# Culture-free Rapid Isolation and Detection of Bacteria from Whole Blood at Clinically Relevant Concentrations

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**Abstract**

Rapid detection and antibiotic susceptibility profiling of bacterial agents in the blood of sepsis patients are crucial for determining appropriate treatment. The low bacteria concentrations and high abundance of blood cells currently necessitate culture-based diagnostic methods, which can take several days. Here, we introduce a method to isolate bacteria from whole blood with high separation power by smart centrifugation, followed by detection through microscopy in microfluidic traps within 2 h without the need for blood culture. We detected *E. coli*, *K. pneumonia*, or *E. faecalis* from spiked samples of healthy human donor blood at clinically relevant concentrations as low as 9, 7 and 32 colony-forming units per ml of blood, respectively. This rapid isolation of living bacteria from blood at clinically relevant concentrations opens possibilities for rapid phenotypic antibiotic susceptibility testing for bloodstream infections without blood culture.

**Keywords:** sepsis, diagnostics, centrifuging, lysis, microscopy, antibiotic treatment

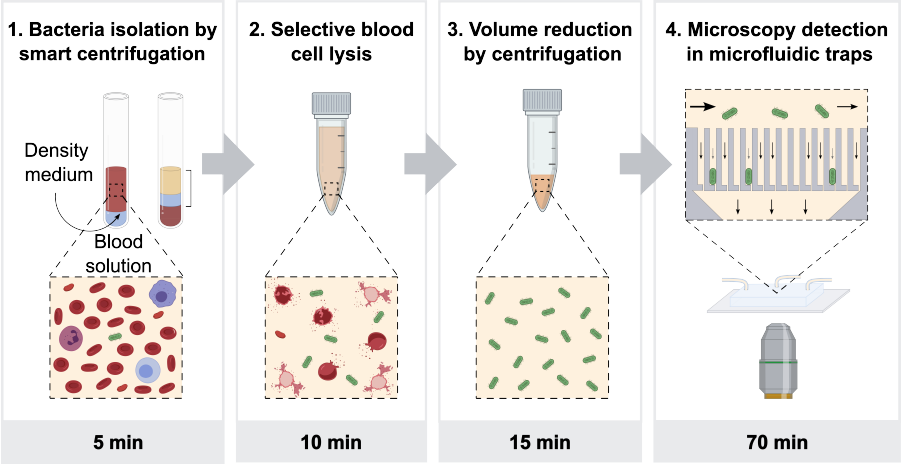


**Fig. 1** Graphical abstract.

# 1 Introduction

Sepsis is a severe medical condition characterised by a systemic host inflammation response to infection [1]. Sepsis has an incidence of approximately 50 million cases annually,[2] causing a significant healthcare burden The mortality of diagnosed patients is around 26% [3]. An estimated 25-30% of sepsis cases involve bloodstream infections (BSI) [4, 5]. The rapid detection and identification of causative organisms in patient blood are important for determining effective antibiotic treatment. The low microbial loads in the bloodstream of patients, as low as one to ten colony-forming units (CFUs) per ml of blood [6], require large sample volumes of blood, which is particularly challenging for pediatric patients [7]. Furthermore, the microorganisms typically need several hours to days of culture before the presence and identification of the pathogens can be confirmed [8, 9]. The latter is problematic, considering the 8% drop in survival rate per hour delayed treatment for patients suffering septic shock [10, 11]. The urgency of the condition, coupled with the latency of the aforementioned diagnosis methods, means that combination therapy with broad-range antibiotics is often prescribed as first-line therapy immediately after the blood drawing [12]. This praxis results in suboptimal treatment [13], contributes to the increase of antibiotic resistance [14], and has also been shown to increase liver toxicity compared to targeted antibiotic monotherapy [15].

The state-of-the-art method for detecting bacteria from sepsis patients is by culturing the blood [16, 17], followed by bacterial identification with genotypic (e.g., polymerase chain reaction), phenotypic (e.g., subcultures), or mass spectrometry methods (e.g., matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF)) [18]. While genotypic methods can identify bacteria at low concentrations without culturing the blood [18],

they do not give susceptibility information [19], limiting clinical impact. Phenotypic and proteomic approaches typically require a higher bacterial load, necessitating culture and therefore long processing time.

