Unlocking rapid phenotypic AST direct-from-blood with automated microbial detection and quantitative growth monitoring

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Introduction

Rapid diagnostic methods can improve quality of care in patients with bloodstream infection (BSI). Low bacterial titres in blood necessitate bacterial enrichment prior to species identification (ID) and phenotypic antibiotic susceptibility testing (AST). However, bacterial enrichment via blood culture (BC) is slow. SepsiSTAT® (Momentum Bioscience Ltd[1]) is an automated system for extracting and enriching bacteria directly from blood (Figure 1A). SepsiSTAT provides rapid detection, Gram status reporting and quantitative growth monitoring so that microbial outputs can be used as soon as the required inoculum is reached, thus allowing much earlier use of existing molecular ID/AST/NGS systems that otherwise rely on positive BC. In this study, we combine the SepsiSTAT system with QuickMIC® AST (Gradientech AB[2], Figure 1B) to demonstrate an automated diagnostic workflow enabling AST results in less than 12 hours, direct from blood.

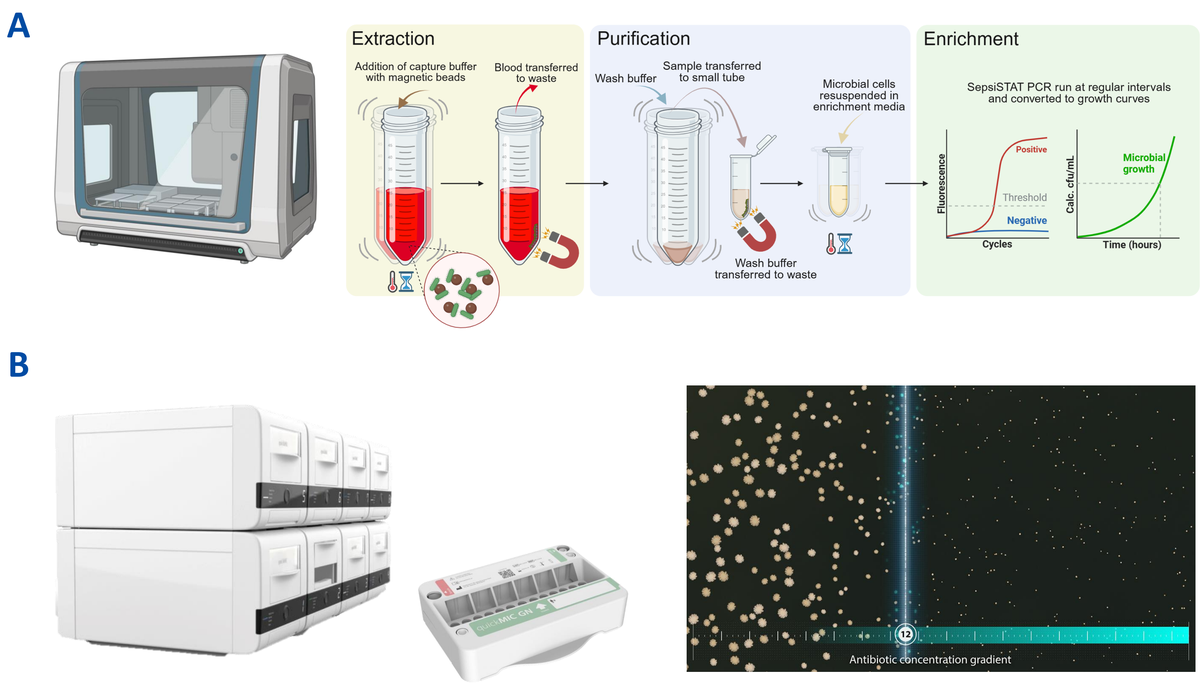

Figure 1: A) SepsiSTAT is a fully automated diagnostic system comprising microbial extraction, purification and enrichment. During the enrichment phase, SepsiSTAT provides rapid detection of BSI (with Gram status reporting) and quantitative growth monitoring via hourly PCR measurements. B) Once a SepsiSTAT sample was enriched to 500,000 CFU/mL, it was transferred to the QuickMIC GN cassette and AST was initiated. Linear MIC values for each antibiotic were read by the QuickMIC software.

Methods

- A selection of 21 Gram-negative strains including *A. baumannii, E. coli, K. aerogenes, K. pneumonia, P. aeruginosa and P. mirabilis* were inoculated into 10-mL human blood samples at a target concentration of ~5 CFU/mL blood (verified by five TVC plates).
- Immediately after inoculation, each sample was processed on the SepsiSTAT system.
- During the enrichment step, automated PCR testing was performed hourly to determine BSI positivity (together with Gram status delineation) and to provide quantitative growth monitoring.
- Once a microbial concentration of 500,000 CFU/mL was reached, as determined by SepsiSTAT's quantitative growth monitoring algorithm, the sample was tested with the QuickMIC Gram-negative (GN) AST panel.
- SepsiSTAT Time-to-Positivity (TTP) and Time-to-Target microbial concentration (TTT) were compared to TTP for equivalently inoculated blood cultures (BD BACTEC™ Plus Aerobic) tested in triplicate.
- Time-to-AST results were compared to pre-determined BC workflow times for the same strains.

