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Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Direct Bacterial Identification from Positive Blood Culture Pellets[▽]

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An ammonium chloride erythrocyte-lysing procedure was used to prepare a bacterial pellet from positive blood cultures for direct matrix-assisted laser desorption–ionization time of flight (MALDI-TOF) mass spectrometry analysis. Identification was obtained for 78.7% of the pellets tested. Moreover, 99% of the MALDI-TOF identifications were congruent at the species level when considering valid scores. This fast and accurate method is promising.

Blood cultures are the best approach to establishing the etiology of bloodstream infections and infectious endocarditis. Moreover, rapid identification of the etiological agents of such severe infections is pivotal to guiding antimicrobial therapy. Thus, the impact of timely microbiology laboratory reporting is maximal at the notification of positive blood cultures (5). Matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) allows identification of both gram-positive (2, 7) and gram-negative bacteria (1, 4) to the species level in a few minutes by measuring the molecular masses of proteins and other bacterial components obtained from whole bacterial extracts. A relatively low crude bacterial load of about 5×10^3 CFU is necessary for reliable MALDI-TOF analysis (3), suggesting that bacterial identification might be done directly with blood culture pellets. We applied a simple procedure for lysing erythrocytes from positive blood cultures and prepared a bacterial pellet for MALDI-TOF MS analysis.

Pellets from positive blood culture vials (Plus aerobic/F, Lytic anaerobic/F, and Peds/F; Becton Dickinson) detected by the Bactec 9240 automated blood culture system (Becton Dickinson) were prepared as follows shortly after the automated system flagged a positive vial. Five milliliters of positive medium was added to 40 ml of sterile H_2O. The sample was mixed and centrifuged at $1,000 \times g$ for 10 min. H_2O and blood cells were removed (Fig. 1A). The pellet was then suspended in 1 ml of a home-made ammonium chloride lysing solution (0.15 M NH_4Cl, 1 mM $KHCO_3$) and centrifuged at $140 \times g$ for 10 min (Fig. 1B and C). When the pellet remained hemorrhagic, the supernatant was discarded and the pellet was washed again with 2 ml of H_2O. MALDI-TOF MS analysis was then directly performed on the bacterial pellet or after an additional extraction step. To extract proteins, 20 μl of the pellet was mixed with 1 ml of 70% ethanol. After a further centrifugation at $13,000 \times g$ for 2 min, the pellet was mixed with 25 μl of 70% formic acid and 25 μl of pure acetonitrile. After centrifugation at $13,000 \times g$ for 2 min, 1 μl of the supernatant containing the bacterial extract was transferred onto the MALDI target plate and dried. Subsequently, both unextracted and extracted samples were overlaid with 1 μl of MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid) and dried in air.

Mass spectra were then acquired by Microflex MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). MALDI BioTyper 2.0 software was used for spectral analysis and comparison with the MALDI BioTyper database. The identification was considered valid at the species level when the score was ≥2, as valid at the genus level when the score was ≥1.7 and <2, and not valid when the score was ≤1.7. The identifications obtained by MALDI-TOF MS analysis were compared with standard biochemical identifications with oxidase, catalase, and commercial identification strips, i.e., Vitek (bioMérieux, France) and API (bioMérieux).

In 9 consecutive weeks, 126 positive blood vials from 78 patients were analyzed. Four blood cultures taken from two patients were excluded from the analysis because they were polymicrobial. For both patients, only one species could be identified by MALDI-TOF MS performed after an extraction step, with scores of 1.93 and 2.33 for *Enterococcus faecalis* and *Klebsiella pneumoniae*, respectively.

