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Rapid antimicrobial susceptibility testing directly from positive blood cultures

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Sepsis results from bloodstream infections and is a leading cause of mortality worldwide, accounting for over 10 million deaths annually and imposing a substantial burden on the global economy. Septic shock is a time-sensitive condition in which mortality increases by approximately 8% for every hour of delayed treatment. A key limitation in current sepsis management is the delayed initiation of targeted antimicrobial therapy, primarily due to time-consuming subculturing steps and slow antimicrobial susceptibility testing (AST) methods. In this study, we present a rapid AST workflow performed directly from positive blood cultures within 3 hours. The method integrates a Smart Centrifugation Device for host cell removal and bacterial concentration, with impedance-based flow cytometry (iFAST) for rapid antimicrobial susceptibility testing. This streamlined, partially automated method has the potential to accelerate sepsis diagnosis and facilitate timely, targeted antimicrobial treatment.

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Introduction

Bloodstream infections (BSIs) often in the form of bacteraemia require prompt diagnosis and treatment if poor outcomes are to be avoided.[1,2] They have the potential to progress into sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to infection.[3] Sepsis is a time-critical condition, associated with high mortality. Globally, sepsis causes over 10 million deaths annually and places a substantial economic burden on healthcare systems, costing the U.S. alone more than 20 billion dollars, primarily due to the need for intensive care unit (ICU) admissions.[4–6] The high mortality is largely due to the fact that the survival rate of patients in septic shock decreases by approximately 8% for every hour of delayed treatment.[7–9]

Diagnosing sepsis remains a significant clinical challenge. Various diagnostic methods are used, including blood cultures to isolate a causative agent, biomarker detection (*e.g.*, C-reactive protein or white blood cell count), and clinical scoring systems such as the quick Sequential Organ Failure Assessment (qSOFA).[10,11] For suspected BSIs, blood cultures are considered the gold standard.[12] In the clinical setting, the blood culture workflow begins with drawing blood from a patient followed by incubating it in a bottle with growth media to grow any organisms that are present. If the presence of viable bacteria is detected in the blood culture, then it is labelled "positive", and the patient is diagnosed with bacteraemia. In patients with bacteraemia, the number of organisms in blood can be as low as 1 colony-forming-unit per millilitre (CFU mL^{-1}),[13] making it necessary to culture bacteria up to 10^8–10^9 CFU mL^{-1}. The drawback of the positive blood culture (PBC) is that it has a long turnaround time, taking typically up to 1 day for commercial blood culture monitoring systems to flag positivity (*e.g.*, BD BACTEC™ FX and BACT/ALERT® 3D, bioMérieux). However, the blood culture is still an essential step in the diagnosis of bacteraemia and has yet to be replaced with a more efficient method (see Fig. 1).

PBCs can only inform the presence of a pathogen in the bloodstream. This information is not enough to initiate targeted antimicrobial therapy, so further tests are necessary, including identification of the causative organism and its susceptibility to different antibiotics. Gram stain is frequently carried out once blood bottle positivity is determined to assist with the choice of antibiotics.[14] Identification at the species level allows the clinician to select the narrowest spectrum and most appropriate antibiotic that is known to be specific against the pathogen. To achieve this, techniques such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), and nucleic acid tests are used. MALDI-TOF MS identifies the genus and species of the organism by comparing a unique fingerprint from the organism to a reference database. Spectra from proteins and lipids belonging to the pathogen are analysed to identify the

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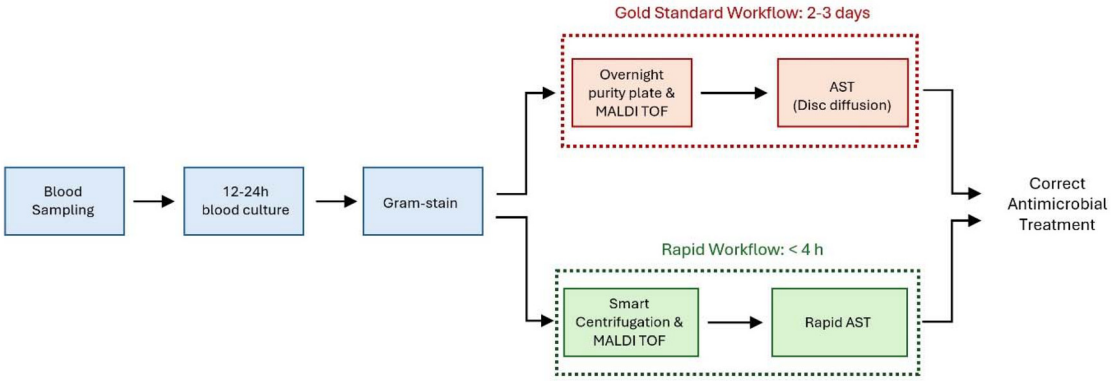

Fig. 1 Example of a typical diagnostic workflow *vs.* rapid workflow. Blood from a positive blood bottle is streaked on a purity plate from which colonies are subcultured for an AST (disc diffusion or broth-microdilution). The rapid workflow replaces the overnight subculture.

organism. This takes no longer than a few minutes per sample. Identification with MALDI-TOF MS has drastically reduced time to pathogen identification, but their clinical benefits are limited without improvements to ASTs.

