



“Paper Machine” for Molecular Diagnostics

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A “Paper Machine” for Molecular Diagnostics

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Abstract

Clinical tests based on primer-initiated amplification of specific nucleic acid sequences achieve high levels of sensitivity and specificity. Despite these desirable characteristics, these tests have not reached their full potential because their complexity and expense limit their usefulness to centralized laboratories. This paper describes a device that integrates sample preparation and Loop-mediated Isothermal Amplification (LAMP) with endpoint detection using a handheld UV source and camera phone. The prototype device integrates paper microfluidics (to enable fluid handling) and a multi-layer structure – or a “paper machine” – that allows a central patterned paper strip to slide in and out of fluidic path, and thus to allow introduction of sample, wash buffers, amplification master mix, and detection reagents with minimal pipetting, in a hand-held, disposable device intended for point-of-care use in resource-limited environments. This device creates a dynamic seal that prevents evaporation during incubation at 65 °C for 1 hour. This interval is sufficient to allow a LAMP reaction for the *Escherichia coli malB* gene to proceed with an analytical sensitivity of 1 target copy. Starting with human plasma spiked with whole,

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3 live *E. coli* cells, this paper demonstrates full integration of sample preparation with LAMP
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5 amplification and endpoint detection with a limit of detection of 5 cells. Further, it shows that the
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7 method used to prepare sample enables concentration of DNA from sample volumes commonly
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9 available from fingerstick blood draw.
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12 13 **Introduction**