Table 1 shows the results of MALDI-TOF MS identification for the 122 remaining analyses. Among these 122 positive blood cultures, as many as 96 (78.7%) bacterial identifications were obtained by MALDI-TOF MS analysis, of which 69 (56.6% of 122) exhibited a score of >2 and 27 (22.1%) exhibited a score of >1.7 and <2. Importantly, 95 (98.95%) of the 96 bacterial identifications were correct at the species level and 1 identification was correct at the genus level only (*Staphylococcus pasteuri* instead of *Staphylococcus caprae*). In the latter case, the score was 1.73. Thus, among the 27 cases with accurate identification at the genus level, 26 (96.3%) of 27 were also accurate at the species level. Moreover, among the 69 valid identifications at the species level (score above 2), no MALDI-TOF MS results were discordant with the conventional identification.

In 26 (21.3%) of the cases, no reliable identification was

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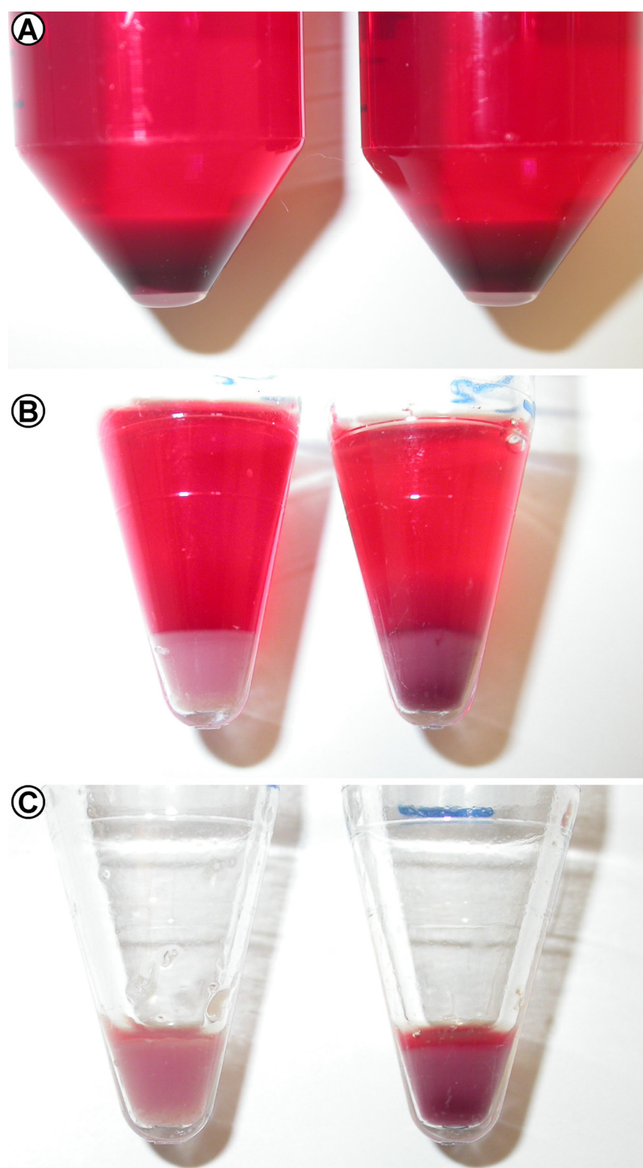

FIG. 1. Ammonium chloride procedure. A positive blood culture with *S. aureus* (aerobic vial) is shown along with a control that was processed in parallel without ammonium chloride (isotonic NaCl control). (A) Hemorrhagic pellets obtained after initial centrifugation, i.e., before the ammonium chloride step. (B) Pellet obtained after erythrocyte lysis with ammonium chloride (left tube) or with the isotonic NaCl control (right tube). (C) Bacterial pellet available for MALDI-TOF MS analysis (left tube). Please note the presence of a large numbers of erythrocytes in the pellet obtained without ammonium chloride (right tube).

obtained (score of <1.7). As many as 21 (80.8%) of these 26 isolates were gram-positive bacteria, mainly streptococci (n = 13) and coagulase-negative staphylococci (n = 5). Most of the unidentified streptococci were *Streptococcus pneumoniae* (eight *S. pneumoniae* isolates were not identified [score of <1.7], the two remaining *S. pneumoniae* isolates being identified correctly but with a low score, i.e., 1.7 to 2). Moreover, among the five gram-negative bacteria with a score below 1.7, four were encapsulated species (two were *K. pneumoniae*, and two were *Haemophilus influenzae*).

