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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. Additionally, smaRT-LAMP did not exhibit false positives in sepsis patients with clinically negative urine cultures. **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

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Research in context

Evidence before this study

Several innovative systems have recently been reported that transform mobile phones into potential clinical point-of-care diagnostic tools; e.g., optical and fluorescence imaging, microtiter assay interpretation, immunologic detection and nucleic acid detection. Although these notable advances broaden access to sophisticated molecular diagnostics, translation to clinical utility remains a significant challenge. PubMed and Google Scholar were searched (up to August 20, 2018) using a variety of key words (e.g., “smartphone urine”, “smartphone detection”, “smartphone pathogen detection”, “smartphone detection clinical utility”), yielding a limited number of reports describing smartphone-based detection systems using patient samples. Therefore, we conclude that there remains an urgent need for rapid, sensitive, and affordable diagnostics for microbial infections at the point-of-care.

Added value of this study

A smartphone-based quantitative platform (smaRT-LAMP) was developed that enabled pathogen ID in urine specimens collected from sepsis patients. A comparative urine bacterial analysis between smartphone-based detection and clinical diagnostics carried out by the hospital managing patient care revealed that smaRT-LAMP matched the hospital diagnosis but in a much shorter time-frame (~1 h vs. 18–28 h). Further, among patients with bacteremic complications of their urinary sepsis (defined as positive blood cultures), the pathogen ID from the urine matched that of the blood, raising the possibility of pathogen diagnosis shortly after hospital admission.

Implications of all the available evidence

Our findings highlight the clinical potential of the smaRT-LAMP assay as a diagnostic tool for urinary tract infections, particularly in the context of resource-limited settings that may lack sophisticated instrumentation or expert clinical diagnosticians. 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 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.

inoculation to time to pathogen ID [10–13]. Additionally, these methods generally require access to specialized laboratory equipment, which can be excessively costly and technologically complex for POC or resource-limited settings.

Among different types of clinical samples, urine samples are particularly attractive because they can be obtained without invasive procedures (such as a blood draw) and the clinically relevant break point defining a positive clinical culture result for urinary tract infections (UTIs) is $\geq 10^5$ CFU/mL [14,15], making timely diagnostic detection potentially simpler. UTIs are among the most common type of infection, and are associated with recurrent illnesses, pyelonephritis with sepsis, renal damage, pre-term birth, and complications from prolonged antimicrobial therapy that include high-level resistance and *Clostridium difficile* colitis [16,17]. Unfortunately, there are presently no direct urine testing methods for pathogen ID approved for human clinical diagnostics [18]. Instead, urine specimens must be cultured before biochemical characterization, and such culture methods are routinely confounded by

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]; microtiter 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/pIB1 (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, YP, 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 1×10^{10} total cells. Cells were resuspended in 0.5 mL TE buffer, 10 μ L 10% SDS, 10 μ L 10 mg/mL DNase-free RNase, mixed and incubated 1 h at 37 °C. Next, 10 μ L 10 mg/mL proteinase 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 m at 16,000 \times 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 m at 16,000 \times 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 μ L 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 WarmStart DNA polymerase was purchased from New England Biolabs (Beverly, MA), deoxynucleotide 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 Sdf I* [47]; *YP inv* [48]; *EC glxK* [49], *KPN fimD* [50], *PA oprI* [50], *SPN lytA* [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 M betaine, 2.8 mM for each of the four deoxynucleotide 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. Lysis protocol

2.4.1. 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 lysate 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 × 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* (10³ cells) or *YP* (5 × 10⁵ 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 (*A*₆₀₀ = 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 TS broth and sub-cultured to mid-log phase (*A*₆₀₀ = 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 (5×10^4 – 5×10^7 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). Institutional Human Subjects Use Committees of the University of California, Santa Barbara and Santa Barbara Cottage Hospital approved studies undertaken herein.

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_t 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 5×10^6 CFU/reaction and below. All steps of this process can be automatically performed by the BactiCount app without using a PC.

2.9. Hardware of smaRT-LAMP platform

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-7079) 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