Identification of bacteria with MALDI-TOF is performed with colonies taken from an overnight purity plate after subculturing on agar. Unpurified blood culture samples can interfere with the mass spectrometry measurements because of the presence of haemoglobin and other blood serum proteins. To address this, improved bacterial isolation protocols have been developed,[15] the most common involving centrifugation of the blood culture sample with[16–21] or without lytic agents.[22,23] Centrifugation protocols often involve multiple steps, with different spin cycles. In parallel, phenotypic antimicrobial susceptibility testing (AST) using disk diffusion or broth-microdilution (BMD) is also performed. Phenotypic AST requires a purified bacterial suspension, as residual RBCs, haemoglobin and other blood components can interfere with antibiotic activity and lead to inaccurate MIC measurements.[24] Normally this is done using colonies but there is a need to accelerate the process in order to facilitate rapid AST, and commercial kits, such as Brucker's Sepsityper®[25,26] rapidly isolate bacteria from PBCs with lysis buffers (and centrifugation) for downstream use. However, lytic agents such as detergents and formic acid can impact the viability of Gram-positive bacteria[27,28] potentially restricting their use for rapid ASTs. The presence of residual detergent, even at low concentrations may also impact the effectiveness of antibiotics, leading to potentially incorrect resistance profiling.[29]

Commercial devices have been developed to streamline and automate the blood processing. For example, the Accelerate Arc System[30] automates the centrifugation-based separation of bacteria from blood. Another device is the VITEK MITUBE; an automated cartridge capable of producing a pellet of bacteria from positive blood cultures.[31] The method yields an inoculum in minutes suitable for MALDI analysis. Further, the FAST-PBC Prep™ cartridge (Qvella) removes blood components using automated sedimentation (via centrifugation).[32,33] A PBC aliquot is loaded into the cartridge and spun for 30 minutes to obtain a "liquid colony" for downstream use, either with MALDI-TOF or phenotypic AST.

Fig. 1 compares a standard clinical workflow with the rapid AST described in this paper. Conventional AST methods rely on overnight incubation resulting in long turnaround times, so that empirical broad-spectrum therapy is continued while awaiting results. This delay contributes to the rise in antimicrobial resistance,[34] which worsens patient outcomes due the decreasing effectiveness of antibiotics. As a result, there has been a growing effort to develop rapid phenotypic AST methods to analyse bacterial growth directly from PBCs.[35,36] Commercially available AST technologies include both genotypic and phenotypic systems. Genotypic methods are generally faster and do not require bacterial growth; for example, the BioFire® BCID panel can deliver results in approximately 1 hour.[37] However, these methods are limited to the detection of known resistance genes and may fail to identify resistance mechanisms not associated with common markers, such as efflux pump-mediated resistance in Gram-negative organisms. Phenotypic systems directly measure the bacterial response to antibiotics and therefore provide a broad assessment of antimicrobial susceptibility.

Systems such as FASTinov,[38] VITEK REVEAL,[39] Phoenix 100 ID/AST,[40] and Alfred 60AST[41] are examples of the shift towards rapid and automated AST systems that have shown success in delivering results from PBCs. These systems measure bacterial growth using methods such as optical turbidity, production of Volatile Organic Compounds (VOC) or fluorescent viability dyes. Bacteria are analysed after extraction from positive blood bottles with reported time-to-result generally in the range of 4–8 hours, with the exception of FASTinov (2 hours) depending on the organism and antibiotic panel. Single-cell analysis methods have also been developed including impedance, high resolution imaging, plasmonic nanosensors and Raman.[42–45] These have the potential to detect antibiotic response potentially delivering results in under 2 hours. However, simple and rapid preparation techniques for the removal of blood components whilst minimising any impact on cell viability are required to make the best use of any rapid AST technology.

In this work we describe a new phenotypic AST workflow that processes a PBC to deliver an actionable AST result in less than 3 hours (see Fig. 1). This streamlined workflow requires a standard centrifuge and a disposable cartridge. It is simple with low hands-on time and with limited impact on the viability of Gram-positive bacteria.

The approach integrates two previously developed technologies: (1) a simple and disposable smart centrifugation device (SCD) for streamlined blood sample preparation,[21] and (2) a novel rapid AST that uses impedance-based flow cytometry (iFAST).[42] As proof of principle the approach was tested using one Gram-negative and one Gram-positive organism (*Escherichia coli* and *Enterococcus faecalis*). The method is shown capable of isolating viable organisms delivering susceptibility results that are comparable with overnight broth-microdilution, in less than 3 hours. Neither the iFAST technology nor the SCD had previously been demonstrated with PBCs, so combining them creates a potential rapid workflow for direct testing from PBCs. Without this integration, the workflow would either require manual centrifugation steps and pipetting or rely on overnight purity plate subculture for bacterial isolation.

Methodology

The Smart Centrifugation Device (SCD) and iFAST workflow have been previously described,[21] see Fig. 2a. The SCD is a small cartridge that isolates and concentrates bacteria whilst eliminating the majority of blood cells (Fig. 2a). The isolated bacteria can then be exposed to antibiotics to perform a rapid AST with the iFAST system (Fig. 2b and c). Each step is described in more detail in the following subsections.

