Electronic Supplementary Information

Assay for *Listeria monocytogenes* cells in whole blood using isotachophoresis and recombinase polymerase amplification

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Contents

S1. Microfluidic device used in ITP-RPA assay

To perform ITP purification, we a used a cyclic olefin copolymer (COC) injection-molded device developed and demonstrated by Marshall et al.[1] In Figure S1, we show an image of the microfluidic device, with labeled reservoirs and channel sections. Capillary barriers are used to aid in sample loading and to create a sharp interface between the sample and separation channels. Steep flat-surface ramps reduce the channel depth by half, before suddenly expanding back to full depth. This "ledge" creates an energetic barrier that stops the flow of a wicking liquid. Images and a more detailed discussion of the device are found in Marshall et al.[1]

The channel is nominally 2 mm wide and 0.15 mm deep, and total channel length is 200 mm. The device is capable of processing 25 µL of sample. The separation channel can hold 30 µL, and the section between the extraction and LE reservoirs holds another 5 µL. We load buffering TE, buffering LE, and channel/separation LE are load into the TE, LE, and extraction reservoirs, respectively, as shown below.

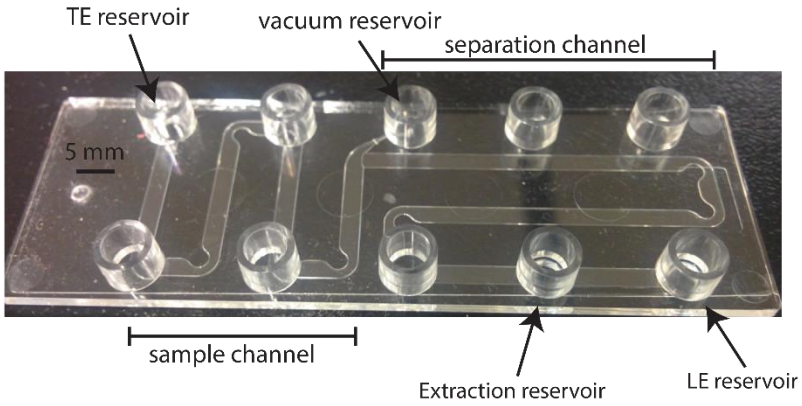

Figure S1. Image of the device used for ITP purification. The sample channel portion holds a total volume of 25 µL, while the separation channel portion can hold 30 µL. The device uses capillary barriers to aid in the loading and create a sharp interface between the channel and separation channels. A more detailed view and explanation of the device design can be found in Marshall et al.[1] We load buffering TE into the TE reservoir, buffering LE into the LE reservoir, and channel LE into the extraction reservoir, and apply vacuum as shown. The vacuum reservoir is also used to dry and rinse the channels between experiments.

S2. Visualizations of ITP purification of bacterial DNA purification whole blood

Following lysis of spiked whole blood using NaOH, Triton X-100, and proteinase K, we quenched the mixture using LE buffer, down to pH 8.1. We then transferred this mixture into the microfluidic device described above. In Figure S2 we show images of the ITP purification process. We dispensed the quenched cell lysate mixture into the TE reservoir, and it passively wicked through the 25 µL portion of the channel. After loading the various LE and TE buffers in the appropriate reservoirs (see Section S1), we applied an electric field between the LE and TE reservoirs to initiate ITP and used AlexaFluor 647 to visualize the ITP peak and so track ITP progress (i.e., only indirect visualization of the zone containing DNA). As analytes progressed through the channel, genomic DNA separated from the contaminants found in whole blood. Eventually, purified DNA was fully separated and reached the extraction reservoir ahead of the contaminants. We manually extracted the DNA using a pipette, and transferred 5 µL of the mixture into the RPA master mix for amplification and detection. The entire process required approximately 40 min. We note that this purification time is constrained by the available power source and Joule heating, and theoretically could be further reduced.

