Electronic supplementary information for

Centrifugal microfluidic lab-on-a-chip system with automated sample lysis, DNA amplification and microarray hybridization for identification of enterohemorrhagic *Escherichia coli* culture isolates

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1 Sequences of oligonucleotide probes

Table S1 Sequences and modifications of oligonucleotide probes

Code	Sequence	5′ position	3′ position
p1	CAA CGC GGC CGC TTT TTT CCT TTT TAA	Cy3	$-NH_2$[a]
p2	CGT GCT GCC TGC CGG AGC AGG CGC AAC	Cy5	$-NH_2$[a]

[a] In conjunction with an internal hexa-ethylene glycol spacer.

Table S2 Oligonucleotide probe[a] sequences used in the EHEC hybridization assay

Probe	Sequence (5′ → 3′)
O26	AAT TAG AAC CAT ACA AAG TTG GAG AAT ATA AAA GCC TGC TAT ATG CAA GC
O45	TAC GTC TGG CTG CAG GGA CTT TCG TTG CGT TGT GCA TGG TGG CAT GGG
O103	CGA ATG TTT TAG CCA TAT CCT CAT CGT TGT TAT CTA TGG TGG GCT TAG TT
O111	TCT TGT ATG TCT GAA TAT TAC CGG TTG TTT CAT CAA TCC TAA TTT TAA TA
O121	GGT CGT GAA ACA GCT CGC TAT CAT GGC GGG ACA ATG ACA GTG CTG GAC TAC A
O145	TTT GGT TTG GTG GTA CTG TGT CCG CGA GTG TGC TTG GAG TGG CTT ATA TT
O157	TAA AAC TAT TAC TAC AGG TGA AGG TGG AAT GGT TGT CAC GAA TGA CAA AA
VT1	ACT GGA TGA TCT CAG TGG GCG TTC TTA TGT AAT GAC TGC TGA AGA TGT TG
VT2	CAC ATA TAT CAG TGC CCG GTG TGA CAA CGG TTT CCA TGA CAA CGG ACA GC
EAE	ACA GTT CCG AAA GCG AAA TGA TGA AGG CTG GAC CTG GTC AGC AGA TCA TT
IAC	CAT AAT ATC ACT CGC GTC CGT TGA AGC TTA CGA TTT CGT C

[a] Oligonucleotide probes are amino-modified at the 5′ position (in conjunction with an internal hexa-ethylene glycol spacer) to mediate covalent attachment on a CNBr-activated Zeonor ZF14-188 surface.

2 PCR assays

Table S3 Primers used in the multiplex EHEC PCR assay

Name	Sequence (5′ → 3′)
O26-F	CAC TCT TGC TTC GCC TGT TG
O26-R	CGC GCT TCT AAT TTC AGC GAT AC
O45-F	TAT GAC AGG CAC ATG GAT CTG TGG
O45-R	TTG AGA CGA GCC TGG CTT TGA TAC
O103-F	TGC TCT ATG CGC TCT TCC TC
O103-R	GCC AGA CAC CTG CAA CCG CA
O111-F	TAC AAG AGT GCT CTG GGC TTC
O111-R	AAC TAA GTG AGA CGC CAC CAG
O121-F	AGG CGC TGT TTG GTC TCT TAG A
O121-R	GAA CCG AAA TGA TGG GTG CT
O145-F	GCT GTG ATG CTA GTG TAT CCG A
O145-R	AAG CCT CGT AGT GCA AGG GC
O157-F	CGG ACA TCC ATG TGA TAT GG
O157-R	TTG CCT ATG TAC AGC TAA TCC
VT1-F	GTG GCA AGA GCG ATG TTA CGG TTT G
VT1-R	ATG ATA GTC AGG CAG GAC ACT ACT C
VT2-F	ACG AGG GCT TGA TGT CTA TCA GGC G
VT2-R	GCG ACA CGT TGC AGA GTG GTA TAA C
EAE-F	GAC CCG GCA CAA GCA TAA GC
EAE-R	CCA CCT GCA GCA ACA ACA GG
IAC-1	CAT AAT ATC ACT CGC GTC CGT TGA AGC TTA
IAC-2	GAC GAA ATC GTA AGC TTC AA