Bacterial isolation

The SCD (Fig. 2a) uses differential centrifugation to isolate bacteria from a PBC in 20 minutes and is adapted from previous work.[21] Briefly, the custom centrifuge-tube separates bacteria based on differences in sedimentation, where blood cells have a higher velocity due to their larger density. The device is similar to a 50 mL centrifuge tube with a top and bottom chamber connected by a siphon-like structure. The top part of the device has a chamber to collect sedimented blood cells, leaving the isolated bacteria in the bottom chamber (Fig. 2aii). A PBC sample is loaded into the top chamber through an opening, the tube is placed into a centrifuge and set to a low spin speed. The compressed air trapped in the bottom chamber prevents liquid from moving to the bottom. In this part of the cycle the blood cells sediment, while bacteria remain in the supernatant. Next, the tube is centrifuged at a slightly higher *g* to transfer the bacteria-rich supernatant from the top to the bottom chamber. Finally, the speed is increased to 3000*g* to pellet the bacteria. When the centrifuge stops, most of the liquid in the bottom chamber is pushed back into the top chamber, leaving a pellet that can be removed with a syringe.

iFAST rapid AST

The iFAST technique measures the electrical properties of thousands of individual particles in an aqueous suspension using single cell impedance-based flow cytometry (Fig. 2b).[42] Bacteria flow through a microfluidic channel containing a microelectrode array. An AC voltage at different frequencies is applied to the electrodes, and as the cells flow through the channel, they obstruct the current between opposing electrodes. This change in current is measured and processed to give the impedance of each cell (magnitude and phase of impedance, *Z*). The iFAST cytometer uses two AC signals at 5 MHz and 40 MHz. The impedance of the cell at 5 MHz is proportional to volume (assuming the particle to be a viable cell with an intact membrane). The higher frequency (40 MHz) signal is influenced by the electrical properties of the cell wall and to a lesser extent the cytoplasm. The cube root of the impedance magnitude (electrical diameter) and electrical phase of each particle is plotted on a scatter diagram as shown in Fig. 3.

The iFAST system performs a susceptibility test by measuring the electrical properties of bacteria before and after two hours exposure to a panel of different antimicrobials. The change in the electrical properties of the bacteria is related to the mode of action of the antibiotics. For example, a β-lactam antibiotic leads to a loss of cell wall integrity; the bacterial membrane becomes compromised resulting in the formation of a spheroplast and eventual cell lysis (Fig. 2bii). This change can be clearly observed in the electrical signals. Bacteriostatic antibiotics inhibit the growth of bacteria which manifests as a significant difference in the count of control and exposed cells after 2 hours.

Materials and methods

PBC sample preparation

Escherichia coli 35218 (Gram-negative) and *Enterococcus faecalis* 29212 (Gram-positive) were obtained from American Type Culture Collection (ATCC). Prior to their use they were removed from −80 °C storage and streaked onto Tryptic Soy Agar (TSA) purity plates. The agar plates were incubated at 35.5 °C overnight to promote growth. The following morning, two 9 mL portions of citrated whole blood, provided by healthy donors were transferred into separate BD BACTEC aerobic blood culture bottles. The blood culture bottles typically contain 35 mL of blood culture media (BCM) and resin beads so that the final blood-to-media ratio was approximately 1 : 4 v/v. With a 10 μL sterile loop, smears of single *E. coli* and *E. faecalis* colonies were transferred into separate microcentrifuge tubes holding 1 mL of BCM. The tubes were vortexed then injected into the aerobic bottles containing 9 mL of whole blood and BCM. The bottles were placed in a BD BACTEC FX incubator, flagging positive 5–8 hours later. To maximise the growth of bacteria, the bottles were removed from the incubator after approximately 16 hours giving esti-

