#P0802

SepsiSTAT: Automated microbial extraction and enrichment for rapid BSI detection and AST, direct from blood

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Introduction

SepsiSTAT is a direct-from-blood workflow for rapid diagnosis of bloodstream infection (BSI) (Figure 1). Starting from 10 mL whole blood, SepsiSTAT provides a concentrated suspension of growing microbial cells, free from blood and antibiotics. Microbial cells are enriched to enable rapid detection of BSI, whilst also providing an enumerated microbial sample for downstream ID/AST. SepsiSTAT is intended as an adjunct to blood culture for patients where rapid results are paramount. Here, we present an automated SepsiSTAT workflow with integrated PCR-based microbial detection, Gram delineation, and quantitative growth monitoring, leading to AST results.

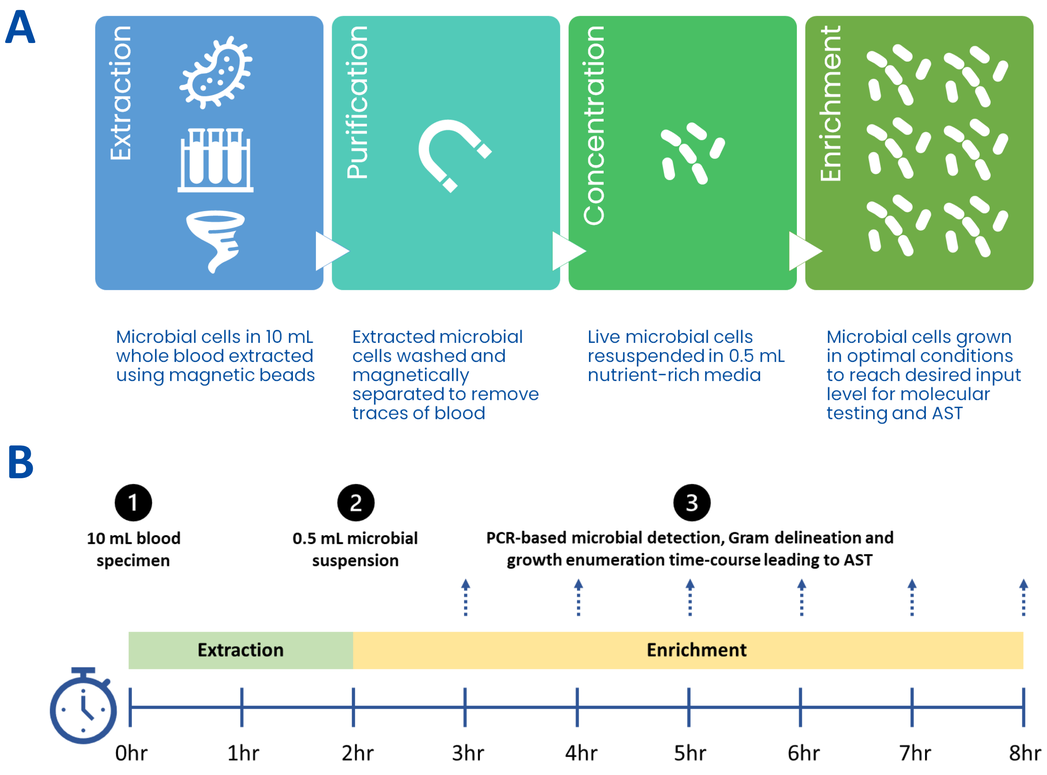

Figure 1: A) SepsiSTAT is a four-step procedure for converting low CFU blood specimens into enriched live microbial suspensions. B) Automated study workflow with timeline starting from the point of inoculation.