Single-cell phenotypic methods, which specifically bypass the requirement for blood culture, offer a quicker alternative but necessitate the initial removal of blood cells.[20–24] Isolation of bacteria from whole blood has been accomplished with inertial [25–27] and elastoinertial microfluidics [28], sedimentation velocity-based separation [29–31], filtration [29, 32], chemical capture [33, 34], magnetic bead-based separation [35], dielectrophoresis [36], or acoustic separation [37]. However, most of these isolation methods suffer limited throughput and low blood cell rejection rates, or have only been demonstrated for bacteria concentrations of 1000 CFU/ml or above, thus necessitating time-consuming bacterial preculture.

This study aims to develop a rapid and high-throughput assay for isolating bacteria from whole blood with high efficiency and minimal dilution to allow integration with microfluidic trapping and rapid microscopy detection. The significance of such a method lies in its potential for rapid identification and drug profiling of bacterial agents in the blood of septic patients within 3-4 hours, as opposed to the current several days. This capability promises timely and appropriate antibiotic treatment and could greatly impact their survival rates.

# Results

## Overall work-flow

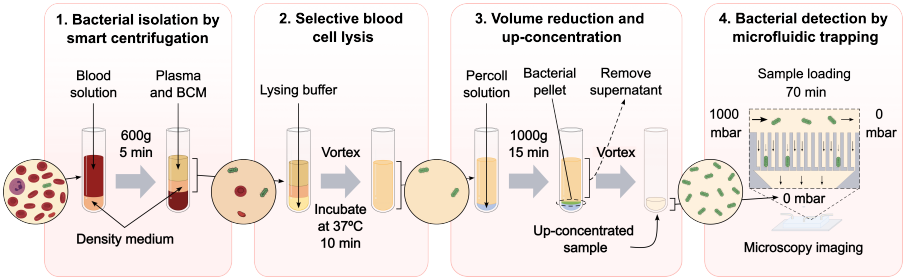


**Fig. 2** Workflow for bacterial detection from blood samples in four assay steps: 1) isolation using smart centrifugation; 2) selective blood cell lysis; 3) volume reduction; and 4) detection in microfluidic traps. BCM is blood culture medium.

Our assay concatenates four steps to isolate and detect bacteria from blood: smart centrifugation, selective blood cell lysis, volume reduction, and microscopy-based detection in microfluidic traps (Figure 2).

For ethical reasons and to allow for repeatability during technical development, this study was conducted with healthy human EDTA donor blood, where we ensured all the blood parameters were within the normal count. The blood

was spiked with bacteria at concentrations, $C$, in the range 4-4000 CFU/ml. To facilitate development and quantification, we used a fluorescent lab strain of *E. coli* in the stationary phase (after overnight culture). The fluorescence facilitated the easy distinction between the *E. coli* test strain and potential contaminating bacteria, none of which were observed. Method evaluation was performed with clinical isolates of *K. pneumoniae*, *E. faecalis*, and *S. aureus* in their exponential growth phase.

Details about materials and methods are provided under "Methods" and all measurement values are tabled in SI.

## Smart centrifugation

The first assay step, which we call smart centrifugation, removes most of the blood cells while recovering most of the bacteria in the supernatant. This step is essential to avoid downstream clogging of the microfluidic device. During blood centrifugation, bacteria are enriched into the supernatant (see SI for a detailed description of bacterial cell trajectories during blood sedimentation). However, when centrifuging pure whole blood, a large fraction of the bacteria become trapped in the remaining plasma inside the blood cell sediment. Our strategy to avoid such bacterial loss is to layer the sample on top of a density medium with a higher density and with a volume sufficient to replace all plasma in the sediment. We adjusted the volumes and densities of both the sample and density medium for optimal bacterial isolation efficiency with minimal dilution.To tune the densities of the blood sample and density medium, we used blood culture medium (BCM) to support bacterial growth throughout the assay.