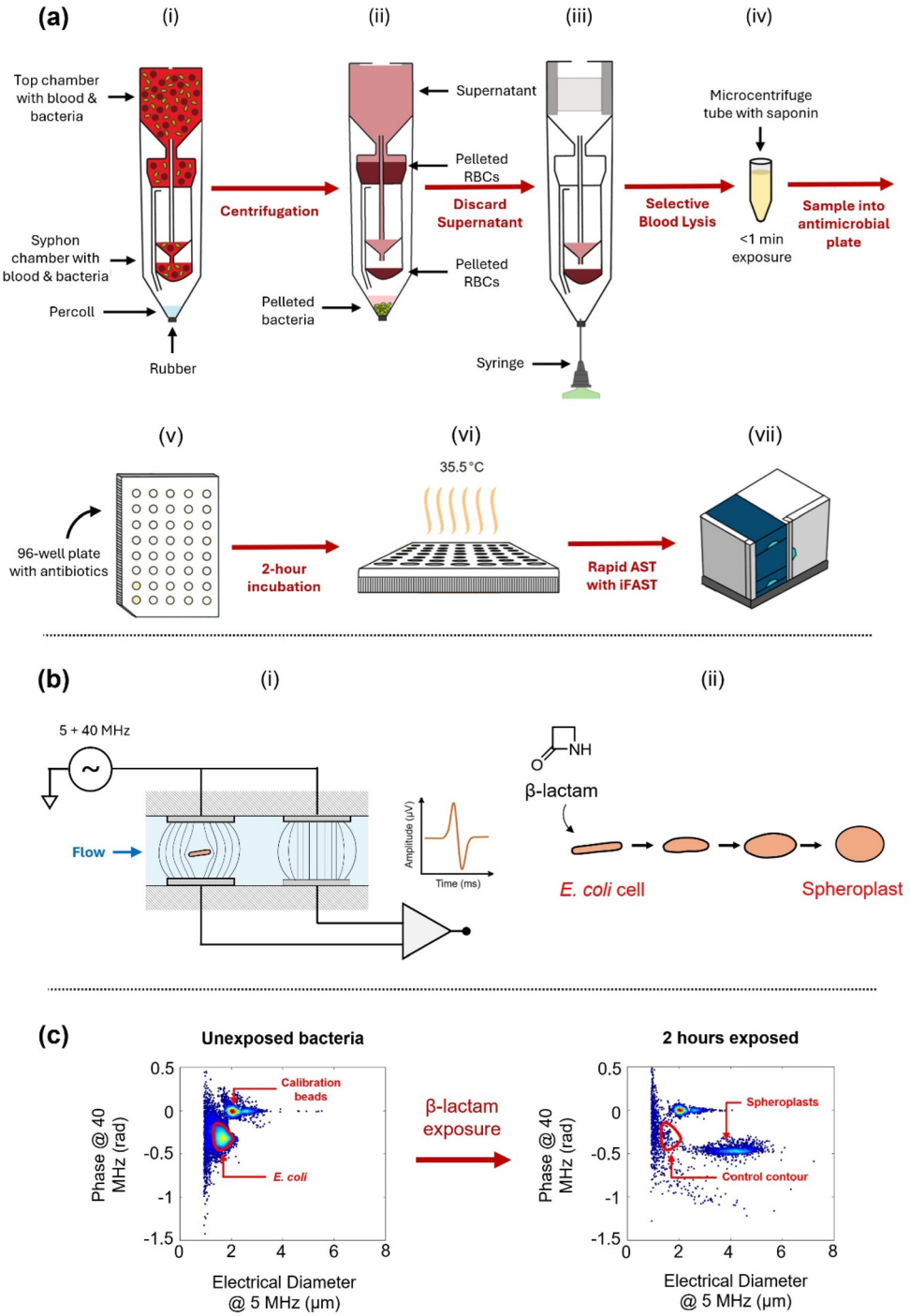

Fig. 2 Overview of the SCD and iFAST impedance cytometer. (a) Workflow for the rapid isolation of bacteria from blood using the SCD. (i) A blood culture aliquot is added into the top chamber. (ii) The device is centrifuged causing RBCs and bacteria to pellet in separate chambers. (iii) The supernatant and RBCs in the top chamber are discarded, and bacteria are removed with a syringe. (iv) The sample is momentarily transferred into a microcentrifuge tube with 0.2% saponin and swiftly diluted before loading into the antimicrobial plate. (b) The iFAST system. (i) Impedance cytometer diagram. An AC voltage at 5 and 40 MHz is applied to a set of electrodes, and the impedance of single cells is measured from the current change. This current is converted to voltage (*I*–*V*) and fed into a differential amplifier to output a signal with an amplitude and phase. (iii) Illustration of the mode of action of a β-lactam antibiotic on a bacterium, as it becomes a spheroplast then finally lyses leading to distinct electrical signatures. (c) Example impedance scatter plot showing change in the population of susceptible bacteria after exposure to a β-lactam antibiotic (cefepime).

