

Rapid Bacterial Identification from Positive Blood Cultures by MALDI-TOF MS Following Short-Term Incubation on Solid Media

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ABSTRACT

This study aimed to assess the performance of the MALDI-TOF MS short incubation method for bacterial identification at short-term incubation times to improve the reporting of blood cultures. MALDI-TOF MS analysis was conducted at intervals of 2, 4, and 6 hours during the development of microbial biomass on solid media until successful identification was achieved, with a final assessment at 24 hours for conventional identification. Species-level identification rates at the 2nd, 4th, 6th, and 24th hours were 57.5%, 83.6%, 93.1%, and 93.1% for Gram-negative bacilli; 12.5%, 42.7%, 76.1%, 97.8% for Gram-positive cocci and 0%, 11.8%, 17.6%, 58.8% for Gram-positive bacilli, respectively. The species-level identification rate was 76.5% for all monomicrobial cultures at the 6th hour. Our results have led us to implement this method into our routine laboratory workflow, and we have started to report rapid identification results for Gram-negative bacteria on the day of blood culture positivity.

Keywords: blood culture, MALDI-TOF MS, short incubation, rapid identification

INTRODUCTION

Sepsis is a life-threatening infection with a mortality rate above 25%. Rapid identification of the microorganisms that cause bloodstream infections is important for choosing targeted and timely antimicrobial treatment (1). The implementation of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology in laboratories has accelerated microbial diagnosis time (2).

Nevertheless, according to the conventional algorithm, 16-24 hours are needed for pathogen identification and at least an additional 24 hr for antimicrobial susceptibility testing (AST) results. There are several rapid culture-independent techniques to detect bloodstream pathogens, but these are expensive for most diagnostic laboratories in developing countries (3). Some in-house extraction methods and commercial kits have been developed to obtain bacterial pellets directly from positive blood cultures.

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Identification can be carried out using MALDI-TOF MS, automated identification (ID)/AST systems, or biochemical tests via bacterial pellets. However, they require manual processing time for lysis and centrifugation steps, additional personnel, and cost (4). Therefore, despite technological developments, implementing these methods in routine laboratory workflows is challenging for many laboratories.

In previous years, a novel method for the rapid identification of microorganisms from positive blood culture (BC) bottles based on MALDI-TOF MS analysis after decreased incubation time on a solid medium was introduced (5). In this study, we evaluated this mass spectrometry-based approach for the identification of bacteria from positive BCs and analyzed the method's performance at short-term incubation times.

MATERIALS AND METHODS

Positive BC bottles (162 BACTEC™ Plus/F Aerobic and 81 BACTEC™ Lytic Anaerobic bottles; BD Diagnostics, USA) were examined over a period of five months (from January 01, 2021, to May 31, 2021) in this study. For positive BC bottles from the same patient, only the initial bottle flagged as positive was included in the study. The BC bottles were incubated in an automated BC system (BACTEC™ FX; BD Diagnostics, USA). Following the positive signal, the Gram staining was performed, and all samples were inoculated onto the Columbia blood agar, chocolate agar, and MacConkey agar. Anaerobic bottles were also inoculated onto the Schaedler agar. The plates were incubated at 36±1 °C in the air with 5% CO₂. Schaedler agars were incubated in the anaerobic jar (Oxoid Ltd., United Kingdom) containing GasPak (Thermo Fisher Scientific, USA). MALDI-TOF MS was performed at time points of 2 hr, 4 hr, and 6 hr from immature biomass grown on solid media until successful identification was achieved.

The analysis was done using the Microflex LT system (Bruker Daltonics, Germany) and MALDI Biotyper 3.3 (Bruker Daltonics) software. If the score was ≥2.0, the microorganism is identified at the species level and ≥1.7 at the genus level. MALDI-TOF MS was conducted at 24th hr for the routine method for identification of microorganisms. The rapid

identification results were compared with those obtained at 24 hr. Gram staining, colony morphology, agglutination with anti-*Brucella* serum, and biochemical tests were used for the routine identification of *Brucella* spp. For all microorganisms, when genus-level identification was not attained, the comparison was based on the Gram staining results. The plates were examined for the presence of polymicrobial growth at 24th hr.

RESULTS

During the study period, a total of 243 positive BCs were analyzed. Overall, a single microorganism grew in 230 BC bottles with a species distribution of 60.0% Gram-positive cocci (GPC), 31.7% Gram-negative bacilli (GNB), 7.4% Gram-positive bacilli (GPB), 0.9% Gram-negative coccobacilli (GNCB). In addition, 27 pathogens were isolated from polymicrobial BCs (n=13). Two different GNBs were detected in four of the polymicrobial growths, two different GPCs in five, one GNB and one GPC in three, and two GNB and one GPC in one.