The density medium was adapted for an as high as possible density, to increase sedimentation differentiation, with a limit determined by the lowest particle density, to enable the sedimentation of all blood cells into the density medium. The densities of RBCs, WBCs and platelets are in the ranges (1.086 - 1.122 g/ml), (1.057 - 1.092 g/ml) and (1.072 - 1.077 g/ml), respectively; as density medium, we chose a 2:1 volumetric mixture of Lymphoprep and BCM, which has a density of 1.051 g/ml. The volume of the density medium was experimentally tuned to be minimal but sufficient to replace all plasma remaining between the sedimented cells. Diluting the blood sample with 25% BCM provided minimal reduction of the bacterial concentration while ensuring a sample density below that of the density medium. Centrifuging time and force were experimentally tuned for optimal enrichment of *E. coli* in the supernatant while removing at least 99.8 % of the RBCs. Optimal conditions involved layering 3 ml of BCM-diluted spiked blood on top of 1 ml density medium and centrifuging for 5 min at 600g in a hanging bucket centrifuge. The relative movements of the RBCs, bacteria and liquid in such a system are described with a linear model in Supplementary Information and illustrated in Figure 3A. After centrifugation, approximately 2.5 ml of clear supernatant containing most bacteria (SI Figure A4) could be removed for further processing.

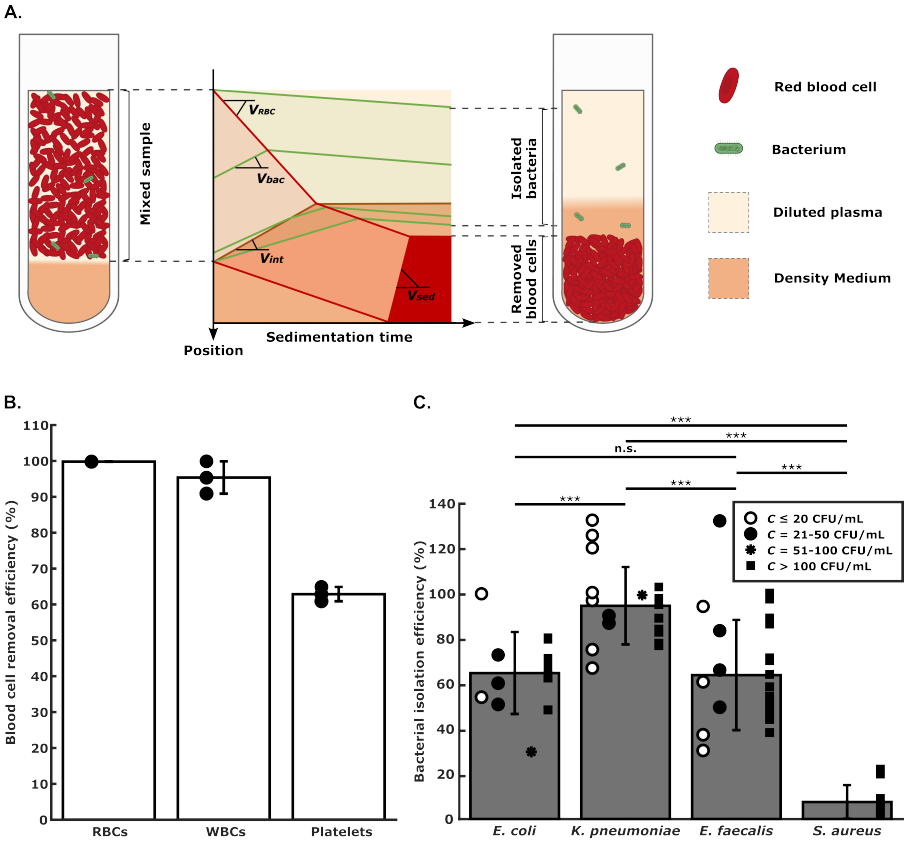


**Fig. 3 Bacterial isolation from blood by smart centrifugation. A. Illustration of liquid and cell movement.** The left and right tubes illustrate the positions of sample liquid, density medium, red blood cells and bacteria before and after smart centrifugation. The middle graph qualitatively illustrates the trajectories (solid lines) of bacteria (green) and red blood cells (red) during centrifugation, from a mixed state (left brackets) to a separated state (right brackets). The slopes of the lines are the particle sedimentation speeds, $v_{RBC}$ and $v_{bac}$, and liquid interface, $v_{int}$, and sedimentation interface velocity, $v_{sed}$, derived in SI. **B. Blood cell removal efficiency**, meaning the fraction of blood cells removed from the supernatant after centrifugation relative to the initial number of blood cells in the sample. **C. Bacterial isolation efficiency**, meaning the number of colony-forming units in the supernatant after centrifugation relative to the initial number of colony-forming units in the spiked sample. Bar heights are mean; error bars are sd; n.s. and *** indicate significance levels $p > 0.05$ and $p \leq 0.001$, respectively; the bacterial concentration $C$ refers to the CFU /ml in the blood sample.