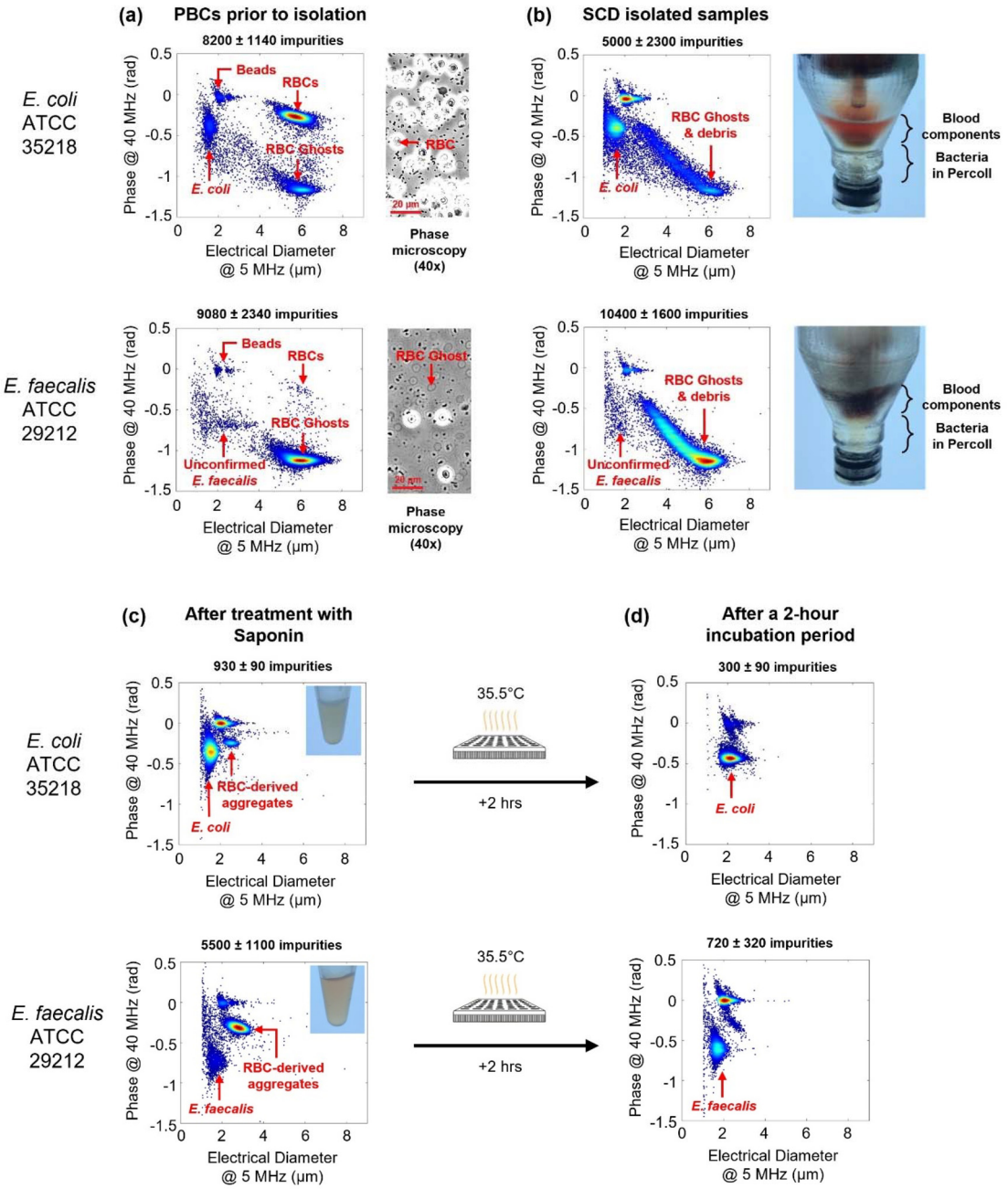

Fig. 3 Electrical impedance scatter plots for *E. coli* and *E. faecalis* samples before and after isolation with the SCD. All samples were diluted in physiological saline for each cytometer run. Each alphabetical subfigure label corresponds to a step carried out chronologically. (a) Impedance scatter plots and phase-contrast microscopy images of samples from a PBC after a 16 h incubation. (b) Scatter plots of the samples isolated with the SCD alongside photographs of the bottom part of the device after the 20-minute spin. (c) Scatter data and photographs of the samples after a short incubation with saponin. (d) Scatter plots for positive control samples (no antibiotic). The photographs shown are from samples aliquoted from the positive control wells on the 96-well antimicrobial plates (EUGNF & EUSTAPF). The counts of (RBC + ghost cells + other non-bacterial particulates) is summarised for each sample.

mated bacterial cell densities of 10^8–10^9 CFU mL^{-1} for *E. coli* and 10^7–10^8 CFU mL^{-1} for *E. faecalis*.

Bacterial isolation with the smart centrifugation device (SCD)

The PBC bottles were strained using 70 μm cell strainers to remove resin beads before loading into the SCD. The SCD tubes are compatible with a programmable centrifuge (Eppendorf 5810 R) without the need for custom buckets. An 8 mL aliquot of PBC was mixed 1 : 1 with BCM, giving a total volume of 16 mL. This mixture was loaded into the top chamber of the SCD. The bottom chamber of the SCD was preloaded with a cushion solution composed of Percoll and BCM

in a 3 : 5 v/v ratio. The SCD was centrifuged in three sequential steps at 100*g*, 200*g*, and 3000*g*. The first step, 100*g* for 10 minutes, sediments most red blood cells (RBCs), the second step, 200*g* for 1 minute, gently transfers the bacteria-rich supernatant into the bottom chamber. Finally, centrifugation at 3000*g* for 10 minutes sediments the bacteria to form a pellet of approximately 0.8 mL. An image of the device after use is shown in Fig. 3b, and the entire process, including the time it takes to load and remove the sample, took ~30 minutes.

Sample recovery and processing

The pellet was collected by piercing the rubber seal at the bottom of the device with a syringe. To remove the remaining RBCs, this pellet (in 0.8 mL Percoll + BCM) was immediately mixed with 0.2 mL of 2% saponin in deionized water, giving a final saponin concentration of 0.2%. The suspension was held in the saponin solution for less than 1 minute to ensure the short exposure of the organisms to the non-ionic detergent. The sample was subsequently diluted 100-fold in saline and aliquoted into a 96-well plate where the cell count was determined with the iFAST cytometer, a process that takes a few minutes.

Antibiotic exposure

As required by EUCAST, a 5 mL bacterial stock suspension at 5 × 10^5 CFU mL^{-1} in MHB was prepared using the iFAST system. ASTs were performed using the iFAST system along with an overnight broth-microdilution (BMD) using 96-well antimicrobial plates with freeze-dried antibiotics (Thermofisher Ltd), one for Gram-negative (EUGNF) and a second for Gram-positive (EUSTAPF). The plates contain 2-fold serial dilutions of solid antibiotics, with approximately 25 antibiotics per plate. The plates require 50 μL samples (at 5 × 10^5 CFU mL^{-1}) to dilute the antibiotics and match the correct concentration. These were loaded using a multichannel pipette. The plates were placed in an incubator for 2 hours at 35.5 °C. After incubation, cells were measured with the iFAST system along with a known amount of 2 μm diameter latex calibration beads. BMD was performed on the same plates after 24 hours following EUCAST guidelines, which involves detecting bacterial growth by eye. Experiments were done in triplicate.

