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Rapid Identification of Bacteria Directly from Positive Blood Cultures by Use of a Serum Separator Tube, Smudge Plate Preparation, and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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We analyzed the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) of smudge plate growth for bacterial identification from 400 blood cultures. Ninety-seven percent of Gram-negative bacilli and 85% of Gram-positive organisms were correctly identified within 4 h; only eight isolates (2.0%) were misidentified. This method provided rapid and accurate microbial identification from positive blood cultures.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is used in clinical microbiology laboratories for rapid identification of microbial isolates grown in culture (1, 2). Implementation of MALDI-TOF MS in the microbiology laboratory in conjunction with antibiotic stewardship has been associated with earlier initiation of effective antimicrobial therapy and lower 30-day mortality in patients with bloodstream infections (3). This technology has also been applied to reduce turnaround times for identification of blood culture isolates directly from positive blood culture broths (4–16). Various techniques have been assessed, generally involving lysis/centrifugation of blood culture pellets in preparation for analysis by MALDI-TOF MS. The current study evaluated a method using "smudge" plates for subsequent analysis with MALDI-TOF MS to simplify sample processing and to improve the ability to rapidly identify bacteria from positive blood cultures.

Blood cultures were collected in Bactec Plus aerobic and anaerobic bottles incubated in the Bactec 9240 system (Becton Dickinson, Franklin Lakes, NJ). We prospectively examined 400 blood cultures that were flagged as positive for bacterial growth between 8:00 a.m. and 3:00 p.m. on weekdays from 1 April to 30 September 2014. A 1- to 2-ml aspirate from the blood culture bottle was used to prepare a Gram stain and was subcultured to blood, chocolate, and MacConkey agar plates. The blood and MacConkey plates were incubated at 35°C in ambient air; chocolate agar plates were incubated at 35°C in 5% CO_2. Brucella agar plates incubated anaerobically were added for subculture from positive anaerobic blood culture bottles. Aerobic and facultative organisms were identified using standard phenotypic methods, including coagulase, oxidase, latex agglutination, streptococcal serotyping, the Vitek 2 system (bioMérieux, Durham, NC), API strips (bioMérieux), and other biochemical tests as appropriate. Anaerobes were identified using the Remel RapID Ana II (Oxoid, Hampshire, United Kingdom).

A smudge plate was prepared when a single morphology was evident on the Gram stain; specimens with more than one bacterial morphology, yeasts, or filamentous fungi were excluded from this study. If two blood culture bottles from the same set were positive, only the aerobic bottle was used for smudge plate preparation, and multiple positive blood cultures obtained within 24 h from the same patient were included in this study only once. For smudge plate preparation, 3 ml of blood culture broth was aspirated from positive blood culture bottles using a sterile syringe and transferred to a 10-ml serum separator tube. The aspirate was centrifuged for 5 min at 3,000 rpm, and the supernatant was discarded with a sterile pipette. The bacterial pellet was transferred to the center of a chocolate agar plate. The inoculum was streaked out to form a 2- by 2-cm lawn, and the plate was incubated at 35°C in 5% CO_2. Smudge plates were incubated for up to 4 h and examined for growth at 1, 2, and 4 h. Growth on the smudge plate was recovered with a 10-μl inoculating loop to obtain a sufficient inoculum to be spotted onto a target slide prepared according to the manufacturer's instructions for analysis using Vitek MS system software version 2.0 (bioMérieux). A single organism identification with a confidence value between 60% and 99.9% was considered acceptable, in accordance with the manufacturer's guidelines. For isolates with multiple identifications with low discrimination, identification at the genus level was accepted if all the identifications were from the same genus. Otherwise, the isolate was considered unidentifiable. Discrepancies with conventional phenotypic identification methods were resolved by 16S rRNA amplification and gene sequencing of colonial growth obtained on routine subculture plates from the positive blood cultures.