Blood cell counting of the supernatant showed the removal of 99.82 ± 0.04% of the RBCs, 95 ± 4% of the WBCs, and 63 ± 2% of the platelets (mean ± sd, n=3; Figure 3B). Agar plate culturing of the supernatant revealed the isolation of 65 ± 16% of *E. coli*, 95 ± 17% of *K. pneumoniae*, 64 ± 24% of *E. faecalis*, or 8 ± 7% of *S. aureus* (mean ± sd, n=10-26; Figure 3C). Recovery exceeding 100% can be attributed to the low plate count numbers, leading to considerable variations [38].

## Selective blood cell lysis

The second assay step removes the remaining blood cells in the sample using a selective lysis mixture of sodium cholate hydrate and saponin [28]. Around 2.5 ml supernatant from the smart centrifugation was mixed with 1 ml of the selective lysing solution and kept in a shaking incubator at 37°C for 10 min, completely lysing remaining RBCs, WBCs and platelets while not affecting bacterial viability.

## Volume reduction

The third assay step enriches the sample and removes the excess lysing buffer in a second centrifugation step. The sample-lysate mixture was layered on top of 0.3 ml high-density liquid, consisting of a 1:2 volumetric mixture of percoll and BCM, and centrifuged for 13 min at 1000g to sediment the bacterial cells on the liquid interface. The supernatant was removed to withhold approximately 0.5 ml liquid containing sedimented bacterial cells, as well as some blood lysate. Sedimenting into a higher-density liquid provided a smoother liquid flow in the microfluidic chip during downstream processing (SI Figure A5), while not significantly affecting the bacterial isolation efficiency (p-value $> 0.05$) (SI Figure A6)

## Detection in microfluidic traps

In the last step, we processed the resuspended sample through a microfluidic device with bacterial traps for 70 min while imaging by microscopy. We used the chip design introduced by Baltekin et al. [23] for cross-flow filtration of the bacteria into individual filter traps. The chips contained in total 8000 microtraps of length 50 µm, height 1.25 µm and width 1.25 µm with a restriction of opening 300 nm at their end. Each microtrap can capture bacterial cells and function as a culture chamber. The microtraps were monitored with fluorescence microscopy to detect *E. coli*; alternatively phase contrast microscopy to detect the clinical isolates of *K. pneumoniae*, *E. faecalis* and *S. aureus*. The 0.5 ml bacterial sample was loaded into the inlet at 1 bar pressure, keeping all outlets at atmospheric pressure.

For *E. coli*-spiked sample, approximately 30% of the sample flowed through the traps with a typical filtrate flow rate of 1-2 µl/min (Figures 4A and A5B). The trapping efficiency, meaning the number of positive traps, $N$, relative to the number of CFUs after smart centrifugation, was 29 ± 6% (mean ± sd, n=5; Figure 4B).

## Overall assay performance

We evaluated the entire assay starting from blood spiked with *E. coli*, *K. pneumonia*, and *E. faecalis*, respectively. All experiments resulted in the successful trapping and subsequent detection of bacteria. The lowest bacterial concentrations, $C$, tested and detected were 9, 7 and 32 CFU/ml, respectively (Figure