Identification rates for monomicrobial cultures are presented in Table 1. In the case of GNB, 57.5%, 83.6%, 93.1%, and 93.1% of the samples were identified at the species level at the 2nd, 4th, 6th, and 24th hours, respectively; these rates were 12.5%, 42.7%, 76.1%, 97.8% for Gram-positive cocci. All *Staphylococcus aureus* isolates were identified at the species level at the 4th hr. Species-level identification rates were low in Gram-positive bacilli and were 0%, 11.8%, 17.6%, and 58.8%, respectively. Species-level identification was achieved in 76.5% of all monomicrobial cultures at the 6th hour. *Brucella*

HIGHLIGHTS

- MALDI-TOF MS analysis after a short period of incubation on solid media is a simple, fast, and reliable method for the same-day identification of pathogens causing sepsis.
- Species-level identification was achieved in 76.5% of all monomicrobial cultures at the 6th hour.
- Identification rates were highest for Gram-negative bacilli and *Staphylococcus aureus*.

Table 1. MALDI-TOF MS identification results of the monomicrobial cultures.

Bacteria	Correct identification rates at different incubation times, n (%)								
	n	2nd		4th		6th		24th	
		Genus	Species	Genus	Species	Genus	Species	Genus	Species
All GPC	138	31 (22.5)	17 (12.3)	102 (73.9)	59 (42.7)	129 (93.5)	105 (76.1)	135 (97.8)	135 (97.8)
<i>Staphylococcus aureus</i>	14	11 (78.6)	11 (78.6)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)
GPC other than <i>S. aureus</i>	124	20 (16.1)	6 (4.8)	88 (71)	45 (36.3)	115 (92.7)	91 (73.4)	121 (97.6)	121 (97.6)
<i>Staphylococcus epidermidis</i>	55	5 (9.1)	0 (0)	41 (74.5)	16 (29.1)	54 (98.2)	44 (80)	55 (100)	55 (100)
<i>Staphylococcus hominis</i>	29	3 (10.3)	2 (6.9)	21(72.4)	13 (44.8)	25 (86.2)	22 (75.9)	29 (100)	29 (100)
<i>Staphylococcus haemolyticus</i>	12	3 (25)	0 (0)	7 (58.3)	3 (25)	12 (100)	6 (50)	12 (100)	12 (100)
<i>Staphylococcus capitis</i>	5	1	0	4	2	5	3	5	5
<i>Staphylococcus cohnii</i>	2	0	0	0	0	1	0	2	2
<i>Staphylococcus simulans</i>	1	0	0	1	1	1	1	1	1
<i>Enterococcus faecalis</i>	5	3	2	4	4	5	4	5	5
<i>Enterococcus faecium</i>	1	1	0	1	1	1	1	1	1
<i>Enterococcus malodoratus</i>	1	0	0	1	0	1	1	1	1
<i>Facklamia hominis</i>	1	0	0	1	0	1	1	1	1
<i>Micrococcus luteus</i>	3	0	0	2	1	3	3	3	3
<i>Rothia mucilaginosa</i>	1	0	0	0	0	1	0	1	1
<i>Streptococcus agalactiae</i>	1	1	1	1	1	1	1	1	1
<i>Streptococcus oralis</i>	2	2	1	2	2	2	2	2	2
<i>Streptococcus pneumoniae</i>	1	0	0	1	0	1	1	1	1
<i>Streptococcus salivarius</i>	1	1	0	1	1	1	1	1	1
<i>Streptococcus constellatus</i> *	1	0	0	0	0	0	0	0	0
<i>Staphylococcus saccharolyticus</i> *	1	0	0	0	0	0	0	0	0
Unidentified anaerobic GPC	1	0	0	0	0	0	0	0	0
GPB	17	0 (0)	0 (0)	3 (17.6)	2 (11.8)	6 (35.3)	3 (17.6)	14 (82.3)	10 (58.8)
<i>Actinomyces</i> sp.	1	0	0	1	0	1	0	1	0
<i>Corynebacterium afermentans</i>	4	0	0	0	0	0	0	1	1
<i>Corynebacterium jeikeum</i>	1	0	0	0	0	0	0	4	4
<i>Corynebacterium</i> sp.	1	0	0	0	0	0	0	1	0
<i>Helcobacillus massiliensis</i>	1	0	0	0	0	1	1	1	1
<i>Microbacterium lacticum</i>	1	0	0	0	0	1	0	1	1
<i>Microbacterium laevaniformans</i>	1	0	0	0	0	0	0	1	1
<i>Microbacterium</i> sp.	1	0	0	0	0	0	0	1	0
<i>Paenibacillus urinalis</i>	2	0	0	2	2	2	2	2	2

