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Rapid Identification of Positive Blood Cultures by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Using Prewarmed Agar Plates

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This study describes an inexpensive and straightforward method for identifying bacteria by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) directly from positive blood cultures using prewarmed agar plates. Different inoculation methods and incubation times were evaluated to determine the optimal conditions. The two methods using pelleted material from positive culture bottles performed best. In particular, the pellet streak method correctly identified 94% of the Gram negatives following 4 h of incubation and 98% of the Gram positives following 6 h of incubation.

The number of people dying from sepsis has almost doubled in the past 20 years, and the frequency of sepsis is expected to rise (1, 2). A rapid microbiological diagnosis allows for prompt therapy with effective antimicrobials, maximizing the chances for survival (3). To decrease the time to identification, attempts have been made to identify bacteria directly from positive blood cultures using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), but the broth medium and blood interfere with mass spectrometry (4, 5). Various extraction protocols have been developed to isolate bacteria from culture vials, but these are laborious and result in decreased accuracy (4–10). An easier and cheaper method has been described; a sample from a positive blood culture bottle is spotted onto a prewarmed agar plate and identified by MALDI-TOF MS following 3 to 5 h of incubation without the need for ethanol-formic acid protein extraction (11). The goals of our study were to evaluate the optimal plating techniques for this method and the ability of this method to rapidly and accurately identify bacteria in positive blood cultures using MALDI-TOF MS.

Blood specimens were inoculated into Bactec Plus culture bottles and incubated in the Bactec FX system (BD Diagnostic Systems, Sparks, MD). Consecutive aerobic blood cultures from new patients that were flagged as positive during the first shift were eligible for inclusion in the study. When a bottle signaled positive, a Gram stain and overnight subculture were performed. Any sample with a Gram-positive stain for yeast or that appeared to be polymicrobial was excluded from the study. In parallel with Gram staining, 1.5 ml of broth was removed using a sterile 3-ml syringe. Trypticase soy agar with 5% sheep blood agar plates (BAP) (BD Diagnostic Systems, Sparks, MD) were prewarmed at 37°C for 1 h. BAP was selected over other medium types because it provides optimal results with our MALDI-TOF MS system (Vitek MS, bioMérieux, Durham, NC), as per the manufacturer, which was confirmed by in-house validation (12). A single drop of the broth was dispensed onto a prewarmed BAP and streaked into four quadrants (direct streak). Another drop was dispensed onto a BAP without streaking (direct spot). The remainder of the 1.5-ml aliquot was transferred into a microcentrifuge tube, and a "flash spin" was performed on a microcentrifuge (approximately 9,600 × *g* for 2 s). The supernatant was transferred into a new microcentrifuge tube and centrifuged at 9,600 × *g* for 1 min. The supernatant was discarded and, using an inoculating loop, and a sample of the pellet was streaked into four quadrants on a prewarmed BAP (pellet streak). A spot of the pellet was dabbed onto a prewarmed BAP (pellet spot). All BAP were incubated in 5% CO_2 atmosphere at 37°C and were removed at 2, 4, 6, 8, and 10 h for analysis. The short incubation times did not produce discernible colonies but rather a visible film on the plate. The surface of each plate was scraped using a 1-μl disposable plastic loop and analyzed by Vitek MS, as previously described (12). Once an acceptable level of identification was achieved, no further time points were analyzed. In most cases, the goal was to obtain species-level identification, but with two organisms, *Achromobacter* and *Salmonella*, a genus-level identification was considered acceptable. Identification by Vitek MS on overnight subculture served as a control, as this is the currently validated method for identifying bacteria from patient samples at our institution. If Vitek MS was unable to provide identification, biochemical testing and 16S rRNA sequencing were available as needed. Calculations of the mean incubation times, associated confidence intervals, and analysis of variance (ANOVA) were performed using QuickCalcs by GraphPad Software, Inc.