Data processing

Analytical software (MATLAB) was used to count bacteria by generating the scatter plots and applying a contour to the population inside them. The contour was defined using a kernel density estimation function, shown in red in Fig. 3. Cell counts obtained from the contours were tabulated, and their concentration (relative to 1 mL) was computed. The cell concentrations were plotted against antimicrobial concentration on a $\log_2$ scale.

Results

AST workflow development and evaluation

Fig. 3a shows example impedance scatter plots of a sample from a PBC before any further processing, along with photographs of the samples. Several different cell populations are visible. For the *E. coli* sample the bacterial population is clearly visible (1–1.5 μm diameter) along with the calibration beads (2 μm diameter). Also, a population of intact RBCs and RBC ghosts (5–7 μm diameter). Comparison of the scatter plot data for *E. coli* with *E. faecalis* show that for the latter there are very few intact RBCs because the organism is haemolytic. This is also evident in the images of the samples.

Fig. 3b shows data after processing a PBC with the SCD but prior to saponin treatment. The *E. coli* and *E. faecalis* scatter plots have a wide distribution of blood debris (between 2–7 μm), also seen in the photographs of the SCD. Enumeration data is shown in Table 1 confirming an increase in cell numbers after isolation with the SCD. The SCD was originally designed to isolate bacteria at low concentrations from whole blood, but in PBCs a large number of RBC ghosts were also concentrated with the bacteria. Because impedance cytometry is sensitive to these particles, a low concentration of saponin (0.2% w/v) was used to solubilise the ghosts and reduce their interference. This shows that saponin treatment

Table 1 Cell counts before and after processing with the SCD, obtained using Miles and Misra (MM) and impedance cytometry (iFAST) for *E. coli* and *E. faecalis*. Columns are CFU mL^{-1} (MM) and cells per mL (iFAST cytometer). Percentage values show recovery with the SCD relative to the corresponding pre-isolation positive blood culture (PBC). Data is for three independent repeats (R1–R3), and the standard error of the mean (SE) is shown. R2 had an error in the data and is discounted

		Before isolation (PBC)		After isolation (SCD sample)					
				MM			iFAST cytometer		
Organism/repeat		MM (CFU mL^{-1})	iFAST cytometer (cells per mL)	(CFU mL^{-1})	%	% SE	(cells per mL)	%	% SE
E. coli 35218	R1	1.8×10^9	1.8×10^9	2.1×10^9	118	11	1.9×10^9	103	41
	R2	1.9×10^8	2.5×10^8	2.4×10^8	125		5.3×10^8	214	
	R3	1.5×10^9	3.2×10^9	1.3×10^9	88		2.7×10^9	83	
E. faecalis 29212	R1	1.1×10^8	1.0×10^9	1.6×10^8	152	26	6.6×10^8	64	19
	R2	1.1×10^8	2.1×10^7	7.9×10^7	74		—	—	
	R3	2.5×10^9	1.7×10^9	3.8×10^9	149		1.7×10^9	102	

could be incorporated into future SCD workflows that are optimised for PBCs.

The centrifuged sample was briefly exposed to saponin (<1 min) and the data after exposure is shown in Fig. 3c. The vast majority of RBC ghosts are destroyed leaving a small population of particles in the 2–3 μm size range. This can be seen from the photographs of the final sample which are almost clear indicating very few RBCs and only a small amount of haemoglobin. Saponin binds specifically to cholesterol,[46] which makes up about 40% of the RBC lipid membrane making the membranes electrically permeable meaning that these particles would appear smaller in the impedance plots. It is also thought that saponin promotes haemolysis by increasing the water transport *via* aquaporins rather than specific binding to the phospholipid membrane.[47] However, for both *E. coli* and *E. faecalis* the data in Fig. 3c clearly indicates that the isolation protocol leaves a visible population of bacteria which does not overlap with the contaminating debris.

To estimate the RBC clearance for each step, the population of intact RBCs, ghost cells, and other debris (excluding the beads) were quantified by gating (in triplicate). For the PBC the total RBC + ghost cells count is similar except that many more ghost cells are evident in the *E. faecalis* sample. After SCD isolation the number of RBCs decreases but the particulate count increases significantly. After a brief saponin exposure the debris count is reduced by a factor of 5 for *E. coli* and by 50% for *E. faecalis* (Fig. 3c). However, in both cases the debris is concentrated into a more focused population (RBC aggregates on the scatter plot) making it easier to gate the bacteria. Finally, after a further 2-hour incubation, the debris count is further reduced.

Prolonged exposure to detergents like saponin can inhibit the growth of Gram-positive bacteria.[48] In this protocol the pellet was exposed to 0.2% saponin for a very short time followed by rapid dilution to very low concentrations (0.002%). To determine if this protocol was detrimental to the growth and viability of the bacteria the cells were counted immediately after isolation, then following a 2-hour incubation in MHB to determine the doubling time. After the two-hour incubation step a clear population of bacteria was obtained that was also measurable with the iFAST impedance cytometer (see Fig. 3d). For *E. coli* an approximate doubling time was calculated to be 30 minutes, and for *E. faecalis* 40 minutes, slightly longer than observed for rapidly dividing cells in mid-exponential phase. Impedance plots for these samples are shown in SI Fig. S2.

