually Tuned | 33 | 33 |
| Trace Thickness -Shell (nm) | Experimental Data | 1000 | 700 |
| Trace Conductivity (S/m) | Experimental Data | 2.3×10^7 | 2.3×10^7 |
| Sample Dimensions (mm) | Manually Measured | 7x7 | 10 x 10 |
| Total Chip Dimensions (mm) | Manually Measured | 15 x 15 | 25 x 25 |
| Microheater Heating Region Dimensions (mm) | Manually Measured | 5 x 9 | 12 x 10 |
| Model Output | | | |
| Resistance (Ω) | - | 6.6 | 8.5 |
| Terminal current (A) | - | 0.18 | 0.14 |
| Bottom of Chip Max Temperature (°C) | - | 71 | 43 |
| Top of Chip Max Temperature (°C) | - | 63 | 35 |
| Max Sample Temperature (°C) | - | 67 | 37 |
| Average Sample Volume Temperature (°C) | - | 63 | 35 |



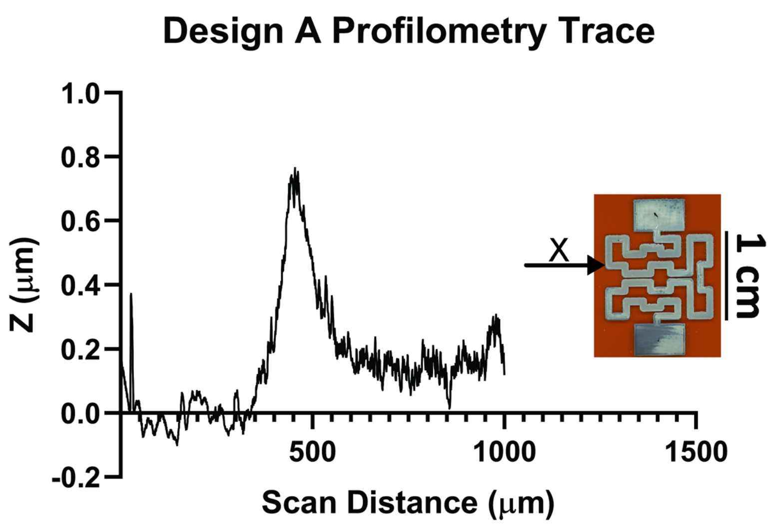




**Figure S3:** Representative graphs of profilometry data. The z height of the single layer nanosilver ink prints varies between the different designs and also appears to vary within a single print as well. There does appear to be some correlation between trace width and z height as thinner designs produced higher z height despite using the same print settings (35μm drop spacing etc.). Other sampling locations on different prints showed z heights between 1 and 1.3 μm.