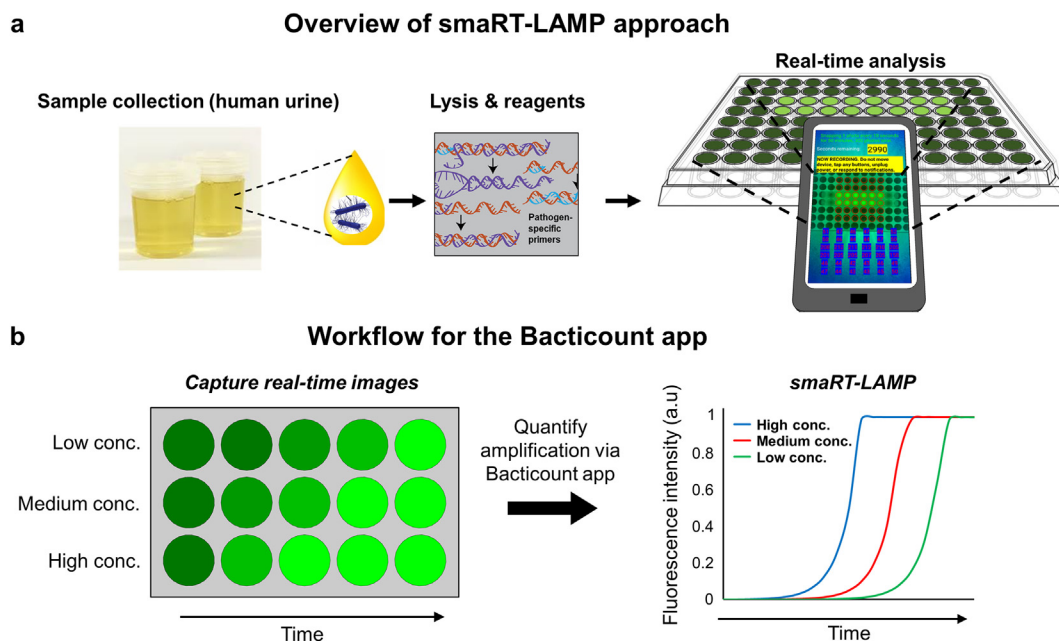


Fig. 1. SmaRT-LAMP direct specimen testing of urine from sepsis patients. (a) Assay schematic for smaRT-LAMP, which entails sample collection, bacterial cell lysis/reagent addition, and real-time analysis via the smartphone. (b) Schematic of workflow for the BactiCount app, which analyzes fluorescence 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).

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 .parr 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 (Supplemental 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 5×10^1 – 5×10^4 copies of the genome. SmaRT-LAMP and qPCR instruments showed equivalent performance in this

Table 1
SmaRT-LAMP intra- and interspecies specificity.

Primer	gDNA template							
	<i>ST</i>	<i>SE</i>	<i>EC</i>	<i>YP</i>	<i>KPN</i>	<i>PA</i>	<i>SPN</i>	<i>SA</i>
<i>ST</i>	+	–	–	–	–	–	–	–
<i>SE</i>	–	+++	–	–	–	–	–	–
<i>EC</i>	–	–	+++	–	–	–	–	–
<i>YP</i>	–	–	–	+++	–	–	–	–
<i>KPN</i>	–	–	–	–	+++	–	–	–
<i>PA</i>	–	–	–	–	–	+++	–	–
<i>SPN</i>	–	–	–	–	–	–	+++	–
<i>SA</i>	–	–	–	–	–	–	–	+++

“+++” denotes amplification of cognate primer-gDNA pairs (10^3 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.

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*), *Yersinia 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 lysis protocol enables quantitative pathogen detection using whole bacterial cells, and if smaRT-LAMP can match the sensitivity of an equivalent LAMP assay performed in an qPCR instrument. *ST* cells were serially diluted into buffer at concentrations ranging from 10^1 to 10^5 CFU/mL. One mL of each dilution sample was then reduced to 2 μ L via sequential centrifugation and subjected to the LAMP protocol. We derived a standard curve through a linear regression fit of T_t vs. $\log_{10}[\text{CFU}]$ (Fig. 2a–d); see Methods). The resultant limit of detection (LOD) was ≤ 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 (≤ 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 lysis procedure works with Gram-positive and -negative pathogens that have large differences in cell envelope structure. Using the same lysis 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 μ L of spiked buffer samples containing 5×10^3 – 5×10^7 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 5×10^3 – 1×10^5 CFU/mL (equivalent