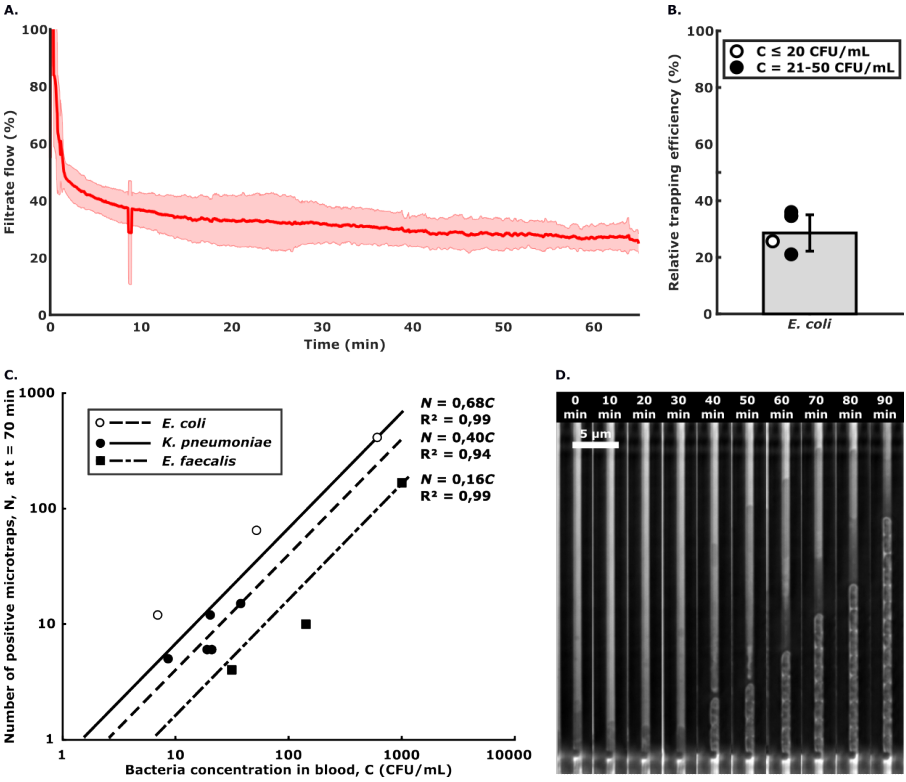


**Fig. 4 Microtrapping and bacterial detection. A. Filtrate flow rate** for *E. coli*-spiked sample, where the solid line is the mean flow through the microtraps relative to the sample input flow and the shaded area indicates one standard deviation (n=5). **B. Trapping efficiency** for *E. coli*-spiked sample, which is the number of bacteria-containing traps after 70 min relative to the colony-forming units in the sample after smart centrifugation (bar height is mean, error bar is sd). **C. Overall assay performance,** where dots represent the number of positive microtraps detected, $N$, for various bacterial concentrations in the 2.25 ml blood sample, $C$, and lines depict the least square fitting linear calibration curves $N = \eta.C$. **D. Timelapse images of bacterial capture and growth in a single microtrap**, showing the capture of one bacterium of *K. pneumoniae* 40 min after sample addition, followed by bacterial cell division.

4C). The least mean square linear curve fit, $N = \eta.C$, to the data allows estimating the overall assay sensitivity, $\eta$. We can infer that the limits of detection, which are the bacterial concentrations for which we expect $N$=1 positive trap, $C_{N=1} = 1/\eta$, are in the range 1-10 CFU/ml. After trapping, the bacterial cells divide inside the microchannels, while the sample is still being loaded (Figure 4D).

Due to the poor isolation during smart centrifugation, we could not detect *S. aureus* (n=2).

# Discussion

We demonstrate the unique isolation and concentration of three sepsis-causing bacterial species from blood, coupled to their detection by microscopy in microfluidic traps within 2 h. These pathogens cover approximately 20% of the bacterial species causing BSIs in the European Union [39] and 35% of those in Japan [40]. The estimated detection limit in the range 1-10 CFU/ml is clinically relevant. Compared to previous work, our approach does not require blood culture and allows the detection of bacteria phenotypically at clinically relevant concentrations.

Smart centrifugation is a simple, inexpensive, high-throughput, and easily parallelizable method to isolate bacteria from whole blood rapidly with a high bacterial isolation efficiency and high blood cell rejection. The method's compatibility with standard lab equipment supports translation to the clinical setting. The bacteria are isolated in a small volume of liquid, similar to the original plasma volume, which facilitates downstream processing.

Bacterial enrichment in the supernatant during centrifugation results from two effects. First, the Stokes terminal velocity of bacteria is a factor $\alpha \simeq 1/30$ times that of blood cells, allowing efficient terminal velocity-based differentiation.[30] Second, the high hematocrit results in downward sedimenting RBCs causing a backflow of the plasma, thereby dragging bacteria upward and further increasing differentiation between the two. Smart centrifugation enhances the separation by extending the sedimentation path of particles through the density medium. For comparison, we experimentally optimised isolation of *E. coli* from spiked whole blood in conventional centrifugation, i.e., without sample dilution or density medium, and achieved 34% ± 7% isolation efficiency in the supernatant (SI Figure A2). Smart centrifugation thus provides a 65% / 34% = 1.9 times improved isolation efficiency for *E. coli* for a comparable blood cell rejection efficiency (SI Table A).

