Supporting Information
For
Microfluidic Inverse Phase ELISA via Manipulation of Magnetic Beads

Hong Chen†, Assem Abolmatty‡∗, Mohammad Faghri†§

†Department of Mechanical, Industrial, and Systems Engineering,
University of Rhode Island, Kingston, RI 02881, USA

‡Department of Plant Sciences,
University of Rhode Island, Kingston, RI 02881, USA

§To whom correspondence should be addressed;
E-mail: faghri@egr.uri.edu; Phone: 401-874- 5180; Fax: 401-874-2355

1 Online video materials

On-line video materials recording the encapsulation of water, the manipulation of magnetic beads and the parallelizing μIPELISA are provided by the following links.

- Encapsulation of water
  (http://www.egr.uri.edu/~hchen/Site/videos/encap.html)
- Manipulation of magnetic beads
  (http://www.egr.uri.edu/~hchen/Site/videos/manip.html)
- Manipulating μIPELISA in multiple microchannels
  (http://www.egr.uri.edu/~hchen/Site/videos/multiplex.html)

∗A. Abolmaaty is affiliated with Food Science department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, as an assistant professor of Food Microbiology, and is currently on sabbaticals at the University of Rhode Island.
Figure S-1: (a) Image of PCR products taken after gel electrophoresis. Lane 1, DNA ladder; Lane 2 to 6, PCR product yielded by $2.5 \times 10^5$, $2.5 \times 10^3$, 25, 2.5, 1.25 copies of the DNA template per PCR reaction. (b) Plot of Dig-dsDNA concentration against DNA templates per PCR reaction.

2 Preparation of biotinylated Dig-dsDNA via PCR

*E. coli* O157:H7 (strain EDL933) was cultured in 50 mL of tryptic soy broth plus 0.5% (w:v) glucose at 37 °C in 250 ml flasks with rotary agitation (200 RPM). Exponentially growing *E. coli* O157:H7 ($1 \times 10^9$ CFU mL$^{-1}$; CFU, colony-forming unit) was harvested by centrifuging broth cultures at 10,000g for 10 min at 4°C. Pellets were washed twice in saline and were then resuspended in DI water for lysis. 100-µL cell suspensions (125, 250, $2.5 \times 10^3$, $2.5 \times 10^5$, $2.5 \times 10^7$ CFU mL$^{-1}$) were lysed using TZ buffer (2.0% Triton X-100 in 0.1 M Tris-HCl buffer plus 2.5 mg/mL sodium azide, pH 8.0) as described by Abolmaaty *et al.* (2000).

To prepare biotinylated digoxigenin-labeled dsDNA (Dig-dsDNA), 10 µL of DNA sample, isolated from different cell concentrations as described above, was added to 90 µL of PCR mixture. The PCR mixture consisted of 2.5 U hot start polymerase (Promega, Madison, WI; U, enzyme unit), 1X PCR reaction buffer (Promega, Madison, WI), 1 mM MgCl$_2$, digoxigenin labeling nucleotide mix (Roche applied science, Mannheim, Germany; 10 mM each dNTP; dNTP, deoxyribonucleotide triphosphate), 250 nM biotinylated forward primer and 250 nM reverse primer in nuclease-free water (Integrated DNA Technology, Coralvilb, IA). The biotinylated forward primer and the reverse primer, listed in Table S-1, were designed for the published nucleotide sequence (424th bp to 603rd bp) of intimin gene (GenBank no. U32312) of *E. Coli* O157:H7. The PCR cycling was conducted on a thermal cycler (Mastercycler ep Gradient S, Eppendorf, Westbury, NY), consisting of initial denaturation at 95 °C for 2 minutes followed by 35 cycles with denaturation at 94 °C for 2 min, annealing at 55 °C for 1 min and extension
Table S-1: Primer sequences and the length of the corresponding amplicon.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>(forward) biotin-5’-GAGCACAATCGCTGTTGTTAGCGT-3’</td>
<td>180 bp</td>
</tr>
<tr>
<td>(reverse) 5’-TGTCGCTTGAACTGATTTCCTCT-3’</td>
<td></td>
</tr>
</tbody>
</table>

Figure S-2: Measurement of the contact angle at side walls. Dashed line, fitting circle of the receding meniscus.

at 72 °C for 1 min. Final extension was performed at 72 °C for 5 min. PCR product (4 µL) was subjected to 2.0% (w:v) agarose gel electrophoreses containing 1 µg mL⁻¹ of ethidium bromide in the electrophoresis buffer (0.2 M tris base, 0.1 M sodium acetate, 0.01 M EDTA, pH 7.8), and digital images of the gel was then captured using an imaging system (BIO-RAD Universal Hood II ChemiDoc™ XRS molecular imager), as shown in Figure S-Ia. The remaining PCR product (96 µL) containing biotinylated digoxigenin-labeled dsDNA was then purified using a PCR purification kit (QIAGEN, Valencia, CA), and was reconstituted in 50 µL of TE buffer (Integrated DNA Technology, Coralville, IA). The concentration of the purified product was measured, using a spectrophotometer (Nanodrop, Thermo Scientific, Wilmington, DE). Overall, PCR reaction containing 1.25, 2.5, 25, 2.5 × 10⁵, 2.5 × 10⁶ copies of the DNA template at the initial yielded 2.59, 2.82, 6.09, 7.78, 14.85 ng µL⁻¹ of purified biotinylated Dig-dsDNA (Figure S-Ib; data are fitted as dashed line, using \( y = 0.2813 \times x^{0.1371}; R^2 = 0.966 \)), respectively.

3 Measurement of the receding contact angle

A circle (dashed line in Figure S-I) diameter, 82 pixel) was fitted to the curve of the projected receding meniscus. By assuming that the meniscus was symmetrically aligned in the microchannel (width, 61 pixel), the contact angle (48 °) was calculated as the angle from the side wall to the the tangent which was at the intersection of the fitted circle and that side wall.
4 Estimation of biotin binding capacities of streptavidin-coated magnetic beads and microplate

For magnetic beads (MBs), the biotin binding capacity was 0.77 pmole mm\(^{-2}\) (converted from the manufacturer’s datasheet). The total surface area of 4-µL suspensions of MBs (3.325 \(\times\) 10\(^5\) µL\(^{-1}\)) to be mixed with Dig-dsDNA was 40.7 mm\(^2\). Thus, 4-µL suspensions of MBs can bind 31.34 pmole of biotin. For a microplate, the typical biotin binding capacity was found in the range of 0.067 to 2 pmole mm\(^{-2}\) (Välimaa and Laurikainen, 2006). The bottom area of the well, which was assumed to be the main place capturing biotinylated Dig-dsDNA, was 40.1 mm\(^2\) (well diameter, 7.15 mm). Thus, the well can bind 2.69 to 80.2 pmole of biotin. The maximum dose of Dig-dsDNA (0.43 pmole, which is equivalent to 49.5 ng of 180-base-pair Dig-dsDNA) was far less the binding capacities of these surfaces.

References