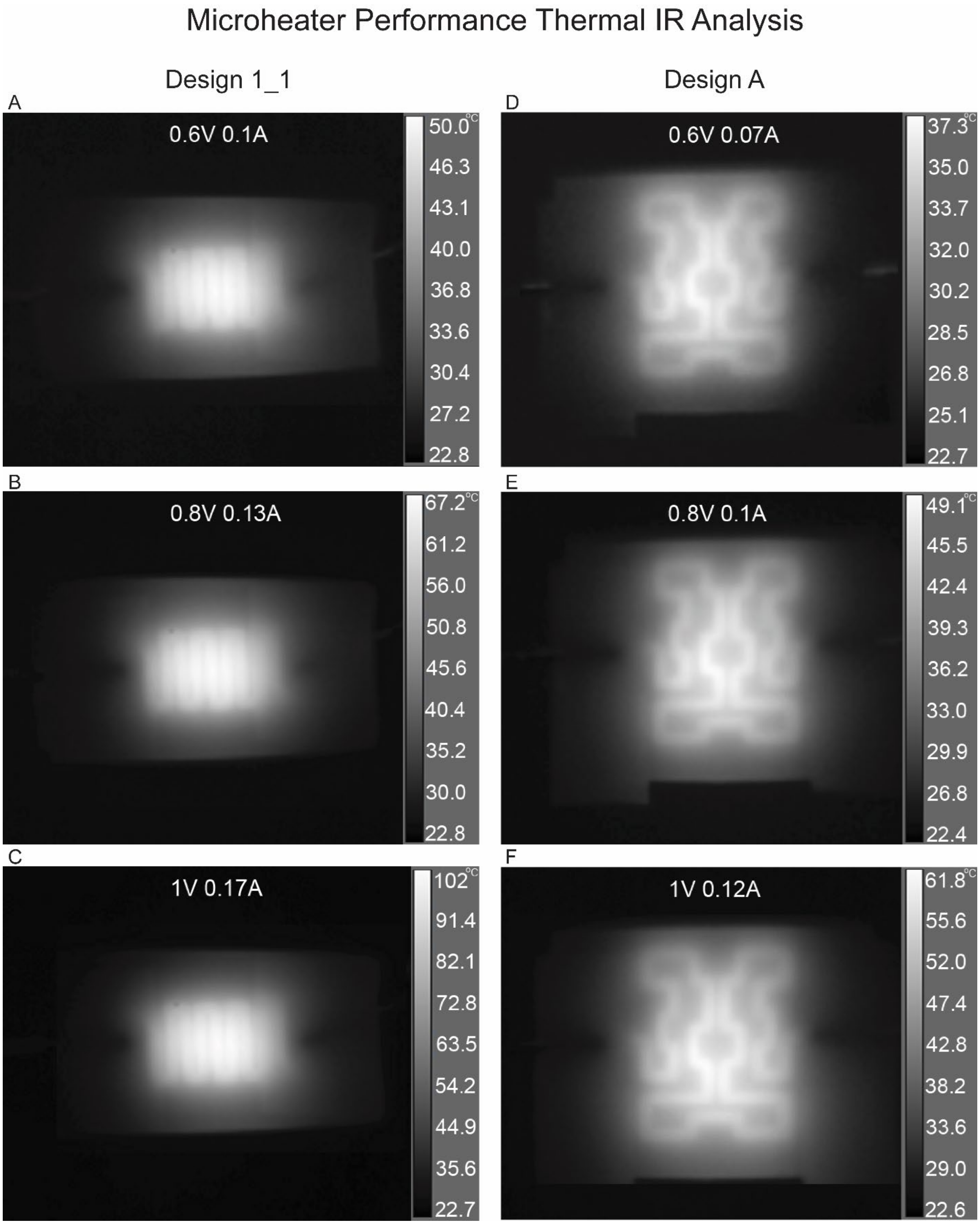



**Figure S4:** Microheater Performance Thermal IR Analysis Designs 1_1 and A. Voltages of 0.6V, 0.8V, 1V were applied to design 1_1 (A, B,C) and Design A (D, E, F) for five minutes each while under video recording with the IR camera.