Continue to Table 1

<i>Pseudoglutamicibacter</i> sp.	1	0	0	0	0	1	0	1	0
<i>Flavonifractor plautii</i> *	1	0	0	0	0	0	0	0	0
Unidentified GPB	2	0	0	0	0	0	0	0	0
GNB	73	52 (71.2)	42 (57.5)	70 (95.9)	61 (83.6)	70 (95.9)	68 (93.1)	71 (97.3)	68 (93.1)
<i>Escherichia coli</i>	23	20 (86.9)	20 (86.9)	23 (100)	23 (100)	23 (100)	23 (100)	23 (100)	23 (100)
<i>Klebsiella pneumoniae</i>	20	16 (80)	11 (55)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
<i>Klebsiella oxytoca</i>	2	2	2	2	2	2	2	2	2
<i>Acinetobacter baumannii</i> / <i>calcoaceticus</i> complex	4	2	0	4	3	4	4	4	4
<i>Acinetobacter johnsonii</i>									
<i>Pseudomonas aeruginosa</i>	1	0	0	1	0	1	1	1	1
<i>Pseudomonas stutzeri</i>	4	3	1	4	2	4	4	4	4
<i>Stenotrophomonas maltophilia</i>	1	0	0	1	0	1	1	1	1
<i>Aeromonas caviae</i>	4	0	0	4	3	4	4	4	4
<i>Aeromonas hydrophilia</i>	1	1	1	1	1	1	1	1	1
<i>Brevimundus</i> sp.	1	1	1	1	1	1	1	1	1
<i>Delftia acidovorans</i>	1	0	0	0	0	0	0	1	0
<i>Enterobacter cloacae</i> complex	1	0	0	1	0	1	1	1	1
<i>Morganella morganii</i>	2	2	2	2	2	2	2	2	2
<i>Proteus mirabilis</i>	1	1	1	1	1	1	1	1	1
<i>Serratia marcescens</i>	1	1	1	1	1	1	1	1	1
<i>Salmonella</i> sp.	1	1	1	1	1	1	1	1	1
<i>Bacteroides fragilis</i>	2	1	0	2	0	2	0	2	0
<i>Bacteroides faecis</i> *	1	1	1	1	1	1	1	1	1
Unidentified GNB	1	0	0	0	0	0	0	0	0
GNCB (<i>Brucella</i> spp.)	2	0	0	0	0	0	0	0	0
Total	230	83 (36.1)	59 (25.6)	175 (76.1)	122 (53)	205 (89.1)	176 (76.5)	220 (95.6)	213 (92.6)

GPC: Gram-positive cocci, GPB: Gram-positive bacilli, GNB: Gram-negative bacilli, GNCB: Gram-negative coccobacilli.

*It is identified at 48th hr from the Schaedler agar incubated under anaerobic conditions.

spp. (n=2) could only be identified by conventional methods at 24 hours because it was not included in the MALDI-TOF MS database. We isolated five anaerobic bacteria, including *Bacteroides fragilis*, *Bacteroides faecis*, *Staphylococcus saccharolyticus*, *Flavonifractor plautii* and an unidentified GPC. Only the *B. fragilis* isolate was identified by rapid ID at 2nd hr from Columbia blood agar. Three anaerobic isolates were identified at the 48th hour from Schaedler agar incubated under anaerobic conditions. One anaerobic GPC could

not be identified by the methods used in our laboratory. In polymicrobial cultures, only one of the pathogens could be identified at the species level at the rate of 46.1%, 69.2%, and 84.6% at the 2nd, 4th, and 6th hours, respectively.

DISCUSSION

The installation cost of a MALDI-TOF MS system is relatively high and often unaffordable, particular-

ly in developing countries. However, if there is already a MALDI-TOF MS in the laboratory used for conventional (24-hour) diagnosis, the short incubation method does not require additional processes and costs. According to our work and the reports in the literature, MALDI-TOF MS, after a short period of incubation on solid media, is a simple, fast, and reliable method for the same-day identification of pathogens causing sepsis.

This study demonstrated that identification rates were higher and the time required for identification was shorter in Gram-negative bacteria and *S. aureus*. Identification rates were lower in other GPC compared to *S. aureus* and were lowest in GPB. Among the five anaerobic isolates, only one *B. fragilis* was identified by rapid ID. The slow growth rate of anaerobes can explain this. Previous studies reported that identification rates vary between 51.9%-96.8% and 85.8%-100% for Gram-positive and Gram-negative bacteria, respectively (6). A major reason for this is that Gram-positive bacteria have slower cell division and growth rates and form smaller biomass in a short duration. In particular, Gram-positive bacilli showed weak protein signals and identification rates were notably lower than those of other bacteria groups. The thick peptidoglycan cell walls of Gram-positive bacteria may lead to suboptimal MALDI-TOF MS spectra (7). Also, species of the bacteria, the culture media, and incubation time may affect the results. Froböse et al. reported that median time to species identification was shortest on MacConkey agar for Gram-negative rods (2.0 h; range:1.9 to 4.2 h) and on Columbia blood agar for Gram-positive cocci (4.0 h; range:1.9 to 25.0 h) (8). However, chocolate agar should be used if fastidious species are expected. Polymicrobial cultures are challenging for all rapid diagnostic methods. There was a mixed growth in 5.3% of our BCs. This highlights the importance of checking cultures at 24th hr for purity.

