Research paper

Smartphone-based pathogen diagnosis in urinary sepsis patients

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Abstract

Background: There is an urgent need for rapid, sensitive, and affordable diagnostics for microbial infections at the point-of-care. Although a number of innovative systems have been reported that transform mobile phones into potential diagnostic tools, the translational challenge to clinical diagnostics remains a significant hurdle to overcome.

Methods: A smartphone-based real-time loop-mediated isothermal amplification (smaRT-LAMP) system was developed for pathogen ID in urinary sepsis patients. The free, custom-built mobile phone app allows the phone to serve as a stand-alone device for quantitative diagnostics, allowing the determination of genome copy-number of bacterial pathogens in real time.

Findings: A head-to-head comparative bacterial analysis of urine from sepsis patients revealed that the performance of smaRT-LAMP matched that of clinical diagnostics at the admitting hospital in a fraction of the time (~1 h vs. 18–28 h). Among patients with bacteremic complications of their urinary sepsis, pathogen ID from the urine matched that from the blood – potentially allowing pathogen diagnosis shortly after hospital admission.

Interpretation: The smaRT-LAMP system is effective against diverse Gram-negative and -positive pathogens and biological specimens, costs less than $100 US to fabricate (in addition to the smartphone), and is configurable for the simultaneous detection of multiple pathogens. SmaRT-LAMP thus offers the potential to deliver rapid diagnosis and treatment of urinary tract infections and urinary sepsis with a simple test that can be performed at low cost at the point-of-care.


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1. Introduction

The World Health Organization and the U.S. Department of Health and Human Services have recently prioritized the development of rapid, accurate, and cost-effective diagnostics that can be used by healthcare providers at the point-of-care (POC) to diagnose bacterial infections [1,2]. Such diagnostic systems are especially needed in less developed countries, where bacterial infections are more prevalent and medical resources are limited [3,4]. The microbial diagnosis of pathogens directly from whole blood has been constrained by the low number of circulating organisms – typically just 1–100 colony forming units (CFU)/mL during infection – and the frequency of false positive results [5]. Moreover, standard culturing practices for pathogen identification (ID) from blood can take 2 to 3 days [6]. To expedite the process, there are numerous molecular methods for the detection of bacteria – including PCR, probe-based direct detection, peptide nucleic acid-based fluorescence in situ hybridization, and matrix-assisted laser desorption ionization–time of flight mass (MALDI) spectrometry analysis [7–9]. However, these clinical techniques require prior sub-culturing in blood culture bottles for bacterial detection (8–24 h), followed by pathogen ID (1.5–24 h), resulting in a total time of ~10–48 h from...
false positive results due to contamination at collection or false negative results due to culture failure [14]. Thus, improved urine-based tests for the rapid detection of pathogens would be highly valuable for improving patient outcomes for UTIs and in potentially fatal conditions arising from septicemia (e.g., pyelonephritis) [19].

A number of innovative systems have recently been reported that transform mobile phones into potential clinical POC diagnostic tools based on various detection modalities. Examples include optical and fluorescence imaging [20], microtiter assay interpretation [21], immunologic detection (e.g. microfluidic chips [22–24]; antibody-conjugated strips [25]) and nucleic acid detection (e.g., microfuge tubes [26,27]; microtitre plates [28]; microfluidic chambers [29]; microfluidic chips [30–36]). Although these are notable advances in terms of broadening access to sophisticated molecular diagnostics, translation to clinical utility using patient-derived samples has been limited (e.g., HIV blood samples [24], influenza throat swabs [25], Chlamydia trachomatis swabs [36]).

We have developed a rapid, quantitative and accessible smartphone-based detection system with clinical utility, achieving timely diagnosis of bacteriuria from human patients. SmART-LAMP performance matched that of standard clinical diagnostics, but within a substantially shorter time-frame and lower cost, thus providing a means for inexpensive and accurate diagnosis of UTIs and urinary sepsis directly from clinical specimens at the POC.

2. Materials and methods

2.1. Bacterial strains and media

Gram-negative bacterial isolates tested included Salmonella sp., Salmonella Typhimurium ATCC 14028 (ST), and S. enteritidis 4973 (SE) [37,38], Escherichia coli (EC) strain ATCC 25922 (EC), Yersinia pseudotuberculosis YPIII/plB1 (YP) [39], Klebsiella pneumoniae strain ATCC 13883 (KPN), and Pseudomonas aeruginosa strain ATCC 10145 (PA). Gram-positive bacterial isolates analyzed included S. aureus USA300 (SA), a community-associated methicillin-resistant isolate causing the most MRSA infections in the U.S. [40], and S. pneumoniae D39 (ser. 2) (SPN) [41]. ST, SE, SP, EC, KPN, and PA [42,43] were streaked from frozen stocks onto Luria-Bertani (LB) agar plates and single colonies were inoculated into LB broth and incubated overnight with shaking at 37 °C. All incubations of YP were at 28 °C. SPN was streaked from frozen stocks onto Todd–Hewitt (TH) broth agar plates containing 2% yeast extract and incubated overnight at 37 °C in a 5% CO2 incubator. Single colonies were inoculated into TH broth containing 2% yeast extract and incubated overnight without shaking at 37 °C in a 5% CO2 incubator. SA was streaked from frozen stocks onto Tryptic Soy (TS) agar plates and incubated overnight at 37 °C. Single colonies were inoculated into TS broth and incubated overnight with shaking at 37 °C.

