Electronic Supplementary Information

A "Paper Machine" for Molecular Diagnostics

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Calculation of Cost of Device

The cost of the device, as shown in Figure 1, was estimated using low-volume pricing for all materials and reagents (Table S1). Once scaled-up for high-volume manufacturing, bulk discounts would further decrease the total cost.

Table S1: Bill of Materials for Device as shown in Figure 1

Materials	**Cost/Device**
Papers	$ 0.06
Magnets	$ 0.48
Films, Lubricant	$ 0.06
Reagents	**Cost/Device**
LAMP Reagents	$ 0.84
Primers	$ 0.26
SYBR Green I	$ 0.14
Total materials cost	***$ 0.60***
Total test cost	***$ 1.83***

Optimization of LAMP Reaction in Paper

The transition from 25 µL LAMP reactions in tubes, to 10 µL reactions run in FTA paper, required optimization of the reaction conditions. Here, we investigated two different versions of the polymerase, the incubation time, and varying concentrations of betaine and the primers (sequences shown in Table S2).

Switching from *Bst* DNA Polymerase, Large Fragment, to *Bst*2.0, as shown in Figure S1a, improved the sensitivity of the reaction at lower concentrations, increasing the detection of samples containing 1 genomic copy from no replicates testing positive with the original polymerase to half (4 of 8 replicates) testing positive with *Bst*2.0. Increasing the amount of *Bst*2.0 from 8 U to 12 and 16 U did not improve the sensitivity of the assay nor did doubling the concentration of primers in the reaction (not shown).

As the system uses LAMP with endpoint detection, allowing the reaction to run longer could increase sensitivity by allowing samples giving weak or undetectable amplification time to generate more product. An additional 30 min (Figure S1b), however, had a detrimental impact. All replicates of the no-template control (NTC) produced false-positive signals, indistinguishable from the true positives.

Optimization of the betaine concentration (Figure S1c) had the greatest effect on the reaction. At the concentration employed in commercially-available kits (Loopamp DNA Amplification Kit, Eiken Chemical Co., Ltd., Taito-Ku, Tokyo, Japan), 0.8 M betaine, LAMP in FTA paper produced false-positives (7 of 8 replicates). Increasing this to 0.9 M betaine eliminated these false-positives. At higher concentrations (1.2 M), betaine inhibited the reaction, producing false-negative results in reactions containing target DNA. Thus, 0.9 M betaine was optimal.

Table S2: LAMP Primer Set

Primer	5' to 3'
F3	GCCATCTCCTGATGACGC
B3	ATTTACCGCAGCCAGACG
BIP	CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT
FIP	CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT
LF	CTTTGTAACAACCTGTCATCGACA
LB	ATCAATCTCGATATCCATGAAGGTG

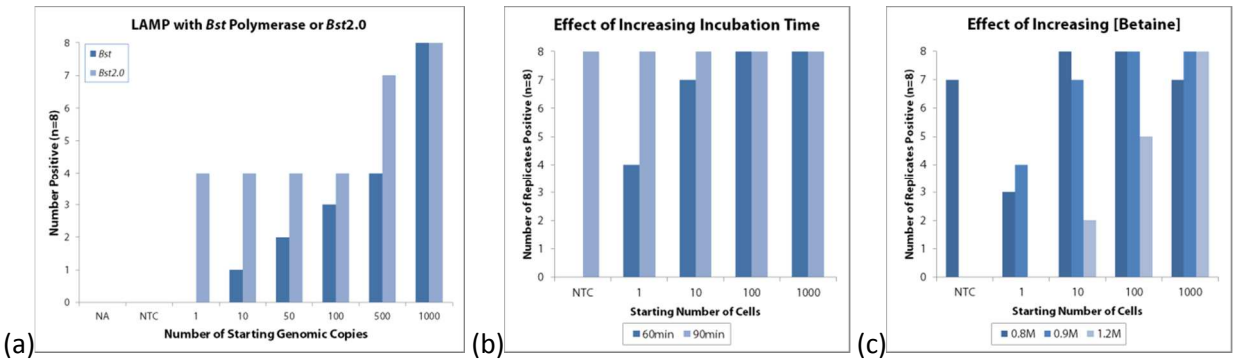

Figure S1: Optimization of LAMP Reaction Conditions in FTA Paper Discs. In order to improve sensitivity and eliminate non-specific amplification we investigated (a) two versions of the polymerase, (b) incubation time, and (c) the impact of the increasing betaine concentration. As we were interested in eliminating false positives resulting from non-specific amplification, replicates were defined as positive if they produced a signal more than 3 times the standard deviation of the no-amplification control (NA).