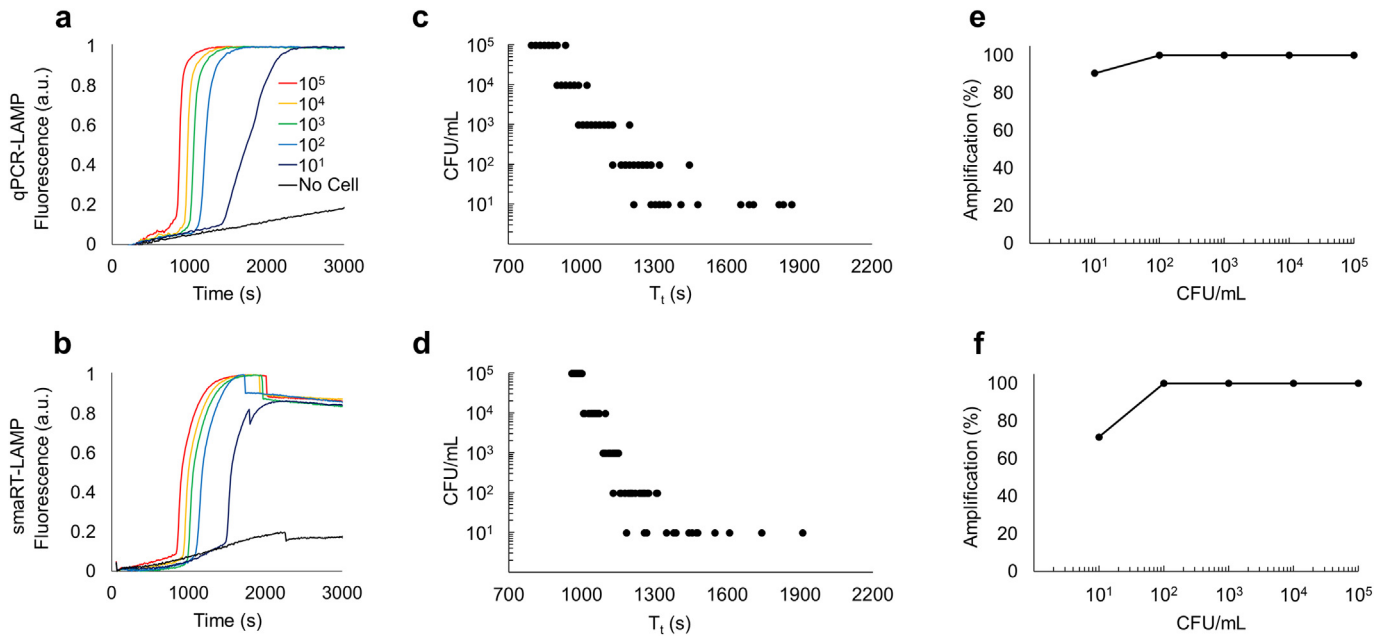


Fig. 2. SmaRT-LAMP quantification of *ST* with performance equivalent to a benchtop laboratory qPCR instrument. (a–d) Normalized representative traces and T_t values of *ST* CFU in buffer at concentrations of 10^1 – 10^5 CFU/mL using qPCR-LAMP and smaRT-LAMP; (e, f), Percentage of total samples amplified at each concentration using qPCR-LAMP or smaRT-LAMP (21 samples/concentration).

to 2–100 CFU/reaction), clear T_t 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 5×10^3 – 5×10^7 CFU/mL. After collecting and processing 2 μ L 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 5×10^3 CFU/mL (Fig. 3a, b) – equivalent to just 2 CFU/reaction – with clear T_t 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 that 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, delaying identification for at least 16 h [18,59,60]. Once again, we demonstrated that smaRT-LAMP could match the performance of the more sophisticated and costly qPCR instrument, achieving a clinically-relevant LOD of 1 – 2×10^4 CFU/mL (Fig. 3c, d), which is within the range needed to demonstrate the clinically relevant break point defining a positive clinical culture result for UTIs (10^5 CFU/mL) [14,15]. Notably, the scattered distribution observed at lower concentrations is a statistical byproduct of the extremely small number of bacteria per sample,

with samples at the lowest concentrations containing on average two or fewer CFU each. We also demonstrated that our assay could achieve equally sensitive performance in testing fecal samples (Fig. 3e, f). As with the blood specimens, we observed excellent reaction efficiency for both urine and fecal samples (Fig. 3g–j). The LOD of smaRT-LAMP is comparable to that of currently used clinical diagnostic technologies for urine and feces, but our system’s capacity for direct specimen testing and minimal sample preparation offers a major advantage in terms of time to treatment.

Finally, we confirmed that smaRT-LAMP direct specimen testing works with diverse pathogens in a variety of specimen types. Specifically, we assessed the performance of smaRT-LAMP with *SPN*, *SA*, *SE*, *EC* and *YP* spiked into murine blood, and with *EC* spiked into donor human urine. SmaRT-LAMP showed strong performance with all of these pathogen-specimen combinations, achieving LODs in the range of 5×10^3 – 1×10^5 CFU/mL (2–40 CFU/reaction) (Fig. 4), which were again comparable with results from qPCR (Supplementary Fig. 5). These data indicate that the smaRT-LAMP platform is compatible with a diverse array of pathogens and biological specimens.

3.5. Pathogen detection in murine models of sepsis

We assessed whether the smaRT-LAMP system was compatible with several murine models of sepsis. Mice were orally infected with *ST* and whole 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 (10^4 – 10^6 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 μ L sample volume, resulting in a ~40 μ L 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 5×10^3 CFU/mL, which is well within the range needed to demonstrate the clinically relevant