14
15 The unparalleled sensitivity and specificity of nucleic acid amplification tests (NAATs),
16
17 led by those based on polymerase chain reaction (PCR), have become increasingly available in
18
19 the developed world for the diagnosis of infectious, genetic, and other diseases. In developing
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21 countries these tests are much less used because of their cost, complexity and requirements for
22
23 expensive equipment and reagents. PCR requires three precisely controlled temperatures to
24
25 enable melting of target DNA, annealing, and elongation of primers, and is typically carried out
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27 using expensive thermocyclers. Additionally, prior to amplification NAATs usually require
28
29 sample preparation involving a series of steps to release the DNA, and to remove the many
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31 inhibitory compounds found in clinical samples^{1,2}. These steps often require the use of complex
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33 laboratory equipment and, therefore, highly trained personnel. This combination of factors –
34
35 expensive and delicate equipment, trained personnel, and complex procedures – has kept NAATs
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37 from being broadly adopted in resource-limited countries (although they are, of course, used to
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39 limited extents in centralized hospitals and government laboratories). Because transporting
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41 patients and/or samples to these central facilities can be difficult, slow, and expensive in
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43 resource-limited environments with under-developed transportation systems, local healthcare
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45 outposts and traveling healthcare workers are the often the primary, or only, providers of
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47 healthcare³⁻⁶.
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3 NAATs intended for use in resource-limited settings must operate within the constraints
4 of those environments. One diagnostic technology that has been successfully implemented at the
5 point of care is lateral flow assays, particularly lateral flow immunochromatographic strips⁶.
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8 These systems require minimal sample preparation and no instrumentation. They typically
9
10 provide a visual result within 10 to 20 minutes⁶. Tests for direct or indirect detection of a range
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12 of pathogens (including *Trypanosoma cruzi*⁷, diphtheria toxin⁸, *Plasmodium falciparum*⁹, and
13
14 *Haemophilis ducreyi*¹⁰) have been reported, tested, and are available in resource-limited settings.
15
16
17 Lateral flow assays have also been used for endpoint detection of PCR and isothermal
18
19 amplification products, either from reactions conducted using standard equipment¹¹⁻¹⁴ or
20
21 integrated into microfluidic systems¹⁵⁻¹⁷. Though lateral flow endpoint detection can simplify the
22
23 final readout of NAATs, the instrumentation required remain (often prohibitively) complicating
24
25 factors, from sample preparation through amplification, and which may include open-channel
26
27 microfluidic systems with external pumps.
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34 Despite the obstacles to implementation of NAATs at the point of care, a number of
35
36 systems are being developed for this type of application. The Cepheid GeneXpert (Sunnyvale,
37
38 CA) is a notable current example. The GeneXpert reduces user handling of samples by
39
40 integrating sample preparation steps with PCR amplification and real-time fluorescence detection
41
42 in the same cartridge¹⁸. Though meeting the ideal of a sample-in, answer-out device, the
43
44 GeneXpert has complex optical and electronic systems required for real-time PCR, and pumps to
45
46 move sample and reagents through the cartridge, and is still not a general solution for resource-
47
48 limited environments¹⁹.
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53 We have chosen to use isothermal amplification methods in the system described here.
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55 These methods simplify molecular diagnostics by eliminating thermocycling, reducing
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3 equipment costs and power requirements, without sacrificing sensitivity or specificity. In
4
5 particular, Loop-mediated Isothermal Amplification (LAMP)^{20,21} has a high tolerance for
6
7 common components present in clinical samples that inhibit PCR and other reactions²²⁻²⁵. This
8
9 robust tolerance toward inhibitors further streamlines assays, because it simplifies sample
10
11 preparation and complexity/expense of equipment²⁶. Although devices integrating LAMP
12
13 amplification with detection have entered the market (for example, the IllumiGene® system;
14
15 Meridian Bioscience, Cincinnati, OH), they have not directly incorporated the steps necessary
16
17 for sample preparation into the instrument²⁷. Further, as products targeted toward developed
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19 world settings, they employ complex optical systems and user interfaces that increase cost and,
20
21 especially, the requirements for maintenance and repair.
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27 By merging the useful characteristics of lateral-flow assays with those of open-channel
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29 microfluidic systems, paper microfluidic platforms using three-dimensional stacked patterned
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31 paper²⁸⁻³¹ or two-dimensional networks of cut paper³²⁻³⁴ allow complex fluid handling without
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33 the need for external pumps and power. Paper as a substrate for diagnostics has other beneficial
34
35 properties as well: (i) It is biocompatible (and has been used for decades for storage of clinical
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37 samples in the form of dried blood spots, and is also widely used as a chromatography medium);
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39 (ii) Because DNA adsorbs to cellulose³⁵, paper provides a simple matrix for its capture and
40
41 isolation; (iii) The cellulose fiber network provides a high surface area, and facilitates storage of
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43 dried reagents³⁶. A paper-based test for liver function (Diagnostics For All) has been successful
44
45 in field trials; this test provides semi-quantitative measurements of liver transaminases from
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47 whole blood^{37,38}. Despite these advances, there have, however, been no demonstrations of
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49 NAATs in complete systems (blood to read-out) in paper microfluidic systems.
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3 In important steps toward the objective of a complete system, Rohrman and Richards-
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5 Kortum have demonstrated Recombinase Polymerase Amplification (RPA) in a paper-based
6
7 device (using sample preparation and detection as separate steps)³⁹. The Ellington group
8
9 demonstrated detection of LAMP products in paper microfluidic devices using their Catalytic
10
11 Hairpin Assembly (CHA) reaction, which uses a series of specially designed oligonucleotide
12
13 hairpins to specifically identify the target and provide an additional signal enhancement. Here,
14
15 the amplification reaction was conducted off-chip⁴⁰.
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20 Our goal is to develop a NAAT device that is compact, inexpensive, and fully integrates
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22 sample preparation, amplification, and detection in a low-cost, single-use format appropriate for
23
24 point-of-care diagnostics in resource-limited settings. To this end, we describe a prototype paper
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26 microfluidic device that enables sample preparation from clinically-relevant matrices, isothermal
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28 amplification, and detection; the cost of materials for the device is US\$ 0.60, and a total cost per
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30 assay of US\$ 1.83 (Table S1). These costs, of course, do not indicate the cost of a fully
31
32 developed product based on this type of system, but they do indicate that the cost of materials
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34 will not limit affordability. Figure 1a shows the multi-layer architecture of device – which we
35
36 hereafter refer to as a “sliding strip;” this design allows for the middle layer – the strip –
37
38 containing the “reaction vessel,” a paper disc in which reaction and washing steps occur, to slide
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40 into different fluidic paths. The system is, thus, a “paper machine”: that is, a device fabricated in
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42 paper, with moving parts, which accomplishes a task.
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48 The linear motion of the sliding strip relative to the stationary surrounding structures, acts
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50 as a valve to control the serial introduction of sample, wash buffer, amplification reagents, and
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52 detection reagents, while also dynamically sealing to prevent evaporation during incubation
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54 steps. The sample preparation steps are simplified by fabricating the reaction disc out of
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3 Whatman FTA® paper, which contains a proprietary blend of lytic reagents dried into the
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5 cellulose matrix. FTA® paper has been shown to lyse a wide range of cells and viruses⁴¹⁻⁴³, and,
6
7 in its normal use, after extensive washing in tubes with vortexing, it can be added directly to
8
9 PCR reactions. Our device replaces this instrumented, multistage, high-volume washing with
10
11 two, through-flow washes before the introduction of LAMP master mix. Also, by exploiting the
12
13 hydrophilicity of paper, we simplify fluid handling by replacing external mechanical systems for
14
15 pumping with fluid flow driven by capillary action. Final detection of the amplified product uses
16
17 SYBR Green I, a fluorescent intercalating dye, and a handheld UV source and camera phone. For
18
19 a proof-of-concept, we used LAMP to amplify a portion of the *malB* gene common to all
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21 *Escherichia coli*⁴⁴.
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27 28 **Materials & Methods**