Table S4 PCR reaction mixture

Master mix components	Concentration (mM)	Volume per reaction (μL)
Water		20.5
Buffer[a]		10
$MgCl_2$[b]	25	3
dATPs[c]	2	2.5
dTTPs[c]	2	2.5
dGTPs[c]	2	2.5
dCTPs[c]	1	4
dCTP-Cy3[c]		1
Primers		3
Polymerase[d]		1
gDNA[e]		1

[a] Solution containing 500 mM KCl (Sigma-Aldrich) and 100 mM of Tris-HCl, pH 8.0 (Sigma-Aldrich). [b] Thermo Fisher Scientific. [c] Invitrogen. [d] Q5 High-Fidelity Polymerase (New England BioLabs). [e] A concentration of 10 ng per reaction was used for bench-top PCR.

Table S5 Primer ratios used in PCR reactions

Primers	Concentration (μM)	Volume (μL)
O45 (F&R)	50	5
O103 (F&R)	50	5
O111 (F&R)	50	5
O145 (F&R)	50	5
VT1 (F&R)	50	5
VT2 (F&R)	50	5
O26 (F&R)	50	7.5
O121 (F&R)	50	7.5
IAC (1&2)	50	7.5
EAE (F&R)	50	10
O157 (F&R)	50	25

Table S6 PCR thermal cycling parameters

Step	Temperature (°C)	Duration (s)
Initiation	97	180
Denaturation	97	15
Annealing	57	10
Extension	72	20

3 XPS analysis of Zeonor substrates

The XPS spectra shown in Fig. S1 reveal the changes in surface composition associated with oxygen plasma treatment of the Zeonor substrate. A C 1s peak at a binding energy of 285.0 eV is revealed as the sole signal for the pristine substrate (Fig. S1A). This finding is expected for a polymer that mainly consists of aliphatic hydrocarbon groups. The high-resolution spectrum suggests that residues of unreacted olefin remain present at the surface. After exposure of the substrate to oxygen plasma, a peak related to O 1s appears at 533.0 eV (Fig. S1B), providing evidence of oxygen enrichment on the surface. For a sample stored at ambient conditions over several days, we determined an O/C ratio of 0.29 based on peak integration and atomic sensitivity factors (for X-ray sources at 57.4°) of 0.711 and 0.296 for O 1s and C 1s, respectively.[1] The satellite peaks emerging at the high-energy side of the hydrocarbon signal indicate heterogeneity in the chemical composition of the surface, which is in agreement with published literature.[2–6] Line deconvolution reveals the presence of (at least) four different functionalities for which binding energies and signal proportions are detailed in Table S7. The peak at 185.7 eV corresponding to C–O moieties accounts for 12.8% of the overall carbon signal. However, this peak also accounts for ether groups present at the surface. Derivatization of hydroxyl groups with a marker molecule such as trifluoroacetic anhydride has been shown to provide an effective way to elucidate their actual percentage on the surface using XPS measurements.[4,5] However, no such effort has been undertaken in this study. According to literature reports, the density of –OH groups can vary depending on the chemical composition of the polymer and the conditions used for plasma oxidation.[5,7] Hydrogen abstraction which results in cross-linking or etching thereby constitutes a limitation to incorporating hydroxyl groups on polymer surfaces using oxygen plasma treatment.[3,6]