Optimization of Wash Purification

The dried lytic agents present on FTA paper are exploited in this device to streamline the sample preparation process. These same agents, however, must be washed away or quenched prior to the application of the amplification reagents as they can denature the polymerase or otherwise inhibit the reaction. In its typical use, FTA paper is washed with large volumes of buffer in tubes, prior to being placed into PCR amplification reactions. Our "paper machine" uses a low-volume, through-flow method. Using *E. coli* spiked into human serum, we developed and optimized the sample preparation process.

FTA Purification Reagent, a proprietary buffer designed for us with FTA paper, was used as the wash buffer. As the current prototype uses liquid LAMP amplification reagents, which necessitates drying of the reaction disc between reagent addition steps, we evaluated the necessity of a second, larger volume, water wash to remove the excess surfactant. As shown in Figure S2a, the assay sensitivity was poor when using only 20 µL of FTA purification reagent as the wash buffer. Reactions containing 1, 10, 100 or 1,000 cells all produced false-negatives in 37.5% to 62.5% of replicates (n = 8 at each

concentration). When following the 20 µL FTA Purification Buffer wash with 40 µL of nuclease-free water reduced the false-negative rate at nearly all starting target numbers.

FTA paper contains EDTA, which improves long-term stability of DNA stored in the paper as it chelates magnesium ions that are required for nuclease activity. As magnesium ions are also required for the activity of *Bst*2.0 DNA polymerase, we investigated supplementing the FTA Purification Buffer with $MgSO_4$ to quench any remaining EDTA chelation sites. Supplementing the FTA Purification Buffer with 8 mM $MgSO_4$, consistently reduced the number of false-negatives compared to the buffer without supplementation (Figure S2b).

Occasionally, it was noted that serum proteins were not fully washed away, which lead to inconsistent signals. We evaluated increasing the wash volumes, maintaining the ratio between the modified FTA purification buffer and the following water wash, to eliminate this. Using 40 µL supplemented FTA Purification Buffer and 80 µL water (2x Volumes) resolved the problem (Figure S2c).

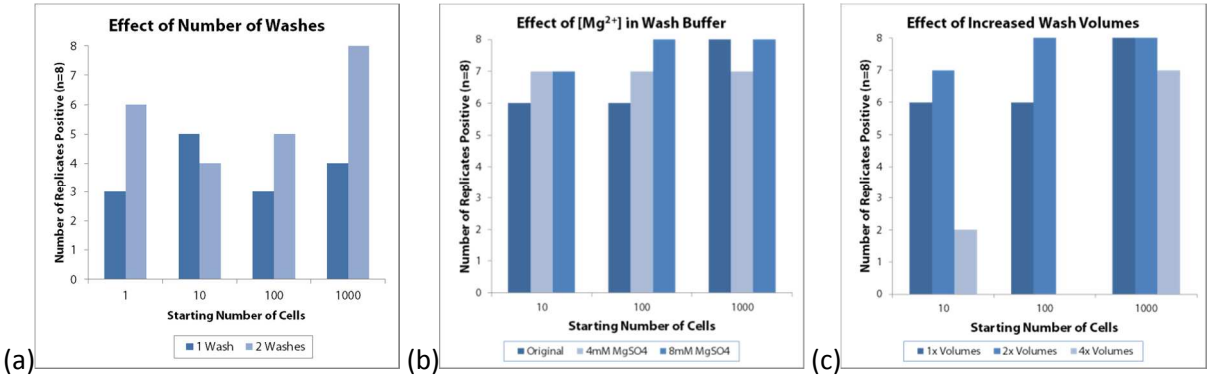

Figure S2: Optimization of Wash-based Purification. As FTA paper contains chemistry that may inhibit LAMP, the wash conditions and the nature of the wash buffer were evaluated to ensure successful amplification. We evaluated (a) the necessity to follow the initial 20 µL FTA Purification Buffer with a 40µL water wash, (b) the impact of increasing the amount of divalent metal ions in the FTA Purification Buffer, and the effect of increasing the original wash volumes. Here replicates were defined as positive if they produced a signal more than 3 times the standard deviation of the no-template control (NTC) run in parallel for each condition.