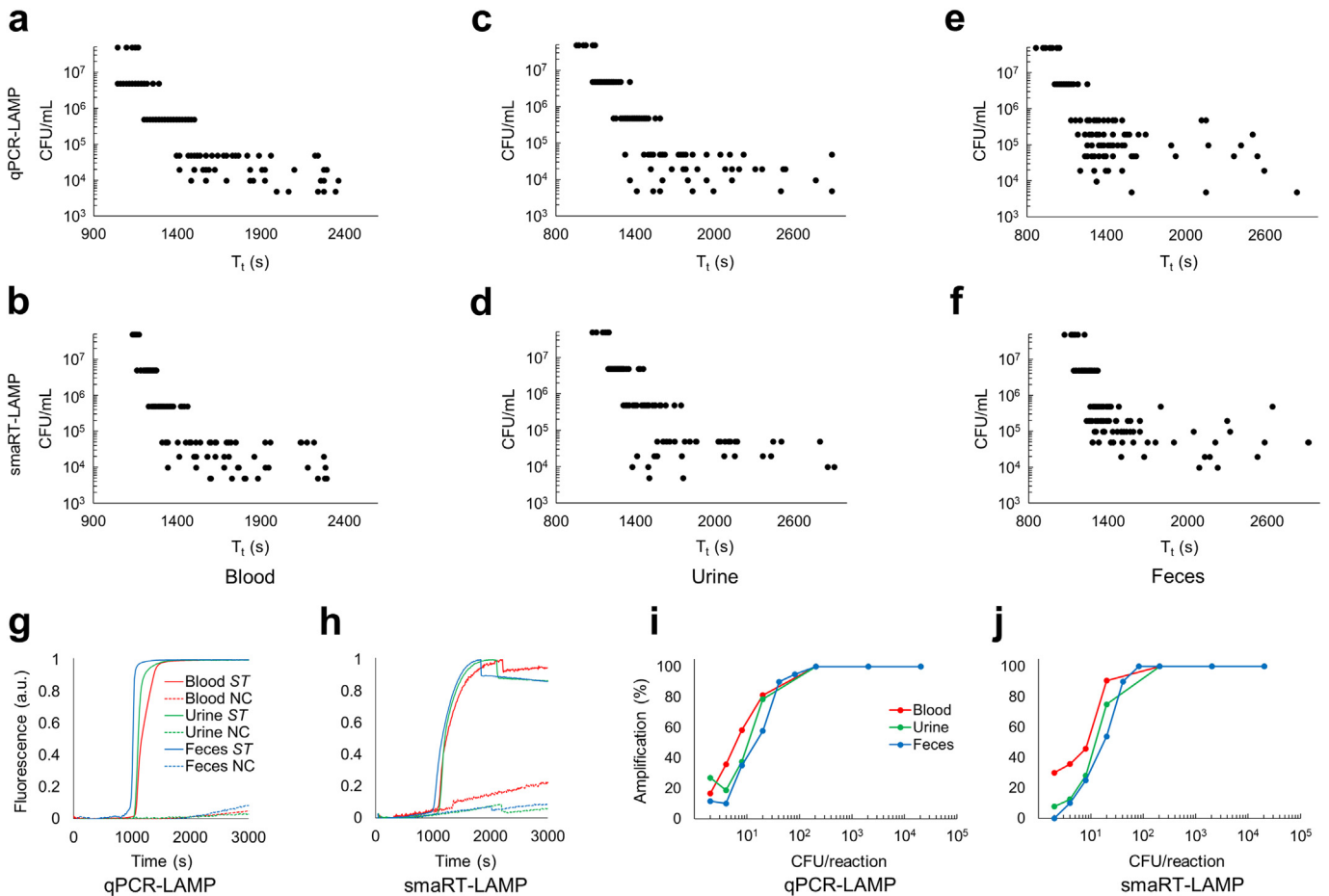


Fig. 3. SmarT-LAMP quantification of *ST* in spiked diverse biological specimens. (a–f) T_1 values for *ST* CFU in murine blood, urine, and feces using qPCR-LAMP and smarT-LAMP. (g, h) corresponding representative traces ($2\text{--}2 \times 10^4$ CFU/reaction); NC, no cell. (i, j) Percentage of total pathogen samples amplifying at each concentration using smarT-LAMP.

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 ($10^5\text{--}10^8$ 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