A limitation of flow cytometry is coincidence where two or more particles appear in the measurement volume at the same time. This was observed when processing undiluted PBC samples where the RBC cell density is very high. In this case the RBCs and ghost numbers were so high that they masked the signals from the bacterial leading to significant undercounting. This effect was also observed when bacteria and blood cells were at similar particle concentrations, for example at bacteria-to-RBC ratios that approximate 1. After saponin exposure there were more bacteria than RBCs or blood-derived particles, and coincidence was minimised, resulting in clear and distinct cell populations, as in Fig. 3c and d. A distinct population of bacteria ensures that they can be enumerated correctly for further analysis.

The efficiency of recovery was estimated by counting the cells with the iFAST cytometer along with Miles and Misra colony counting.[49] Table 1 compares the cell numbers before and after isolation (absolute numbers and relative percentages). The "Before Isolation" column is data for each of the unprocessed PBCs. Enumeration of bacteria in each blood culture bottles was determined after positivity but prior to isolation with the SCD. The "After isolation" column shows the enumeration of bacteria after isolation with the SCD and a short exposure to saponin. The percentage of recovered bacteria exceeds 100% in some instances, meaning that the concentration of bacteria had increased after isolation.

Phenotypic AST

The Susceptible/Resistant (S/R) profile for the two species was determined using the new AST workflow highlighted in Fig. 2. Overnight BMD was performed for a gold-standard comparison. Fig. 4 summarises results for four different antimicrobials per bacterium, selected from the EUGNF and EUSTAPF antimicrobial panels, along with their respective BMD results. Fig. 4a and c show consecutive scatter plots for succesive antimicrobial concentrations. The Ampicillin plots remain unchanged for the four different concentrations, indicating that *E. coli* is resistant. The EUCAST breakpoint of Ampicillin for *Enterococcus* is 4 μg mL^{-1} but the scatter plot shows no change in cell distribution across the tested range of antimicrobial concentrations. This is consistent with the overnight BMD results that show growth for all four antimicrobial concentrations (Fig. 4b, right hand axis). In order to determine the S/R profile of the bacteria from the iFAST data, the bacterial counts inside a defined contour (in red) were calculated for each antibiotic and concentration. The mean of three biological repeats is plotted in Fig. 4b and d (left hand axis).

The gated bacteria populations after antibiotic exposure were compared to a positive control (unexposed) population. A significant shift in cell shape, size, count or membrane electrical properties occurs for susceptible cells,[42] manifest as a change in electrical diameter or phase. For example, Tobramycin and Gentamicin are aminoglycosides that disrupt protein synthesis, leading to cell death (smaller electrical size) and a reduced cell count. Ceftazidime is a β-lactam that inhibits bacterial cell wall synthesis, causing morphological changes such as spheroplast formation or filamentation. Linezolid is a bacteriostatic antibiotic which inhibits bacterial growth and therefore the number of cells does not increase in time when compared with a control. The S/R results were determined from the number of cells within the red contour in Fig. 4 and correlate with overnight BMD results (no growth observed at the minimum inhibitory concentration) and EUCAST breakpoints. This data indicates that a short exposure to 0.2% saponin did not skew the AST data, as bacterial growth was not significantly inhibited by the short exposure to detergent.

