

## Study of a novel method to assist in early reporting of sepsis from the microbiology laboratory

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### Abstract

**Introduction:** Microbiology laboratories must provide accurate blood culture reports with rapid turnaround time (TAT) to effectively manage patients with sepsis. In this study three methods are compared for reporting blood culture results: a manual method that included use of a serum separator tube (SST), the conventional manual, and an automated method for identification and susceptibility (ID/AST).

**Methodology:** Broth from positive blood culture bottles was added to an SST and then centrifuged. The pellet obtained was used to directly inoculate biochemical tests for identification and agar plates for AST on the first day of positivity. Biochemicals and AST plates were read the next day and final results reported on the second day at 24 hours. For conventional disk diffusion testing, the newly positive blood culture broth was also inoculated on solid media on the first day and incubated overnight. The next day AST by was performed as well as biochemical tests from pure colonies. These colonies were also used to inoculate panels for ID/AST using the automated MicroScan 40SI System. These results were recorded on the third day and results reported at 48 hours.

**Results:** The study included 851 samples Out of 106 (12.4%) positive blood cultures, 102 were included in the study; Comparison of the 3 methods showed good correlation. Identification was correctly reported in 95 (93.1%) isolates. The overall AST error rate was 3.8%.

**Conclusions:** The use of SST and direct from pellet inoculation reduced TAT for identification and AST results between 18 and 24 hours.

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### Introduction

Sepsis imposes a significant burden on our health-care system and it is the tenth leading cause of death in the United States [1]. Rapid etiological diagnosis with prompt and adequate antimicrobial therapy is crucial for the successful management of sepsis. Routine laboratory diagnosis relies on automated blood culture methods with continuous monitoring for growth. However, the time to determine blood culture positivity and the growth on subcultured media can take longer than 48 hours to identify and perform antimicrobial susceptibility tests (ID/AST) on the pathogen. Both culture-dependent and independent methods are available for microbial ID/AST. Growth dependent automated systems are valued for their capability of performing both ID and AST from isolated colonies within 24 hours. Rapid, culture-independent methods include molecular tests (PCR) [2] but these require sophisticated equipment, which is too expensive for most diagnostic laboratories in developing countries.

The purpose of the current study was to validate the serum separator tube (SST) method to obtain faster results from blood cultures without incurring any major additional cost. This study compared the ID/AST results, TATs and cost per test of the following three different methods:

i) Conventional manual method of ID and AST (Kirby Bauer disk diffusion) from isolated bacterial colonies.

ii) Automated method for ID/AST using MicroScan 40 SI (Siemens, Frimley, Camberley, UK) from isolated bacterial colonies.

iii) Manual ID and disk diffusion method with the SST directly from pelleted positive blood culture broths.

The aim of the study was to determine if the addition of SST to the routine blood culture methods would be a cost-effective way to reduce the turnaround time (TAT) for final results.

## Methodology

The study was conducted in the laboratory of a tertiary care hospital in Delhi (national capital region), India, over a period of three months. Blood samples from admitted patients with clinical diagnosis of septicaemia were inoculated in Bactec Plus Aerobic bottles (Becton Dickinson, Sparks, Maryland, USA). These bottles were incubated in the Bactec 9050 instrument (Becton Dickinson, Sparks, Maryland, USA). Once blood culture bottles signalled positive for growth, they were removed from the instrument and Gram stains were prepared to select only samples with a single microscopic morphology for this study. The samples were processed using the following methods: rapid serum separator tube (SST) method, conventional manual and automated methods

### *Rapid serum separator tube (SST) method*

Broth (5mL) from a newly positive blood culture bottle was mixed well, transferred to a SST (BD Vacutainer SST II Advance, Becton Dickinson Sparks, Maryland, USA) and centrifuged (2000g, 10 minutes) to separate bacterial cells from the broth by centrifugation. The supernatant was decanted and the pellet was suspended in Phoenix ID broth (Becton Dickinson, Sparks, Maryland, USA) and adjusted to the 0.5 McFarland turbidity standard using the Phoenix Nephelometer (Becton Dickinson, Sparks, Maryland, USA). This sediment was used to inoculate biochemical tests [3] for bacterial identification and used to perform AST by the disk diffusion method. The biochemical tests included sugar fermentation, indole, methyl red, Voges-Proskauer, citrate, phenylalanine deaminase, urease, triple sugar iron, amino acid decarboxylase and arginine dihydrolase in addition to oxidase, antisera typing and motility for identification of Gram-negative bacteria. Optochin susceptibility, catalase and coagulase tests were performed for Gram-positive bacteria. These ID and AST plates were incubated at 37°C overnight. In addition, the suspension was subcultured to 5% sheep blood agar (BA), chocolate agar (CA) and nutrient agar (NA), which were prepared in-house. ID/AST results were available on the second day and ID/AST results were reported at 24 hours.