Spiked Blood

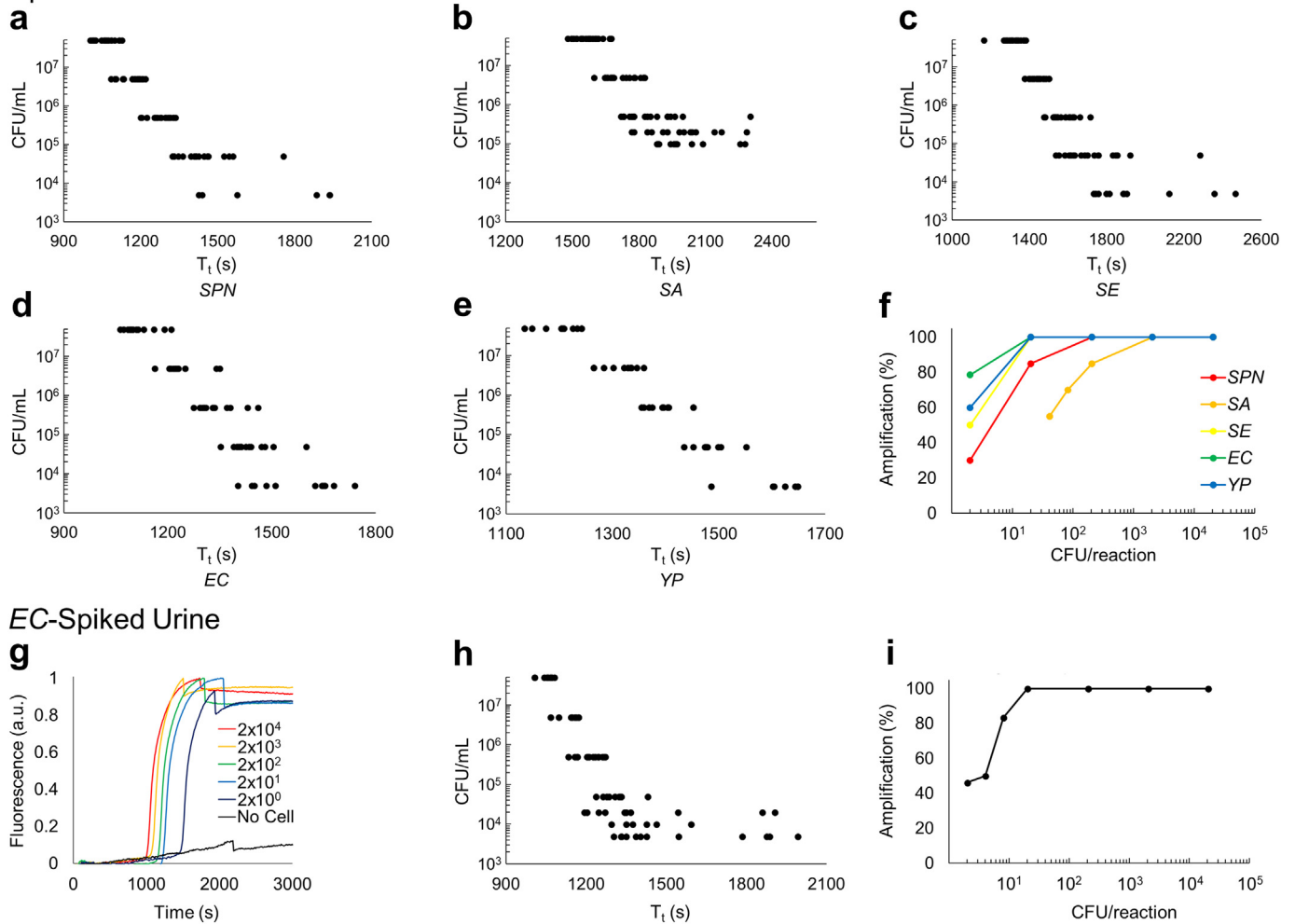


Fig. 4. SmarT-LAMP quantitation of diverse pathogens in spiked murine whole blood and human donor urine. (a–e) T_t values for SPN, SE, EC, YP ($2\text{--}2 \times 10^4$ CFU/reaction); SA ($4 \times 10^1\text{--}2 \times 10^4$ CFU/reaction). (f) Percentage of total pathogen samples amplifying at each concentration in smarT-LAMP. (g, h) Representative traces and T_t values for EC in spiked human donor urine ($2\text{--}2 \times 10^4$ CFU/reaction). (i) Percentage of total EC samples amplifying at each concentration in smarT-LAMP (≥ 10 samples/concentration).

quantitatively detect diverse pathogens in blood, urine and feces, with an LOD that matches what can be achieved with a 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.

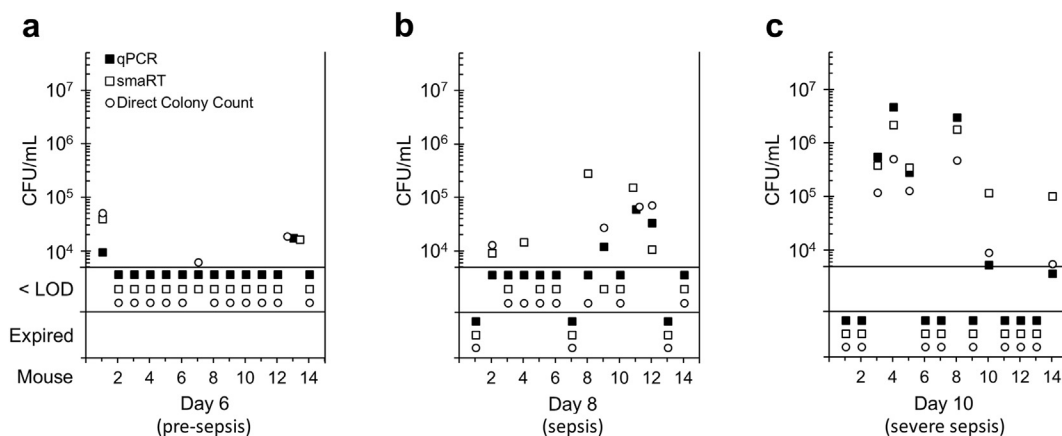


Fig. 5. SmarT-LAMP detection and quantitation of *Salmonella* in whole blood of septic mice. (a–c). Mice were orally infected with ST via gastric intubation at a dose of 2×10^7 cells and whole blood was sampled at days 6 (pre-sepsis), 8 (sepsis), and 10 (severe sepsis) post-infection. CFU were determined by qPCR-LAMP (closed boxes), smarT-LAMP (open boxes), and direct colony count (circles). $n = 14$ mice.

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

Patient	Pathogen ID		Urine CFU/mL		
	Urine	Blood	qPCR-LAMP	smaRT-LAMP	Colony Count
002	PA	PA	2.8×10^6	5.0×10^6	3.0×10^5
006	KPN	KPN	2.9×10^7	1.2×10^7	1.0×10^7
009	EC	–	1.8×10^7	4.4×10^7	8.0×10^6
010	EC	EC	1.5×10^4	8.3×10^5	9.4×10^5
011	EC	–	1.2×10^5	2.5×10^5	1.0×10^7
012	EC	EC	1.5×10^8	6.4×10^8	1.9×10^8
013	EC	–	2.2×10^4	6.6×10^4	4.3×10^7
014	EC	–	1.5×10^5	1.1×10^6	8.5×10^7
015	EC	EC	7.2×10^4	1.4×10^5	4.8×10^8
019	EC	EC	6.2×10^7	2.2×10^8	1.3×10^7

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 (5×10^4 – 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., pyelonephritis) [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, pyelonephritis 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 μ L 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>.