Our study has limitations as it used data from a single institution and was unable to compare this method with other protocols for the direct identification of microorganisms from positive BC bottles. The number of isolates included in the study was relatively low, and the interpretation of the results was limited. The constrained number of anaerobic samples represents another limitation in our study. Also, this study did not examine the clinical significance of ID from short-term cultures in determining effective antimicrobial treatment. Delport et al. reported that using a short-term incubation method by MALDI-TOF MS for identification from BCs is correlated with a decrease in the length of hospitalization and a reduction in mortality associated with bacteremia (9). Providing rapid ID results using MALDI-TOF MS without a rapid method for AST may have limited effect on directed appropriate antimicrobial therapy. However, rapid identification of the infectious agents causing bloodstream infection could be helpful for the clinician to establish an effective empirical antimicrobial therapy for patients who are in particularly critical conditions. Besides that, it is possible to combine this rapid identification method with rapid AST methods (10). Future studies should aim to combine this method with a rapid AST method and analyze whether such identification with MALDI-TOF MS leads to better clinical outcomes.

In conclusion, the application of MALDI-TOF MS following a short culture period is easy and provides same-day results. We observed a high percentage of categorical agreement with the standard identification algorithm used in our laboratory for GNB (>93.1%, 6th hr). As an outcome of our study, we have started to use the short incubation method at the 6th hour for the same-day identification of Gram-negative pathogens from positive BCs in our laboratory.



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REFERENCES

- 1 Niederman MS, Baron RM, Bouadma L, Calandra T, Daneman N, DeWaele J, et al. Initial antimicrobial management of sepsis. *Crit Care*. 2021;25(1):307. [\[CrossRef\]](#)
- 2 Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin Microbiol Infect*. 2010;16(11):1614-9. [\[CrossRef\]](#)
- 3 Peri AM, Harris PNA, Paterson DL. Culture-independent detection systems for bloodstream infection. *Clin Microbiol Infect*. 2022;28(2):195-201. [\[CrossRef\]](#)
- 4 Gray TJ, Thomas L, Olma T, Iredell JR, Chen SC. Rapid identification of Gram-negative organisms from blood culture bottles using a modified extraction method and MALDI-TOF mass spectrometry. *Diagn Microbiol Infect Dis*. 2013;77(2):110-2. [\[CrossRef\]](#)
- 5 Idelevich EA, Schüle I, Grünastel B, Willenweber J, Peters G, Becker K. Rapid identification of microorganisms from positive blood cultures by MALDI-TOF mass spectrometry subsequent to very short-term incubation on solid medium. *Clin Microbiol Infect*. 2014;20(10):1001-6. [\[CrossRef\]](#)
- 6 Tsai YW, Lin TC, Chou HY, Hung HY, Tan CK, Wu LC, et al. Shortening the time of the identification and antimicrobial susceptibility testing on positive blood cultures with MALDI-TOF MS. *Diagnostics (Basel)*. 2021;11(8):1514. [\[CrossRef\]](#)
- 7 McElvania Tekippe E, Shuey S, Winkler DW, Butler MA, Burnham CA. Optimizing identification of clinically relevant Gram-positive organisms by use of the Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system. *J Clin Microbiol*. 2013;51(5):1421-7. [\[CrossRef\]](#)
- 8 Froböse NJ, Idelevich EA, Schaumburg F. Short incubation of positive blood cultures on solid media for species identification by MALDI-TOF MS: which agar is the fastest? *Microbiol Spectr*. 2021;9(1):e0003821. [\[CrossRef\]](#)
- 9 Delport JA, Strikwerda A, Armstrong A, Schaus D, John M. MALDI-ToF short incubation identification from blood cultures is associated with reduced length of hospitalization and a decrease in bacteremia associated mortality. *Eur J Clin Microbiol Infect Dis*. 2017;36(7):1181-6. [\[CrossRef\]](#)
- 10 Cherkaoui A, Renzi G, Azam N, Schorderet D, Vuilleumier N, Schrenzel J. Rapid identification by MALDI-TOF/MS and antimicrobial disk diffusion susceptibility testing for positive blood cultures after a short incubation on the WASPLab. *Eur J Clin Microbiol Infect Dis*. 2020;39(6):1063-70. [\[CrossRef\]](#)