With 4 h of smudge plate incubation, 358 blood culture isolates (89.5%) were correctly identified by the Vitek MS: 343 (85.8%) to

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TABLE 1 Rapid identification of bacteria in 400 blood cultures using smudge plate preparation and MALDI-TOF MS system

Organism	No. of isolates	No. with correct ID[a] (%)			No. with discordant ID	No. with no ID
		Species level	Genus level	Combined		
Total	400	343 (85.8)	15 (3.7)	358 (89.5)	8	34
Gram-positive organisms	248	197 (79.4)	13 (5.2)	210 (84.7)	7	31
Staphylococcus aureus	58	56 (96.6)	0	56 (96.6)	1	1
CoNS[b]	95	76 (80.0)	0	76 (80.0)	2	17
Enterococcus faecalis	18	18 (100)	0	18 (100)	0	0
E. faecium	7	6 (85.7)	0	6 (85.7)	0	1
Streptococcus pneumoniae	7	7 (100)	0	7 (100)	0	0
S. viridans group	9	4 (44.4)	0	4 (44.4)	1	4
S. anginosus group	7	3 (42.9)	0	3 (42.9)	1	3
S. bovis group	3	3 (100)	0	3 (100)	0	0
S. pyogenes	2	1 (50.0)	0	1 (50.0)	0	1
S. agalactiae	5	5 (100)	0	5 (100)	0	0
S. dysgalactiae	2	1 (50.0)	0	1 (50.0)	0	1
S. suis	1	1 (100)	0	1 (100)	0	0
Bacillus species	21	9 (42.9)	10 (47.6)	19 (90.5)	1	1
Paenibacillus species	4	0	3 (75.0)	3 (75.0)	0	1
Listeria monocytogenes	2	1 (50.0)	0	1 (50.0)	1	0
Corynebacterium species	1	0	0	0	0	1
Lactobacillus casei	1	1 (100)	0	1 (100)	0	0
Dermabacter hominis	1	1 (100)	0	1 (100)	0	0
Microbacterium flavescens	1	1 (100)	0	1 (100)	0	0
Gram-negative organisms	152	146 (96.1)	2 (1.3)	148 (97.4)	1	3
Enterobacteriaceae	133	130 (97.7)	2 (1.5)	132 (99.2)	0	1
Escherichia coli	78	78 (100)	0	78 (100)	0	0
Klebsiella species	25	25 (100)	0	25 (100)	0	0
Enterobacter species	10	10 (100)	0	10 (100)	0	0
Proteus species	10	10 (100)	0	10 (100)	0	0
Serratia marcescens	7	7 (100)	0	7 (100)	0	0
Raoultella ornithinolytica	1	0	1 (100)	1 (100)	0	0
Salmonella species (nontyphi)	1	0	1 (100)	1 (100)	0	0
Pseudomonas aeruginosa	10	10 (100)	0	10 (100)	0	0
Stenotrophomonas maltophilia	2	2 (100)	0	2 (100)	0	0
Campylobacter jejuni	1	0	0	0	0	1
Moraxella atlantae	1	0	0	0	0	0
M. osloensis	1	1 (100)	0	1 (100)	0	1
Brevundimonas vesicularis	1	0	0	0	0	1
Sphingobacterium species	1	0	0	0	0	1
Anaerobic organisms						
Clostridium clostridioforme	1	1 (100)	0	1 (100)	0	0
C. ramosum	1	1 (100)	0	1 (100)	0	0
Propionibacterium acnes	1	1 (100)	0	1 (100)	0	0
Bacteroides fragilis	2	2 (100)	0	2 (100)	0	0
B. thetaiotaomicron	1	1 (100)	0	1 (100)	0	0

[a] ID, identification.
[b] CoNS, coagulase-negative *Staphylococcus*.