**Table S4: Microheater reusability repeated heating statistical summary**

| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 6 | Trial 7 | Trial 8 | Trial 9 | Trial 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of values | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 |
| Minimum | 25.00 | 24.10 | 24.20 | 25.90 | 25.10 | 26.70 | 24.80 | 24.70 | 24.40 | 24.80 |
| 25% Percentile | 68.40 | 68.20 | 68.30 | 68.80 | 68.50 | 68.70 | 68.20 | 68.45 | 68.40 | 68.20 |
| Median | 69.20 | 69.30 | 69.30 | 69.60 | 69.40 | 69.50 | 69.00 | 69.30 | 69.30 | 69.20 |
| 75% Percentile | 69.50 | 69.50 | 69.70 | 69.80 | 69.80 | 69.90 | 69.20 | 69.50 | 69.60 | 69.50 |
| Maximum | 69.60 | 69.70 | 69.80 | 70.00 | 69.90 | 70.00 | 69.70 | 69.50 | 69.70 | 69.50 |
| Mean | 67.87 | 67.74 | 67.90 | 68.27 | 68.08 | 68.28 | 67.75 | 67.88 | 67.88 | 67.76 |
| Std. Deviation | 4.52 | 4.89 | 4.71 | 4.55 | 4.59 | 4.31 | 4.68 | 4.70 | 4.87 | 4.67 |
| Std. Error of Mean | 0.15 | 0.16 | 0.16 | 0.15 | 0.15 | 0.14 | 0.16 | 0.16 | 0.16 | 0.16 |
| Lower 95% CI | 67.57 | 67.42 | 67.59 | 67.98 | 67.78 | 68.00 | 67.44 | 67.57 | 67.56 | 67.46 |
| Upper 95% CI | 68.17 | 68.06 | 68.21 | 68.57 | 68.39 | 68.56 | 68.05 | 68.19 | 68.20 | 68.07 |

**Table S5: Tukey's multiple comparisons test for microheater repeated heating reusability test**

| Test | Mean Diff. | 95.00% CI of diff. | Significant? | Adjusted P Value | |
|---|---|---|---|---|---|
| Trial 1 vs. Trial 2 | 0.1326 | -0.5614 to 0.8266 | No | 0.9999 | A-B |
| Trial 1 vs. Trial 3 | -0.03052 | -0.7245 to 0.6635 | No | >0.9999 | A-C |
| Trial 1 vs. Trial 4 | -0.4046 | -1.099 to 0.2894 | No | 0.7065 | A-D |
| Trial 1 vs. Trial 5 | -0.2143 | -0.9083 to 0.4797 | No | 0.9935 | A-E |
| Trial 1 vs. Trial 6 | -0.4075 | -1.102 to 0.2864 | No | 0.6974 | A-F |
| Trial 1 vs. Trial 7 | 0.1225 | -0.5715 to 0.8165 | No | >0.9999 | A-G |
| Trial 1 vs. Trial 8 | -0.01065 | -0.7046 to 0.6833 | No | >0.9999 | A-H |
| Trial 1 vs. Trial 9 | -0.01088 | -0.7049 to 0.6831 | No | >0.9999 | A-I |
| Trial 1 vs. Trial 10 | 0.1084 | -0.5856 to 0.8024 | No | >0.9999 | A-J |
| Trial 2 vs. Trial 3 | -0.1632 | -0.8571 to 0.5308 | No | 0.9992 | B-C |
| Trial 2 vs. Trial 4 | -0.5372 | -1.231 to 0.1568 | No | 0.2966 | B-D |
| Trial 2 vs. Trial 5 | -0.3469 | -1.041 to 0.3470 | No | 0.8572 | B-E |
| Trial 2 vs. Trial 6 | -0.5402 | -1.234 to 0.1538 | No | 0.2888 | B-F |
| Trial 2 vs. Trial 7 | -0.01010 | -0.7041 to 0.6839 | No | >0.9999 | B-G |
| Trial 2 vs. Trial 8 | -0.1433 | -0.8373 to 0.5507 | No | 0.9997 | B-H |
| Trial 2 vs. Trial 9 | -0.1435 | -0.8375 to 0.5505 | No | 0.9997 | B-I |
| Trial 2 vs. Trial 10 | -0.02420 | -0.7182 to 0.6698 | No | >0.9999 | B-J |
| Trial 3 vs. Trial 4 | -0.3740 | -1.068 to 0.3200 | No | 0.7927 | C-D |
| Trial 3 vs. Trial 5 | -0.1838 | -0.8778 to 0.5102 | No | 0.9980 | C-E |
| Trial 3 vs. Trial 6 | -0.3770 | -1.071 to 0.3170 | No | 0.7848 | C-F |
| Trial 3 vs. Trial 7 | 0.1531 | -0.5409 to 0.8470 | No | 0.9995 | C-G |
| Trial 3 vs. Trial 8 | 0.01987 | -0.6741 to 0.7139 | No | >0.9999 | C-H |
| Trial 3 vs. Trial 9 | 0.01964 | -0.6743 to 0.7136 | No | >0.9999 | C-I |
| Trial 3 vs. Trial 10 | 0.1390 | -0.5550 to 0.8329 | No | 0.9998 | C-J |
| Trial 4 vs. Trial 5 | 0.1902 | -0.5038 to 0.8842 | No | 0.9974 | D-E |
| Trial 4 vs. Trial 6 | -0.002997 | -0.6970 to 0.6910 | No | >0.9999 | D-F |
| Trial 4 vs. Trial 7 | 0.5271 | -0.1669 to 1.221 | No | 0.3237 | D-G |
| Trial 4 vs. Trial 8 | 0.3939 | -0.3001 to 1.088 | No | 0.7379 | D-H |
| Trial 4 vs. Trial 9 | 0.3937 | -0.3003 to 1.088 | No | 0.7386 | D-I |
| Trial 4 vs. Trial 10 | 0.5130 | -0.1810 to 1.207 | No | 0.3636 | D-J |
| Trial 5 vs. Trial 6 | -0.1932 | -0.8872 to 0.5008 | No | 0.9970 | E-F |