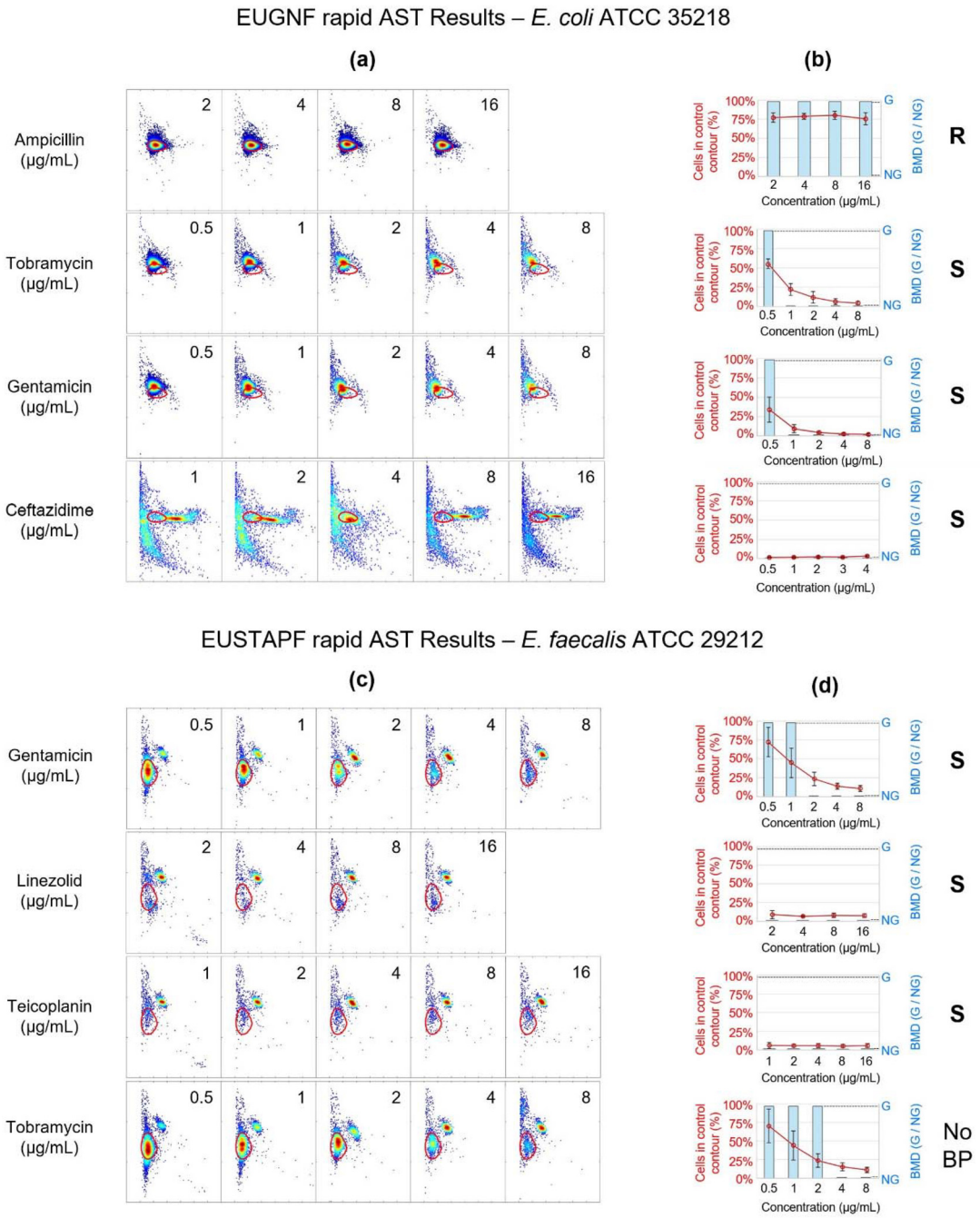

Fig. 4 Antimicrobial susceptibility test (AST) results for *E. coli* and *E. faecalis*. (a) & (c) iFAST AST scatter plots comparing the positive control cell count with counts for each dilution of antimicrobial. (b) and (d) 24-hour broth microdilution (BMD) reference AST shown as binary "growth" (G) or "no growth" (NG), (right hand axis). EUCAST breakpoints for "Susceptible" (S), "Resistant" (R) are also shown. 'NO BP' corresponds to no clinical breakpoint from EUCAST. The vertical axis corresponds to the phase at 40 MHz, and the values range from 0.25 to −0.25 rad. The horizontal axis corresponds to the electrical diameter at 5 MHz and ranges from 0 to 7 µm.

Clinical blood samples can differ from laboratory spiked whole blood taken from healthy volunteers, blood to BCM ratios in bottles might also vary, as blood is drawn *via* venesection and can fall short of or exceed the typical 8–10 mL range. The properties of blood can also change from patient to patient, such as hematocrit, differences in blood viscosity, variations in white blood cell counts, or increased concentrations of triglycerides. White blood cells (WBCs) do not interfere with identification and gating of bacteria because their concentration is several orders of magnitude lower than RBCs and they are much larger. Clinical samples from bacteremia patients are likely to have lower haematocrit

which should make processing the samples and subsequent analysis easier, but further validation using blood cultures from patients with sepsis are required. Furthermore, different types of bacteria might alter the blood culture composition, as was observed here with *E. faecalis* that is haemolytic and leads to the formation of RBC ghosts. High priority pathogens responsible for BSIs include *K. pneumoniae* and *S. aureus*, and blood cultures containing these organisms may be different. Future work will focus on validation of the method with clinical isolates for complete optimisation of the workflow.

Conclusion

This paper describes an end-to-end workflow for a rapid phenotypic AST directly from positive blood cultures (PBCs). A Smart Centrifugation Device (SCD) was coupled with a rapid impedance-based AST platform (iFAST), accelerating the current standard workflow for bacteraemia and sepsis from the standard 2–3 days to as short as 3 hours. The SCD requires a single programmable spin cycle to produce a bacterial pellet suitable for iFAST or MALDI-TOF identification. A short incubation in saponin to remove remaining ghosts did not significantly inhibit bacterial growth as the rapid AST results are in agreement with classical broth microdilution. The rapid AST S/R profiles correlate with broth-microdilution (BMD) results showing the potential application of this workflow in a clinical environment. Future work will focus on the clinical validation with PBCs from patients with bacteraemia, as this methodology needs to account for the composition and variability of true clinical blood samples. A selection of bacterial species would also need to be trialled, as organisms behave differently in blood culture bottles; for example, *S. aureus* is known to aggregate and may adhere to RBCs. In addition, either a chemical or mechanical solution would be required within the SCD to address the RBC ghost limitation. Full integration with the iFAST technology would also require development of a custom centrifugation module to produce a final bacterial suspension that is similar to the broth suspensions currently used in the iFAST workflow.

Conflicts of interest

Hywel Morgan and Daniel Spencer own equity in iFAST Diagnostics Ltd.

Ethics statement

The whole blood used for this research was purchased from Cambridge Bioscience Ltd Research Donors, an HTA-licensed clinic based in London, UK. The samples were used strictly for the purposes of this study and were destroyed after use.

Data availability

The original data for figures presented in this article, including for supplementary Fig. 2 is available at https://doi.org/10.5258/SOTON/D3752. Further information is also supplied in the supplementary information (SI). Supplementary information: Fig. S1: SCD diagram. Fig. S2: Start and end points for bacteria after growth in MHB measured with the iFAST cytometer. See DOI: https://doi.org/10.1039/d6an00259e.

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