2.2. gDNA preparation

gDNA was prepared by growing bacteria as described above and pelleting approximately 1 x 10¹⁰ total cells. Cells were resuspended in 0.5 mL TE buffer, 10 μL 10% SDS, 10 μL 10 mg/mL DNase-free RNase, mixed and incubated 1 h at 37 °C. Next, 10 μL 10 mg/mL protease K was added and samples were incubated 2 h at 65 °C. Samples were then extracted with an equal volume of chloroform/isoamyl alcohol and spun 5 min at 16,000 x g in a microcentrifuge. The aqueous phase was transferred to a fresh tube and DNA was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) and spun 5 min at 16,000 x g. The aqueous phase was transferred to a fresh tube and DNA was extracted with 2.5 vol 100% ethanol and 0.1 vol 3 M sodium acetate. Precipitate was washed once with 70% ethanol, supernatant was removed and pellet was dried briefly in a DNA speedvac. Pellets were resuspended in 100 μl ultrapure H2O, aliquoted and stored at –20 °C until use.
2.3. LAMP reaction conditions

2.3.1. LAMP reagents

Betaine, calcein, KCl, MgSO4, MnCl2, (NH4)2SO4, and Triton X-100 were purchased from MilliporeSigma (St. Louis, MO). Bst 2.0 WarmStar DNA polymerase was purchased from New England Biolabs (Beverly, MA), deoxyxynucleotide triphosphates from Promega (Madison, WI), Tris (pH 7.5) from Invitrogen (Carlsbad, CA), Nuclease-free water, DMSO, NaOH, and polysorbate 20 from Thermofisher (Waltham, MA). Tris (pH 8.8) was purchased from VWR (Radnor, PA), and PCR tubes with optically clear lid strips from Bio-Rad (Hercules, CA). Primers were purchased from Integrated DNA Technologies (Coralville, IA).

2.3.2. Oligonucleotide primers

Supplemental Table S2 provides a list of oligonucleotide primer sequences. All synthetic oligos were purchased from Integrated DNA Technologies (Coralville, IA). Previously designed primers targeting the recF gene of Salmonella sp. [44] were employed with the addition of loop primers chosen to accelerate the reaction by priming strand displacement synthesis [45]. The set of primers consisted of two outer (F3 and B3), two inner (FIP and BIP), and two loop primers (F-Loop and B-Loop). Additional published primer sets were selected for other pathogens: ST rfbj [46]; SE SdfI [47]; VP inv [48]; Ec gdxK [49]; KPN fimD [50]; PA oipL [51] (a F-Loop primer was developed for the SPN lytA set); SA 16S rRNA [52].

2.3.3. Reaction conditions

We generated a 2× LAMP reagent “master mix” containing 40 mM Tris (pH 8.8), 20 mM KCl, 16 mM MgSO4, 20 mM (NH4)2SO4, 0.2% v/v polysorbate 20, 1.6 mM betaine, 2.8 mM each for the four deoxyxynucleotide triphosphates, 0.58 U/μL of Bst WarmStart DNA polymerase, 0.4 μM each of F3, B3 primers, 3.2 μM each of FIP, BIP primers, 1.6 μM each of F-Loop, B-Loop primers, 750 μM MnCl2, and 37.6 μM calcein. All reactions were conducted at 65 °C for 50 m.

2.4. Purified gDNA

20 μL of purified pathogen gDNA stock was diluted to specified concentrations, mixed 1:1 with 20 μL of LAMP master mix (at 2× final concentration), and split into 19 μL aliquots between qPCR-LAMP and smaRT-LAMP.

2.4.2. CFU in buffer and blood

For analysis of CFU in buffer and blood, a modified alkaline treatment was used [53]. A 2 μL sample was vortexed for 15 s after mixture with 78 μL [54] of lysis mix (50 mM NaOH and 0.5% Triton X-100 in the final 80 μL lysisate volume), then pulse-spun for 3 s on a microcentrifuge, and heated at 100 °C for 10 m on an aluminum heat block. After cooling on ice for 2 m, samples were centrifuged for 2 m at 16,000 x g and 40 μL of supernatant removed to another tube. To neutralize, 6.4 μL of 1 M Tris-HCl (pH 7.5) was added, vortexed briefly, and centrifuged for 2 m. 40 μL of supernatant was added to tubes containing 40 μL of 2× LAMP master mix and mixed by pipetting. The resultant lysate was split into two 38 μL aliquots that were analyzed by a Bio-Rad thermocycler and smaRT-LAMP with the BactiCount app.