The study included 137 positive blood cultures obtained from inpatients at the University of Chicago Medicine; 134 were monomicrobial. Table 1 outlines the identification results of the four different inoculation methods along the incubation time points. The only difference in the identification rates between 8 and 10 h of incubation was the identification of a *Gemella* sp. by the direct spot method; therefore, the 10-h data were not included in Table 1. All four methods were able to achieve >90% identification fol-

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TABLE 1 Correct identification rates of blood cultures with prewarmed plates by direct and pellet methods

Organism(s) (*n*)[a]	Direct streak (no. identified [%])				Direct spot (no. identified [%])			
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h
All cultures (134)	66 (49)	107 (80)	122 (91)	127 (95)	64 (48)	110 (82)	124 (92)	127 (95)
Gram positives (87)	41 (47)	70 (80)	81 (93)	85 (98)	43 (49)	69 (79)	81 (93)	84 (96)
GPC (84)	40 (48)	69 (82)	80 (95)	84 (100)	42 (50)	68 (80)	80 (95)	84 (100)
Enterococcus spp. (8)	4 (50)	6 (75)	8 (100)	—[b]	1 (12)	5 (62)	6 (75)	8 (100)
Staphylococcus aureus (33)	24 (73)	32 (97)	32 (97)	33 (100)	24 (73)	31 (94)	33 (100)	—
CoNS (33)	7 (21)	22 (67)	31 (94)	33 (100)	14 (42)	23 (70)	32 (97)	33 (100)
Streptococcus spp. (10)	5 (50)	9 (90)	—	10 (100)	3 (30)	9 (90)	—	—
GPB (3)[c]	1 (33)	—	—	—	1 (33)	—	—	—
Gram negatives (47)	25 (53)	37 (79)	41 (87)	42 (89)	21 (45)	41 (87)	43 (91)	—
Enterobacteriaceae (34)	16 (47)	27 (79)	31 (91)	32 (94)	16 (47)	32 (94)	—	—
Escherichia coli (17)	12 (71)	16 (94)	17 (100)	—	11 (65)	17 (100)	—	—
Klebsiella pneumoniae (9)	2 (22)	6 (67)	7 (78)	8 (89)	2 (22)	8 (89)	—	—
Other (8)[d]	2 (25)	5 (62)	7 (87)[e]	—	3 (37)	7 (87)[f]	—	—
Non-*Enterobacteriaceae* (13)	10 (77)	11 (85)	—	—	6 (46)	10 (77)	—	—
Pseudomonas aeruginosa (9)	8 (89)	9 (100)	—	—	5 (56)	8 (89)	9 (100)	—
Other (4)[g]	2 (50)[h]	—	—	—	1 (25)	2 (50)	3 (75)[i]	—

Organism(s) (*n*)	Pellet streak (no. identified [%])				Pellet spot (no. identified [%])			
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h
All cultures (134)	91 (68)	119 (89)	129 (96)	—	91 (68)	120 (90)	126 (94)	129 (96)
Gram positives (87)	48 (55)	76 (87)	85 (98)	—	48 (55)	76 (87)	82 (94)	85 (98)
GPC (84)	48 (57)	75 (89)	84 (100)	—	48 (57)	75 (89)	81 (96)	84 (100)
Enterococcus spp. (8)	7 (87)	—	—	—	7 (87)	8 (100)	—	—
S. aureus (33)	17 (51)	29 (88)	33 (100)	—	18 (55)	27 (82)	31 (94)	33 (100)
CoNS (33)	16 (48)	30 (91)	33 (100)	—	16 (48)	31 (94)	33 (100)	—
Streptococcus spp. (10)	8 (80)	—	10 (100)	—	7 (70)	9 (90)	—	10 (100)
GPB (3)[c]	0 (0)	1 (33)	—	—	0 (0)	1 (33)	—	—
Gram negatives (47)	43 (91)	43 (91)	44 (94)	—	43 (91)	44 (94)	—	—
Enterobacteriaceae (34)	32 (94)	—	33 (97)	—	32 (94)	33 (97)	—	—
E. coli (17)	17 (100)	—	—	—	17 (100)	—	—	—
Klebsiella spp. (9)	8 (89)	—	9 (100)	—	8 (89)	9 (100)	—	—
Other (8)[d]	7 (87)[j]	—	—	—	7 (87)[j]	—	—	—
Non-*Enterobacteriaceae* (13)	12 (92)	—	—	—	12 (92)	—	—	—
P. aeruginosa (9)	9 (100)	—	—	—	9 (100)	—	—	—
Other (4)[g]	3 (75)[k]	—	—	—	3 (75)[k]	—	—	—