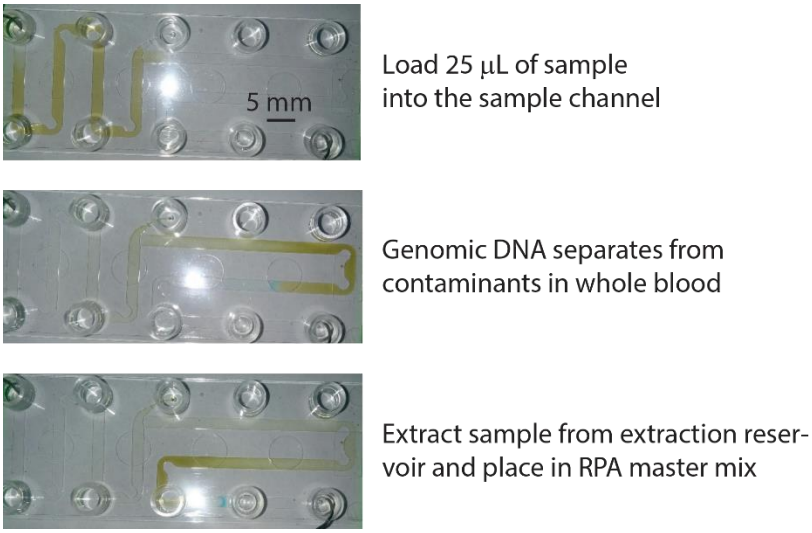

Figure S2. Images of an ITP experiment using whole blood. We first load 25 µL of sample into the sample channel. The sample is 10-fold diluted infected whole blood mixed with quenching LE buffer. We apply electric field between the TE and LE reservoirs, initiating ITP. We use AlexaFluor 647 as an ITP tracking dye. Genomic DNA begins to separate from the contaminants found in whole blood. Eventually purified genomic DNA reaches the extraction reservoir ahead of the contaminants. At that point, we manually extract DNA using a pipette, and place in the RPA master mix.

S3. Estimating ITP purification time

Electromigration velocity of a species migrating under an applied electric field is determined by the electrophoretic mobility of the species as well as the magnitude of the applied electric field. In the LE zone, this relation can be written as:

$$U_{ITP} = \mu_{LE} E^{LE}. \tag{S1}$$

U_{ITP} is the observed velocity, μ_{LE} is the effective electrophoretic mobility of the LE, and E_{LE} is the electric field in the LE zone. We relate velocity to total channel length and total assay time by the simple relation,

$$U_{ITP} = \frac{L_{channel}}{t_{tot}}, \tag{S2}$$

where $L_{channel}$ is the total length of the channel swept by ITP, and t_{tot} is the total ITP purification time. We only consider current in zones away from the ITP interface, and neglect bulk flow, and so can neglect advective and diffusive currents, and relate conductivity and electric field through Ohmic law:

$$j = \sigma^{LE} E^{LE}. \tag{S3}$$

Here, j is current density. Conductivity in the LE zone is expressed by:

$$\sigma^{LE} = \sum_i \sum_{z=z_i}^{z_n} F z \mu_{i,z} c_{i,z}, \tag{S4}$$

where F is Faraday's constant, z is valence of a given ionization state of a species, $\mu_{i,z}$ is the electrophoretic mobility of species i at valence z, and $c_{i,z}$ is the concentration of species i at valence z. Here, we use monovalent LE (Cl^-) and counterion species (Tris). This allows us to recast eq S3 as:

$$\sigma = F\left(\mu_{LE} c_{LE} + \mu_{CI} c_{CI}\right), \tag{S5}$$

where μ_{CI} is the electrophoretic mobility of the counterion, and c_{CI} is the concentration of the counterion. To satisfy electroneutrality, $c_{LE} = c_{CI}$, and we further simplify eq S4 as:

$$\sigma = Fc_{LE}\left(\mu_{LE} + \mu_{CI}\right). \tag{S6}$$

For channels with constant cross-sectional areas, we substitute eqs S2 and S6 into eq S1, and algebraically manipulate to obtain:

$$t_{tot} = \frac{L_{channel} Fc_{LE}}{j}\left(\frac{\mu_{LE} + \mu_{CI}}{\mu_{LE}}\right) = \frac{\forall_{channel} Fc_{LE}}{I}\left(\frac{\mu_{LE} + \mu_{CI}}{\mu_{LE}}\right). \tag{S7}$$