Analytical Sensitivity of LAMP in Device

The data shown in Figure 3a is the result of quantifying the mean gray value of the reaction discs shown in Figure 3b using ImageJ and setting a cut-off value to bin the replicates as positive and negative. This cut-off value is the mean of the NTC replicates plus three times their standard deviation as shown in Figure S3, which shows the mean gray value signals for all conditions.

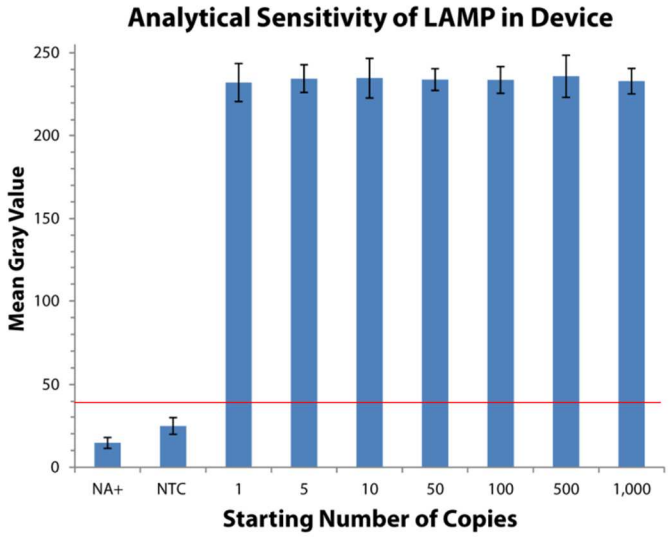

Figure S3: Average signals used to bin replicates for analytical sensitivity of LAMP in the device. The image of the reaction discs shown in Figure 3b was analyzed using ImageJ to produce the data shown here. The error bars are the standard deviation of n = 8 replicates and the red line indicates the cut-off value used to bin the replicates as positive or negative. This value is the limit of detection (LOD) defined as the mean of the NTC plus 3 times the standard deviation.

Restriction Endonuclease Agarose Gel Electrophoresis Analysis

To confirm that fluorescent signal is the result of the specific template-driven reaction, a restriction endonuclease analysis was performed. Here, a positive reaction run in a tube was digested with a restriction enzyme, *PvuII*, which cuts the concatenated high-molecular weight products of the inner primer pathway (FIP, BIP) giving bands at 133 and 80 bp and products of the loop primer pathway

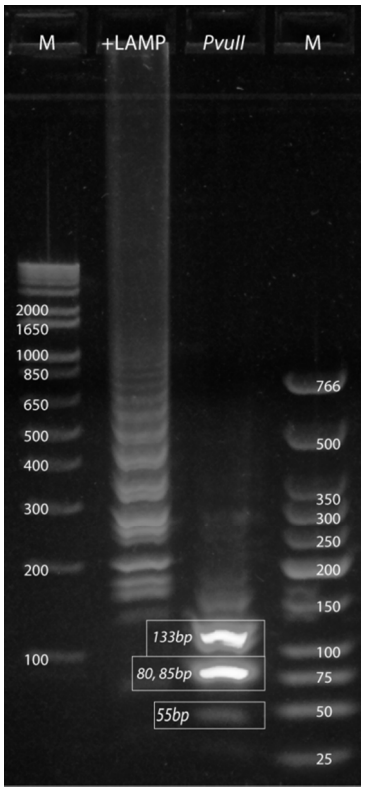

Figure S4: Restriction Endonuclease Digest for Specificity Confirmation. To determine that fluorescent signal is the result of specific, template-driven amplification and not primer artifacts, a positive reaction was digested with a restriction enzyme, *PvuII*, and analyzed by AGE, lane 3 (c). The presence bands at 133, 80, 85, and 55 bp indicate the specific reaction. This confirms that the LAMP banding pattern in lane 2, the undigested positive, can be identified as the specific pattern for further comparison.

(LF, LB) producing additional bands at 85 and 55bp. This digested product and the original product were analyzed by agarose gel electrophoresis (AGE), Figure S4, confirming the absence of primer cross-reactivity. Further, this result identifies the banding pattern of the undigested product as the desired, specific reaction and this pattern was used to confirm the specificity of subsequent results.

Integrated Assay with Live *E. coli* Spiked into Human Plasma

As described above, replicates were binned as positive or negative using mean gray value and the mean plus three times the standard deviation of the NTC as the cut-off giving the data shown in Figure 4b. The average mean gray values for all replicates at each cell number, as well as the average signal for just those scored positive at each cell number, are shown in Figure S5.