Even though our method was optimised for *E. coli*, we achieved even higher isolation efficiency of *K. pneumoniae*. Isolation efficiency of *S. aureus*, however, was much lower. Isolation efficiency of *E. coli* and *E. faecalis* were not significantly different. We found no significant differences in bacterial isolation efficiencies between samples spiked with the same strain at different concentrations (SI Figure A1).[1] We speculate that several factors can cause the recovery differences between the species, as well as the large variation in recovery between different measurements with the same strain. These factors include differences in Stokes radius between the bacterial species [30], differences in interaction between the blood cells and the bacteria, differences in viscosity and hematocrit between the blood samples, variations in the immune response of the patient blood to the bacteria (reducing the bacterial count), or variations in bacterial growth phase (compared to *K. pneumonia*, *E. coli* were spiked in stationary phase) or growth rate (*E. faecalis* have a slower growth

[1] With the exception of *E. coli*, where we found a significantly lower isolation efficiency for concentrations from 21 - 100 CFU/ml compared to > 100 CFU/ml ($p \leq 0.05$). However, the statistical power of this finding is low considering the very low number of measurements.

rate than *K. pneumonia*). We speculate that the low isolation efficiencyof *S. aureus* is caused by their binding to blood cells or plasma protein, leading to their rapid sedimentation [31]. Indeed, spiking *S. aureus* in a sample liquid with the same density as diluted blood - but without blood cells - resulted in 54 ± 6% (n=6) isolation efficiencyby smart centrifugation, compared to 8 ± 7 % (n=10) isolation efficiencyfrom spiked blood (SI Figure A3).

Based on our reading of the literature, smart centrifugation has superior recovery and throughput for *E. coli*, data for which are presented for most tests (Table 1).

**Table 1** Key performance parameters of smart centrifugation and other separation methods for *E. coli*.

| | Bacteria isolation efficiency | RBC removal efficiency | Blood processed (ml) | Time (min) | Throughput (µl/min) | Minimum concentration $C$ detected (CFU/ml) | Volume concentration (Vf / Vi) |
|---|---|---|---|---|---|---|---|
| **Smart centrifugation** | 64% | 99.98% | 2.25 | 5 | 450 | 9 | 1.1 |
| Filter based centrifugation [29] | 26-40% | 99.4% | 1 | 60 | 16.7 | 10 | 18 |
| Compact Disk [30] | 40% | 94% | 7 | 1 | 7000 | 10 | 0.6 |
| Dextran sedimentation [31] | 50-60% | >90% | 10 | 30 | 333 | 10-100 | 0.4 |
| Elastoinertial Separation [28] | >65% | 100% | 1 | 40 | 25 | 40 CFU | 1.5 |
| Inertial lift forces [41] | >80% | 90% | 0.15 | 4 | 37.5 | $>10^5$ | 50 |
| Dielectrophoreis [42] | 87.2% | 100% | 0.05 | 75 | 0.67 | 10000 | 0.01 |
| SAW [43] | 88% | 99.6% | 0.012 | 600 | 0.02 | $4.4.10^7$ | 25 |
| Acoustophoresis [37] | 75% | 99.99% | 1 | 50 | 20 | $5.10^8$ | 3 |

The concentrated bacterial sample was loaded onto the microfluidic chip at 1000 mbar. At higher pressures, we observed bacterial loss through the 300 nm constrictions of the microtraps. Sedimenting into a higher-density liquid during the prior volume reduction step reduced the compression of bacterial cells and lysate and allowed more homogeneous resuspension, leading to lower on-chip clogging. The trapping of bacterial cells and blood cell debris in the traps increases the fluidic resistance of the filter and decreases the through-filter flow over time. The chips enabled processing 0.5 ml sample within 70 to 90 min, during which 30% of the sample flows through the microtraps (filtrate flow) and the rest flows past the filter directly to the waste outlet (retentate flow). The low filtrate flow rate is thus a major contributor to bacterial loss between isolation and detection.