[a] GPC, Gram-positive cocci; CoNS, coagulase-negative staphylococci; GPB, Gram-positive bacilli.
[b] —, no change in the identification rate at the subsequent incubation time point.
[c] A *Bacillus pumilus* organism identified at the time noted; two *Corynebacterium* spp. not identified by Vitek MS.
[d] A *Pantoea* sp. identified by Vitek MS on overnight subculture only.
[e] At 2 h, an *Enterobacter cloacae* and *Salmonella* sp. were identified; at 4 h, *Citrobacter freundii*, *Proteus mirabilis*, the 2nd *Salmonella* sp., and *Serratia marcescens* were identified; at 6 h, the 2nd *S. marcescens* was identified.
[f] At 2 h, *C. freundii*, *S. marcescens*, and *Salmonella* sp. were identified; at 4 h, *E. cloacae*, *P. mirabilis*, the 2nd *Salmonella* sp., and the 2nd *S. marcescens* organism were identified.
[g] A *Moraxella* sp. identified by Vitek MS on overnight subculture only.
[h] At 2 h, two *Stenotrophomonas maltophilia* organisms were identified; an *Achromobacter* sp. was not identified to the genus level.
[i] At 2 h, an *S. maltophilia* sp. was identified; at 4 h, the 2nd *S. maltophilia* organism was identified; at 6 h, an *Achromobacter* sp. was identified.
[j] At 2 h, *C. freundii*, *E. cloacae*, *P. mirabilis*, two *Salmonella* spp., and two *S. marcescens* organisms were identified.
[k] At 2 h, an *Achromobacter* sp. and two *S. maltophilia* organisms were identified.

lowing 6 h of incubation. The pellet streak method achieved the highest accuracy (96%) at 6 h; pellet spot achieved the same at 8 h. However, this difference was not found to be statistically significant, as described below.

There were 87 monomicrobial cultures that contained Gram-positive organisms (Table 1). The pellet streak method was able to provide an identification for all 84 (100%) Gram-positive cocci in the shortest time frame (6 h). The pellet spot and direct streak methods did not identify all 84 Gram-positive cocci until 8 h of incubation, but this difference was not statistically significant. The streptococci included two *Streptococcus agalactiae*, two *Streptococcus pyogenes*, and one *Gemella morbilliform* organism but no *Streptococcus pneumoniae* organisms. All five alpha-hemolytic streptococci were identified correctly to the species level except one *Streptococcus oralis* organism, which was identified as *Streptococcus mitis*. However, *S. oralis* is considered to be a member of the *S. mitis* group, so this was considered an acceptable species-level identification. Three Gram-positive rods were included in this study. A *Bacillus pumilus* organism was correctly identified at 2 h by both direct methods and at 4 h by both pellet methods. Two cultures grew out *Corynebacterium* spp. but were not able to be identified by Vitek MS, even from overnight culture. There were no incorrect identifications of any Gram-positive organisms by Vitek MS.

Forty-seven monomicrobial blood cultures containing Gram-negative organisms were studied (Table 1). Of the four methods, the pellet spot was able to provide a species-level identification for 44 (94%) of the Gram-negative organisms at the earliest time point, 4 h. Both pellet methods achieved correct identification for 31 (91%) of the 34 *Enterobacteriaceae* family members after only 2 h of incubation; the direct pellet method required 4 h and the direct streak method 8 h. A culture with *Pantoea agglomerans* required overnight incubation for identification by Vitek MS. Two *Salmonella* species were also correctly identified by all four methods. Of the 13 non-*Enterobacteriaceae* family members, 12 (92%) were identified with only 2 h of incubation by both pellet methods. One culture with *Moraxella* spp. required overnight incubation for identification by Vitek MS. There was only one incorrect identification. A *Klebsiella pneumoniae* organism was initially identified as *Salmonella* sp. following 4 h of incubation using the direct streak method. The same sample was correctly identified as *K. pneumoniae* at 2 h by both pellet methods and the direct spot method at 4 h. Because three of the four methods identified the sample as *K. pneumoniae*, the direct streak sample was reanalyzed following a total of 6 h of incubation and was correctly identified as *K. pneumoniae*, which was confirmed by identification from overnight subculture.