the species level and 15 (3.7%) to the genus level only (Table 1). The confidence value was 99.9% for 304 isolates (76.0%) and ≥95% for 354 isolates (88.5%). Of the 152 Gram-negative bacilli, 148 (97.4%) were correctly identified within 4 h of smudge plate incubation. A total of 132 (99.2%) of the *Enterobacteriaceae* and all 10 of the *Pseudomonas aeruginosa* blood culture isolates were correctly identified. Overall, 210 (84.7%) of 248 Gram-positive organisms were correctly identified, and of these, all but 13 were identified correctly to the species level. A total of 56 (96.6%) of the 58 *Staphylococcus aureus* isolates were correctly identified to the species level, as were 24 (96.0%) of the enterococci isolates. However, only 25 (69.4%) of the streptococci and 26 (83.9%) of the aerobic Gram-positive bacilli were correctly identified. All six anaerobic blood culture isolates were correctly identified to the species level (Table 1). In most cases, failure to correctly identify the blood culture isolate was because no Vitek MS identification was generated. An incorrect identification was observed in only 8 (2.0%) of the 400 isolates (Table 2). Five (62.5%) of the incorrect identifications would have been recognized as errors based on observing a mismatch between the MALDI-TOF MS identification and the Gram stain morphology of the organism from the blood culture aspirate.

TABLE 2 Incorrect identification of bacteria in blood cultures using smudge plate preparation and MALDI-TOF MS system

Correct identification[a]	MALDI-TOF MS identification	Confidence value	Gram stain	Incubation period (h)
Staphylococcus aureus	*Prevotella buccalis*	99.9	Gram-positive cocci	1
S. warneri	*Staphylococcus aureus*	99.9	Gram-positive cocci	1
S. haemolyticus	*Lactobacillus previs*	99.9	Gram-positive cocci	1
Listeria monocytogenes	*Paenibacillus* species	99.9	Gram-positive bacilli	1
Streptococcus intermedius	*Globicatella sanguinis*	99.8	Gram-positive cocci	1
S. sanguinis	*Globicatella sanguinis*	99.7	Gram-positive cocci	4
Corynebacterium striatum	*Chryseobacterium indogenes*	99.9	Gram-positive bacilli	1
Brevundimonas vesicularis	*Rothia dentocariosa*	99.9	Gram-negative bacilli	4

[a] Correct identification using 16S rRNA gene sequencing.

Most (64.3%) of the blood culture isolates were correctly identified after only 1 h of smudge plate incubation; with 2 h of incubation, 310 isolates (77.5%) were correctly identified (Table 3). A total of 115 (86.5%) of the *Enterobacteriaceae* were identified after 1 h of incubation, and 122 (91.7%) were identified after 2 h of incubation. All *P. aeruginosa* isolates were identified within 2 h. Gram-positive blood culture isolates generally required longer incubation, as only 128 (51.6%) and 173 (69.8%) of the isolates were identified with 1 and 2 h of incubation, respectively.

Several studies have shown that MALDI-TOF MS analysis of samples obtained directly from positive blood cultures can correctly identify 66% to 97% of aerobic and anaerobic organisms causing monomicrobial bacteremia (4–16). As in the current study, the best results have been obtained with Gram-negative organisms. Difficulty in obtaining an accurate identification of Gram-positive organisms directly from blood cultures has been attributed to suboptimal inoculum size; with low inoculum, fewer proteins are available for analysis, leading to misidentification or a lack of identification (2). The procedures that have been employed are often laborious, typically requiring an extraction procedure with centrifugation, lysis, or filtration of the specimen. A commercial lysis kit, Sepsityper (Bruker Daltonics), was developed to facilitate extraction and purification of the bacterial proteome, but it is relatively costly and still requires at least 20 min of processing time (9–12, 15).