| | | | | | |
|---|---|---|---|---|---|
| Trial 5 vs. Trial 7 | 0.3368 | -0.3571 to 1.031 | No | 0.8778 | E-G |
| Trial 5 vs. Trial 8 | 0.2037 | -0.4903 to 0.8977 | No | 0.9956 | E-H |
| Trial 5 vs. Trial 9 | 0.2034 | -0.4905 to 0.8974 | No | 0.9956 | E-I |
| Trial 5 vs. Trial 10 | 0.3228 | -0.3712 to 1.017 | No | 0.9034 | E-J |
| Trial 6 vs. Trial 7 | 0.5301 | -0.1639 to 1.224 | No | 0.3155 | F-G |
| Trial 6 vs. Trial 8 | 0.3969 | -0.2971 to 1.091 | No | 0.7292 | F-H |
| Trial 6 vs. Trial 9 | 0.3967 | -0.2973 to 1.091 | No | 0.7299 | F-I |
| Trial 6 vs. Trial 10 | 0.5160 | -0.1780 to 1.210 | No | 0.3549 | F-J |
| Trial 7 vs. Trial 8 | -0.1332 | -0.8272 to 0.5608 | No | 0.9999 | G-H |
| Trial 7 vs. Trial 9 | -0.1334 | -0.8274 to 0.5606 | No | 0.9998 | G-I |
| Trial 7 vs. Trial 10 | -0.01410 | -0.7081 to 0.6799 | No | >0.9999 | G-J |
| Trial 8 vs. Trial 9 | -0.0002220 | -0.6942 to 0.6938 | No | >0.9999 | H-I |
| Trial 8 vs. Trial 10 | 0.1191 | -0.5749 to 0.8131 | No | >0.9999 | H-J |
| Trial 9 vs. Trial 10 | 0.1193 | -0.5747 to 0.8133 | No | >0.9999 | I-J |