There were three polymicrobial samples that were included in the study because the Gram stain appeared monomicrobial. The first was a blood culture positive for Gram-negative bacilli that grew *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. The *P. aeruginosa* culture was identified at 4 h by the direct spot method and at 2 h by the other three methods. The *S. maltophilia* culture was identified only from overnight subculture. The second culture contained two different strains of coagulase-negative staphylococci, *Staphylococcus hominis*, and *Staphylococcus epidermidis*. The *S. hominis* culture was identified by all four inoculation methods following 4 h of incubation, but the *S. epidermidis* culture required overnight subculture to be isolated and identified. The third culture was positive for Gram-negative bacilli by Gram stain but grew *P. aeruginosa* and *S. epidermidis* on overnight subculture. Only the *P. aeruginosa* culture was identified at 2 h by all four inoculation methods. Our method was able to identify at least one organism present in all three polymicrobial specimens without any incorrect identifications. Other molecular methods for rapidly identifying organisms present in positive blood cultures have all been limited in their ability to detect all of the organisms present in polymicrobial specimens (13).

For each inoculation method, the incubation time at which each monomicrobial sample was correctly identified was averaged. The mean incubation times with associated 95% confidence intervals are as follows: 3.33 h (3.04 to 3.61 h) for direct spot; 3.32 h (3.08 to 3.57 h) for direct streak; 2.75 h (2.52 to 2.98 h) for pellet spot; and 2.69 h (2.52 to 2.86 h) for pellet streak. Both pellet methods had statistically shorter mean incubation times than those for both direct methods (P value < 0.05 by ANOVA). However, there was no statistical difference in the mean incubation times between the pellet spot and pellet streak or between the direct spot and direct streak methods. The mean incubation times for the identification of monomicrobial blood cultures containing Gram-positive cocci were 3.48 h (3.10 to 3.85 h) for direct spot; 3.48 h (3.08 to 3.88 h) for direct streak; 2.75 h (2.52 to 2.98 h) for pellet spot; and 3.12 h (2.79 to 3.44 h) for pellet streak. For Gram negatives, they were 3.05 h (2.71 to 3.38 h) for direct spot; 3.02 h (2.57 to 3.48 h) for direct streak; 2.04 h (1.95 to 2.13 h) for pellet spot; and 2.09 h (1.91 to 2.27 h) for pellet streak. Statistical analysis did not detect a significant difference between the mean incubation times required to identify Gram-positive cocci, but it did with Gram-negative organisms. Both pellet methods required statistically shorter mean incubation times for identifying Gram-negative organisms than those for both direct methods (P value < 0.05 by ANOVA), but there was no statistical difference between the pellet spot and pellet streak or between the direct spot and direct streak methods.

Over the last decade, several platforms have been introduced to rapidly identify bacteria in positive blood culture bottles through molecular techniques, such as nucleic amplification, *in situ* hybridization, and/or microarray technology (14). These systems are limited by the number of pathogens they can detect and require costly consumables ranging in price from tens to hundreds of dollars per sample. The estimated cost of analyzing 150 positive blood cultures using nucleic acid-based technology is between $10,000 and $20,000, assuming the organism is able to be detected by the platform (15–17). In comparison, MALDI-TOF MS is able to identify a much wider range of pathogens with a lower operating cost than these nucleic acid-based detection systems. The cost of identifying a bacterial colony following overnight incubation from solid medium without any extraction is estimated to be $1 to 2 per sample and was demonstrated to be significantly cheaper than automated phenotypic-based identification systems (18). Our method requires an additional blood agar plate and microcentrifuge tube per sample, which would still keep the cost of identification by our method to <$2 per sample. Therefore, 150 positive blood cultures could be analyzed by our method for <$300, which is significantly cheaper than the molecular methods described above.