2.4.3. CFU in urine and feces

Urine and feces samples were analyzed similar to buffer and blood, with the omission of centrifugation and pellet-removal steps. After heating at 100 °C and cooling on ice, the 80 μL lysates were neutralized with 12.8 μL of 1 M Tris-HCl (pH 7.5), vortexed briefly to mix, and 40 μL of lysate was mixed with 40 μL 2× LAMP master mix before splitting into two aliquots for analysis.

2.5. Preparation of pathogen samples in spiked buffer and uninfected murine specimens

For LOD buffer analysis, serial dilutions of S. typhimurium cells (101 to 105 CFU/mL) were spiked into buffer. Briefly, 1 mL samples of the stated concentrations were reduced to 2 μL via sequential centrifugation. Blood from uninfected mice was collected by tail bleed into BD Microtainer PST tubes with lithium heparin (Becton Dickinson, cat. no. 365985). Urine was collected into sterile microfuge tubes. Feces (0.1 g) was collected into sterile microfuge tubes, resuspended in 0.3 mL reaction buffer, and the mixture was pulse-spun in a microcentrifuge for 5 s to pellet large particulates. Bacteria were diluted into reaction buffer (20 mM Tris, pH 7.5), or spiked into blood, feces, or urine, collected from uninfected mice, respectively, at specified concentrations. Mice: 8–12 wk. old male and female C57BL/6J mice (Jackson Labs, Bar Harbor, ME) were used for all infections and blood, urine, and feces specimen collections.

2.6. Animal infection protocols and specimen collection

2.6.1. Gram-negative pathogens

All Gram-negative strains were grown overnight in LB. ST and SE bacterial strains were pelleted by centrifugation, washed, and suspended in sterile 0.2 M sodium phosphate buffer (pH 8.1). Mice were orally infected with ST via gastric intubation at a dose of 2×107 cells (20× LD50) and whole blood was sampled at days 6 (pre-sepsis), 8 (sepsis), and 10 (severe sepsis) post-infection. For intraperitoneal (i.p.) infections, a 20× LD50 dose of SE (105 cells) or VP (5×106 cells) in 100 μL 0.15 M NaCl was administered and whole blood was collected from the tail vein of septic mice at day 5 post-infection. EC was suspended in sterile phosphate buffered saline (PBS) and mice were infected via the i.p. route at a dose of 1–2×107 bacteria (20× LD50) in 100 μL volume. Blood was taken for analyses at 48 h post-infection (severe sepsis). A dose of 20× LD50 ensures that virtually all infected animals will undergo sepsis.

2.6.2. Gram-positive pathogens

SPN cultures were diluted 1:10 into fresh TH broth and sub-cultured to mid-log phase (A600 = 0.4), pelleted in a microfuge at 16,000 × g for 2 m, washed, and suspended in 0.15 M NaCl. i.p. injection of 1 to 2×104 cells (20× LD50) was done in 100 μL 0.15 M NaCl. Whole blood was collected from the tail vein of septic mice at 48 h post-infection into microtainer tubes. SA cultures were diluted 1:100 into fresh TH broth and sub-cultured to mid-log phase (A600 = 0.4), pelleted in a microfuge at 16,000 × g for 2 m, washed, and suspended in 0.15 M NaCl. Intravenous (i.v.) injection into the retroorbital sinus of 1–2×108 cells (20× LD50) was done in 100 μL 0.15 M NaCl. Whole blood was collected from the tail vein of septic mice at 48 h post-infection into microtainer tubes. Institutional Animal Care and Use Committee of the University of California, Santa Barbara approved studies undertaken herein.

2.7. Urine specimens from human sepsis patients

Human specimens were collected at Santa Barbara Cottage Hospital, Santa Barbara, CA. Patients were selected who met the clinical criteria for sepsis based on fever, increased heart rate, and/or elevated white blood cell count, and had a suspected urinary source of their severe infection. Some of these patients had severe sepsis, with evidence of end-organ dysfunction or septic shock. Upon presentation at the hospital, urine and blood specimens were collected from patients before antibiotic administration. A comparative urine bacterial analysis was performed between smaRT-LAMP and clinical diagnostics carried out by the hospital managing patient care. Pathogen ID in the urine and blood of sepsis patients was determined by the hospital microbiology laboratory. The bacterial load in urine specimens was assessed by both
direct colony count, and smaRT-LAMP utilizing primer sets directed against the urine pathogen identified in the clinical setting. The bacterial load in the urine of human sepsis patients with clinically negative urine cultures (below the standard threshold for infection of $10^5$ CFU) [14,15] was determined by the hospital microbiology laboratory (clinical culture) versus an academic laboratory examining CFU by direct colony count, qPCR-LAMP, and smaRT-LAMP, utilizing E. coli primer sets. A linear fit of standard curves with a clinically relevant bacterial burden ($5 \times 10^7$–$5 \times 10^8$ CFU/mL) was used to determine LAMP-based CFUs. LAMP-based assays were sometimes inhibited in cloudy urine specimens (precipitated phosphate crystals and/or pyuria) [15], but inhibition was relieved by a 1:10 dilution of the specimen in 20 mM Tris-HCl (pH 7.5).

