Supplementary information for:

DNA extraction from bacteria using a gravity-driven microcapillary siphon

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1. SUPPLEMENTARY METHODS

1.1. Calculating DNA recovery efficiency from *E. coli* K12 MG1655 in PBS.

The recovery efficiency was calculated as follows:

Recovery efficiency = observed DNA concentration / expected DNA concentration (S1)

The observed DNA concentration was given by the experimental results obtained with NanoDrop™ 2000 spectrophotometer. For a more accurate determination of the recovery efficiency, we used the normalised DNA concentration to eliminate any noise interference. The normalised concentration was given by the average DNA concentration in the bacterial samples minus the average value given by the negative controls due to the presence of co-purified contaminants into the samples.

The expected DNA concentration was determined as follows. Firstly, the molecular weight of *E. coli* genome was calculated multiplying the average molecular weight of a nucleotide base pair by the length of the genome and adding 157.9 g/mol to the total:

Molecular weight = (607.4 g/mol x 4,639,221) + 157.9 g/mol = 2,817,862,993.30 g/mol (S2)

The number of moles of *E. coli* genome copies (gc) in 1 ng of DNA were calculated by diving 1 x 10^{-9} (units: g) by the molecular weight of the genome:

Moles of gc in 1 ng = (1 x 10^{-9} g / 2,817,862,993.30 g/mol) / 1 ng = 3.55 x 10^{-19} mol/ng (S3)

Next, gc of *E. coli* in 1 ng of DNA were calculated multiplying the number of moles by the Avogadro's constant:

gc in 1 ng = 3.55 x 10^{-19} mol/ng x 6.022 x 10^{23} mol^{-1}= 213,708.05 ng^{-1} (S4)

It was assumed that 1 colony-forming unit (CFU) contains one gc; since 213,708.05 gc have a mass of 1 ng, then 213,708.05 CFU contain 1 ng of genomic DNA (213,708.05 CFU/ng) as per equation (S4). For the manual protocol, the expected concentration in ng/µl was calculated by multiplying the bacterial CFU per ml by the sample volume loaded for the protocol (0.2 ml) and dividing by 213,708.05 CFU/ng. Finally, it was divided by the volume of elution buffer used for the DNA recovery (200 µl):

DNA expected (manual) = (2.325×10^9 CFU/ml $\times$ 0.2 ml / 213,708.05 CFU/ng) / 200 µl = 10.88 ng (S5)

For the microcapillary siphons, we initially evaluated the total CFU of bacterial loaded through the microcapillaries. This was calculated multiplying the bacterial CFU per ml by the sample volume loaded for the protocol (0.05 ml). The mix for the bacteria lysis and DNA binding (bacterial sample, proteinase K, lysis buffer, binding buffer, Magazorb reagents with magnetic beads) resulted in a volume of approximately 200.0 µl; however, only 88.5 µl of this volume were confirmed to flow through the siphon during the priming and enrichment step of 120 seconds, therefore the bacterial CFU used for the microfluidic protocol were multiplied by a factor 88.5 µl / 200.0 µl:

CFU in microfluidic siphon = (2.325×10^9 CFU/ml $\times$ 0.05 ml) x 88.5 µl / 200 µl = 5.14×10^7 CFU (S6)

The estimated CFU were divided by 213,708.05 CFU/ng (gc in ng) and finally divided by the volume of elution buffer used for the DNA recovery (48.0 µl):

DNA expected (microfluidic) = (5.14×10^7 CFU / 213,708.05 CFU/ng) / 48.0 µl = 5.01 ng (S7)

2. SUPPLEMENTARY RESULTS

2.1. Hydrophilic coating of FEP-Teflon® microcapillary films

The inner surface of FEP-Teflon® microcapillary films was coated with high-molecular weight PVOH as described in section **2.3** of the main manuscript. The efficiency of the coating was evaluated by dipping a 10 mm microcapillary strip into a cuvette filled with 1 ml of distilled water. The rise of the

water was measured in each microcapillary from the liquid level as equilibrium liquid height (H_{eq}) using a ruler (**Fig. S1**). The experiment was done in triplicate.