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31 Oligonucleotide primers were ordered from Eurofins Operon MWG (Huntsville, AL)
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33 with HPLC purification and suspended in 1X Tris-EDTA. *Bst* 2.0 DNA polymerase, 10X
34
35 Isothermal Amplification Buffer, dNTPs, DNA/DNAse –free stocks of MgSO₄, and restriction
36
37 endonucleases were purchased from New England Biolabs (Ipswich, MA) and betaine from
38
39 Sigma-Aldrich (St. Louis, MO). Pooled human plasma in sodium heparin derived from whole
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41 blood donations was acquired from Valley Biomedical Products and Services, Inc. (Winchester,
42
43 VA). Pre-cast 2% agarose gels and SYBR Green I were purchased from Invitrogen. All other
44
45 reagents were supplied by Fisher Scientific.
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50 51 **Fabrication of magnetic sliding-strip devices and confirmation of evaporation-resistance**

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53 The magnetic material used to fabricate the devices is 0.75 mm thick synthetic rubber-
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55 bonded ferrite magnetic sheeting with 3 mm bands of alternating poles on both faces obtained
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3 from McMaster-Carr (Robbinsville, NJ). The magnetic sheets, as well as all laminate layers,
4
5 were cut using a design file from Adobe Illustrator and a Graphtec CraftRoboPro (Graphtec
6
7 Irvine, CA) knife plotter. A thin film of Krytox® LVP lubricant (DuPont Wilmington, DE) is
8
9 applied with a roller to the inner faces of the top and lower layers as well as to both sides of the
10
11 sliding strip. The reaction disc is 4.75 mm diameter and consists of Whatman FTA® paper while
12
13 the wash vias are 6.35 mm diameter discs cut from Ahlstromm 226 paper. The various layer
14
15 were stacked, laminated, and pressed as illustrated in Figure 1a.
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20 The attractive force produced by the magnetic material forms an evaporation-resistant
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22 seal in combination with the thin film of lubricant applied to the layer interfaces. This seal was
23
24 tested by applying 10 μL of water to the paper reaction disc within a sliding-strip device, which
25
26 was pre-dried to remove any ambient moisture, and slid to seal. The mass of the device before
27
28 and after the addition of the water was recorded to serve as the baseline. This device was then
29
30 incubated at 65 °C for 1 hour, weighed, and slid to unseal the disc. The device was returned to
31
32 the incubator to completely evaporate any water remaining and the final mass recorded. The
33
34 masses of the device after incubation and after complete evaporation were used to determine the
35
36 amount of water lost during incubation by comparison to the baseline.
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42 ***E. coli* Culture**

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44 We used *E. coli* BL21(DE3)pLysS as a model organism in this work. Cells were cultured
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46 overnight at 37 °C, shaking, from frozen glycerol stocks in LB broth containing 34 $\mu\text{g/mL}$
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48 chloramphenicol. We determined the concentration of cells by measuring the optical density at
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50 600 nm and comparing this value to a growth curve. When directly used to spike sample, the
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52 culture was centrifuged, the supernatant aspirated, and the pellet resuspended to the desired
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3 concentration with fresh media, twice, to remove any free DNA. This stock of cells was then
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5 serially diluted in human plasma for experiments.
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10 11 **LAMP Amplification**

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14 The primer set (Table S2) targeting the *malB* gene of *E. coli*, including loop primers, as
15 described by Hill *et al.* were employed in this work⁴⁴. LAMP was performed using the *Bst* 2.0
16 DNA Polymerase, an *in silico* designed homologue of *Bst* DNA Polymerase I, Large Fragment,
17 commonly used in this reaction, and its supplied buffer⁴⁵. In transitioning the reaction from tubes
18 to paper, the concentration of betaine, primers, and enzyme were optimized, as was the
19 incubation time, to minimize non-specific reactions and maximize sensitivity, as shown in Figure
20 S1 and discussed in the supplementary information. The final reactions contained 1X Isothermal
21 Amplification Buffer (10 mM Tris-HCl, 10 mM (NH₄)₂SO₄, 50 mM KCl, 2 mM MgSO₄, 0.1%
22 Tween-20) supplemented with an additional 6 mM MgSO₄, 0.9 M betaine, 1.4 mM each dNTP,
23 1.6 μM each inner primer (FIP, BIP), 0.8 μM each loop primer (LF, LB), 0.2 μM each outer
24 primer (F3, B3), and 0.32 U of polymerase. The optimal incubation was 65 °C for 60 min. The
25 reaction was always run with two negative controls – a no-template control (NTC) and a no-
26 amplification control (NA+), containing a heat-inactivated enzyme and 1,000 copies of target or
27 1,000 cells, as appropriate. Reactions in tubes were carried out at 25 μL; in paper, the reaction
28 volume was 10 μL.
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49 50 **Product Analysis by Gel Electrophoresis**