2.8. Data analysis

Real-Time LAMP traces were automatically generated at the end of each run for each sample by the qPCR thermocycler and the Bacticount app. Trace files were transferred to a personal computer (PC), where MATLAB was used (described in detail in the Supplementary Methods section) to find the maximum of the derivative taken over a coarse time stepper (i.e., a chosen length of time over which to average the derivative). The resultant $T_i$ value was linearly related to the logarithm of the input concentration and used to determine the concentration of bacteria in septic murine samples using standard curves with a minimum of 10 reaction replicates per concentration at $5 \times 10^6$ CFU/reaction. All experiments were performed in low-profile 0.2–mL PCR strips (Bio-Rad cat. no. TLS-0801) covered with optical flat strips (Bio-Rad cat. no.TLS-0803). Sample tubes were placed in an aluminum sample block (LightLabs cat. no. A-7029) on a hot plate (HP30A digital aluminum hotplate, Torrey Pines Scientific, Carlsbad, CA). A cardboard box large enough to cover the hot plate was painted black and two flexible cables of 96 W, 480 nm, 672 lm, 96-LEDs (DealeXtreme cat. no. 180563) were affixed to the inside top cover of the box. LEDS were powered using a single output DC power supply (UA8001A, Agilent Technologies, Santa Clara, CA). A Samsung Galaxy S7 smartphone (Samsung Electronics Co., Ltd.) was outfitted with a 520 ± 10 nm bandpass filter (Edmund Optics cat. no. 65–699) for visual detection of emitted green light (Supplementary Fig. 1). All qPCR reactions were performed on a Bio-Rad CFX96 qPCR Thermocycler.

2.10. Development and function of bacticount android application

2.10.1. Bacticount android application

The Bacticount smartphone application was built on a Samsung Galaxy S7 phone using the developer tools in Android Studio IDE, Android SDK (Android), and OpenCV library. The app can be downloaded and installed from the Google Play Store; the user is then prompted to install the “OpenCV Manager” application, which is employed to handle complex algorithms such as image rendering, histogram generation, and back-calculations. Upon opening the app, the user is initially presented with an option for a step-by-step tutorial. In addition to the tutorial, the user is given a choice to “Start Bacterial Analysis”; when selected, the user is prompted to pick the correct sample type (Blood, Urine, or Feces). The user can then follow a three-step analysis procedure: 1) record a standard curve for the pathogen in spiked samples; 2) record a sample reaction from unknown analytes; and 3) select and view results to analyze a sample reaction using a specific standard curve to instantly determine bacterial burden (Supplemental Fig. 2a–c).

2.10.2. Running standard curve and unknown sample reactions

When running a standard curve or unknown sample reaction, the app launches a specialized viewfinder, allowing the user to carefully center the reaction vials in the view-frame of the phone’s camera, such that their intensity can be analyzed over time. After entering a name, the user must load samples and press “start”, which begins a timer to correct for lost reaction time while setting up the box and aiming the camera (Supplemental Fig. 2d, e). When the user selects “Begin Recording Amplification,” the application proceeds to take one photograph of the amplification reaction every 10 s over the course of a 50 m period (Fig. 1b, Supplemental Fig. 2f). The app performs image analysis of the photograph, allowing the user to carefully center the reaction vials in the view-frame of the phone’s camera, such that their intensity can be analyzed over time.

![Overview of smaRT-LAMP approach](image1.png)

**Fig. 1.** SmRT-LAMP direct specimen testing of urine from sepsis patients. (a) Assay schematic for smRT-LAMP, which entails sample collection, bacterial cell lysis/reagent addition, and real-time analysis via the smartphone. (b) Schematic of workflow for the Bacticount app, which analyzes fluorescence data collected continuously from multiple samples through the phone’s camera (left panel), and then uses these data to automatically determine the genome copy-number of bacterial pathogens in real time (right panel).

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processing for each of the vials outlined in the viewfinder to extract the average green intensity of each pixel, which is stored in a matrix. For the “1. Record Standard Curve” option, the software also prompts the user to align each reference sample with a provided sample map so that the input starting concentrations of DNA are known. The standard curve is determined through a linear regression fit of $T_t$ vs. $\log_{10}[\text{conc}]$, which is stored as a .pasc file for determining the results in future tests. When the user has selected the “2. Record Sample” option, the app will record traces for each sample, to be analyzed later. The numerical sample traces and collected time-stamped photos are saved as a .part file and as .jpeg files, respectively, which may be extracted by the user to any computer.

2.10.3. Automated data analysis

When the user selects “3. Select and view results”, the app will prompt the user to choose a standard curve that has been recorded as outlined in the previous section with known standard concentrations. After data processing and analysis (described in the Supplementary Methods section), the $T_t$ of unknown test samples are related to their initial concentrations via the standard curve. On its final screen, the app displays the number of bacterial CFU in each reaction vial (Supplementary Fig. 2 g–i).