**Design 1_1 Microheater, LAMP Amplification of *V. cholerae* Sample Chip Heating Model**

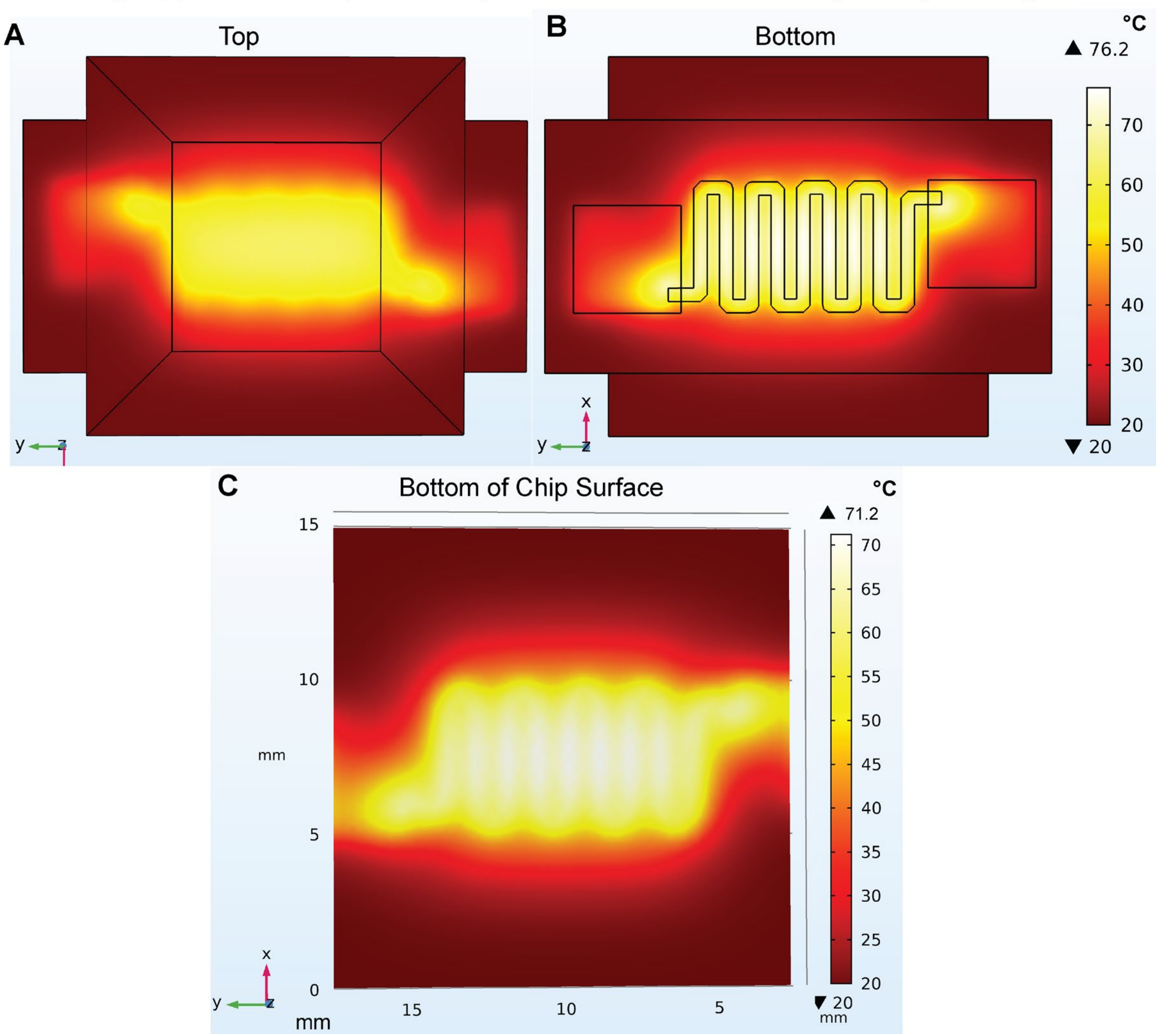



**Figure S5:** COMSOL Model of Design 1_1 Microheater and LAMP sample assembly, showing heat distributions for the entire outer surface (A and B), the bottom surface of the sample chip (in contact with the heater) (C) Each figure intensity (color gradient) is specific to the temperature scale to the right of each evaluation, please note that the scales are different for each.