In summary, we address the critical limitation in state-of-the-art BSI diagnosis: the long blood culture time to reach positive culture [8, 9]. We here demonstrated the detection of bacteria in microtraps without blood culture at clinically relevant bacterial concentrations in 2 h. Since the cells are living it is possible to do phenotypic AST [23] or species ID with FISH [44].

# Methods

## Blood Sample Preparation

Blood in EDTA tubes from healthy donors was purchased from the blood bank (Blodcentralen, Stockholm, Sweden) or Uppsala University Hospital

(Akademiska Sjukhuset, Uppsala, Sweden). The blood samples were stored at 4 °C, and used for experiments no later than two days after collection. Samples presenting a milky supernatant (< 10%), i.e., indicating abnormally high concentrations of triglycerides in the blood, were discarded. Before processing the samples, they were put at room temperature, and blood samples were spiked with respective bacteria concentrations of approximately $10^3$, $10^2$, and 10 CFU/ml. The concentration of the spiking solution was quantified by plate counting (n=3). The spiking volume was always less than 5% of the total blood solution volume.

## Bacterial Strains

We used three clinical isolates that were randomly collected from a clinical microbiology laboratory in Sweden, covering both gram-negative and gram-positive species. As gram-positive representatives, *S. aureus* (DA70300) and *E. feacalis* (DA70208) were used, and as gram-negative, *K. pneumoniae* (DA72206). We also used *E. coli* (EL3041) cells harbouring a plasmid expressing mVenusNB fluorescence proteins.

Bacteria were stored for long term at -80 °C in standard glycerol solution and were incubated overnight at 37 °C in BD BACTEC Plus Aerobic medium (BD, USA), referred to as Blood Culture Medium (BCM), prior to use, and later diluted to approximately $10^4$, $10^3$, and $10^2$ CFU/ml and used to spike blood with different concentrations and quantified for isolation. For detection experiments with *E. coli* cells harbouring a plasmid expressing mVenusNB fluorescence proteins, the above-mentioned protocol was followed. For detection experiments with clinical isolates, 2 µL of bacteria from an overnight culture were spiked in 2 ml of BD BACTEC Plus Aerobic Medium, and cultured for 2-4 h depending on the species. They were then diluted to approximately $10^4$, $10^3$, and $10^2$ CFU/ml and used to spike blood with different concentrations.

## Smart Centrifugation

Lymphophrep (STEMCELL Technologies, Canada), a density medium with 1.077 g/ml density, was mixed with BD BACTEC Plus Aerobic medium (BD, USA) in a volumetric ratio of 2:1, and 1 ml of the solution, with 1.051 g/ml density, was poured into a 15 ml Falcon centrifuge tube. 3 ml of spiked blood mixed with BD BACTEC Plus Aerobic medium (BD, USA) (3:1 Blood:BD BACTEC Plus Aerobic medium) was gently placed over the density media. The tube was centrifuged at 600g for 5 min in a hanging bucket centrifuge. The supernatant was removed and mixed with the lysing solution.

## Conventional centrifugation for bacteria isolation

To establish a control measurement, we centrifuged 4 ml of whole blood without any dilution and density media. The sample, after being spiked, underwent centrifugation at 500g for 4 min, resulting in the separation of clean plasma at the top. This process effectively settled most of the blood cells.

## Selective Lysing Solution

The selective lysing solution was prepared by mixing sodium chocolate hydrate (Sigma-Aldrich, USA) and saponin (Sigma-Aldrich, USA). Concentrations of 2% (W/V) sodium cholate hydrate and 1% (W/V) saponin were prepared by dissolving the chemicals in BD BACTEC Standard Aerobic medium.

## Microfluidic Platform Fabrication

The microfluidic chip design and fabrication were previously reported [23, 45]. A silicon wafer mold was fabricated according to design specifications by the company ConScience AB, Sweden. The wafer was once silanized for 30 min prior to polydimethylsiloxane (PDMS; Sylgard 184, DOW, USA) replication. Subsequently, a 10:1 w/w PDMS:curing agent mixture was poured on the wafer and baked at 80°C overnight. Holes on the PDMS ports 2.0, 2.1, 2.2, 5.1 and 5.2 (see chip design in [23, 45]) were made using a 0.5 mm puncher. Then, the PDMS stamps were cleaned in IPA before covalent bonding to a glass coverslip (No. 1.5, Menzel-Gläser, Germany) after plasma treatment, followed by heat curing for 1 h at 80°C.