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52 To confirm that positive signals were the result of specific, template-driven reactions,
53 LAMP products were analyzed by agarose gel electrophoresis (AGE). First, a positive reaction
54 conducted in a tube using 1,000 copies of the target was digested using restriction endonuclease
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3 *PvuII*, which recognizes a sequence in region F1 of the target, and run on a 2% agarose gel with
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5 a DNA ladder and the un-digested product to determine if the observed LAMP banding pattern
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7 was the result of template-specific amplification and to mark said banding pattern as the specific
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9 reaction product for future comparison.
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12 13 **General Sliding-Strip Operation** 14

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16 The sliding-strip devices enable the serial operations of sample preparation – including
17
18 cell lysis, DNA isolation and purification – as well as LAMP amplification and detection. Unless
19
20 otherwise noted, the procedure for sliding-strip assays, illustrated in Figure 4a, began with the
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22 application to the reaction disc of 10 μL sample through the sample port. The strip was then slid
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24 to align the reaction disc with the wash port and 40 μL of FTA Purification Buffer and 80 μL of
25
26 nuclease-free water were sequentially applied and allowed to transit through the disc to the paper
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28 vias and finally to the removable wash pad. The disc was then dried completely the strip slid to
29
30 align the disc with the amplification reagent port, and 10 μL of LAMP Master Mix was applied.
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32 The strip was then slid again, sealing the disc (by the magnetic force and the lubricant film)
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34 within the amplification zone. The device was placed in an incubator at 65 °C. After a 60 min
35
36 incubation time, the devices were removed from the incubator and the strip was slid to the
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38 detection window, and the disc was dried completely. For detection, 10 μL of 100X SYBR
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40 Green I in 1X TE was applied to the disc and it was excited using a handheld shortwave UV
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42 source and imaged using a camera phone. Images were then processed, converted to grayscale,
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44 and analyzed using Image J to quantify mean pixel intensity as gray value. The mean of the NTC
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46 replicates plus 3 times their standard deviation was used as the LOD, and the threshold value, for
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48 determining positive and negative reactions. In some cases, for ease of imaging and data
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3 presentation of multiple samples and replicates, the reaction discs were removed from the device
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5 and arrayed before imaging.
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8 9 **Determination of Analytical Sensitivity**

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11 The analytical sensitivity of amplification and detection in the sliding-strip device was
12 determined using a double-stranded 200 bp analog of the *malB* target sequence produced by
13 Integrated DNA Technologies, Inc (Coralville, IA) as two single-stranded DNA Ultramers®, the
14 forward strand and the reverse complement. These strands were then combined to yield an
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The concentration was confirmed by spectrometry using the absorbance at 280 nm. Serial
dilutions were prepared, and 10 μL applied to dry reaction discs, which had been pretreated with
10 μL human plasma, 40 μL of FTA Purification Buffer, and 80 μL of nuclease-free water,
through the amplification reagent port of the sliding-strip devices. The discs were then dried
completely, and the general sliding-strip operations outlined above were followed starting from
the application of LAMP Master Mix.

40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 **Integrated Assay with Spiked Plasma Samples**

To examine the effectiveness of the sliding-strip device for sample preparation from
complex matrices, the assay was challenged with samples of human plasma spiked with whole,
live *E. coli* prepared from overnight culture as described above. All steps in the general sliding-
strip operation outlined previously were followed. Here, parameters such as the number of
washes, wash volumes, and wash buffer composition were optimized as shown Figure S2.