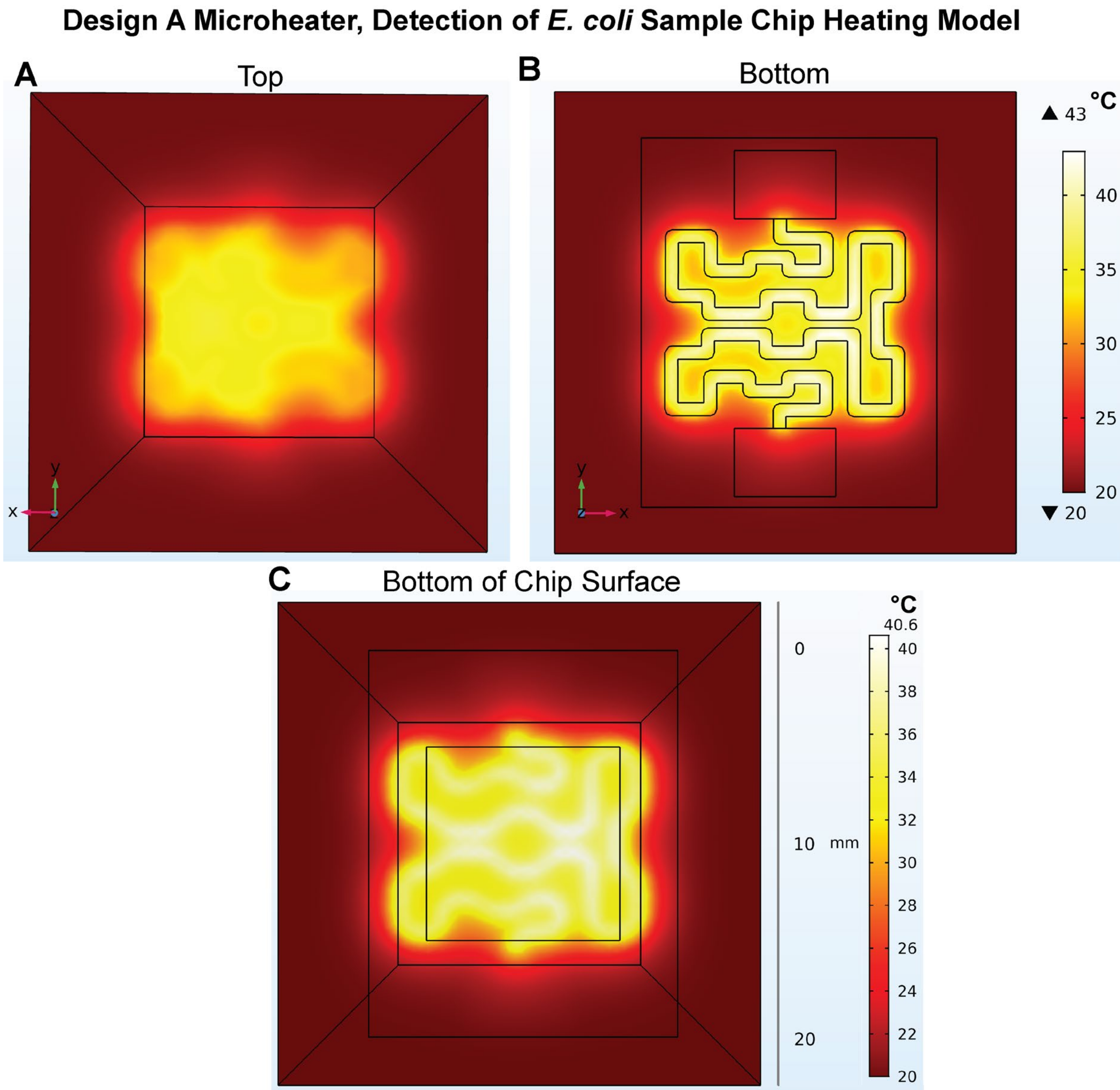



**Figure S6:** COMSOL Model of Design A microheater and *E. coli* sample chip, showing heat distributions for the entire outer surface (A and B), the bottom surface of the sample chip (in contact with the heater) (C). Each figure intensity (color gradient) is specific to the temperature scale to the right of each evaluation, please note that the scales are different for each.