## Microfluidic Flow Control

The microfluidic device was mounted on the microscope and connected to the sample reservoirs with flexible plastic tubing (TYGON, Saint-Gobain, North America). OB1 CONTROLLER (Elveflow, France) flow control units were used to pressurize reservoirs.

The reservoirs connected to ports 2.1, 2.2, 5.1 and 5.2 were first pressurized at 500 mbar for chip priming with water infused with 0.085 g/l of Pluronic F108 (Sigma-Aldrich, USA) and was decreased to 0 mbar. Then the reservoir connected to inlet port 2.0 was pressurized at 500 mbar for priming and at 1000 mbar for sample loading.

## Volume reduction

Percoll (Sigma-Aldrich, USA), a density medium having a density of 1.125-1.135 g/ml was mixed with BD BACTEC Plus Aerobic medium (BD, USA) in a volumetric ratio of 1:2, and 0.3 ml of the solution, with approximately 1.04 g/ml density, was poured into a 15 ml Falcon centrifuge tube. The supernatant infused with the lysing solution was gently placed over the density medium. The tube was centrifuged at 1000g for 13 min in a fixed rotor centrifuge. All excess liquid above 0.5 ml was removed, without removing the pellet.

## Optical setup

Images of all the microtraps contained in the microfluidic device were acquired using a Nikon Ti, inverted microscope. For imaging experiments containing clinical strains, a CFI plan Apo lambda 100x (1.45 NA, oil) objective was

used. Phase contrast images were acquired by using DMK 38UX304 (the imaging source) camera, with an exposure time of 20 ms with an interval of 10 min, for a maximum of 90 min. Cells expressing mVenus fluorescence proteins were captured using a filter cube consisting of a Di02-R488 (Semrock, USA) dichroic mirror, a FF02-482/18 (Semrock, USA) excitation filter, and a FF01-524/24 (Semrock, USA) emission filter and CFI Plan Apo lambda DM Ph2 20x objective. Each fluorescence image was acquired with an exposure time of 500 ms with an interval of 5 min, for a maximum of 90 min. A constant temperature of 37$^{o}$C was maintained during the measurements using a temperature-controllable unit (Okolab, Italy). The imaging setup was operated by micromanager 1.4 version software.

## Bacteria Quantitation

All bacterial sample quantitation was performed by plate counting after plating a sample aliquot on agar plates and overnight culture at 37 °C in the incubator. The agar plates were prepared by dissolving LB broth with agar (Miller) (Sigma-Aldrich, USA) in deionized water at 40 g/L concentration followed by autoclaving and placing in Petri dishes.

For on-chip bacteria quantitation of the clinical isolates, we employed ImageJ software image analysis of phase-contrast microscopy images. Each microtrap was analyzed manually and determined positive if filled with at least one bacterium. The same analysis pipeline was performed for fluorescence microscopy images of the *E. coli* cells expressing mVenus fluorescence proteins.

## Blood Cell Quantitation

The concentrations of the blood cells in whole blood before dilution and in the supernatant after smart centrifugation were measured using a haematology analyzer (Swelab Alfa Plus, Boule Diagnostics, Sweden).

## Flow characterization

The ports 2.1 and 2.2 were coupled together, and ports 5.1 and 5.2 were coupled together, using a PEEK union T-connector (Thermo Fisher Scientific, USA). Flow measurements were taken from both couplers using a SLI Liquid Flow Sensor (80 µL/min) (Sensirion, Switzerland). Two independent flow measurements were acquired with a sampling time of 1 s, for a maximum time of 90 min, using the Sensor Viewer Software (Sensirion, Switzerland). The data was analysed using MATLAB.

## Statistical analysis

Data analyses were performed with R. The data samples were tested for normality using the Shapiro-Wilk test; variance between normal populations was studied using the F-test; and statistically significant differences between groups were studied using a t-test, for samples following a normal distribution,

or a Mann Whitney test for samples not following a normal distribution. A two-sided p-value of $\leq 0.05$ was considered statistically significant.

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