A previously described smudge plate method involved direct inoculation of an aliquot of the blood culture broth onto an agar plate without prior centrifugation (15). Using this protocol, 76% of Gram-negative bacilli were correctly identified to the species level after 2 h of incubation, and 95% were identified after 4 h of incubation. Results were poor for identification of Gram-positive cocci, with only 19% correctly identified after 4 h of incubation. In our study, we demonstrate the feasibility of a method requiring minimal sample manipulation, with a single centrifugation step utilizing a serum separator to facilitate removal of blood cells. The recovered microorganisms are then planted onto an agar (smudge) plate, which is incubated for a few hours to obtain sufficient growth for MALDI-TOF MS analysis. Advantages of this protocol include its simplicity and speed, requiring only 5 to 10 min for preparation of the smudge plate. Results are available within 1 to 4 h, and the procedure is easily incorporated into routine laboratory workflow. With implementation of this procedure in our laboratory, technologists examine the smudge plates once after 2 to 4 h of incubation during hours of operation (8:00 a.m. to 10:00 p.m.) daily. Results are reported only for identifications with high confidence values (>98.0%) and if the identification is consistent with the organism's Gram stain morphology from the blood culture broth aspirate.

The procedure accurately identified the vast majority of Gram-negative organisms, as well as certain streptococcal and enterococcal species. Identification of Gram-positive bacilli was still suboptimal, and the number of anaerobic blood culture isolates in this study was too small to draw any conclusions. As previously reported in other studies, there were only a few (2%) mistaken identifications (6, 7, 11, 14, 16), and most of these would have been recognized as erroneous by comparing the MALDI-TOF MS results with colonial and Gram stain morphology. Perhaps the most clinically significant errors in this study were the misidentification of an isolate of *S. aureus* and a *Listeria monocytogenes*. Fortunately, in both cases, the Gram stain morphology from the positive blood

TABLE 3 Correct identification of bacteria in blood cultures using smudge plates with different incubation periods

Bacteria	No. of isolates	Cumulative no. with correct identification (%) at:		
		1 h	2 h	4 h
Total	400	257 (64.3)	310 (77.5)	358 (89.5)
Gram-positive organisms	248	128 (51.6)	173 (69.8)	210 (84.7)
Staphylococci	153	81 (52.9)	112 (73.2)	132 (86.3)
Enterococci	25	20 (80.0)	22 (88.0)	24 (96.0)
Streptococci	36	11 (30.6)	16 (44.4)	25 (69.4)
S. pneumoniae	7	4 (57.1)	7 (100)	7 (100)
S. agalactiae	5	3 (60.0)	5 (100)	5 (100)
Gram-positive bacilli	31	16 (51.6)	22 (71.0)	26 (83.9)
Gram-negative organisms	152	127 (83.6)	136 (89.5)	148 (97.4)
Enterobacteriaceae	133	115 (86.5)	122 (91.7)	132 (99.2)
Pseudomonas aeruginosa	10	9 (90.0)	10 (100)	10 (100)

cultures would have alerted the technologist to question the MALDI-TOF MS identification result. Importantly, use of MALDI-TOF MS directly from positive blood cultures has had a beneficial impact in influencing the selection of appropriate antimicrobial therapy and thereby improving outcomes in patients with bloodstream infections, especially those caused by Gram-negative bacilli (17–19).