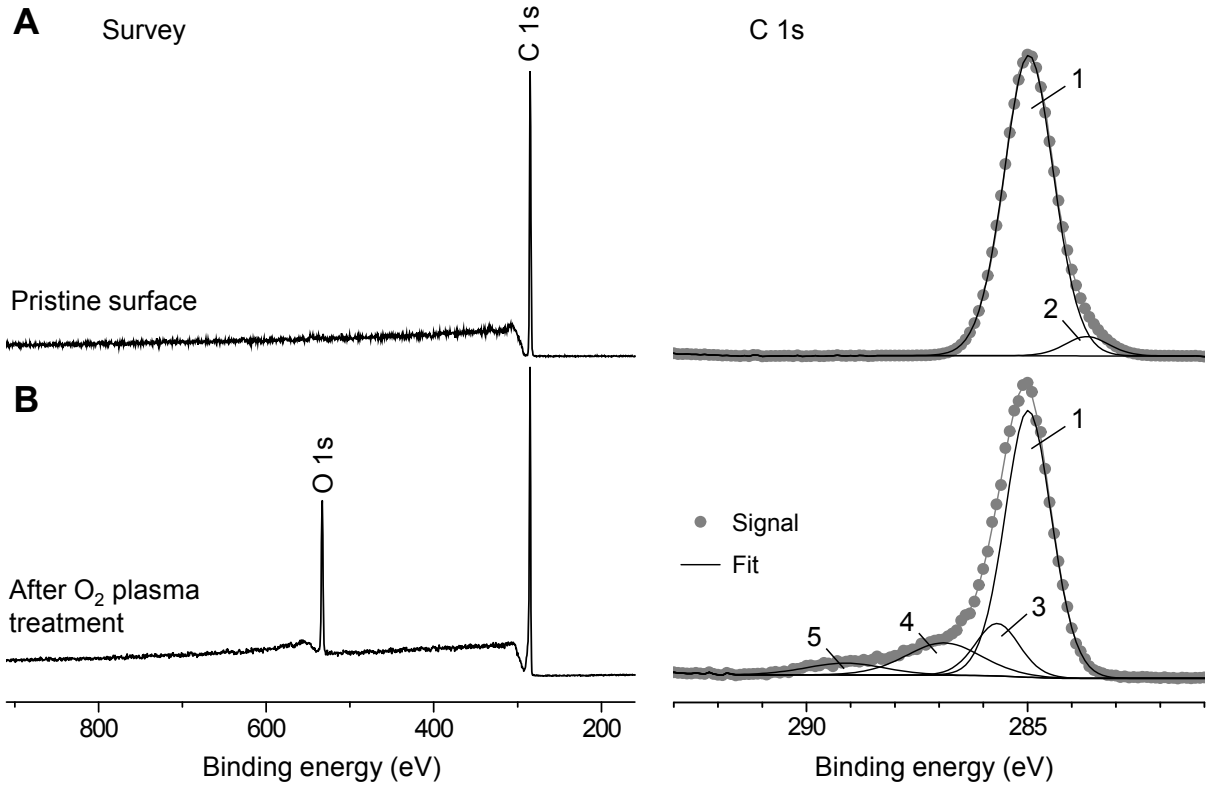

Fig. S1 XPS spectra of Zeonor ZF14-188 before and after O_2 plasma treatment. Survey and C 1s high-resolution spectra acquired before (A) and after exposure to oxygen plasma (B). Fit curves were assigned as follows: (1) aliphatic carbon (C–C); (2) unsaturated carbon (C=C); (3) carbon attached to one oxygen (C–O); (4) carbon double bonded to oxygen (C=O) or attached to two oxygen atoms (O–C–O); and (5) carbon in carboxylic acids (COOH) or esters.

Table S7 Line deconvolution of the C 1s signal for O_2 plasma-treated Zeonor ZF14-188

Peak #	Binding energy (eV)	Peak area (%)	Assignment
1	285.0	68.7	Aliphatic carbon
3	285.7	12.8	Carbon bonded to hydroxyl or ether groups
4	286.9	13.4	Carbon in ketones
5	289.1	5.1	Carbon in carboxylic acid groups

4 Fabrication of µCSs

Elastomeric open through-hole µCSs (Fig. 3B) were fabricated from Versaflex CL30 (GLS Corp., McHenry, IL) using hot embossing.[8] The mold was prepared by photolithography using two levels of SU-8 resist (GM1040, Gersteltec) on a 4″ silicon wafer (Silicon Quest International). The wafer was first baked on a hot plate at 200 °C for 10 min. SU-8 resist was applied through spin coating, which was followed by a pre-bake at 65 and 95 °C for 5 and 15 min, respectively, using a temperature ramp of 2 °C/min. Resist was exposed to UV light with a wavelength of 365 nm (Hg i-line) at 280 µJ/cm^2 using two complementary, high-resolution transparency photomasks (FineLine Imaging) on a 6200 mask aligner (EV Group). The post-exposure bake was done using the same conditions as for the pre-bake. Resist features were developed in PGMEA for 2 min. The wafer was rinsed with PGMEA and isopropanol and dried with a stream of nitrogen gas. Resultant resist pattern was hard-baked at 130 °C for 2 h. Finally, the SU-8 master mold was coated with a thin, anti-adhesive layer formed from 1*H*,1*H*,2*H*,2*H*-perfluorooctyl-trichlorosilane (Sigma-Aldrich) using deposition from the vapor phase under reduced pressure. Versaflex CL30 (received in the form of pellets) was extruded at a temperature of 165 °C to form sheets of several meters in length, ~15 cm in width and 150 µm in thickness. For the fabrication of the open through-hole membranes, a circular piece (4″ in diameter) was cut from the extruded, 150-µm sheet and placed between the SU-8 mold and an unstructured counter plate. The counter plate consisted of a silicon wafer coated with a thin (20 to 100 µm) layer of PDMS deposited by spin-coating a degassed prepolymer mixture of 10:1 (w/w) followed by curing at 200 °C for 2 h. Hot embossing was performed using an EVG520 system at 170 °C, an applied force of 10 kN, and a pressure of 10^{-2} mbar.