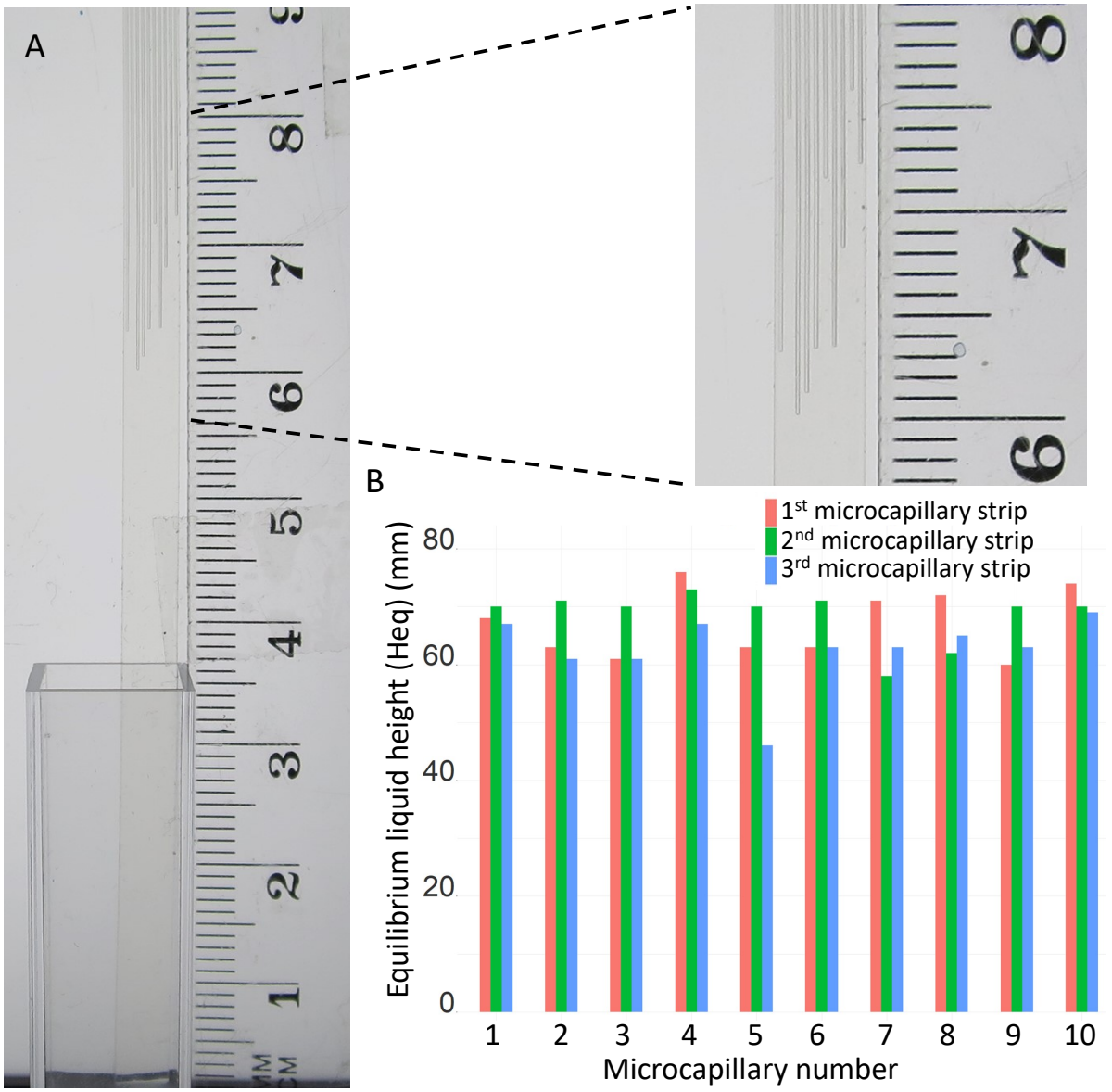

Fig. S1. (A) Rising of distilled water in hydrophilic microcapillaries. (B) H_{eq} of liquid rise from water level per individual microcapillary. The experiment was performed for 3 microcapillary strip, and each strip was made of 10 individual microcapillaries, for a total of 30.