### *Conventional manual and automated methods*

Conventional manual and automated ID/AST methods were considered the gold standard to which

the SST method was compared. With the conventional systems, newly positive Bactec bottles were subcultured on BA, CA, Mac-Conkey agar and NA and incubated at 37°C overnight. The next day isolated colonies from solid media were used as inoculum to perform the following tests:

1. Standardized biochemicals (as used in the SST method) and AST by Kirby Bauer disk diffusion method [3,4] were used for ID.

2. ID/AST panels were inoculated with colonies mixed with 25 mL of inoculum water and adjusted with the MicroScan Turbidity Meter (Siemens, Frimley, Camberley, UK) to reach a final turbidity of the 0.5 McFarland standard. This suspension was then inoculated into the panels by the Renok system Inoculator-D (Siemens, Frimley, Camberley, UK). The MicroScan ID/AST (Siemens, Frimley, Camberley, UK) panels contained some wells with biochemicals, chromogenic substrates, and pH changes for bacterial identification and other wells were reserved for concentrations of antimicrobial drugs to provide minimum inhibitory concentration (MIC) breakpoint values that could be interpreted as susceptible, intermediate or resistant. The ID/AST breakpoint panels inoculated for Gram-negative bacteria were type NBPC 34 (Siemens, Frimley Camberley, UK) and those for Gram-positive bacteria were type 23. The MICroSTREP plus (Siemens, Frimley, Camberley, UK) panel was used for identification of *Streptococci*. All panels were placed in the automated MicroScan Walkaway 40 SI System (Siemens, Frimley, Camberley, UK) for results. In both manual and automated conventional methods, reporting was completed in 48 hours since an additional 18 to 24 hours was required for bacterial growth on media plates that had been subcultured from blood broths.

### *Comparison of SST to the conventional methods*

Bacterial identification results with the SST method were grouped in three different categories: (i) correct identification to the species level; (ii) misidentification at either the genus or species level; and (iii) no identification provided [5].

The AST results by SST method were compared with those from the two conventional methods and data was categorized as very major errors (false susceptibility), major errors (false resistance) and minor errors (resistant to intermediate, intermediate to susceptible, susceptible to intermediate or intermediate to resistant) [6]. The conventional