Here, I is the current in the channel and $\forall_{channel}$ is the total volume of channel swept by ITP. Using eq S7, we can calculate the expected ITP purification time. We estimate an LE concentration of approximately 50 mM in the sample channel (40 mM from the quenching buffer and approximately 10 mM from diluted blood) and 35 mM in the separation channel. The total channel volume is 60 µL, and we apply a constant current of 105 µA using a Keithley 2410 current source. We estimate the mobility of our LE (Cl^-) to be 79 x 10^{-9} $m^2V^{-1}s^{-1}$ and of our counterion (Tris) to be 11 x 10^{-1} $m^2V^{-1}s^{-1}$. We insert these values into eq S7 and find an expected assay time of just under 40 min, consistent with our experimental observations.

S4. Effect of proteinase K on assay performance

Persat et al.[2] noted that proteinase K was necessary for successful nucleic acid purification from whole blood using ITP. Without using proteinase K, their assay's estimated extraction efficiency was on par with control experiments. They hypothesized that DNA binding proteins like histones significantly reduced DNA mobility and caused the complex to not focus in ITP. Proteinase K is a broad range protease, and nonspecifically degrades proteins. Proteinase K degrades DNA-binding proteins, thereby releasing DNA and allowing it to focus.

We experimentally demonstrated the effect of proteinase K on ITP purification and RPA detection, as shown in Figure S3. In these experiments, we spiked purified *L. Monocytogenes* genomic DNA into whole blood. We measured fluorescent signal after 15 min of RPA for both sets of experiments. RPA was unsuccessful when proteinase K was excluded from our lysis buffer. On the other hand, including 1 mg/mL proteinase K in our lysis buffer allowed for efficient degradation of proteins and focusing of DNA. Our findings are consistent with those of Persat et al.,[2] and further validate the importance of proteinase K in successful ITP purification of genomic nucleic acids from whole blood.