References

- [1] Magill S, Dumyati G, Ray S, Fridkin S. Evaluating epidemiology and improving surveillance of infections associated with health care, United States. *Emerg Infect Dis* 2015;21(9):1537–42.
- [2] World Health Organization. *Worldwide Country Situation Analysis: Response to Antimicrobial Resistance*; 2015.
- [3] Caliendo A, Gilbert D, Ginocchio C, Hanson K, May L, Quinn T, et al. Better tests, better care: improved diagnostics for infectious diseases. *Clin Infect Dis* 2013; 57(suppl_3):S139–S170.
- [4] Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive nontyphoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet* 2012;379(9835):2489–99.
- [5] Opota O, Jaton K, Greub G. Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. *Clin Microbiol Infect* 2015;21(4):323–31.
- [6] Andrews J, Ryan E. Diagnostics for invasive Salmonella infections: current challenges and future directions. *Vaccine* 2015;33:C8–C15.
- [7] Beal S, Ciurca J, Smith G, John J, Lee F, Doern C, et al. Evaluation of the nanosphere verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. *J Clin Microbiol* 2013; 51(12):3988–92.
- [8] Leli C, Cenci E, Cardaccia A, Moretti A, D'Alò F, Pagliochini R, et al. Rapid identification of bacterial and fungal pathogens from positive blood cultures by MALDI-TOF MS. *Int J Med Microbiol* 2013;303(4):205–9.