REFERENCES

1. **Clark AE, Kaleta EJ, Arora A, Wolk DM.** 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev **26:**547–603. http://dx.doi.org/10.1128/CMR.00072-12.
2. **Croxatto A, Prod'hom G, Greub G.** 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev **36:**380–407. http://dx.doi.org/10.1111/j.1574-6976.2011.00298.x.
3. **Huang AM, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J, Collins CD, Nagel JL.** 2013. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis **57:**1237–1245. http://dx.doi.org/10.1093/cid/cit498.
4. **Stevenson LG, Drake SK, Murray PR.** 2010. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol **48:**444–447. http://dx.doi.org/10.1128/JCM.01541-09.
5. **Moussaoui W, Jaulhac B, Hoffmann AM, Ludes B, Kostrzewa M, Riegel P, Prévost G.** 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identifies 90% of bacteria directly from blood culture vials. Clin Microbiol Infect **16:**1631–1638. http://dx.doi.org/10.1111/j.1469-0691.2010.03356.x.
6. **Christner M, Rohde H, Wolters M, Sobottka I, Wegscheider K, Aepfelbacher M.** 2010. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption–ionization time of flight mass spectrometry fingerprinting. J Clin Microbiol **48:**1584–1591. http://dx.doi.org/10.1128/JCM.01831-09.
7. **Prod'hom G, Bizzini A, Durussel C, Bille J, Greub G.** 2010. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J Clin Microbiol **48:**1481–1483. http://dx.doi.org/10.1128/JCM.01780-09.
8. **Ferreira L, Sánchez-Juanes F, Porras-Guerra I, García-García MI, García-Sánchez JE, González-Buitrago JM, Muñoz-Bellido JL.** 2011. Microorganisms direct identification from blood culture by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Microbiol Infect **17:**546–551. http://dx.doi.org/10.1111/j.1469-0691.2010.03257.x.
9. **Martiny D, Dediste A, Vandenberg O.** 2012. Comparison of an in-house method and the commercial Sepsityper™ kit for bacterial identification directly from positive blood culture broths by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry. Eur J Clin Microbiol Infect Dis **31:**2269–2281. http://dx.doi.org/10.1007/s10096-012-1566-1.
10. **Buchan BW, Riebe KM, Ledeboer NA.** 2012. Comparison of the MALDI Biotyper system using Sepsityper specimen processing to routine microbiological methods for identification of bacteria from positive blood culture bottles. J Clin Microbiol **50:**346–352. http://dx.doi.org/10.1128/JCM.05021-11.
11. **Lagacé-Wiens PRS, Adam HJ, Karlowsky JA, Nichol KA, Pang PF, Guenther J, Webb AA, Miller C, Alfa MJ.** 2012. Identification of blood culture isolates directly from positive blood cultures by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry and a commercial extraction system: analysis of performance, cost, and turnaround time. J Clin Microbiol **50:**3324–3328. http://dx.doi.org/10.1128/JCM.01479-12.
12. **Chen JHK, Ho P-L, Kwan GSW, She KKK, Siu GKH, Cheng VCC, Yuen K-Y, Yam W-C.** 2013. Direct bacterial identification in positive blood cultures by use of two commercial matrix-assisted laser desorption ionization–time of flight mass spectrometry systems. J Clin Microbiol **51:**1733–1739. http://dx.doi.org/10.1128/JCM.03259-12.
13. **Rodrıguez-Sánchez B, Sánchez-Carrillo C, Ruiz A, Marın M, Cercenado E, Rodríguez-Créixems M, Bouza E.** 2013. Direct identification of pathogens from positive blood cultures using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. Clin Microbiol Infect **20:**O421–O427. http://dx.doi.org/10.1111/1469-0691.12455.
14. **Foster AGW.** 2013. Rapid identification of microbes in positive blood cultures by use of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system. J Clin Microbiol **51:**3717–3719. http://dx.doi.org/10.1128/JCM.01679-13.
15. **Idelevich EA, Schule I, Grünastel B, Wüllenweber J, Peters G, Becker K.** 2014. 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 **20:**1001–1006. http://dx.doi.org/10.1111/1469-0691.12640.
16. **La Scola B, Raoult D.** 2009. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One **4:**e8041. http://dx.doi.org/10.1371/journal.pone.0008041.
17. **Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G.** 2013. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study. Clin Infect Dis **56:**1101–1107. http://dx.doi.org/10.1093/cid/cis1204.
18. **Martiny D, Debaugnies F, Gateff D, Gérard M, Aoun M, Martin C, Konopnicki D, Loizdou A, Georgaia A, Hainault M, Chantrenne M, Dediste A, Vandenberg O, Van Praet S.** 2013. Impact of rapid microbial identification directly from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on patient management. Clin Microbiol Infect **19:**E568–E581. http://dx.doi.org/10.1111/1469-0691.12282.
19. **Vlek ALM, Bonten MJM, Boel CHE.** 2012. Direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry improves appropriateness of antibiotic treatment of bacteremia. PLoS One **7:**e32589. http://dx.doi.org/10.1371/journal.pone.0032589.