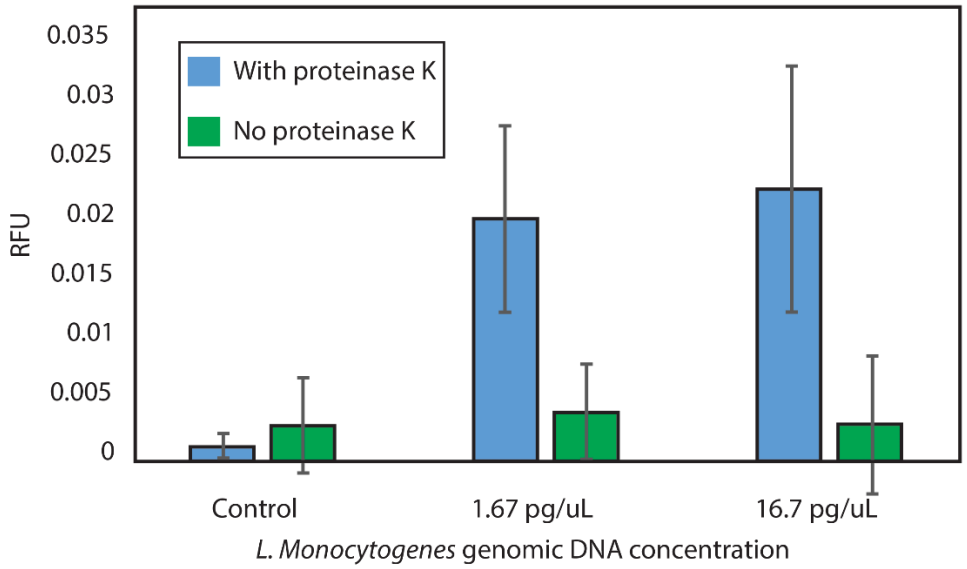

Figure S3. Experimental demonstration of the effect of proteinase K on ITP purification of spiked *L. Monocytogenes* genomic DNA in whole blood. We plot fluorescence measurements following 15 min of amplification with RPA. In experiments with proteinase K, we added 1 mg/mL proteinase K into the lysis buffer. Lysis in the presence of proteinase K resulted in significantly higher fluorescent signal, indicating successful ITP purification and RPA detection. On the contrary, excluding proteinase K resulted in no amplification. Together, these experiments demonstrate the importance of proteinase K on ITP purification of genomic DNA in whole blood. Uncertainty bars represent 95% confidence on the mean (as determined from Student's t-distribution). This result is consistent with the finding of Persat et al.[2] They hypothesized that proteinase K degrades DNA-binding proteins like histones, allowing DNA to focus in ITP. For experiments with proteinase K, these results are from N = 6, 7, and 6 repetitions, respectively. For experiments without proteinase K, these results are from N = 5, 10, and 3 repetitions, respectively.

S5. Threshold times for ITP-RPA *L. Monocytogenes* assay

In the main paper, we showed the final fluorescence signal as a positive detection of a sequence above some minimum level. The use of final fluorescence signal has been used successfully to indicate RPA-based detection of a sequence above some minimum level by, for example, Crannell et al.,[3] Rohrman and Richards-Kortum,[4] Borysiak et al.,[5] and Cordray et al.[6] In this section, we present an alternate measure of fluorescence signal in the form of threshold times. In Figure S4, we show the results of the assay using purified genomic DNA. For experiments using chemically inactivated *L. Monocytogenes* cells spiked directly into whole blood, the threshold amplification times were 6.48 +/- 1.50 min and 2.70 +/- 1.23 min for 2 x 10^4 and 2 x 10^5 cells/mL, respectively. The uncertainty here represents 95% confidence on the mean (as determined from Student's t-distribution).

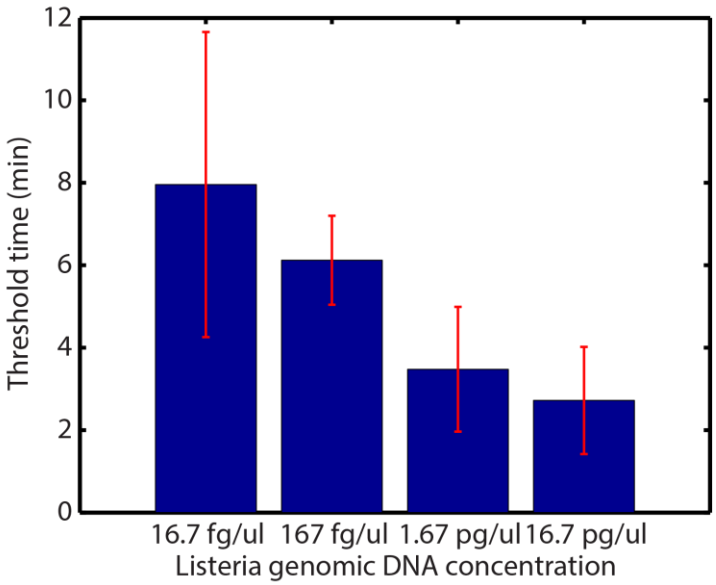

Figure S4. Results of the ITP-RPA assay using purified *L. Monocytogenes* genomic DNA spiked into whole blood. We here quantify threshold times as determined by the Opticon Monitor software. Error bars represent 95% confidence on the mean (from Student's t-distribution).

References

1. L. A. Marshall, A. Rogacs, C. D. Meinhart and J. G. Santiago, *Journal of chromatography. A*, 2014, **1331**, 139-142.
2. A. Persat, L. A. Marshall and J. G. Santiago, *Analytical chemistry*, 2009, **81**, 9507-9511.
3. Z. A. Crannell, B. Rohrman and R. Richards-Kortum, *PloS one*, 2014, **9**, e112146.
4. B. Rohrman and R. Richards-Kortum, *Analytical chemistry*, 2015, **87**, 1963-1967.
5. M. D. Borysiak, K. W. Kimura and J. D. Posner, *Lab on a chip*, 2015, **15**, 1697-1707.
6. M. S. Cordray and R. R. Richards-Kortum, *Malar J*, 2015, **14**, 472.