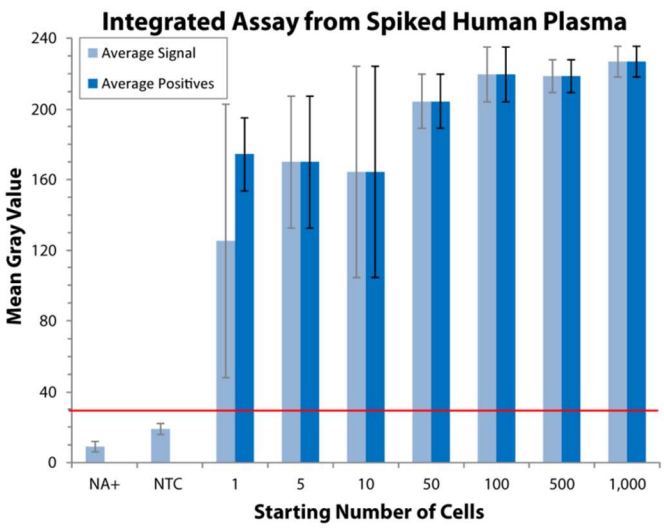

Figure S5: Assay Integrated with Device. The average mean gray value at each condition across all replicates (n = 6) and just those scored positive are shown. The red line indicates the cut-off value for binning, calculated as the mean plus 3 times the standard deviation of the NTC. The only condition containing template DNA that did not score all replicates positive is 1 cell, which scored 4 replicates positive.

Most Probable Number Analysis

The spiked samples tested in the assay integrated with the device were derived from a dilution series beginning with a sample determined to contain 100,000 cells/µL by spectrometry. To determine if the observed result – scoring 4 of 6 replicates nominally containing 1 cell as positive – is consistent with the expected outcome resulting from stochastic sampling error we employed a most probable number (MPN) analysis. To do so, we converted each concentration into a volume of the original stock in the 10

µL applied to the reaction disc – i.e. discs inoculated with 1,000 cells contained 0.01 µL of the original – and entered these, along with the number of replicates positive with n = 6, into a MPN Excel spreadsheet available online as an appendix to the US FDA Bacteriological Analytical Manual (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm).This calculation gives the lowest tested sample as 1 cell/disc with a 95% confidence interval of 0.4 to 2.8 cells/disc. This indicates that the 2 replicates scored negative that should have contained 1 cell likely did not contain a cell due to sampling error and, therefore, were truly negative, not falsely negative.

Effect of Increased Sample Volumes

As with the data from the analytical sensitivity and integrated assay experiments, the data shown in Figure 5 for the effect of increased sample volume is the result of binning replicates of each condition as positive and negative based on mean gray value and a cut-off value set as the mean of the NTC replicates plus three times their standard deviation. The average signal of all replicates and just those scored positive at each condition is shown in Figure S6.

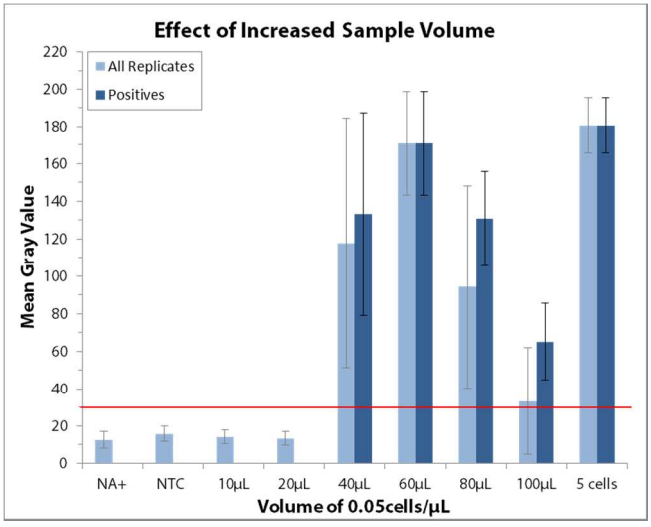

Figure S6: Effect of Increased Sample Volume. The average signal across all replicates at each condition (n = 8) as well as the average signal from the positive replicates is shown with error bars representing the standard deviations. The red line indicates the cut-off value for binning, calculated as the mean plus 3 times the standard deviation of the NTC.