3. Results

3.1. Overview of the SmaRT-LAMP system

The SmaRT-LAMP procedure can be performed with freshly collected biological specimens (e.g., blood, urine, or feces), which are then lysed with a simple NaOH and detergent treatment and subsequent heating procedure (Fig. 1a). The resultant lysate is combined with a pre-mixed LAMP reaction mixture that will generate a fluorescent signal in response to successful amplification (see Methods). These samples are then placed in an inexpensive apparatus consisting of a platform that can simultaneously accommodate up to 36 samples, a single-temperature heat block, and an LED light source (Supplementary Fig. 1). The entire detection system can be fabricated for less than $100 US, not including the smartphone (Supplementary Table 1).

The streaming image data from SmaRT-LAMP are collected in real-time and analyzed by a smartphone running the Bacticount app (Fig. 1b, Supplementary Fig. 2), which we developed for the Android operating system and have made freely available through the Google Play store. We derive the template DNA copy number by using a ‘coarse derivative’ algorithm [55] to convert the fluorescence data into a time-to-threshold parameter ($T_t$) – the time at which the rate of fluorescence increase is fastest. This $T_t$ measurement indicates the exponential phase of the LAMP reaction and is linearly proportional to the logarithm of the template DNA copy number [56]. Thus, we can quantitatively determine the concentration of gDNA in a sample based on a standard curve of $T_t$ measurements derived from samples of known concentration. As shown below, our coarse derivative algorithm is robust and produces highly reproducible data, even with fluctuations in background fluorescence, camera recalibrations, and shifts in the relative position between the sample and the smartphone camera.

3.2. SmaRT-LAMP sensitivity and intra- and interspecies detection

We tested whether the sensitivity of SmaRT-LAMP can match that of a LAMP assay performed in a real-time quantitative PCR instrument (qPCR) for detecting gDNA of Salmonella Typhimurium (ST). We used a set of six primers designed to target the highly conserved recF gene [44,57]. These primers are specific to Salmonella sp., and thus are not expected to hybridize to DNA of unrelated pathogens. We compared the SmaRT-LAMP and qPCR instrument for measuring the gDNA of ST over a broad range, from $5 \times 10^{-5} - 5 \times 10^9$ copies of the genome. SmaRT-LAMP and qPCR instruments showed equivalent performance in this assay, as evidenced by the $T_t$ dose dependency, trace quality and reaction time (Supplementary Fig. 3).

Strain discrimination is imperative for clinical diagnostics and treatment, and we demonstrated that the SmaRT-LAMP platform is compatible with both intra- and interspecies detection and strain discrimination. We tested this by employing primer sets specific to gDNA templates from eight different Gram-negative and -positive pathogens, including ST, S. enteritidis (SE), Escherichia coli (EC), Klebsiella pneumoniae (KPN), Pseudomonas aeruginosa (PA), Versinia pseudotuberculosis (YP), Streptococcus pneumoniae (SPN) and Staphylococcus aureus (SA) (Supplementary Table 2). SmaRT-LAMP achieved robust interspecies detection and strain discrimination of these Gram-negative and -positive pathogens, with each of the eight primer sets amplifying only the gDNA of their cognate template but not any of the seven other templates (Table 1). SmaRT-LAMP can distinguish between ST and SE, which are serovars of the same Salmonella subspecies (S. enterica subsp. enterica) that are 99% identical at the DNA sequence level [58]. It should be noted that the reduced primer sensitivity for ST relative to the other pathogens tested is not indicative of a failure of the reaction but rather the limited number of sequences available to design LAMP primer sets that distinguish Salmonella subsp. that are closely related at the DNA level (e.g., ST vs. SE).

3.3. Pathogen detection using whole bacterial cells

We next assessed whether our LAMP protocol enables quantitative pathogen detection using whole bacterial cells, and if SmaRT-LAMP can match the sensitivity of an equivalent LAMP assay performed in a qPCR instrument. ST cells were serially diluted into buffer at concentrations ranging from $10^2$ to $10^5$ CFU/mL. One mL of each dilution sample was then reduced to 2 μL via sequential centrifugation and subjected to the LAMP protocol. We derived a standard curve through a linear regression fit of $T_t$ vs. $\log_{10}[\text{CFU}]/\text{mL}$ (Fig. 2–d); see Methods). The resultant limit of detection (LOD) was $\pm 10$ CFU/mL for both SmaRT-LAMP and qPCR. We also observed excellent reaction efficiency in terms of the percent of samples that were successfully amplified at 10 CFU/mL (70 and 90%, respectively; Fig. 2e, f). Such a low LOD ($\pm 10$ CFU/mL) obtained from sequential centrifugation may have clinical utility for swabbing infection sites, medical devices and other potentially contaminated surfaces.