53 54 55 56 57 58 59 60 **Collecting and Concentrating DNA from Large Sample Volumes**

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3 To examine the ability to isolate DNA from larger volumes of dilute samples, we applied
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5 10, 20, 30, 40 or 50 μL of a 0.05 cell/ μL sample of *E. coli* in human plasma to the reaction discs.
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8 After each sample wicked through the reaction disc, the general sliding-strip operation procedure
9
10 was followed as described.
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12 13 14 **Results & Discussion**

15 16 17 **Fabrication of Sliding-Strip Devices with Evaporation-Resistant Dynamic Seals**

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19 Nucleic acid-based diagnostic tests require serial operations to be performed on a
20
21 complex sample in order to yield reliable results. DNA must be released from cells, isolated from
22
23 the cell debris, proteins, and other sample components, purified of these potential polymerase
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25 inhibitors, amplified, and detected. Our device is designed to perform these operations –
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27 typically done using laboratory equipment – with minimal user steps and at a fraction of the cost.
28
29 The core architecture of the sliding-strip device, illustrated in Figure 1a and b, consists of three
30
31 major layers: the top layer, which provides ports to access a reaction disc; the middle layer,
32
33 which consists primarily of the reaction disc housed in the sliding strip that is linearly actuated to
34
35 serially address the ports; and the bottom layer, which contains paper vias to contact the reaction
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37 disc and the wash pad to transit waste wash out of the device. Each of these layers is fabricated
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39 out of a low-cost, flexible magnetic substrate, the attractive force of which, when combined with
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41 the thin film of lubricant, creates a dynamic seal that allows for extended incubation at 65 °C
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43 with negligible evaporation (Figure 2). As shown in Figure 1c, these devices are small – only
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45 0.225 cm thick and the prototype device, capable of running one sample and two controls, is 4.7
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47 cm wide and 10 cm from the top to the end of the strip-pull when it is in the starting position –
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49 and will ultimately include an inexpensive electronic heater. This, in combination with on-board
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51 storage of lyophilized reagents, enables point-of-care use.
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Analytical Sensitivity in Paper

To assess the performance of the LAMP reaction when conducted in buffer contained within a paper disc that is housed in the sliding strip, we examined serial dilutions of the 200 bp dsDNA target sequence in nuclease-free water. By drying these samples onto reaction discs, which had been treated with human plasma and washed, we replicated the condition of the discs that would exist in the full assay prior to amplification and isolated potential amplification inhibition from sample preparation losses, both of which could yield false negative results. As shown in Figure 3a, the reaction proceeds uninhibited and reactions starting with as few as 1 copy yield a statistically significant signal over the no template control (NTC) ($n=8$, $p < 1E-11$). As is evident in Figure 3b, a camera phone image of the reaction discs under UV excitation, these optimal conditions and idealized sample produce a binary result – common with endpoint detection of isothermal amplification reactions.

Though no sequence homology exists between primers in the set, in sub-optimal conditions mismatches may be tolerated. To confirm that fluorescent signal is the result of the specific template-driven reaction, a restriction endonuclease analysis was performed as described in the supporting information and shown in Figure S4.

Integrated Assay with Spiked Plasma Samples

The assay integrated with the sliding-strip device, was challenged with whole, live *E. coli* serially diluted in human plasma. The samples were subjected to all of the steps of sample preparation, in order as described in the Materials & Methods section, by linear actuation of the sliding strip to the appropriate ports for application of the wash buffer, LAMP Master Mix, and detection reagents, as detailed in Figure 4a. Upon imaging, positive results were obtained from four of six samples containing as few as 1 cell (Figure 4b), this outcome is consistent with

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2
3 stochastic sample error as predicted by the most probable number (MPN) method (described in
4
5 the supporting information).
6

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8 As shown in Figure S5, statistical significance ($p < 0.001$) is achieved for all
9
10 concentrations over 5 cells per sample (e.g. 0.5 cells/ μL or 500 cells/mL).
11

12 13 **Collecting and Concentrating DNA from Larger Sample Volumes** 14

15
16 Our intended use of a product based on this system includes direct application of 30 μL
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18 of finger-stick blood, the average volume obtained from a safety lancet. As the limit of detection
19
20 of 500 cells/mL was achieved using a 10 μL sample volume, we investigated the effects of larger
21
22 sample volumes. By varying the volume of a sample containing 0.05 cells/ μL (an order of
23
24 magnitude lower than the above established limit of detection) in increments of 10 μL applied to
25
26 different reaction discs, as shown in Figure 5, we noted an increase in the number of replicates
27
28 resulting in positive amplification with 10 and 20 μL volumes (approximately 0.5 and 1 cell/disc,
29
30 respectively) producing no positive signals, 40 μL (approximately 2 cells/disc) producing 7
31
32 positive replicates, and 60 μL (approximately 3 cells/disc) yielding positive signals in all 8
33
34 replicates. This demonstrates that the additional cells present in the larger volume samples are
35
36 being successfully lysed and their DNA captured by the cellulose fibers of the paper reaction
37
38 discs. This concentration effect is limited. Volumes over 60 μL degrade these gains, with 80 and
39
40 100 μL volumes (approximately 4 and 5 cells/disc, respectively) dropping to 5 and 3 positive
41
42 replicates, respectively. This decline is likely due to washing effects; both washing away of the
43
44 dried lysis chemistry of the FTA[®] paper disc, preventing the release of additional target, and
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46 washing away of previously captured target.
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53 54 55 **Conclusions** 56 57 58 59 60