Methods

- Human blood samples were inoculated with clinically prevalent bacterial and fungal species, including the ESKAPE panel. Target inoculum level was 5 CFU/mL blood. Input levels were confirmed using Total Viable Counts (TVC).
- Blood samples (10 mL blood + 10 mL collection medium) were immediately loaded onto the SepsiSTAT system and run on a 16-sample basis: six replicates for each of two species and four No Spike Control (NSC) samples.
- Paired blood culture bottles (BACTEC™ Plus Aerobic) were prepared simultaneously in triplicate and loaded immediately onto the BD BACTEC™ FX system.
- On the SepsiSTAT system, microbial cells were extracted into 0.5 mL Enrichment Media. Enriching microbial samples were tested hourly using Momentum's proprietary qPCR test to delineate Gram-positive, Gram-negative, and Candida, and to predict microbial concentration (CFU/mL) based on pre-determined standard curves for each channel.
- Microbial growth was automatically monitored, and AST (E-test, bioMérieux) was performed once a predicted concentration of 10^5-10^7 CFU/mL was reached. TVC analysis was performed to evaluate enumeration accuracy.

Results

Time to Positivity

- Fourteen clinically prevalent microbial species were evaluated. All replicates were successfully extracted with average input levels ranging from 0.8 to 11.2 CFU/mL blood.
- Average time to positivity[1] (TTP) was 5.36 hours (95% CI: 5.19 - 5.54) for SepsiSTAT *vs* 12.52 hours (95% CI: 11.58 - 13.47) for paired blood cultures. SepsiSTAT detection averaged from 1.84x faster (*E. faecium*) to 3.36x faster (*P. mirabilis*) (Table 1 and Figure 2). Note, SepsiSTAT TTP includes PCR protocol currently taking ~100 minutes.

		Input (CFU/mL blood)		Average TTP (HH:MM:SS)	
Species	Strain#	Average	Median	SepsiSTAT	Blood Culture
A. baumannii	NCTC 12156	3.2	2.6	04:57:04	11:14:00
C. albicans	ATCC 10231	11.2	10.2	06:55:01	21:25:20
E. cloacae	ATCC 13047	5.5	5.0	05:18:33	11:36:20
E. coli	ATCC 25922	5.5	5.6	04:56:53	10:02:20
E. faecalis	NCTC 13779	8.4	8.2	04:57:06	11:58:40
E. faecium	ATCC 19434	8.2	8.4	05:51:40	10:47:20
K. aerogenes	ATCC 10006	4.0	3.4	04:56:43	09:42:00
K. pneumoniae	NCTC 10004	5.9	6.4	04:57:06	09:47:40
P. aeruginosa	ATCC 27853	5.6	5.8	05:18:23	14:13:20
P. mirabilis	NCTC 10975	5.5	4.8	04:57:11	16:18:20
S. marcescens	ATCC 8100	0.8	0.6	04:56:43	12:24:20
S. aureus	ATCC 12973	7.2	6.0	05:07:54	11:33:00
S. agalactiae	NCTC 6175	9.4	8.4	07:16:38	16:20:20
S. pyogenes	NCTC 13739	5.6	5.6	05:07:41	10:07:20
Polymicrobial - E. coli	ATCC 25922	6.5	5.6	05:07:51	09:48:20
Polymicrobial - S. aureus	ATCC 12973	5.4	4.2	04:57:07	09:48:20

Table 1: SepsiSTAT results (n = 6 per species) compared to paired blood culture (n = 3 per species). Sample input levels (CFU/mL blood: five input TVC plates per species) are shown together with average TTP values (HH:MM:SS).