5 Patterning of oligonucleotide probes

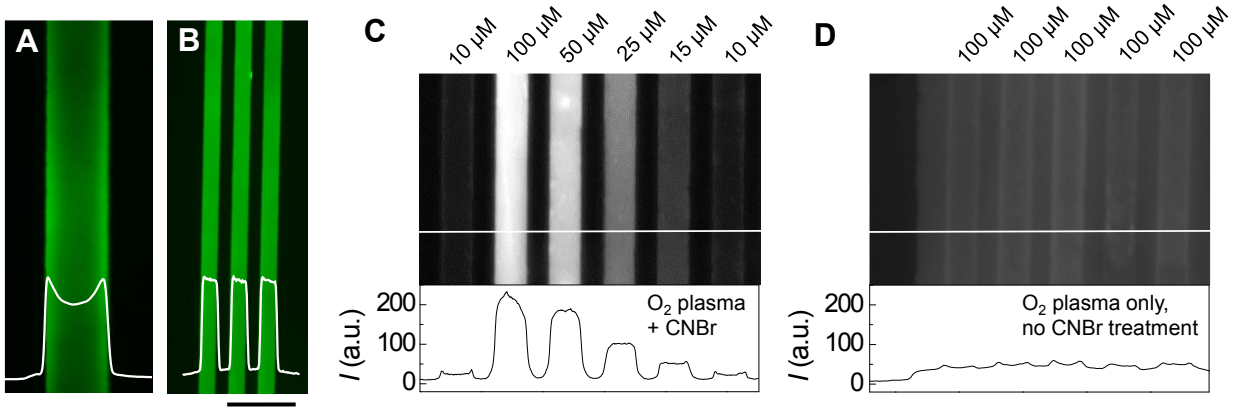

Fig. S2 Fluorescence micrographs and intensity profiles of lanes produced by patterning Cy3-conjugated oligonucleotides (p1) on Zeonor ZF14-188 substrates. (A, B) Lanes obtained by using a µCS with larger and smaller channel widths. Edge effects are commonly observed for wider channels. Scale bar: 200 µm. (C) Gradient pattern obtained by incubation of the substrate with solutions containing five different concentrations of p1 (e.g., ranging from 10 to 100 µM in PBS). (D) Control sample produced on an oxygen plasma-treated Zeonor ZF14-188 substrate without CNBr activation using a 100 µM solution of p1 in PBS.

6 Thermal interface

Table S8 Characteristics of thermal interface materials tested in this work

Interface	h_t (μm)	κ_t (W/m K)	$\frac{h_t}{\kappa_t}$ $(10^{-6}\ K/W)$	Comments
None (air gap)	Variable (~100)	0.024	4166	Poor reproducibility
Tgrease 2500 Thermal Grease[a]	Variable (~100)	3.8	26	High viscosity (2.5×10^6 cP); slow creep during heating and rotation of the platform (800 rpm)
Silicone Heat Sink Compound[b]	Variable (~100)	0.92	108	Low viscosity; rapid creep during heating and rotation of the platform (800 rpm)
Ti900 Thermal Pad[c]	120	1.8	66.7	Good adhesion; expensive
PC99 Thermal Pad[c]	60	6.0[d]	10	Good adhesion; risk of air pocket formation
Li98CN Thermal Tape[c]	180	2.0	90	Good adhesion, relatively easy to apply
T62-1 Graphite Sheet[c]	160	15 in *z*-direction (400 in *x*- and *y*-directions)	10.7[e]	Poor adhesion; one side adhesive only; hardness 80 (Shore A)
Bergquist Gap Pad 3000S30[f]	500	3.0	166.7	Poor adhesion; very soft (hardness 30, Shore 00)