2.2. Reagents' flow rate at different duration of beads' isolation step

Flow rates for washing and elution buffers were calculated at different durations of isolation step by weighting the volume of the fluid using a laboratory scale. For this experiment, 105 mm-long microcapillary siphon were used configured with a *H*/*L* of 1.75, where H is the height difference between the top and bottom ends of the siphon and *L* is the length of the microcapillary strip. The siphon was primed with PBS only (no beads) prior to isolation step, where the magnetic particles were captured. By extending the isolation step, more beads were captured, decreasing flow rates for both washing and elution buffer (**Fig. S2**).

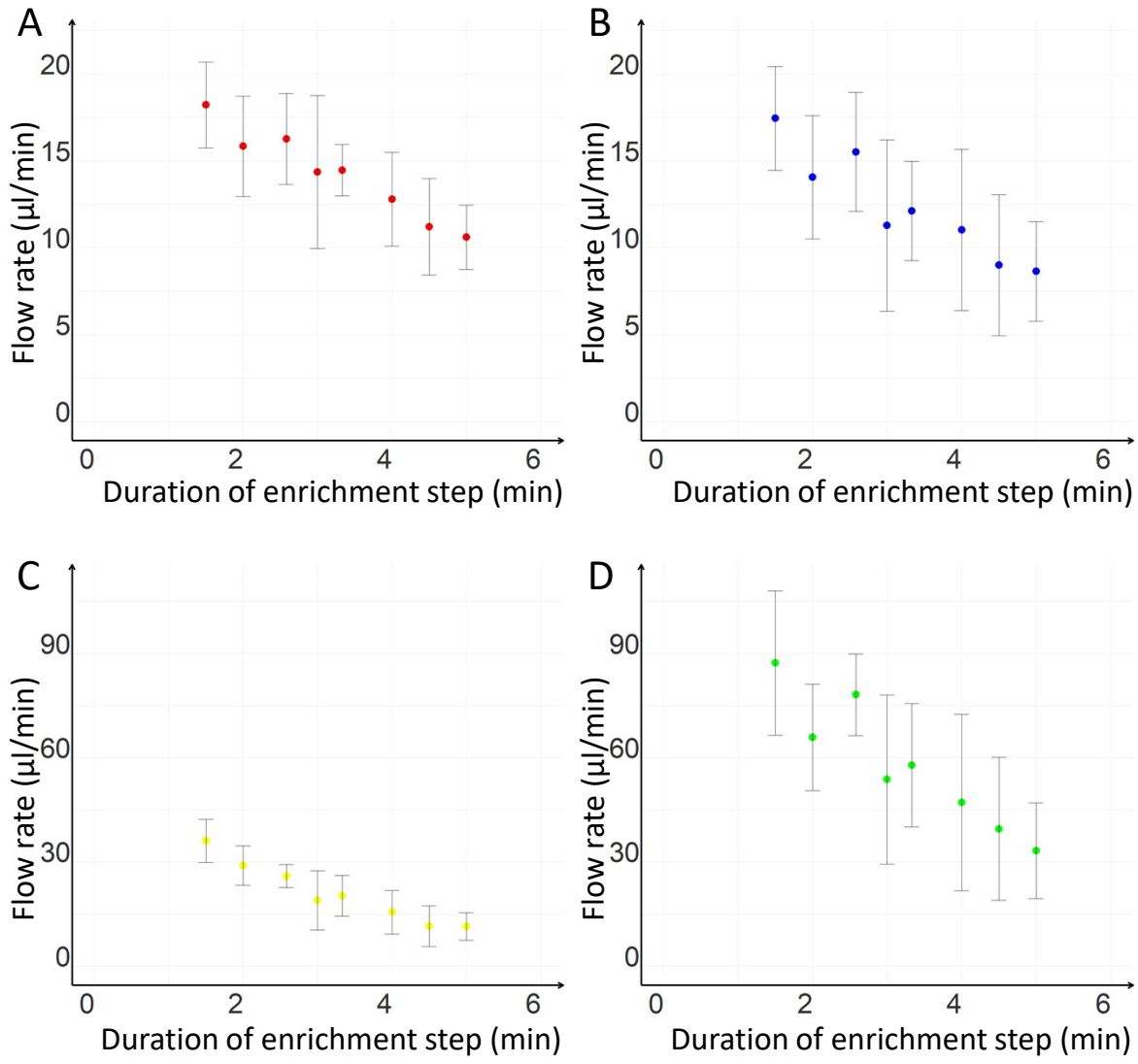

Fig. S2. Flow rates of washing buffer during (A) first and (B) second wash and for elution buffer during (C) first and (D) second elution at increasing duration of enrichment step.