- [9] Ly T, Gulia J, Pyrgos V, Waga M, Shoham S. Impact upon clinical outcomes of translation of PNA FISH-generated laboratory data from the clinical microbiology bench to bedside in real time. *Therapeut Clin Risk Manag* 2008;4(3):637–40.
- [10] Blaschke A, Heyrend C, Byington C, Fisher M, Barker E, Garrone N, et al. Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system. *Diagn Microbiol Infect Dis* 2012;74(4):349–55.
- [11] Cockerill III F, Wilson J, Vetter E, Goodman K, Torgerson C, Harmsen W, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004;38(12):1724–30.
- [12] Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M. The era of molecular and other non-culture-based methods in diagnosis of sepsis. *Clin Microbiol Rev* 2010;23(1):235–51.
- [13] Salimnia H, Fairfax M, Lephart P, Schreckenberger P, Desjarlais S, Johnson J, et al. Evaluation of the FilmArray blood culture identification panel: results of a multicenter controlled trial. *J Clin Microbiol* 2016;54(3):687–98.
- [14] Rabinovitch A, Sarewitz S, Woodcock S, Allinger D, Azar M, Dynek D, et al. Urinalysis diagnosis, transportation, and preservation of urine specimens: approved guideline. In: Wayne Pa, editor. Contract No.: NCCLS Document GP16-A22nd ed.; 2001.
- [15] Simerville J, Maxted W, Pahlira J. Urinalysis: a comprehensive review. *Am Fam Physician* 2005;71(6):1153–62.
- [16] Flores-Mireles A, Walker J, Caparon M, Hultgren S. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2015;13(5):269.
- [17] National Institutes of Health. Bladder Health for Older Adults. NIH; 2018.
- [18] Davenport M, Mach K, Shortliffe L, Banaei N, Wang T-H, Liao J. New and developing diagnostic technologies for urinary tract infections. *Nat Rev Urol* 2017;14(5):296.
- [19] Seymour C, Gesten F, Prescott H, Friedrich M, Iwashyna T, Phillips G, et al. Time to treatment and mortality during mandated emergency care for sepsis. *N Engl J Med* 2017;376:2235–44.
- [20] Greenbaum A, Luo W, Su T-W, Göröcs Z, Xue L, Isikman S, et al. Imaging without lenses: achievements and remaining challenges of wide-field on-chip microscopy. *Nat Methods* 2012;9(9):889–95.
- [21] Feng S, Tseng D, Di Carlo D, Garner O, Ozcan A. High-throughput and automated diagnosis of antimicrobial resistance using a cost-effective cellphone-based microplate reader. *Sci Rep* 2016;6:srep39203.
- [22] Liu X, Lin T-Y, Lillehoj PB. Smartphones for cell and biomolecular detection. *Ann Biomed Eng* 2014;42(11):2205–17.
- [23] Cho S, San Park T, Nahapetian T, Yoon J-Y. Smartphone-based, sensitive μ PAD detection of urinary tract infection and gonorrhoea. *Biosens Bioelectron* 2015;74:601–11.
- [24] Laksanasopin T, Guo T, Nayak S, Sridhara A, Xie S, Olowookere O, et al. A smartphone dongle for diagnosis of infectious diseases at the point of care. *Sci Transl Med* 2015;7(273):273re1–re1.
- [25] Yeo S-J, Choi K, Cuc B, Hong N, Bao D, Ngoc N, et al. Smartphone-based fluorescent diagnostic system for highly pathogenic H5N1 viruses. *Theranostics* 2016;6(2):231.
- [26] Jiang Y, Riedel T, Popoola J, Cai S, Ellington A, Bhadra S. Portable platform for rapid in-field identification of human fecal pollution in water. *Water Res* 2018;131:186–95.
- [27] Priye A, Bird S, Light Y, Ball C, Negrete O, Meagher R. A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. *Sci Rep* 2017;7:44778.
- [28] Kong J, Wei Q, Tseng D, Zhang J, Pan E, Lewinski M, et al. Highly stable and sensitive nucleic acid amplification and cell-phone-based readout. *ACS Nano* 2017;11(3):2934–43.
- [29] Im H, Castro C, Shao H, Liang M, Song J, Pathania D, et al. Digital diffraction analysis enables low-cost molecular diagnostics on a smartphone. *Proc Natl Acad Sci* 2015;112(18):5613–8.
- [30] Chen W, Yu H, Sun F, Ornob A, Brisbin R, Ganguli A, et al. Mobile platform for multiplexed detection and differentiation of disease-specific nucleic acid sequences, using microfluidic loop-mediated isothermal amplification and smartphone detection. *Anal Chem* 2017;89(21):11219–26.
- [31] Damhorst G, Duarte-Guevara C, Chen W, Ghonge T, Cunningham B, Bashir R. Smartphone-imaged HIV-1 reverse-transcription loop-mediated isothermal amplification (RT-LAMP) on a chip from whole blood. *Engineering* 2015;1(3):324–35.
- [32] Ganguli A, Ornob A, Yu H, Damhorst G, Chen W, Sun F, et al. Hands-free smartphone-based diagnostics for simultaneous detection of Zika, Chikungunya, and Dengue at point-of-care. *Biomed Microdevices* 2017;19(4):73.
- [33] Liao S-C, Peng J, Mauk M, Awasthi S, Song J, Friedman H, et al. Smart cup: a minimally-instrumented, smartphone-based point-of-care molecular diagnostic device. *Sens Actuators B* 2016;229:232–8.
- [34] Snodgrass R, Gardner A, Jiang L, Fu C, Cesarman E, Erickson D. KS-Detect-validation of solar thermal PCR for the diagnosis of Kaposi's sarcoma using pseudo-biopsy samples. *PLoS One* 2016;11(1):e0147636.
- [35] Stedtfeld R, Tourlousse D, Seyrig G, Stedtfeld T, Kronlein M, Price S, et al. Gene-Z: a device for point of care genetic testing using a smartphone. *Lab Chip* 2012;12(8):1454–62.
- [36] Shin D, Athamanolap P, Chen L, Hardick J, Lewis M, Hsieh Y, et al. Mobile nucleic acid amplification testing (mobiNAAT) for Chlamydia trachomatis screening in hospital emergency department settings. *Sci Rep* 2017;7(1):4495.
- [37] Heithoff DM, Shimp WR, House JK, Xie Y, Weimer BC, Sinsheimer RL, et al. Intraspecific variation in the emergence of hyperinfectious bacterial strains in nature. *PLoS Pathog* 2012;8(4):e1002647.
- [38] Heithoff DM, Shimp WR, Lau PW, Badie G, Enioutina EY, Daynes RA, et al. Human Salmonella clinical isolates distinct from those of animal origin. *Appl Environ Microbiol* 2008;74(6):1757–66.
