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Comparison among four proposed direct blood culture microbial identification methods using MALDI-TOF MS

Ali M. Bazzi[a,*], Ali A. Rabaan[b], Zeyad El Edaily[a], Susan John[a], Mahmoud M. Fawarah[a], Jaffar A. Al-Tawfiq[c,d]

[a] *Microbiology Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia*
[b] *Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia*
[c] *Specialty Internal Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia*
[d] *Indiana University School of Medicine, Indiana, USA*

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Summary Matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry facilitates rapid and accurate identification of pathogens, which is critical for sepsis patients.

In this study, we assessed the accuracy in identification of both Gram-negative and Gram-positive bacteria, except for *Streptococcus viridans*, using four rapid blood culture methods with Vitek MALDI-TOF-MS. We compared our proposed lysis centrifugation followed by washing and 30% acetic acid treatment method (method 2) with two other lysis centrifugation methods (washing and 30% formic acid treatment (method 1); 100% ethanol treatment (method 3)), and picking colonies from 90 to 180 min subculture plates (method 4). Methods 1 and 2 identified all organisms down to species level with 100% accuracy, except for *Streptococcus viridans*, *Streptococcus pyogenes*, *Enterobacter cloacae* and *Proteus vulgaris*. The latter two were identified to genus level with 100% accuracy. Each method exhibited excellent accuracy and precision in terms of identification to genus level with certain limitations.

* Corresponding author at: P.O. Box 76, Room 281-C, Building 62, Dhahran Health Center, Saudi Aramco, Dhahran 31311, Saudi Arabia. Tel.: +966 13 877 6636; fax: +966 13 877 6741.
E-mail addresses: ali.bazzi@jhah.com, bazziamh@gmail.com (A.M. Bazzi).

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Introduction

Sepsis is one of the leading causes of death of both adults and children worldwide, imposing a heavy human and economic burden in both the developed and developing world [1—3]. In the United States, for example, incidence of sepsis is 0.5 per 1000 in children and 0.7—2.3 per 1000 in adults, with mortality rates being estimated at 10%—27% and 25%—50%, respectively [2,4]. Sepsis-related mortality in the United States is greater than that of stroke [5], and it is responsible for over five million pediatric deaths worldwide each year and has a particularly devastating impact in resource-poor countries [2,6]. Early recognition of causative infective agents is key to effective treatment of sepsis; time from triage and qualification to administration of appropriate antimicrobials are critical determinants of mortality [4,7]. The first 24 h has been identified as critical in terms of delivery of effective antimicrobial treatment. Each hour of delay in administration of appropriate antibiotics is associated with a decrease in survival of 7.6% over 6 h, while administration of inappropriate antibiotics has been associated with an approximately fivefold decreased survival rate [8,9]. As a result, clinicians initially assign patients with presumed bacterial infections to empiric broad-spectrum antibiotics. Due to increasing antibiotic resistance rates, however, approximately 20% of septic shock patients are initially assigned to inappropriate antimicrobials with serious consequences in terms of mortality rates [9—12]. Thus, techniques to decrease turnaround time in identification of causative bacterial agents are vital for the reduction of mortality due to sepsis.

Current standard protocols for microbial identification have a turnaround time of approximately 18—24 h from when signal-positive samples are identified and involve the overnight sub-culture of signal-positive samples on solid medium to obtain isolated colonies for identification and antibiotic susceptibility testing [13—15]. Use of more rapid identification technology, such as Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS), has the potential to expedite identification of microbial species and help guide appropriate antibiotic treatment choices [15—17]. However, if MALDI-TOF-MS could be applied directly to blood culture, rather than to isolated colonies grown overnight, this application would further reduce the turnaround time. Various direct culture methods have been used with a degree of success in microbial identification to genus or species level using Vitek MALDI-TOF-MS (bioMérieux), including lysis filtration [16—18], Sepsityper (Bruker) or in-house saponin-based bacterial extraction [19,20] or serum separator [21]. However, in general, these methods have been more successful in identification of Gram-negative compared to Gram-positive bacteria. The methods have shown difficulty in differentiation of *Staphylococci* from each other [19—21]. The objective of this study was to compare our proposed direct method of lysis centrifugation followed by washing and 30% acetic acid treatment along with our modified lysis centrifugation followed by washing and 30% formic acid treatment method, lysis centrifugation followed by washing and 100% ethanol treatment method, and picking colonies from 90 to 180 min subculture plates in order to determine the accuracy and precision of each method. These methods were all compared to a gold standard method based on testing isolated colonies from overnight cultures in terms of accurate identification of both Gram-negative and Gram-positive bacteria using Vitek MALDI-TOF-MS.

Materials and methods

Samples

A mixture of 120 monomicrobial positive blood culture vials (60 Gram-negative samples that included 35 patient and 25 seeded vials, and 60 Gram-positive samples that included 42 patients and 18 seeded vials, containing various microorganisms that have been already identified using Vitek 2), including aerobic Bactec plus, anaerobic Bactec lytic, and Bactec pediatric plus, were assessed between October 2015 and December 2015. The aim of using the seeded vials was to make the number of most common blood culture isolates equal to five for each. This sample size was chosen to conform to FDA/CLSI guidelines laid down by the American Society of Microbiology (ASM Cumitechs 31A) [22]. For Gram-negative: three *Enterobacter aerogenes* ATCC 15038; two *Enterobacter cloacae* subsp. *cloacae* ATCC® 49141; two *Pseudomonas aeroginosa* ATCC 27853; four *Stenotrophomonas maltophilia* ATCC® 51331; three *Acinetobacter baumannii* ATCC® 19606; two *Proteus mirabilis* ATCC® 7002; three *Proteus vulgaris* ATCC® 29905; three *Citrobacter koseri* ATCC® BAA-895; three *Bacteroides fragilis* ATCC® 25285. For Gram-positive: one *Staphylococcus aureus* ATCC 25923; three *Enterococcus faecium* ATCC® 51559™; two *Streptococcus pyogenes* ATCC 19615; four *Staphylococcus capitis* ATCC® 27840; two *Streptococcus agalactiae* ATCC® 51487; two *Staphylococcus lugdunensis*

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