2.3. Determination of particles' size

The size of the magnetic particles used in this experiment was determined on a Zetasizer instrument (Malvern Panalytical). The magnetic beads were diluted in distilled water, and three dilutions were tested (1:510, 1:1177, 1:2354). Each solution was tested in 5 technical replicates and outliers were identified and removed using the Tukey's fences method. The mean diameter obtained were between 130-260 nm, with particles size slightly lower at larger dilutions, likely due to fewer clusters (**Fig. S3**).

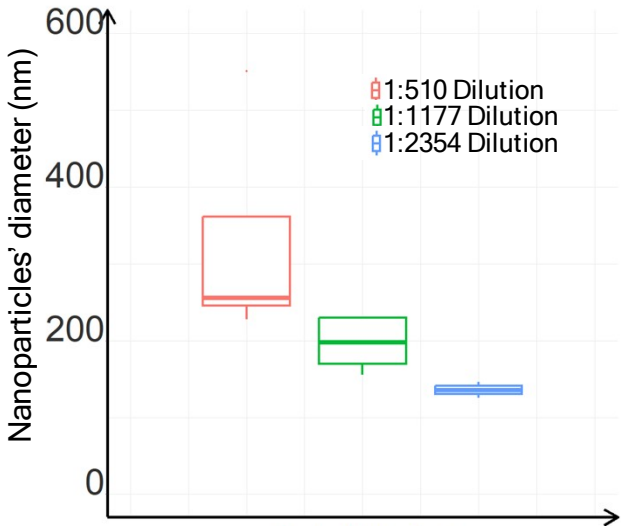

Fig. S3. Determination of nanoparticles' diameter (nm) in three dilutions with distilled water. The plot shows median values, interquartile range, min and max values.

2.4. Desing for operation of multiple siphons

The gravity-driven siphon is a suitable solution for multiplex testing, as shown in **Fig. S4**. Magnetic beads were simultaneously isolated in 8 microcapillary strips, with no increase in bulkiness and maintaining a suitable size for in-field testing. For this design, 72 mm-long microcapillary strips and Greiner Bio-One 8-well polystyrene round-bottom strip plates (Greiner Bio-One, 767001) were used. For the isolation of magnetic beads, two magnets (Promega, Z5332) were placed in a single case to easily remove and add the magnets when required.