This is the first study employing prewarmed plates for rapid identification with the Vitek MS system. Other methods have been reported for rapid identification with Vitek MS using complex extraction methods or the Sepsityper kit. Chen et al. (8) reported identifying 81.5% and 88% of blood cultures analyzed to the species and genus levels, respectively, using the Sepsityper kit with the Vitek MS system, and the cost was approximately $15 per sample (8). Another study by Foster (9) used a detergent-based method for the rapid identification of blood cultures with Vitek MS and achieved 92% identification to the genus level and 88.1% to the species level. While these and other methods can provide species-level identification rates of >80%, they utilize complex methods requiring skilled personnel. Our prewarmed plate method using a bacterial pellet is cheaper and requires only two spin steps, without any additional reagents. The method does require an incubation time of 2 to 4 h for Gram-negative organisms and 4 to 6 h for Gram-positive organisms to achieve appropriate identification rates; other methods, while more complex, provide results within 30 to 60 min.

Others have investigated the use of solid medium for rapid identification by MALDI-TOF MS. Haigh et al. (19) examined the use of incubation with prewarmed plates but not beyond 4 h, and they used a direct spot method only. They achieved a 92% identification rate with Gram-negative bacilli at 2 h. The Gram-positive identification rates were much lower: only 55% of staphylococci were identified at 4 h. The study also incorporated spectral modification of the Bruker Biotyper MS instrument by eliminating the 2- to 3-kDa range of analyzed spectra, which some laboratories

may not be able to replicate. A recent publication by Idelevich et al. (20) also used incubation on solid medium to identify positive blood cultures by MALDI-TOF MS. However, the study did not prewarm the plates. They achieved species-level identification of Gram-positive cocci in only 64% of the samples following 6 h of incubation and 94% species-level identification of Gram-negative bacilli following 4 h of incubation using the Biotyper MS. Our study indicates that using prewarmed plates can decrease the incubation time required for identification by 2 h.

This study has a few limitations. A larger number of blood cultures would have allowed a more thorough assessment of our method of rapid identification. Also, shorter time intervals (e.g., every hour) during incubation could have been assessed. We did not include yeast samples, because they are difficult for MALDI-TOF MS systems to analyze directly from blood culture without additional processing (5, 21).

This study demonstrates the use of prewarmed agar plates as an effective, inexpensive, and straightforward method for obtaining a rapid identification of bacteria in positive blood cultures with MALDI-TOF MS. Prewarmed BAP are inoculated with a sample from a positive blood culture using a pellet-based method following Gram stain. Gram-negative organisms are incubated for a minimum of 2 to 3 h and Gram-positive organisms for a minimum of 4 to 6 h. MALDI-TOF MS analysis is typically performed at regular intervals in most laboratories (e.g., every 3 h). Plates that have incubated for a sufficient time would be included in the next scheduled MALDI-TOF MS analysis. Specimens that are not identified would be reincubated and reanalyzed at the next MALDI-TOF MS analysis cycle. Overnight growth of colonies would still be required to identify organisms not identifiable by this method (e.g., polymicrobial specimens, Gram-positive rods, anaerobes, and yeast) and to perform susceptibility testing. That being said, methods have been developed using pelleted bacteria from positive blood cultures to assess antimicrobial resistance by MALDI-TOF MS (22, 23). A valuable future study would be to employ our pellet method in parallel with a method for the rapid detection of antimicrobial resistance. In theory, MALDI-TOF MS would be able to provide not only rapid identification but also meaningful data regarding susceptibility. As we continue to find new ways to decrease the time to the identification of pathogens and resistance, the next challenge will be working with clinicians to have these rapid results yield an improvement in patient outcomes.

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