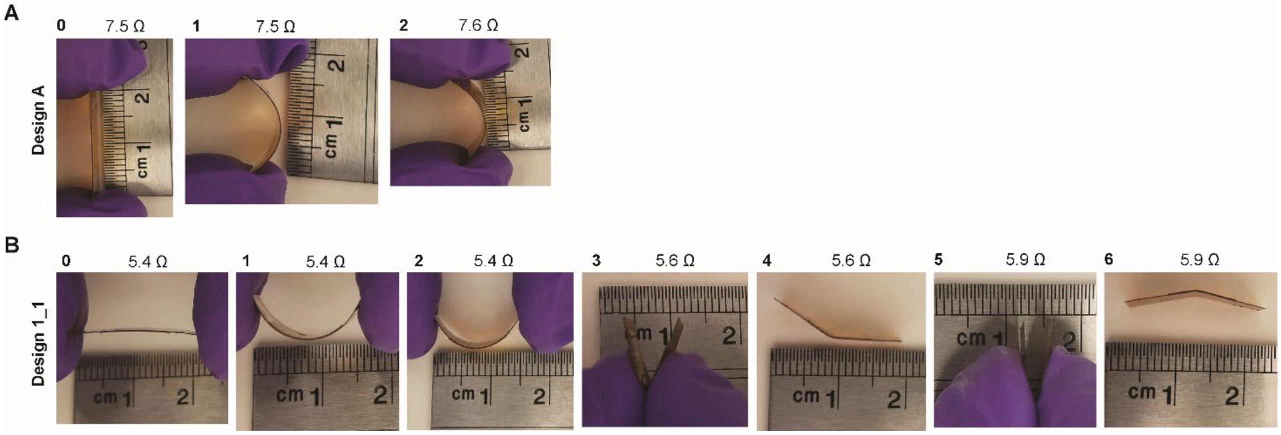



**Figure S7:** (A) Bending of Design A microheater for the durability test. Strain was calculated based on ΔL/L for the sample in each direction and resistance was measured after each deformation. Microheater traces for Design A tolerate bending resulting in a strain of Ɛ= 0.16 with only 0.1 Ohm change in resistance after bending in both the concave and convex directions. (B) A series of more aggressive bending on a single Design 1_1 microheater resulted in only minor resistance increases after each treatment. Strains for each bending treatment were Ɛ1=0.25, Ɛ2=0.25, Ɛ3=0.8, Ɛ4=0.7. The resistance was measured before and after each treatment using the two-point probe method (shown in Figure S6).

**Table S6:** Material cost estimations per individual microheater for Design D and Design 1_1.

| Item | Cost (USD) per microheater (Design D) | Cost (USD) per microheater (Design 1_1) |
|---|---|---|
| **AgInk** | 0.035 | 0.01 |
| **Substrate** | 0.52 | 0.15 |
| **Filter** | 0.02 | 0.006 |
| **Total $** | **0.58** | **0.17** |


**Figure S8:** Low resistance microheaters are possible to create using the technique described in the main text. The design here has fewer serpentine turns resulting in a shorter path length. Specs: trace width: 0.529mm, path length: 24.264 mm, total heating area: 4 mm x 8.8 mm.