We then tested whether our LAMP procedure works with Gram-positive and -negative pathogens that have large differences in cell envelope structure. Using the same LAMP protocol, we assessed the performance of both amplification platforms against Gram-positive SPN and SA, as well as Gram-negative SE, EC and YP. Briefly, 2 μL of spiked buffer samples containing $5 \times 10^{-5} - 5 \times 10^5$ CFU/mL of bacteria were processed and assessed in the SmaRT-LAMP and qPCR instruments as described above. Both platforms showed strong performance against all five pathogens, with a LOD of $5 \times 10^{-4} - 1 \times 10^3$ CFU/mL (equivalent

**Table 1**

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<th>SmaRT-LAMP intra- and interspecies specificity.</th>
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*+* denotes amplification of cognate primer-gDNA pairs ($10^5$ gDNA copies) without amplification of non-cognate primer-gDNA pairs ($10^5$ gDNA copies). *-* denotes amplification of cognate primer-gDNA pairs ($10^5$ gDNA copies) without amplification of non-cognate primer-gDNA pairs ($10^5$ gDNA copies). *-* represents no amplification.
to 2–100 CFU/reaction), clear Tt dose dependency, and strong reaction efficiency (Supplementary Fig. 4). These data indicate that the smart-LAMP system is compatible with a diverse array of pathogens.

### 3.4. Pathogen detection in spiked murine whole blood, urine, and feces

Next, we examined the performance of smart-LAMP and qPCR-LAMP in diverse biological specimens and tested whether bacterial detection can be achieved at clinically-relevant concentrations. Briefly, ST was serially diluted in murine whole blood, urine, and feces over a range of $5 \times 10^3$–$5 \times 10^7$ CFU/mL. After collecting and processing 2 μL aliquots from these samples, half of each lysate reaction was subjected to LAMP in both the smart-LAMP and qPCR platforms.

Direct specimen testing in whole blood is problematic due to the low bacterial load typically observed in circulation of sepsis patients (1–100 CFU/mL) [5]. As a result, clinical detection methods require samples to first be incubated in blood culture bottles, resulting in a total time of ~10–48 h from inoculation to time to pathogen ID [10–13]. Both smart-LAMP and qPCR instruments were able to achieve this level of detection sensitivity of ST from whole blood, with an LOD of $5 \times 10^3$ CFU/mL (Fig. 3a, b) – equivalent to just 2 CFU/reaction – with clear Tt dose dependency and strong reaction efficiency (Fig. 3i, j).

Importantly, smart-LAMP offers the potential to achieve rapid direct detection of clinically-relevant signatures of bacterial infection in urine. Urine can offer an early readout of patients with UTIs, and potentially in cases of sepsis – particularly those who have a suspected urinary source – since infected urine is associated with a much higher bacterial load than blood ($\geq 10^5$ CFU/mL) [14,15]. However, there is currently no direct specimen testing method approved for urine, principally due to the challenges of microbial contamination at the point of collection [14,18]. Thus, the gold standard of care entails a bacterial culture step, to the challenges of microbial contamination at the point of collection.

We assessed whether the smart-LAMP system was compatible with several murine models of sepsis. Mice were orally infected with ST and blood was sampled at day 6 (pre-sepsis); day 8 (sepsis), and day 10 post-infection (severe sepsis). smart-LAMP enabled pathogen detection via direct specimen testing of whole blood at the 3 infection time points, with an LOD of $10^4$ CFU/mL, equivalent to 4 CFU/reaction (Fig. 5a–c). Similarly, pathogen detection was observed in several other murine models of sepsis (SPN, SA, SE, YP, EC) (Supplementary Fig. 6). However, the CFUs in circulation for all sepsis models tested ($10^9$–$10^8$ CFU/mL) were well-above the range needed for clinical utility in humans (1–100 CFU/mL) [5]. It should be noted that the relatively high LOD in circulation is not indicative of a failure of the reaction but rather a physical limitation of the 2 μL sample volume, resulting in a $\sim 40$ μL reaction volume that is near the maximum allowable with the current platform (see Methods). Thus, we redirected subsequent efforts on pathogen detection in urine with an LOD of $5 \times 10^3$ CFU/mL, which is well within the range needed to demonstrate the clinically relevant
break point defining a positive clinical culture result for UTI patients ($\geq 10^5$ CFU/mL) [14,15].