**Table 1.** Antibiotic susceptibility (%) of bacteria isolated from blood cultures

Antibiotics	<i>Escherichia coli</i>	<i>Salmonella sp</i>	<i>Pseudomonas sp</i>	<i>Acinetobacter sp</i>	<i>Klebsiella pneumoniae</i>	<i>Citrobacter freundii</i>	<i>Morgenella morgani</i>	<i>Enterobacter cloacae</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus pneumoniae</i>
Ampicillin	0	92.8	NT	NT	0	0	0	0	5.7	50	NT
Amikacin	85	NT	46.6	0	66.6	75	100	100	NT	NT	NT
Amoxicillin/clavulanic acid	30	92.8	NT	NT	33.3	50	100	50	77.1	50	100
Ampicillin/Salbactam	15	92.8	NT	0	33.3	50	100	50	77.1	50	NT
Aztreonem	20	NT	33.3	0	33.3	50	100	50	NT	NT	NT
Cephalothin	10	NT	NT	NT	33.3	25	0	50	NT	NT	NT
Cefazoline	15	NT	NT	NT	33.3	25	0	50	77.1	50	NT
Cefuroxime	15	NT	NT	NT	33.3	25	0	50	NT	NT	NT
Cefotaxime	15	92.8	13.3	0	33.3	50	100	50	77.1	50	100
Ceftriaxone	15	92.8	13.3	0	33.3	50	100	50	77.1	50	100
Ceftazidime	15	92.8	33.3	0	33.3	50	100	50	NT	NT	NT
Cefepime	20	100	40	0	33.3	50	100	100	77.1	50	100
Ciprofloxacin	10	85.7	40	0	33.3	50	100	50	37.1	50	NT
Ertapenem	95	NT	NT	NT	100	100	100	100	NT	NT	NT
Gentamicin	35	NT	33.3	0	33.3	75	100	50	28.5	50	NT
Imipenem	95	NT	60	20	100	100	100	100	NT	NT	NT
Levofloxacin	10	85.7	40	0	33.3	50	100	50	37.1	50	100
Moxifloxacin	10	85.7	40	NT	33.3	50	100	50	80	50	NT
Piperacillin/Tazobactam	75	92.8	60	NT	100	100	100	100	NT	NT	NT
Meropenem	95	NT	60	20	100	100	100	100	NT	NT	NT
Tobramycin	50	NT	46.6	0	66.6	75	0	50	NT	NT	NT
Tetracycline	15	92.8	NT	20	33.3	50	0	0	77.1	50	100
Trimethoprim/Sulfamethaxole	15	92.8	NT	NT	33.3	25	0	0	80	100	100
Penicillin	NT	NT	NT	NT	NT	NT	NT	NT	5.7	50	100
Oxacillin	NT	NT	NT	NT	NT	NT	NT	NT	77.1	50	NT
Clindamycin	NT	NT	NT	NT	NT	NT	NT	NT	80	100	100
Linezolid	NT	NT	NT	NT	NT	NT	NT	NT	100	100	NT
Vancomycin	NT	NT	NT	NT	NT	NT	NT	NT	100	100	NT
Erythromycin	NT	NT	NT	NT	NT	NT	NT	NT	42	50	100

methods were considered the reference methods and hence were used for patient reporting.

TAT for each test was defined from the time a blood culture bottle signalled positive to the final release of the ID/AST report.

Cost per test by all the three different methods was calculated.

## Results

A total of 851 samples were included in the study. Of these, 106 (12.4%) blood cultures were positive, 69 (65%) samples were positive within 12 to

24 hours, and 24 (22.6%) were positive between 24 to 48 hours.

Out of 106 positive blood culture samples, 102 were included in the study as two were of mixed morphology on Gram stain and two grew yeast. Sixty-four (60.3%) were identified as Gram-negative bacteria, and 38 (35.8%) were Gram-positive bacteria.

### *Identification of positive blood cultures from Bactec*

The isolates identified during the study were

**Table 2.** Discordant AST results

Antibiotics	Conventional methods	SST	Errors
	<i>Pseudomonas aeruginosa</i>		
Ciprofloxacin	36.3%	27.2%	1
Meropenem	63.6%	54.5%	1
			Minor errors: 2 (1.9%)
	<i>Staphylococcus aureus</i>		
Penicillin	5.7%	2.8%	1
Levofloxacin	37.1%	34.2%	1
			Major errors: 2 (1.9%)

*Escherichia coli* 20 (19.6%), *Salmonella typhi* 10 (9.8%), *Pseudomonas aeruginosa* 11 (10.7%), *Pseudomonas fluorescens* 4 (3.9%), *Acinetobacter sp* 5 (4.9%), *Salmonella paratyphi A* 4 (3.9%), *Klebsiella pneumoniae* 3 (2.9%), *Citrobacter freundii* 4 (3.9%), *Morganella morganii* 1 (0.9%), *Enterobacter cloacae* 2 (1.9%), *Staphylococcus aureus* 35 (34.3%), *Staphylococcus epidermidis* 2 (1.9%) and *Streptococcus pneumoniae* 1 (0.9%). Identification by all three methods was comparable. The SST method correctly identified 95 (93.1%) of the isolates; however, 6 (5.8%) could not be identified to the species level (4 *P. fluorescens* and 2 *S. epidermidis*) and one isolate (< 1%) of *S. pneumoniae* could not be identified.

#### Antimicrobial susceptibility test

The disk diffusion AST method was used with both SST and the conventional manual method. The susceptibility testing for the conventional automated method was performed using MicroScan Walkaway 40SI panels. The AST results of all isolates tested by both conventional methods are shown in Table 1.