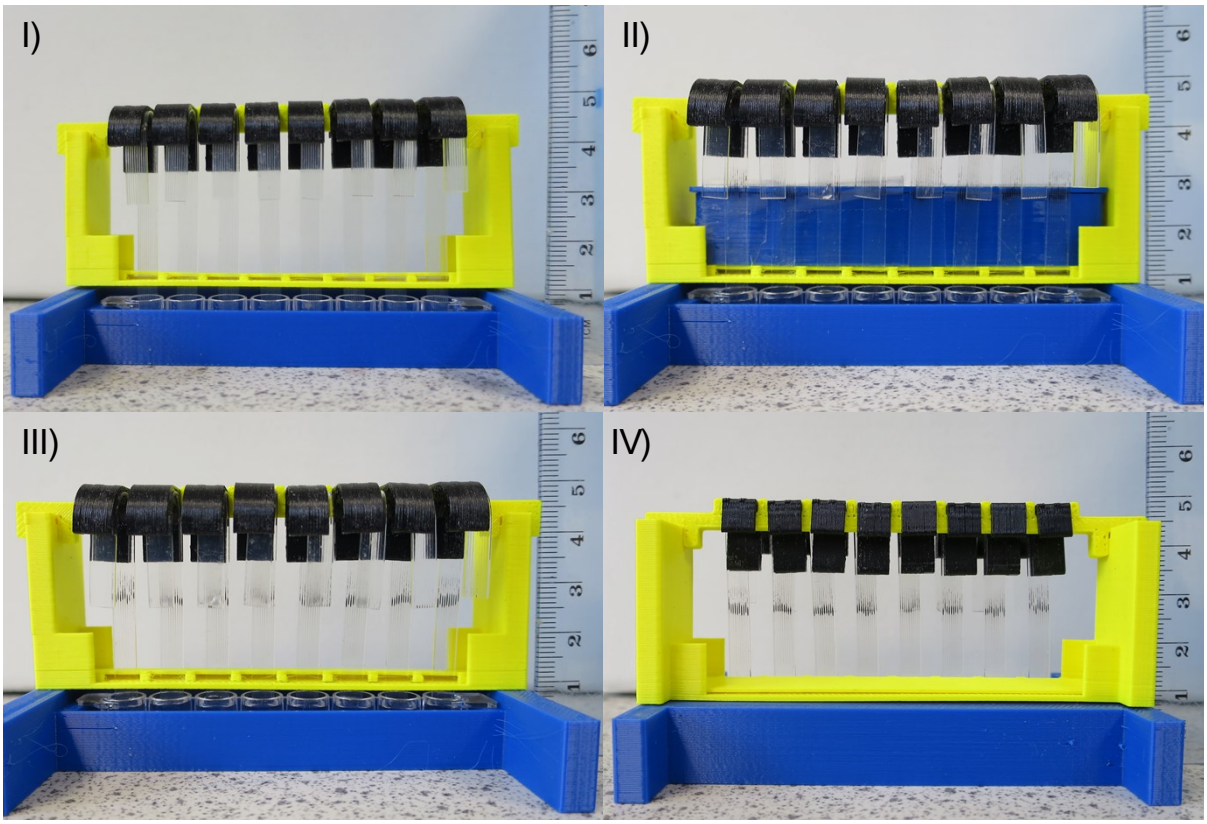

Fig. S4. Portable design for simultaneous operation of 8 gravity-driven microcapillary siphons. I) Empty microcapillary siphons. II) Siphons' holder for multiplex testing with magnets in place (blue case on yellow support) after magnetic particles isolation. III) Front view of the device showing captured particles. IV) Rear view of the device showing captured particles.

2.5. PCR and gel electrophoresis for extracted DNA

DNA was extracted from 2.325x10^9 CFU/ml *E. coli* K12 MG1655 in PBS using the protocol described in the main manuscript for the microcapillary siphon protocol. Sterile PBS was used as negative control and the experiment was repeated in triplicate for both bacterial and control samples. The same process was repeated through the standard manual extraction as per user manual. A PCR protocol was run to evaluate the integrity of the DNA, and the amplification products were run on 1% agarose electrophoresis gel. All reagents were used as detailed in the main manuscript. As shown in **Fig. S5**, all bacterial samples showed a bright band of the expected size (552bp), and all negative controls

were clear, proving the effectiveness of both microfluidic and manual protocol for DNA extraction. The size of the amplicon was confirmed using a DNA ladder.

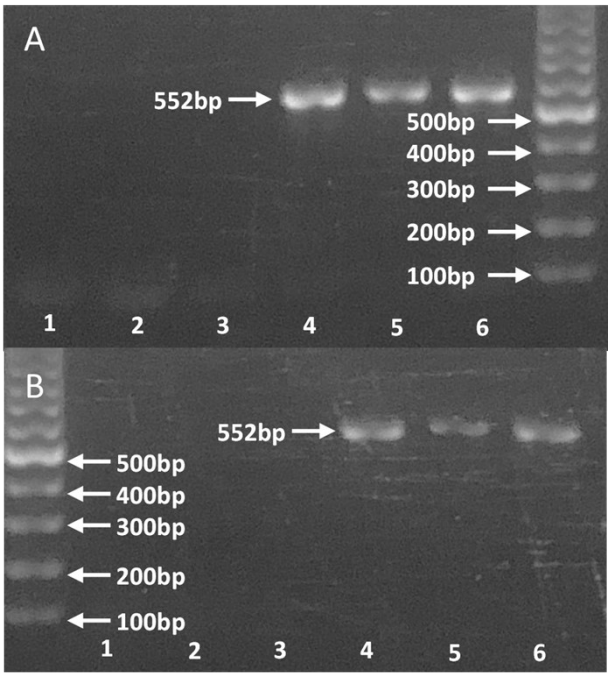

Fig S5. (A) Gel electrophoresis after microfluidic DNA extraction and amplification (lanes 1-3 for negative controls, lanes 4-6 for bacterial samples). (B) Gel electrophoresis after manual DNA extraction and amplification (lanes 1-3 for negative controls, lanes 4-6 for bacterial samples).