3.6. Quantitative pathogen diagnosis in urine of human sepsis patients

Based on its strong performance with spiked murine urine samples, we assessed whether smaRT-LAMP may have immediate clinical utility for the POC analysis of urine specimens from human patients. We selected patients who met the clinical criteria for sepsis based on fever, increased heart rate, and/or elevated white blood cell count, and had a suspected urinary source of their severe infection. Some of these patients had severe sepsis, with evidence of end-organ dysfunction or septic shock. Briefly, upon presentation at the hospital, urine and blood specimens were collected from patients before antibiotic administration. A comparative urine bacterial analysis was performed between smaRT-LAMP and clinical diagnostics carried out by the hospital managing patient care. Pathogen ID in the urine and blood of sepsis patients was determined by the hospital microbiology laboratory. The bacterial load in urine of ten patient specimens was assessed by both direct colony count, and smaRT-LAMP utilizing primer sets directed against the urine pathogen identified in the clinical setting. SmaRT-LAMP achieved rapid and accurate detection of EC, KPN, and PA in urine specimens of sepsis patients ($10^2$–$10^8$ CFUs), matching that of the more cumbersome and expensive qPCR analysis (Table 2). Importantly, both smaRT-LAMP and qPCR-LAMP systems achieved a diagnosis in ~1 h, a fraction of the time required for clinical diagnostics by the hospital microbiology laboratory (18–28 h). Moreover, since false positive results are a primary concern due to contamination at collection [14], the bacterial load in urine of sepsis patients with clinically negative urine cultures was determined by the hospital microbiology laboratory (clinical culture) versus an academic laboratory examining CFU by direct colony count, qPCR-LAMP, and smaRT-LAMP. The bacterial load discerned by qPCR-LAMP and smaRT-LAMP direct specimen testing matched the low- or non-detectable- bacterial load obtained by clinical culture or direct colony count in all five cases (Supplementary Table S3). These data demonstrate the feasibility of improving time to detection and quantitation in clinical settings and at the POC. Further, in the six patients who had bacteremic complications (defined as positive blood cultures) of their urinary sepsis, the pathogen ID from the urine matched that of the blood in all six cases (patient 002, 006, 010, 012, 015, 019). This concordance demonstrates the applicability of smaRT-LAMP to even the most severe cases of sepsis, with the advantage of accurate and rapid diagnosis at the POC in these cases, and the potential to greatly accelerate directed therapy for urinary tract infections. Notably, time to treatment was the significant factor associated with positive patient outcomes in emergency care for sepsis [19]. SmaRT-LAMP thus offers the potential to deliver rapid diagnosis and treatment of urinary tract infections and urinary sepsis.

4. Discussion

Efforts to improve global public health will benefit immensely from accurate, rapid, affordable and user-friendly methods for detecting microbial pathogens at the POC. Toward this goal, we have developed smaRT-LAMP, a rapid, portable diagnostic platform that can achieve sensitive and accurate bacterial detection with performance comparable to gold-standard clinical methodologies based on costly, specialized instrumentation. We have demonstrated that smaRT-LAMP can
quantitatively detect diverse pathogens in blood, urine and feces, with an LOD that matches what can be achieved with an qPCR instrument-based assay. Perhaps most importantly, we demonstrated that our platform can achieve robust detection of different bacterial pathogens in urine specimens collected from sepsis patients, matching the hospital diagnosis but in a much shorter time-frame (~1 h vs. 18–28 h). These results highlight the clear clinical potential of the smaRT-LAMP assay as a diagnostic tool for UTIs, particularly in the context of resource-limited settings that may lack sophisticated instrumentation or expert clinical diagnosticians.

Fig. 4. SmaRT-LAMP quantitation of diverse pathogens in spiked murine whole blood and human donor urine. (a–e) Time values for SPN, SE, EC, YP (2–2×10⁴ CFU/reaction); SA (4×10¹–2×10⁴ CFU/reaction). (f) Percentage of total pathogen samples amplifying at each concentration in smaRT-LAMP. (g, h) Representative traces and T values for EC in spiked human donor urine (2–2×10⁴ CFU/reaction). (i) Percentage of total EC samples amplifying at each concentration in smaRT-LAMP (≥10 samples/concentration).

Fig. 5. SmaRT-LAMP detection and quantitation of Salmonella in whole blood of septic mice. (a–c). Mice were orally infected with ST via gastric intubation at a dose of 2×10⁷ cells and whole blood was sampled at days 6 (pre-sepsis), 8 (sepsis), and 10 (severe sepsis) post-infection. CFU were determined by qPCR-LAMP (closed boxes), smaRT-LAMP (open boxes), and direct colony count (circles). n = 14 mice.

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Table 2
Comparative bacterial analysis of urine from sepsis patients using smaRT-LAMP versus standard clinical diagnostics.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pathogen ID</th>
<th>Urine CFU/mL</th>
<th>qPCR-LAMP</th>
<th>smaRT-LAMP</th>
<th>Colony Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>PA</td>
<td>2.8 × 10^8</td>
<td>5.0 × 10^6</td>
<td>3.0 × 10^7</td>
<td></td>
</tr>
<tr>
<td>006</td>
<td>KPN</td>
<td>2.9 × 10^7</td>
<td>1.2 × 10^7</td>
<td>1.0 × 10^7</td>
<td></td>
</tr>
<tr>
<td>009</td>
<td>EC</td>
<td>–</td>
<td>1.8 × 10^7</td>
<td>8.0 × 10^6</td>
<td></td>
</tr>
<tr>
<td>010</td>
<td>EC</td>
<td>1.5 × 10^6</td>
<td>8.3 × 10^5</td>
<td>9.4 × 10^5</td>
<td></td>
</tr>
<tr>
<td>011</td>
<td>EC</td>
<td>–</td>
<td>1.2 × 10^6</td>
<td>1.0 × 10^6</td>
<td></td>
</tr>
<tr>
<td>012</td>
<td>EC</td>
<td>–</td>
<td>1.5 × 10^6</td>
<td>6.4 × 10^5</td>
<td>1.9 × 10^6</td>
</tr>
<tr>
<td>013</td>
<td>EC</td>
<td>–</td>
<td>2.2 × 10^5</td>
<td>6.5 × 10^4</td>
<td>4.3 × 10^5</td>
</tr>
<tr>
<td>016</td>
<td>EC</td>
<td>1.5 × 10^6</td>
<td>1.1 × 10^5</td>
<td>8.5 × 10^4</td>
<td></td>
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<tr>
<td>015</td>
<td>EC</td>
<td>7.2 × 10^5</td>
<td>1.4 × 10^4</td>
<td>4.8 × 10^4</td>
<td></td>
</tr>
<tr>
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<td>EC</td>
<td>6.2 × 10^5</td>
<td>2.2 × 10^5</td>
<td>1.3 × 10^5</td>
<td></td>
</tr>
</tbody>
</table>