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4 We designed, fabricated, and tested an inexpensive technology for molecular diagnostics
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6 that enables sample preparation steps of cell lysis, DNA isolation, concentration, and purification
7
8 integrated with LAMP amplification and detection. The basis for fabrication uses a patterned
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10 strip that contains the reaction mixture in a paper disc, and slides this disc from station to station
11
12 to accomplish addition of sample, lysis of cells (if relevant), adsorption of DNA, washing, and
13
14 addition of reagents. This simple sliding motion of the strip in the “paper machine,” in
15
16 conjunction with the ability of paper to cause the transport of water by capillary wicking,
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18 replaces most of the equipment needed to conduct NAATs on the benchtop – centrifuges, vortex
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20 mixers – and, in a consumer product, pipettors. The assembly is held together using synthetic
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22 rubber-bonded ferrite magnets and laminates. The compressive force of the magnets on the strip
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24 and thin film of lubricant between the layers creates a dynamic seal that resists evaporation, and
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26 allows for incubation of the amplification reaction at 65 °C for 1 hour.
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32 Using a dsDNA analog of the target region within the *malB* gene of *E. coli*, we
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34 demonstrated the LAMP reaction can be run within the cellulose fiber matrix of a paper disc with
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36 an analytical sensitivity of a single copy of the target sequence. When integrated with the entire
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38 procedure for sample preparation, and beginning from whole, live *E. coli* cells in human plasma,
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40 we were able to detect consistently, 5 cells, and presence of the organism was detected on 4 out
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42 of 6 samples containing 1 cell.
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46 We also demonstrated the ability to concentrate target in the reaction disc by applying
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48 larger sample volumes. This may permit less precise metering of sample in a product based on
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50 this system (e.g. direct application of a finger-stick blood drop), making it more user friendly and
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52 eliminating the need for pipettors. Further, the use of larger sample volumes may improve
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3 detection at low concentrations by increasing the probability of capturing the target in the sample
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5 volume.
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8 The current prototype uses an inexpensive detection scheme – excitation of an
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10 intercalating fluorophore with a UV source and camera-phone-based imaging – that can be
11
12 adapted directly to a marketable product. This system replaces complicated and expensive optics,
13
14 such as those found in a fluorimeter. The requirement of even this simple equipment, and the
15
16 incubator required to heat the device, may limit use in some environments. To address this,
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18 current work is focused on replacing this detection scheme with one that produces a colorimetric
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20 signal visible with the unaided eye in ambient light. Similarly, we are designing and testing a
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22 low-cost, electronic heater built-in to the device to eliminate the incubator.
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27 The use of liquid amplification reagents in this proof-of-concept will prevent application
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29 in resource-limited environments as they have a limited shelf-life in the absence of refrigeration.
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31 Ongoing work is focused on lyophilizing these reagents on-board to address this problem.
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34 A key metric in the development of this device is cost; in order for a product based on
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36 this system to be widely adopted in resource-limited countries it must be affordable. This “paper
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38 machine,” as presented, is low-cost through its use of paper microfluidics and an isothermal
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40 amplification reaction with endpoint detection. Through continued development of this system,
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42 we envision a a total-disposable, non-instrumented device that can dramatically reduce the cost
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44 of nucleic acid diagnostic tests for infectious diseases, genetic conditions, and cancer and bring it
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46 to the point-of-care in resource-limited settings.
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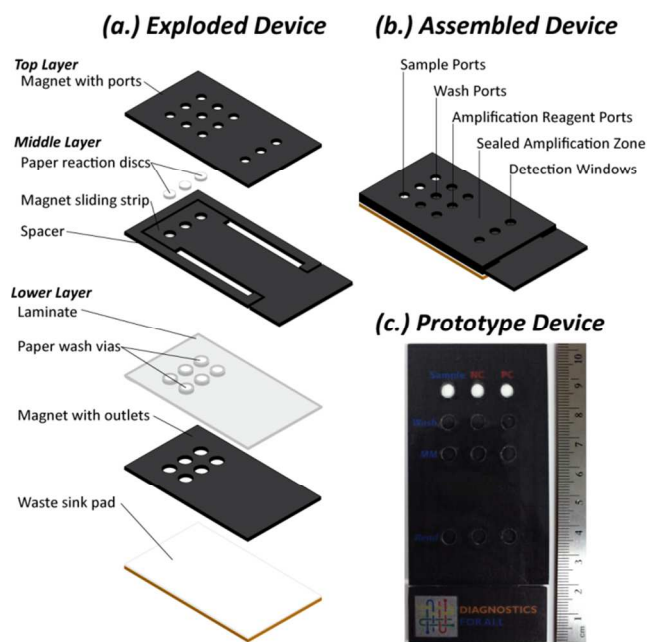