**Table S7: POC heating methods analysis**

| METHOD | COMPATIBILITY FOR POC USE | RAMP-UP TIME | TEMP. ACCURACY | POWER SOURCE | MAXIMUM INCUBATION TIME | HEATER COST (USD) | INTERFACE /POWER COSTS | REUSABLE | REF |
|---|---|---|---|---|---|---|---|---|---|
| **HOT PLATE** | Not suitable | ~5 min. | Moderate (± 5 °C) | Electrical Grid | Grid dependent | $287 - $531 | N/A | Yes | 1 |
| **CHEMICAL HEATER** | Moderate | 5-10 min. | Poor, highly variable | None | 19.0 ± 5.0 minutes | $0.41 | $1.61 (foam housing) | No | 2 |
| **PCB** | Good | ~3 min. | Highly accurate (± 1.5 °C) | Battery /Electrical Grid | Battery/Grid dependent | <$1/cm$^2$ | Accompanying control units vary from simple boards to entire desktop computers. | Yes | 3,4 |
| **OMEGA POLYIMIDE HEATER** | Moderate | Unknown | Unknown | DC Power Source | Grid dependent | $52 | $167 DC Power Supply | Yes | 5 |
| **INKJET PRINTED MICROHEATER** | Excellent | 5-10 min. | Good (± 2.5 °C from median of desired Temp. window) | Battery or DC Power Source | Battery dependent (30 min. to 16 h) | <$0.58/heater | $1.45 for one NiMH AA rechargeable battery | Yes | N/A |

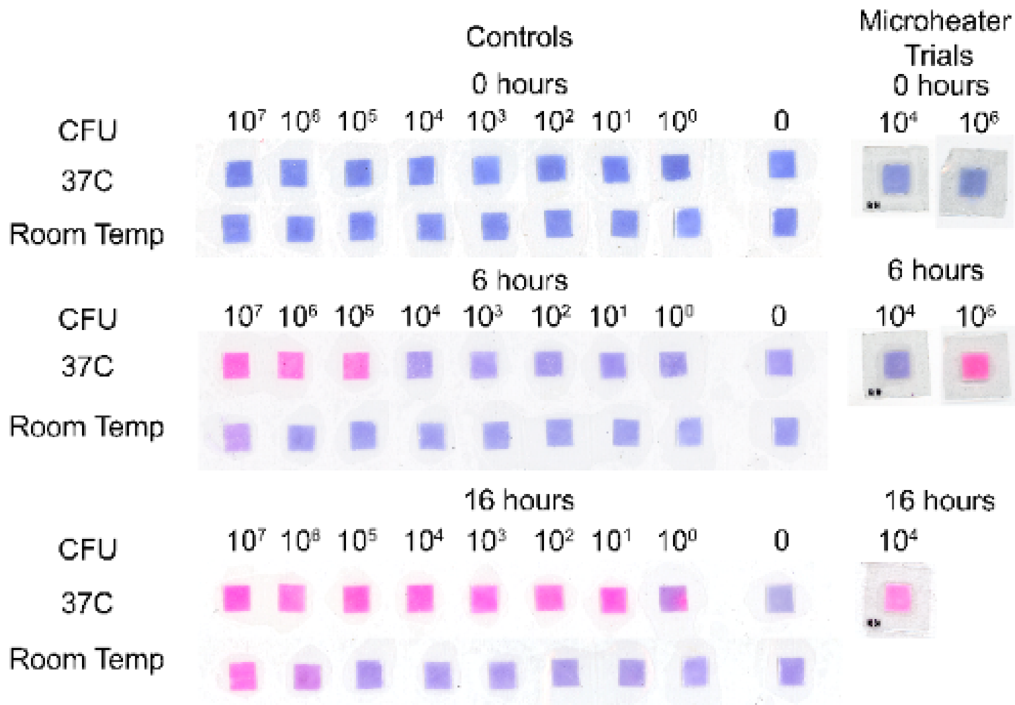



**Figure S9:** Detection of serial dilutions of *E. coli* using PrestoBlue™. LOD of 37°C after 6 hours is $10^5$ colony-forming unit (CFU). After 16 hours limit of detection (LOD) of 37°C is $10^1$ CFU. LOD of room temperature samples was $10^6$ CFU after 16 hours. Design A microheater (8 Ohms) was used for these trials. Color change for microheater incubations closely match incubator results while room temperature samples did not. Incubator and microheater performance data suggest the microheater incubation could have a similarly low LOD of $10^1$ CFU.

## REFERENCES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