2.6. Melt-curves from real-time experiments

The real time experiments described in section **2.8** of the main manuscript were performed on a StepOnePlus Real-Time PCR (Applied Biosystems) using SYBR green to track DNA amplification. SYBR green does not discriminate between specific and non-specific amplified products; however, it was possible to detect the presence of non-specific amplifications conducting the melt curve analysis, that consists of increasing the temperature to denature the dsDNA and record the change of fluorescence over time. The denaturation of dsDNA causes changes in the fluorescence emissions, since SYBR green drastically decreases its fluorescence once released from the dsDNA. It is very common to observe non-specific amplification products during the last cycles of a real-time PCR protocol, particularly for samples with low analyte concentration. For this reason, melt curves should

be taken into consideration when analysing data from real-time PCR. The instrument performs 120 fluorescence readings during the melt curve analysis.

Fig. S6 shows the melt curves of DNA extracted from *E. coli* K12 MG1655 in PBS. As shown in the curves for the highest values of $\log_{10}$CFU, a clear peak between readings 90 and 94 is expected for the desired product. Negative control and 0.68 $\log_{10}$CFU showed multiple peaks, linked to non-specific amplification events. **Fig. S7** shows the melt curves of DNA extracted from *E. coli* K12 MG1655 in sheep blood. In addition to the main peak, it is clear the presence of a second peak, with the height of the peak increasing at low bacterial concentration. This is likely due to the interference of genomic DNA derived from the blood cells. Finally, **Fig. S8** shows the melt curves of DNA extracted from *E. coli* K12 MG1655 in river water. Similarly, the intensity of non-specific peaks is inversely proportional to the bacterial concentration.

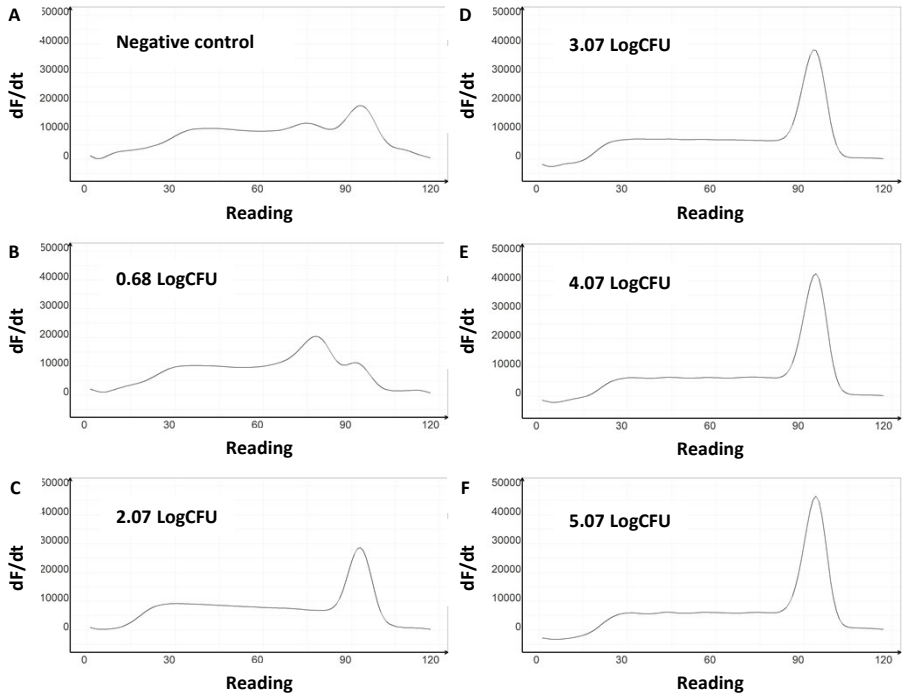

Fig S6. Melt curve for DNA extracted from *E. coli* in PBS using the microfluidic siphon.