[a] Laird, London, UK. [b] Techspray, Kennesaw, GA. [c] t-Global Technology, Lutterworth, UK. [d] Estimated from the thermal resistance (at 30 psi) and tape thickness values disclosed in the product datasheet. [e] Corresponds to the *z*-direction only. [f] Henkel, Düsseldorf, Germany.

7 Numerical modeling of heat transfer

2D finite element transient numerical analysis of heat transfer was done by simulation using COMSOL Multiphysics Modeling Software (COMSOL, Inc., Burlington, MA). Materials and parameters considered in the analysis are detailed in Table S9.

Table S9 Materials and properties used in the numerical heat transfer analysis

Material	Thickness (μm)	Density (kg/m^3)	Thermal conductivity (W/m K)	Heat capacity (J/kg K)
Aluminum	1250	2700	237	897
Thermal interface (variable)	100	2500	0.02–3.8	1200
Zeonor	188	1010	0.1	1300
TPE	1000	890	0.25	1300
Teflon	n.a.	2200	0.25	1172
Water	100 (hybridization chamber) 500 (PCR chamber)	998	0.58	4183
Air	100	1.205	0.0257	1005

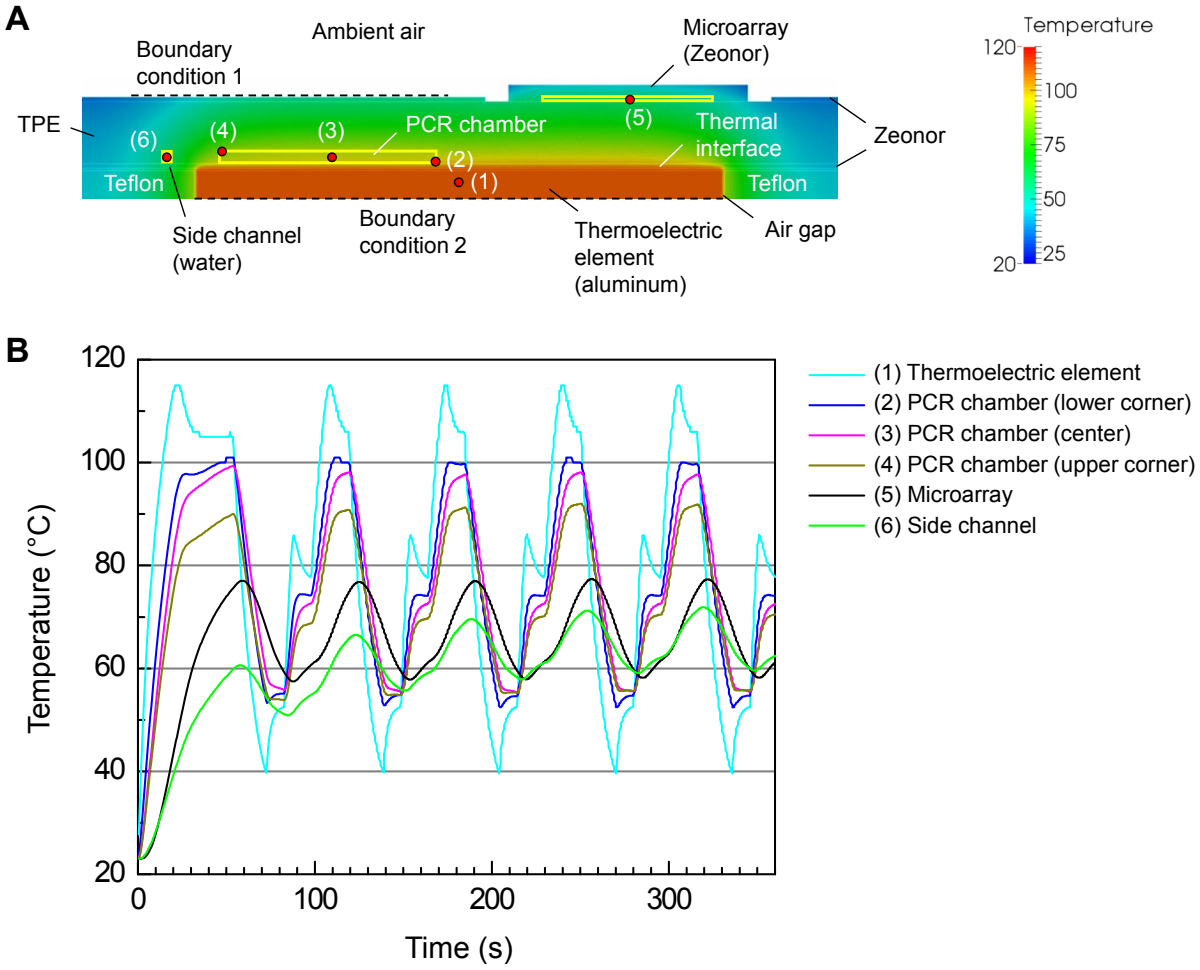

Fig. S3 Simulated heat distribution inside the cartridge. (A) Representation of the 2D model (cross-sectional view) used to calculate the respective temperature gradients during heating and cooling cycles. The image depicts the system when the thermoelectric element reaches the set-temperature of 115 °C. Locations of interest include (1) the thermoelectric element; (2) the lower right-hand corner, (3) the center, and (4) the upper left-hand corner of the PCR chamber (filled with water); (5) the DNA microarray (hybridization chamber filled with water); and (6) a small side channel (further apart from the thermoelectric element). For boundary condition 1, the temperature was derived from an experimental measurement (variable over time). For boundary condition 2, an individual convection heat transfer coefficient of 100 W/m^2 K was considered for air flowing at 10 m/s above the cartridge. (B) Plot of the calculated temperature profiles for each of the six locations. A thermal resistance of 3.3 K cm^2/W was considered for the interface.