The correlation of all AST methods showed discordant results with two isolates. One isolate of *P. aeruginosa* was intermediate to ciprofloxacin and meropenem by the SST method and was susceptible by both conventional methods (minor error, 1.9%). An isolate of *S. aureus* was resistant to penicillin and levofloxacin by the SST method and susceptible by both conventional methods (major error, 1.9%). These data are summarized in Table 2.

#### Turnaround time

The TAT of the SST method was 24 hours after a blood culture bottle signalled positive by the Bactec

instrument, whereas 48 hours were needed when using conventional manual and automated methods.

#### Cost

The approximate costs per test were \$5.34, \$14.46 and \$5.69 for the conventional manual method, conventional automated method, and SST, respectively. The cost of the SST method was comparable to the manual conventional method. However, the automated ID/AST method was more than twice the price per test compared to the other methods studied.

#### Discussion

Blood cultures are a critical diagnostic test to guide management of patients with sepsis and they are important for instituting prompt and appropriate antimicrobial therapy. The significance of providing rapid and reliable information to clinicians when a blood culture first becomes positive and reduction in TAT to final results has been well documented [7,8]. Although conventional blood culture methods are the gold standard, the turnaround time to results for ID/AST is delayed by 18 to 24 hours to permit colonial growth from subcultures of positive blood culture bottles. The SST method of direct inoculation for positive blood broth reduces TAT by 18 to 24 hours; however, the major limitation is the inability to rapidly identify polymicrobial bacteremia.

The ability of the MicroScan Walkaway (Siemens, Frimley, Camberley, UK) to identify isolates to the species level and provide MIC breakpoints to antimicrobial agents makes the reporting system more reliable, standardized, user friendly and less prone to handling error. However, the requirement for subculturing to obtain isolated bacterial colonies increases the TAT by one day. In addition, there are considerable capital and recurring costs inherent in this automated system. Several studies have reported some

success with ID/AST testing in automated systems directly from newly positive blood culture broths, although this technique is not standardized recommended protocol and requires further study [9,10,11]. Similarly, the adaptation of the SST method to automated ID/AST systems also deserves evaluation to determine the accuracy of this approach.

The cost per test was evaluated for all the three methods and clearly, the SST method was comparable to the conventional manual method; however, the automated system was more than twice the price. The intangible costs of rapid blood culture testing cannot easily be assigned a monetary value; however, they include reduced TAT for results, which can lead to improved patient management and to the selection of appropriate antimicrobial agents. Resources for health research are limited in developing countries and should be directed at finding effective and economical test algorithms.

Although conventional ID/AST methods remain the gold standard, the SST modification has the main advantage of providing ID/AST results one day earlier. The major limitation of the protocol includes the exclusion of polymicrobial blood culture. The SST method correctly identified 95 (93.1%) of isolates to species level; however, one isolate of *S. pneumoniae* could not be identified and 6 (5.8%) could not be assigned a species identification. A similar study done by Trenholme *et al.* also reported discrepancies in identifying the isolates [12].

Few discrepancies were observed in AST results by the SST method. An overall category error rate of 10% had been reported as acceptable performance, including 1.5% very major errors and 3% major errors [5]. The data from the current study resulted in no very major errors, 1.9% major errors (one isolate of *S. aureus*) and 1.9% minor errors (one isolate of *P. aeruginosa*). Discrepancies in AST results using the SST method might be a result of low bacterial concentrations in the direct inoculation of blood culture broth. Other factors such as the presence of red blood cells, cellular debris and constituents of blood culture media or technical error may be additional confounding factors [13]. Some studies have reported discrepancies in results when isolates were directly inoculated from blood culture broths [5,6,12,14,15].

Resource-limited countries need inexpensive, quick and accurate methods for the laboratory diagnosis of sepsis. The SST method as described in this study offers one approach for consideration,

reporting that it effectively reduced TAT to results by 18 to 24 hours with a negligible increase in price. This method has the potential to improve patient care and decrease hospital cost. Limitations of the study include the lack of data on fastidious organisms and the limited sample size. Standardization and validation with a larger number of positive blood cultures over a longer period of time is necessary to authenticate the value of the SST method.

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