Results

SepsiSTAT provides rapid detection of BSI

- SepsiSTAT microbial inputs ranged from 1.6 to 15.3 CFU/mL blood (average 6.0 CFU/mL blood) across strains (Table 1).
- SepsiSTAT TTP ranged from 4h43m to 6h56m (average 5h19m), compared to 8h34m to 15h03m (average 10h38m) for equivalently inoculated BC.
- In addition to positive detection, SepsiSTAT also confirmed Gram status for each strain.

Table 1: Summary of results for combined SepsiSTAT-to-QuickMIC workflow. Microbial input levels are shown for each species. Workflow durations from the point of inoculation to Time-to-Positivity, Time-to-Target microbial concentration and Time-to-AST result are shown (n=1 per strain). Time-to-Positivity for equivalently inoculated BC samples (n=3) are also shown.

			Average time from inoculation (HH:MM)			
Species	No. strains	Ave. input (CFU/mL)	BC TTP	SepsiSTAT TTP	SepsiSTAT TTT	QuickMIC AST result
Acinetobacter baumannii	1	2.8	09:37	04:43	07:10	11:21
Escherichia coli	9	4.0	09:50	05:08	07:07	11:06
Klebsiella aerogenes	1	5.4	09:41	04:45	06:09	10:08
Klebsiella pneumoniae	5	6.8	09:39	05:21	06:50	10:50
Pseudomonas aeruginosa	4	10.6	13:57	06:06	08:41	13:11
Proteus mirabilis	1	4.6	11:29	04:46	07:13	11:27
Average	**21 total**	**6.0**	**10:38**	**05:19**	**07:19**	**11:26**

Quantitative growth monitoring provides accurate inocula for AST

- SepsiSTAT Time-to-Target microbial concentration (500,000 CFU/mL) ranged from 5h30m to 8h48m (average 7h19m) across strains (Table 1).
- Microbial concentration, as measured by QuickMIC at the start of AST, ranged from 133,812 to 5,442,564 CFU/mL (average 874,580 CFU/mL) compared to the target of 500,000 CFU/mL as determined by SepsiSTAT (Figure 2).

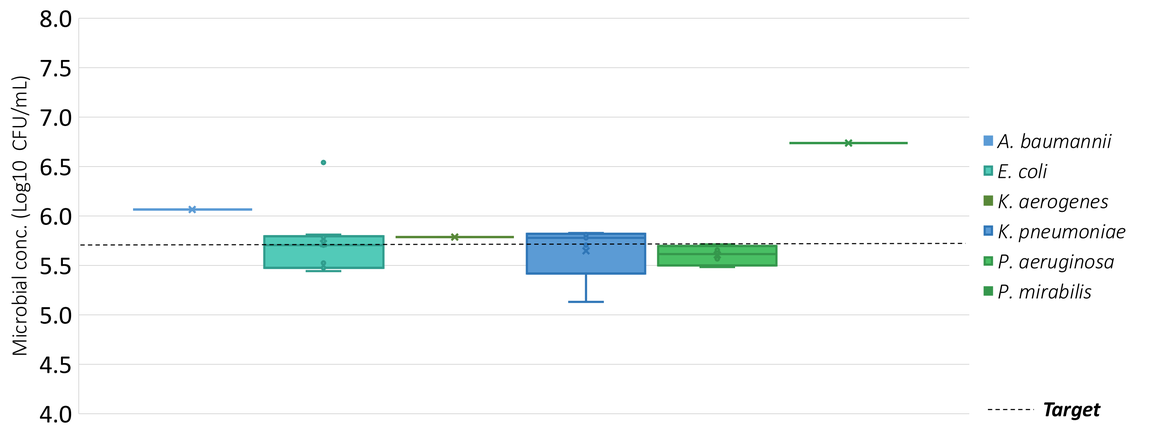

Figure 2: Box-whisker plot of microbial concentrations (Log10 CFU/mL) for SepsiSTAT outputs at the start of AST, as measured by QuickMIC software. The target microbial concentration of 500,000 CFU/mL is annotated with a black dotted line. Min and max (whiskers), lower and upper quartiles (box), median (line), mean (X) and outliers (points) are shown.