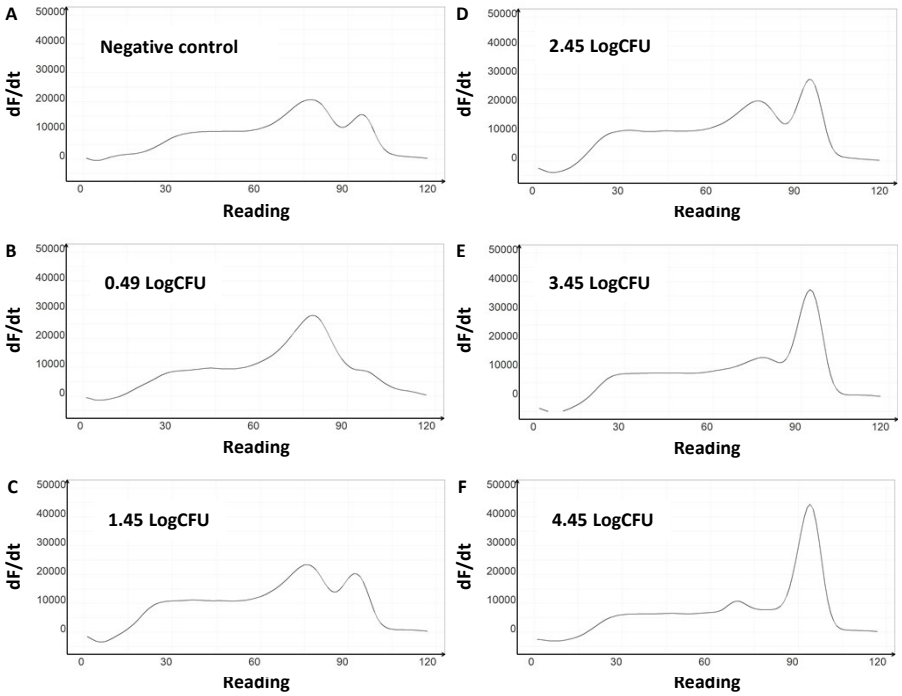

Fig S7. Melt curves for DNA extracted from *E. coli* in sheep blood using the microfluidic siphon.

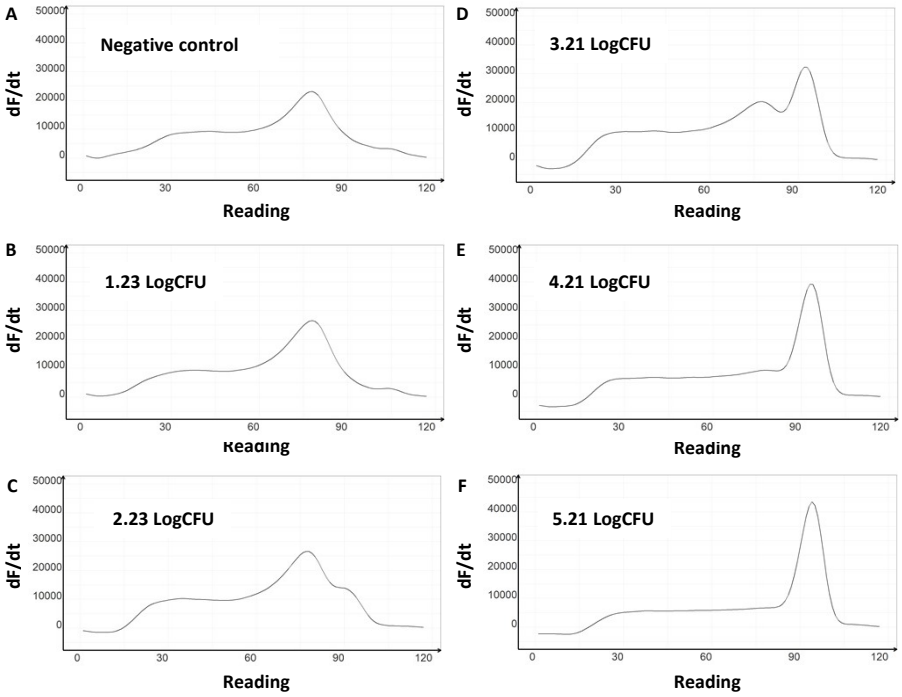

Fig S8. Melt curves for DNA extracted from *E. coli* in river water using the microfluidic siphon.