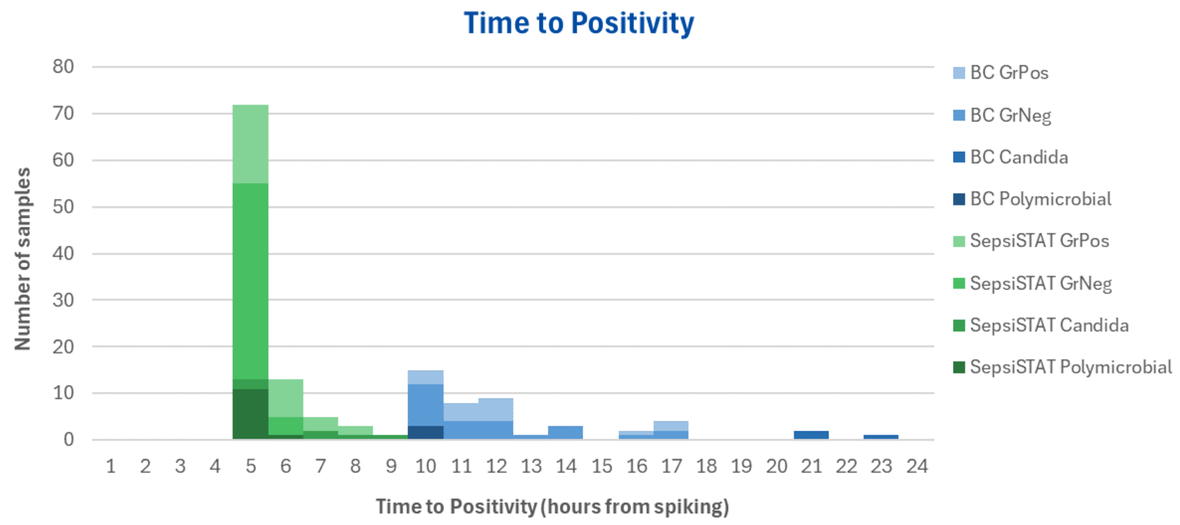

Figure 2: TTP results are shown for all replicates of SepsiSTAT and blood culture, colour-coded as Gram-negative, Gram-positive, Candida or polymicrobial.

Growth Monitoring and Enumeration

- Microbial enumeration equations were applied to PCR data in real-time to predict when samples reached 10^5-10^7 CFU/mL, considered a suitable range for rapid AST methods.
- Figure 3 shows average calculated CFU/mL values over time, and average measured CFU/mL values (derived from TVC analysis) at the point of AST. For all species tested, a microbial concentration of 10^5-10^7 CFU/mL was reached 6 - 8 hours from time of spiking.
- Paired-sample comparisons of calculated *vs* measured CFU/mL demonstrated no significant difference for 11/14 species ($p > 0.05$, paired-sample t-test). *K. pneumoniae* showed the greatest paired fold-difference of all species (3.6-fold, data available on request). In comparison, quantification using McFarland standards is reported to vary up to 6.2-fold within species and over 100-fold across species[2].
- E-test MIC values matched those from McFarland standards (data available on request).