The SepsiSTAT-to-QuickMIC workflow provides AST results in under 12 hours

- Time-to-AST results ranged from 9h16m to 13h49m (average 11h26m, Table 1) compared to an average time of 22h12m for pre-determined BC (data not shown).
- SepsiSTAT-to-QuickMIC Essential Agreement (EA) and Categoric Agreement (CA) values were comparable to EA/CA values obtained for pre-determined BC, relative to broth microdilution (BMD) reference values (Figure 3).

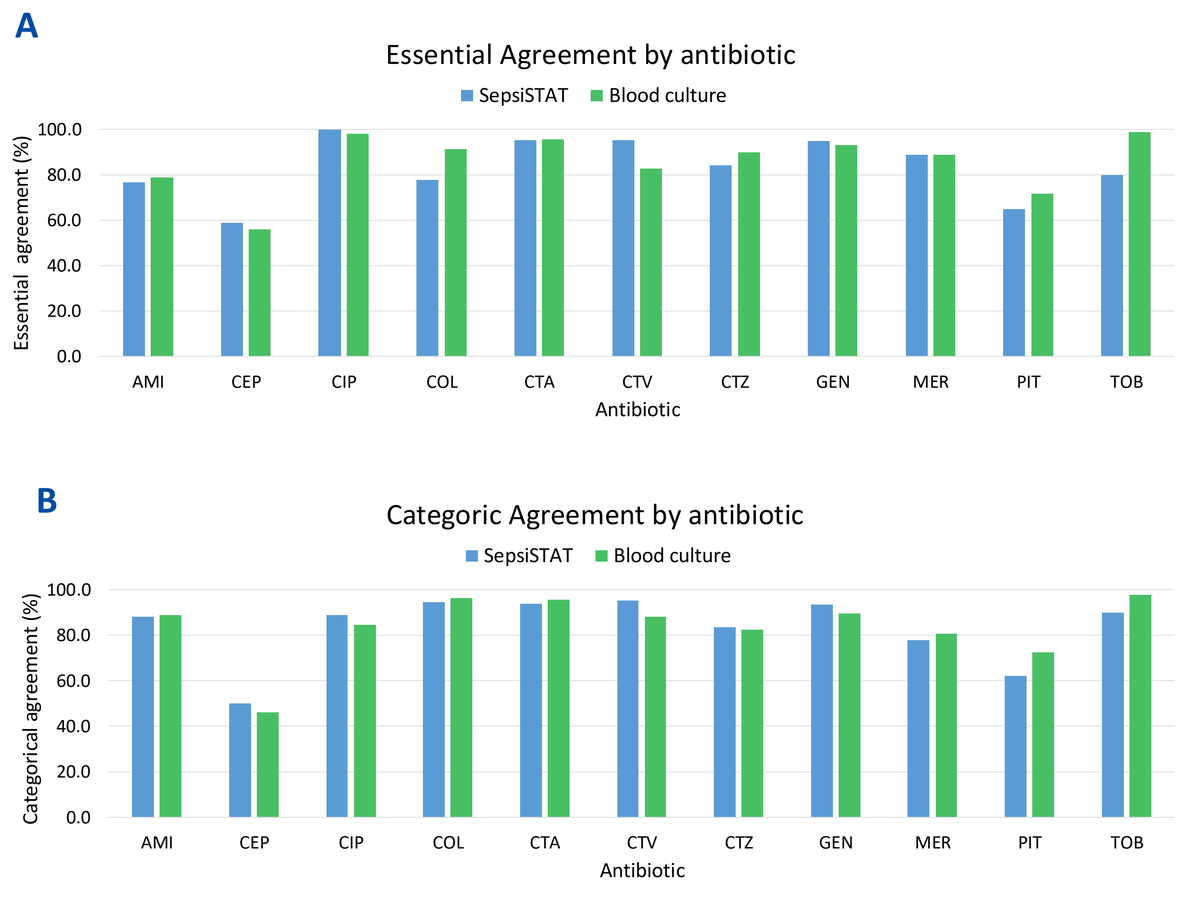

Figure 3: A) Essential Agreement and B) Categoric Agreement of QuickMIC-derived MIC values for SepsiSTAT and pre-determined BC samples, shown as percentage of samples with MIC values matching BMD reference values.

Discussion

- This study demonstrates substantial progress in the development of a rapid, automated, and direct-from-blood diagnostic pipeline for BSI.
- SepsiSTAT successfully extracted bacteria at low inocula in blood (~5 CFU/mL), whilst also providing monitored microbial enrichment so that QuickMIC AST could be initiated as early as possible, taking advantage of the AST system's low inoculation capability (from 1 x 10^5 CFU/mL).
- QuickMIC MIC values from SepsiSTAT were obtained substantially faster than those from BC and produced equivalent Essential and Categoric Agreement relative to BMD reference standards.
- The 'two-box' automated workflow presented here showcases a rapid alternative to BC, providing direct-from-blood AST results in under 12 hours.

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