8 Testing of bacterial viability

The viability of cells was determined using LIVE/DEAD *Bac*Light Bacterial Viability Kit (L7012; Thermo Fisher Scientific, Waltham, MA). Stains A and B were thawed at RT. Bacterial suspensions were prepared by pipetting 100 µL of lysate, 50:50 (v/v) mixture of lysate and control cells, and control cells into duplicate wells of a black 96-well flat-bottom microplate. 6 µL of Component A stain was mixed with 3 µL of Component B in a borosilicate glass culture tube. 2X stain solution was prepared by adding the 9 µL of component mixture to 2.0 mL of nuclease-free ultrapure water (Invitrogen, Waltham, MA). The solution was vortexed for 10 s. 100 µL of the 2X stain was pipetted into each well, followed by gentle mixing. The plate was covered with aluminum foil and incubated for 15 min at room temperature. Fluorescence measurements were obtained using a Synergy 2 Multi-Mode Microplate Reader (BioTek, Winooski, VT).

Table S10 Viability test for *E. coli* O157:H7

Sample[a]	Green fluorescence, 520 nm (a.u.)[b]		Red fluorescence, 620 nm (a.u.)[b]	
	Test 1	Test 2	Test 1	Test 2
Lysate from cartridge	41 710	38 026	53 342	50 800
50:50 (v/v) mixture of lysate (cartridge) and live control sample	64 680	62 894	49 988	50 002
Lysate from heat block	40 817	36 981	50 408	51 186
50:50 (v/v) mixture of lysate (heat block) and live control sample	57 528	58 006	50 442	50 768
Live control sample	70 476	69 063	48 844	48 600

[a] Samples were prepared from suspensions containing 1×10^6 cfu/mL. [b] Fluorescence values are corrected against background from empty wells on the same plate. A total of 10 measurements was performed for each data point.