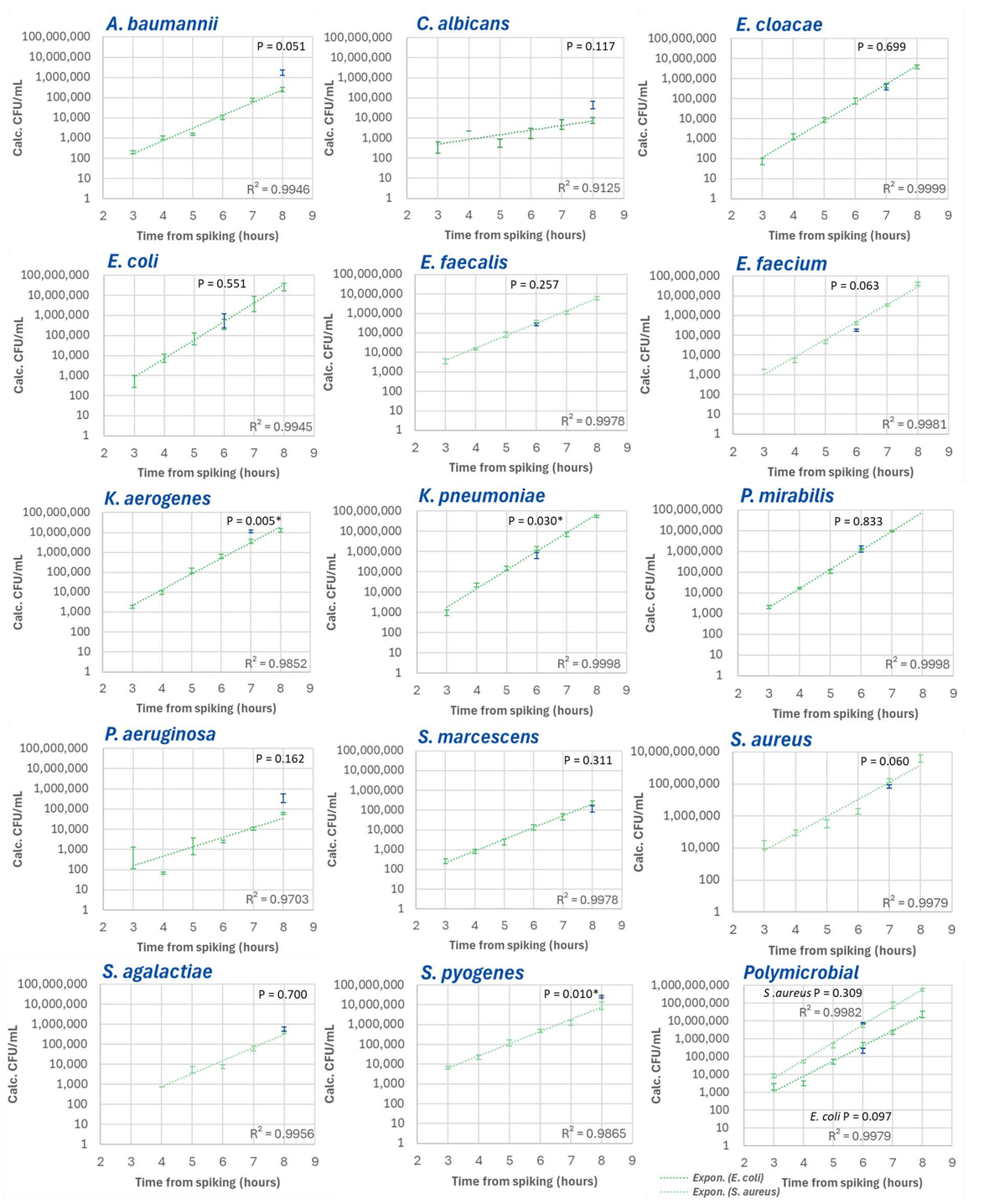

Figure 3: Microbial concentration (CFU/mL) was calculated for each time point using PCR data and enumeration equations for each delineation channel (Gram-positive, Gram-negative and Candida). Average calculated CFU/mL values (green) were plotted alongside average measured CFU/mL values derived from TVC analysis (blue). Error bars represent SEM. P values are shown for each species (denotes $p < 0.05$, paired-sample t-test)*

Discussion

- The automated SepsiSTAT system with integrated microbial detection, Gram delineation, and growth enumeration has been designed to substantially reduce time to result for existing diagnostic systems which currently rely on positive blood culture.
- SepsiSTAT determines Gram status to inform third-party ID/AST panel selection and provides a quantified ready-to-use microbial sample.
- Manual SepsiSTAT workflows have been demonstrated in combination with several downstream ID/AST applications, including FilmArray ID (bioMérieux), nanopore sequencing ID/AMR[3], rapid phenotypic AST[4] (QuickMIC, Gradientech) and MALDI ID. The automated SepsiSTAT system with integrated microbial detection and growth monitoring is expected to further shorten time to result for these applications.
- Momentum Bioscience Ltd is preparing automated SepsiSTAT for clinical use, fitting current laboratory workflows and bringing detection, molecular Gram, ID and AST within a 4 to 12-hour timeframe.

1. Positivity defined as positive reading on BACTEC FX system for blood culture, and positive PCR result for SepsiSTAT.
2. Zapata et al Curr Microbiol (2015) DOI 10.1007/s00284-015-0801-2
3. Moragues-Solanas et al BMC Medical Genomics (2024) 17:71 https://doi.org/10.1186/s12920-024-01835-5
4. Pärssinen 2023 ECCMID abstract A rapid workflow for bacterial isolation and phenotypic AST direct from blood

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