Pathogen ID in the urine and blood of sepsis patients was determined by the hospital microbiology laboratory. The bacterial load in urine specimens was determined by direct colony count, and by direct specimen testing via qPCR-LAMP and smaRT-LAMP utilizing primer sets directed against the urine pathogen identified in the clinical setting. A linear fit of standard curves with a clinically relevant bacterial burden (5 × 10^−5–5 × 10^7 CFU/mL) was used to determine LAMP-based CFUs. “−” denotes no pathogen was isolated from blood cultures. qPCR-LAMP and smaRT-LAMP values depict an average of a minimum of 3 determinations from each specimen.

SmaRT-LAMP requires little more than a smartphone, hot plate, LED lights, low force mini-centrifuge, and a cardboard box, making our approach highly affordable and accessible. Indeed, the entire detection system can be fabricated for less than $100 US (in addition to the smartphone), and can readily be configured for the simultaneous detection of multiple pathogens. SmaRT-LAMP thus offers the potential to leverage a widely available consumer technology to affordably deliver state-of-the-art nucleic acid diagnostics technology for accurate, quantitative pathogen detection at the POC.

Early diagnosis and intervention enabled by smaRT-LAMP direct urine testing could prove highly advantageous in a number of clinical contexts. These include cases with clinical manifestations indicating UTI (among the most common types of infection) [16,17] and potentially fatal conditions arising from septicemia (e.g., pylonephritis) [15,19]. Such an assay could also prove useful for monitoring pregnant women with asymptomatic bacteriuria that receive antibiotics to reduce the risk of acute cystitis, pylonephritis and/or miscarriage [61]. Early intervention is also essential for accelerating directed therapy and encouraging the judicious use of antibiotics to minimize the emergence of multidrug-resistant strains that have limited treatment options (e.g., MRSA, extended-spectrum β-lactamase–producing (ESBL) and carbapenem-resistant (CRE) Enterobacteriaceae, and multidrug-resistant PA and Acinetobacter sp. [62–64]. SmaRT-LAMP may complement clinical UTI diagnostic practices such as colorimetric dipstick assays, microscopy, lateral flow assays (approved for veterinary use) that are rapid (1–2 h) but do not identify the pathogen, and MALDI-TOF mass spectrometry that rapidly identifies the pathogen but requires bacterial culture (~18–28 h) and expensive instrumentation [18].

There are numerous opportunities to further extend the utility of the smart-LAMP platform in the future. First, the LOD in diverse biological specimens could be improved simply by increasing the sample volume used in the assay (e.g., from 2 μl to ≥1 mL). Although this will increase the cost of reagents and the size of the peripheral apparatus, the LOD will scale linearly with sample volume, potentially making it possible to detect 1–100 CFU from a 1 mL blood specimen. Such sensitivity could enable extremely early-stage diagnosis and intervention, particularly in the context of multidrug-resistant pathogens for which treatment options are highly limited. Second, multiplexed detection of pathogens could be readily achieved with appropriate LAMP primers that can be designed from whole-genome databases [65,66]. Finally, the utility of our system for field applications could be further improved with lyophilized reagents, which will be especially useful in resource-limited areas where refrigeration is impractical [67,68]. We therefore believe that smaRT-LAMP holds exciting potential to bring state-of-the-art nucleic acid diagnostics technology within easy reach of non-expert smartphone users.

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Conflicts of interest

The authors declare no competing financial interests.

Author contributions

Experiments were conducted by L.B., D.M.H., G.N.F., A.Z., J.C. Data was analyzed by L.B., D.M.H., S.P.M., L.N.F., J.D.M., J.C.F., H.T.S., and M.J.M. The manuscript was prepared by L.B., D.M.H., S.P.M., H.T.S. and M.J.M. The study was planned and directed by H.T.S and M.J.M.

Ethics statement

All animal experimentation was conducted following the National Institutes of Health (NIH) guidelines for housing and care of laboratory animals and performed in accordance with institutional regulations after pertinent review and approval by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara. Specimens from human patients were obtained following NIH guidelines for human subjects and performed in accordance with institutional regulations after pertinent review and approval from institutional Human Subjects Use Committee of the University of California, Santa Barbara and Cottage Health Institutional Review Board.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.09.001.

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