Figure 1: Schematic of Sliding-Strip Device. The exploded view (a) shows the three major layers of the sliding-strip architecture and their components. The assembled device (b) can accommodate a single sample and controls. A prototype device (c) contains three reactions discs – one sample and a negative (NC) and positive (PC) control – with ports to add the samples, wash buffer, amplification Master Mix (MM) and to add detection reagents and visual the signal (Read).

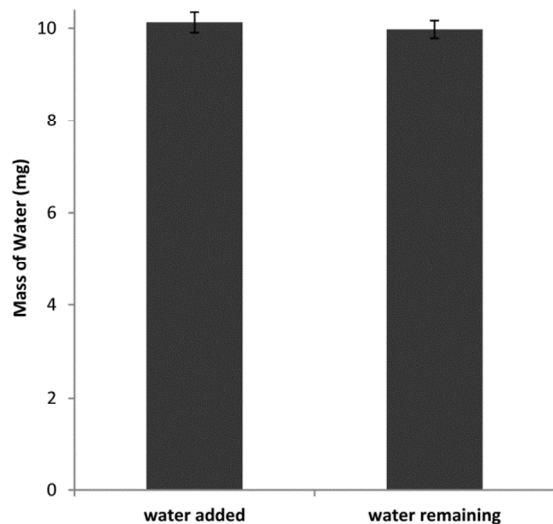
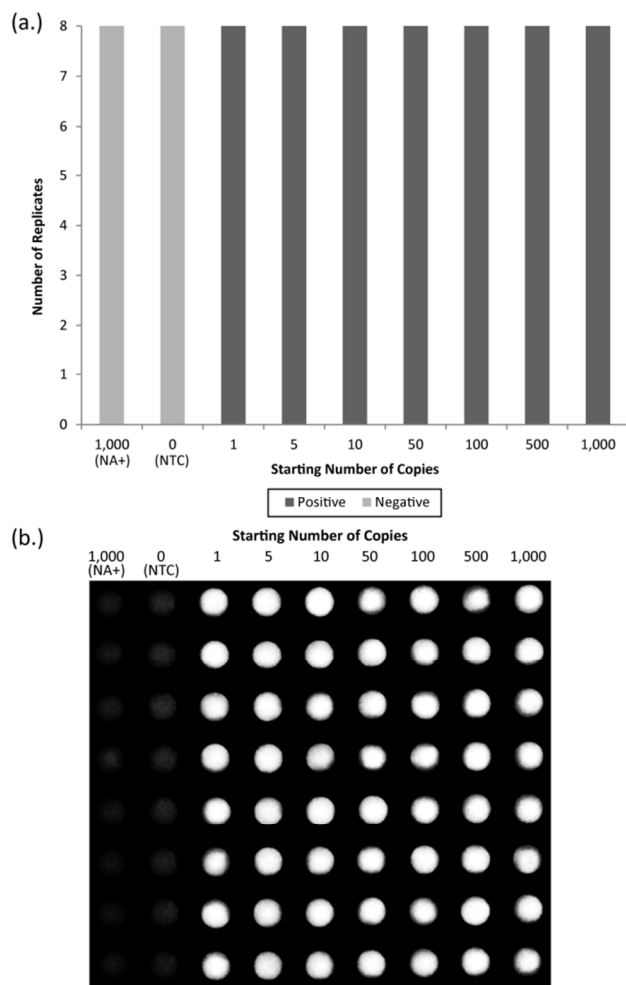


Figure 2: Demonstration of Evaporation-Resistance. The magnetic substrate used to fabricate the device, in conjunction with the thin film of lubricant, creates an evaporation-resistant seal, with an average loss of less than $0.02 \mu\text{L}$ of $10 \mu\text{L}$ water applied ($n = 4$) after 1 hour of heating at $65 \text{ }^\circ\text{C}$.