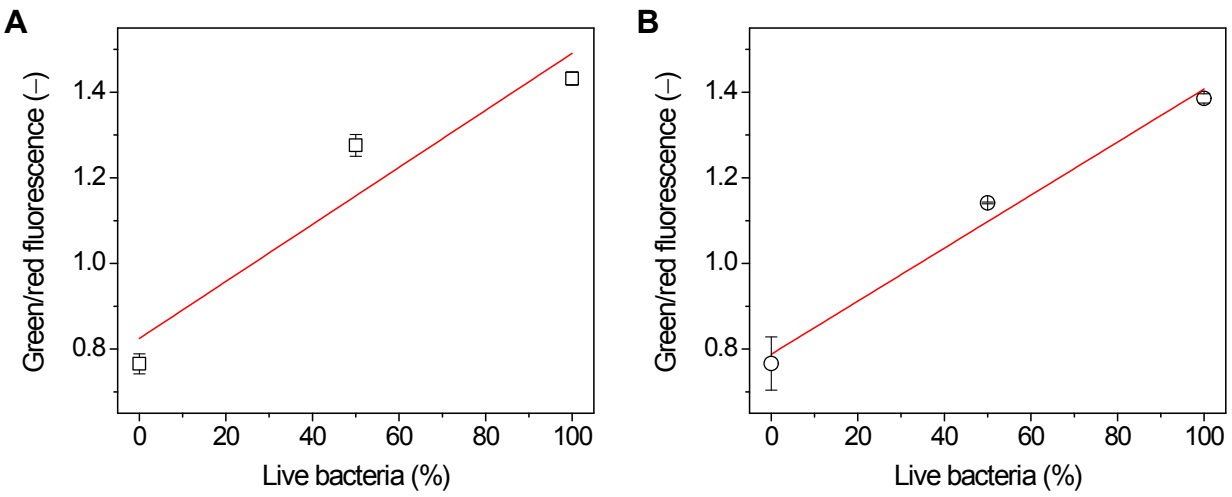

Fig. S4 Plot of green/red fluorescence ratios as a function of the percentage of live bacteria derived from the viability test in Table S10. Lysates were produced (A) on the microfluidic cartridge and (B) on the heat block. The lines represent a linear fit of the respective data. Slopes of the fit curves correspond to 0.0067 ± 0.002 (R^2 = 0.828) and 0.0062 ± 7.59 × 10^{-4} (R^2 = 0.970), respectively.

9 Hybridization assays

Table S11 Identification key for EHEC microarray hybridization assays

Serovar	Microarray signal										
	VT1	VT2	EAE	IAC	O26	O45	O103	O111	O121	O145	O157
O26	x		x	x	x						
O45				x		x					
O103	x		x	x			x				
O111	x		x	x				x			
O121		x	x	x					x		
O145	x		x	x						x	
O157	x	x	x	x							x

A stock solution of 2X hybridization buffer was prepared by adding 800 µL of a 10% (w/v) SDS solution (Sigma-Aldrich) and 4.0 mL of a 10% (w/v) *N*-lauroyl sarcosine sodium salt solution (Sigma-Aldrich) to 60 mL of DI water. The mixture was then combined with 100 mL 20X SSC solution (VWR International), followed by adding Bio-Rad protein blocking reagent. The solution was stirred vigorously for 1 h (until well dissolved) before water was added to obtain a total volume of 200 mL.

Off-chip hybridization assays were performed using an elastomeric µCS that was re-enforced on the top surface using a hard thermoplastic polymer layer (Zeonor 1060R, 1 mm in thickness) in order to fit the system with metallic connectors that were glued into inlet ports. In this way, solution can be infused into the channels with the help of a syringe pump and elastic tubing.

Proof-of-concept demonstrations were performed with Cy3-conjugated *E. coli* gene targets (obtained by multiplexed PCR amplification performed off-chip) on plastic supports (ZF14-188) using a micromosaic-like assay format.[9,10] To effectuate hybridization, 3 µL of each

target solution was combined with 3 μL of 2X hybridization buffer in a tube. The tube was heated at 100 °C for 2 min in a heat block to denaturate the amplicons. The entire volume was then injected into the microfluidic hybridization unit (using a micropipette) and incubated with the array at 42 °C. The liquid samples were moved manually (using a micropipette) until the entire volume has passed across the array. Each channel was rinsed with 6 μL of 0.5X SSC buffer (VWR International) followed by DI water at the end. Upon removal of the μCS from the Zeonor ZF14-188 substrate, the microarray was inspected using fluorescence microscopy.

10 References

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