- [39] Kubicek-Sutherland J, Heithoff D, Ersoy S, Shimp W, Mahan M. Immunization with a DNA adenine methylase over-producing *Yersinia pseudotuberculosis* vaccine confers robust cross-protection against heterologous pathogenic serotypes. *Vaccine* 2014;32(13):1451–9.
- [40] Diekema D, Richter S, Heilmann K, Dohrn C, Riahi F, Tendolkar S, et al. Continued emergence of USA300 methicillin-resistant *Staphylococcus aureus* in the United States: results from a nationwide surveillance study. *Infect Control Hosp Epidemiol* 2014;35(03):285–92.
- [41] Lanie J, Ng W-L, Kazmierczak K, Andrzejewski T, Davidsen T, Wayne K, et al. Genome sequence of Avery's virulent serotype 2 strain D39 of *Streptococcus pneumoniae* and comparison with that of unencapsulated laboratory strain R6. *J Bacteriol* 2007;189(1):38–51.
- [42] Ersoy S, Heithoff D, Barnes L, Tripp G, House J, Marth J, et al. Correcting a fundamental flaw in the paradigm for antimicrobial susceptibility testing. *EBioMedicine* 2017;20:173–81.
- [43] Kubicek-Sutherland J, Heithoff D, Ersoy S, Shimp W, House J, Marth J, et al. Host-dependent induction of transient antibiotic resistance: a prelude to treatment failure. *EBioMedicine* 2015;2(9):1169–78.
- [44] Patterson A, Heithoff D, Ferguson B, Soh H, Mahan M, Plaxco K. Microfluidic chip-based detection and intraspecies strain discrimination of *Salmonella* serovars derived from whole blood of septic mice. *Appl Environ Microbiol* 2013;79(7):2302–11.
- [45] Nagamine K, Hase T, Notomi T. Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Mol Cell Probes* 2002;16(3):223–9.
- [46] Okamura M, Ohba Y, Kikuchi S, Takehara K, Ikedo M, Kojima T, et al. Rapid, sensitive, and specific detection of the O4 group of *Salmonella enterica* by loop-mediated isothermal amplification. *Avian Dis* 2009;53(2):216–21.
- [47] Yang JL, Ma G-P, Yang R, Yang SQ, Fu LZ, Cheng AC, et al. Simple and rapid detection of *Salmonella* serovar Enteritidis under field conditions by loop-mediated isothermal amplification. *J Appl Microbiol* 2010;109(5):1715–23.
- [48] Horisaka T, Fujita K, Iwata T, Nakada A, Okatani A, Horikita T, et al. Sensitive and specific detection of *Yersinia pseudotuberculosis* by loop-mediated isothermal amplification. *J Clin Microbiol* 2004;42(11):5349–52.
- [49] Diaz L, Jenkins D, Li Y, McNealy T, Tzeng T-R, Walter N, editors. Electroflotation of *Escherichia coli* Improves Detection Rates by Loop-mediated Isothermal Amplification. 2017 ASABE Annu Intl Meeting. Amer Soc Agricultural and Biological Engineers; 2017.
- [50] Kang Y, Deng R, Wang C, Deng T, Peng P, Cheng X, et al. Etiologic diagnosis of lower respiratory tract bacterial infections using sputum samples and quantitative loop-mediated isothermal amplification. *PLoS One* 2012;7(6):e38743.
- [51] Kim D, Kilgore P, Kim E, Kim S, Anh D, Dong B, et al. The enhanced pneumococcal LAMP assay: a clinical tool for the diagnosis of meningitis due to *Streptococcus pneumoniae*. *PLoS One* 2012;7(8):e42954.
- [52] Xu Z, Li L, Chu J, Peters B, Harris M, Li B, et al. Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. *Food Res Int* 2012;47(2):166–73.
- [53] Beige J, Lokies J, Schaberg T, Finckh U, Fischer M, Mauch H, et al. Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. *J Clin Microbiol* 1995;33(1):90–5.
- [54] International Telecommunication Union. The World in 2014 - ICT Facts and Figures; 2014.
- [55] Tichopad A, Dilger M, Schwarz G, Pfaffl M. Standardized determination of real-time PCR efficiency from a single reaction set-up. *Nucleic Acids Res* 2003;31(20):e122–e.
- [56] Mori Y, Kitao M, Tomita N, Notomi T. Real-time turbidimetry of LAMP reaction for quantifying template DNA. *J Biochem Biophys Methods* 2004;59(2):145–57.
- [57] Edwards R, Olsen G, Maloy S. Comparative genomics of closely related salmonellae. *Trends Microbiol* 2002;10(2):94–9.
- [58] Thomson N, Clayton D, Windhorst D, Vernikos G, Davidson S, Churcher C, et al. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Res* 2008;18(10):1624–37.
- [59] De Rauw K, Detemmerman L, Breynaert J, Piérard D. Detection of Shiga toxin-producing and other diarrheagenic *Escherichia coli* by the BioFire FilmArray® Gastrointestinal Panel in human fecal samples. *Eur J Clin Microbiol Infect Dis* 2016;35(9):1479–86.
- [60] Khare R, Espy M, Cebelinski E, Boxrud D, Sloan L, Cunningham S, et al. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. *J Clin Microbiol* 2014;52(10):3667–73.
- [61] Delzell J, Ledevre M. Urinary tract infections during pregnancy. *Am Fam Physician* 2000;61(3):713–20.
- [62] Gupta N, Limbago B, Patel J, Kallen A. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011;53(1):60–7.
- [63] Kanj S, Kanafani Z. Current concepts in antimicrobial therapy against resistant gram-negative organisms: extended-spectrum β -lactamase-producing enterobacteriaceae, carbapenem-resistant enterobacteriaceae, and multidrug-resistant *Pseudomonas aeruginosa*. *Mayo Clin Proc* 2011;86(3):250–9.
- [64] Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18(3):268–81.
- [65] Loman N, Constantinoiu C, Chan J, Halachev M, Sergeant M, Penn C, et al. High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. *Nat Rev Microbiol* 2012;10(9):599–606.
- [66] Peacock S. Health care: Bring microbial sequencing to hospitals. *Nature* 2014;509(7502):557–9.
- [67] Hayashida K, Kajino K, Hachaambwa L, Namangala B, Sugimoto C. Direct blood dry LAMP: a rapid, stable, and easy diagnostic tool for Human African Trypanosomiasis. *PLoS Negl Trop Dis* 2015;9(3):e0003578.
- [68] Yoshikawa T, Matsuo T, Kawamura Y, Ohashi M, Yonekawa T, Kanda H, et al. Direct detection of human herpesvirus 6B by the LAMP method using newly developed dry-reagents. *J Virol Methods* 2014;201:65–7.