37 **Figure 3: Analytical Sensitivity of LAMP in Sliding-Strip Device.** All reactions containing as
38 few as 1 copy of the 200 bp dsDNA target yielded detectable amplification (a). Positive
39 replicates are those producing signals greater than the limit of detection (LOD) calculated as the
40 average plus three times the standard deviation of the no template control (NTC) ($n = 8$).
41 Average signal data is presented in Figure S3. The data is generated by photographing the discs
42 under UV excitation (b) after application of 10 μL of 100x SYBR Green I and quantifying the
43 mean gray values with ImageJ.
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(a.) *Cross section of Sliding Strip***Integrated Assay Procedure**

1. Apply sample to reaction disc.



2. Slide strip to move reaction disc to wash port.
Apply wash buffers to reaction disc.



3. Slide strip to move reaction disc to reagent port.
Apply Master Mix to reaction disc.



4. Slide strip to sealed amplification zone and incubate.



5. Slide strip to move reaction disc to detection window.
Apply SYBR Green I, excite with UV, and image.



(b.)

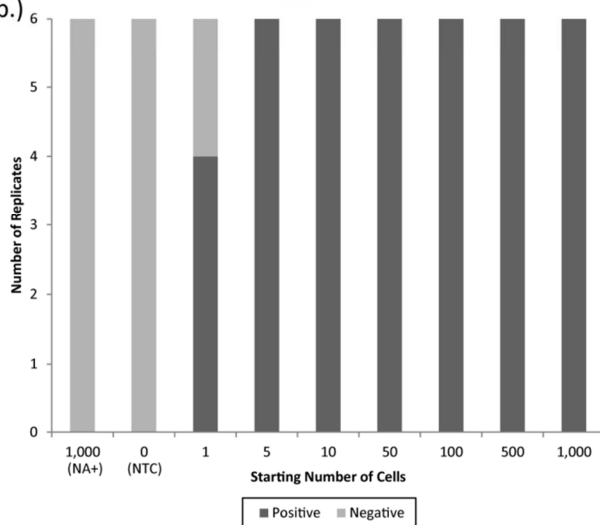


Figure 4: Assay Integrated with Sliding-Strip Device using Spiked Plasma. The entire bioanalytical assay, from sample preparation through amplification and detection, was integrated with the sliding-strip devices. Visualized in cross-section (a), the sample consisting of whole, live *E. coli* spiked into human plasma was applied to the reaction disc, and then washing, reagent addition, incubation, and detection were controlled in series via linear actuation of the sliding

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3 strip. As before, the replicates were binned as positive and negative (b), based on mean gray
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5 values quantified via ImageJ (Figure S5) (n = 6).
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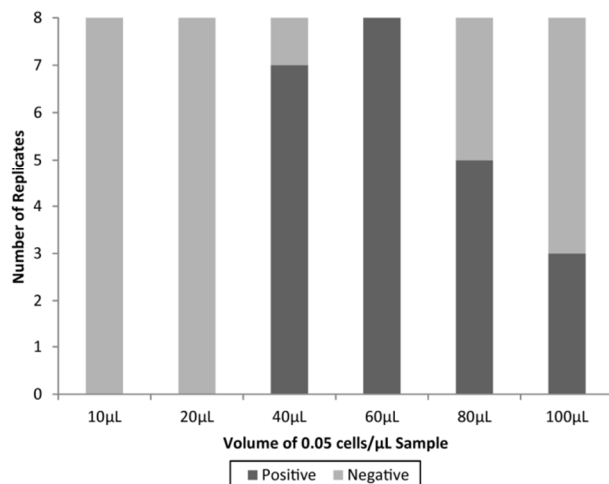


Figure 5: Collection and Concentration of Target from Larger Sample Volumes. Samples of 0.05 cells/μL *E. coli* in human plasma were applied in 10 μL increments to reactions discs in sliding-strip devices and processed through the integrated sample preparation, amplification and detection procedures of the device (n = 8). Signals from all replicates of 10 and 20 μL (~0.5 and 1 cell applied to discs) samples remained below the LOD, determined as the mean plus 3 times the standard deviation of a NTC run in parallel, and were thus scored as negative. With 40 and 60 μL samples (~2 and 3 cells) the number of replicates scoring positive, above the LOD, increased to 7 and 8, respectively. Sample volumes over 60 μL began to reduce the number of replicates positive likely as a result of washing effects. Average signal and average positive signal data is shown in Figure S6.