Our results showed that a simple procedure such as lysing erythrocytes from positive blood culture pellets with an ammonium chloride solution allowed efficient identification of bloodstream isolates by MALDI-TOF MS. Indeed, identification was obtained for 78.7% of the blood culture pellets analyzed. Moreover, 100% of the MALDI-TOF MS identifications were congruent at the species level when only considering valid scores greater than 2, as proposed by the manufacturer. This demonstrates the usefulness of the lysis step since, in contrast, when performing MALDI-TOF MS on blood culture pellets without the lysis procedure, we did not obtain any accurate identification. In addition, 99% of the MALDI-TOF MS identifications matched the conventional identifications at the species level when considering both scores of 1.7 to 2 and those greater than 2. This excellent performance of a coupled ammonium chloride lysis procedure and MALDI-TOF MS analysis was unexpected, since in a recent study comparing

TABLE 1. Direct bacterial identification from positive blood culture pellets using MALDI-TOF MS

Identification	No. of isolates	No. (%) correctly identified		No. (%) not reliably identified (score, <1.7)
		High score (≥2)[a]	Low score (1.7–2)[b]	
Gram-negative bacilli	46	38 (83)	3 (7)	5 (11)
Escherichia coli	15	14	1	
Klebsiella pneumoniae	9	6	1	2
Klebsiella oxytoca	4	3	1	
Pseudomonas aeruginosa	4	4		
Enterobacter cloacae	3	3		
Citrobacter koseri	2	2		
Haemophilus influenzae	2			2
Morganella morganii	2	2		
Bacteroides distasonis	1			1
Citrobacter freundii	1	1		
Fusobacterium necrophorum	1	1		
Proteus vulgaris	1	1		
Serratia marcescens	1	1		
Gram-positive cocci	74	31 (42)	23 (31)	20 (27)
Staphylococcus aureus	25	20	5	
Staphylococcus epidermidis	23	6	13	4
Streptococcus pneumoniae	10		2	8
Streptococcus agalactiae	5	2	1	2
Staphylococcus hominis	2	2		
Streptococcus dysgalactiae	2			2
Enterococcus faecalis	1		1	
Finegoldia magna	1			1
Micromonas micros	1			1
Staphylococcus caprae	1		1	
Staphylococcus haemolyticus	1			1
Streptococcus bovis	1			1
Streptococcus pyogenes	1	1		
Other: *Brevibacterium casei*	2		1	1
Total	122	69 (57)	27 (22)	26 (21)

[a] Correct identification to the species level.
[b] Correct identification to the species level of all but one isolate, for which MALDI-TOF MS identification was congruent with conventional identification at the genus level only (identified as *S. caprae* by Vitek and as *S. pasteuri* by MALDI-TOF MS).

MS identification of routine bacterial strains with conventional identification, only 84.1% of the strains were correctly identified (6). The better result we observed is likely due to the different setting studied, i.e., blood cultures versus a large variety of different samples. Indeed, in blood cultures, the most frequently recovered pathogenic species that represent more than 50% of all cases of bacteremia are species accurately identified by MALDI-TOF MS such as *Enterobacteriaceae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and enterococci.

The lower yield of valid MALDI-TOF MS results with streptococci and staphylococci might be due (i) to the close relatedness of the different species of streptococci belonging to the *S. mitis* group (i.e., *S. pneumoniae*, *S. mitis*, *S. sanguinis*, *S. oralis*, . . .), (ii) to some relatedness of different coagulase-negative staphylococci, (iii) to the cell wall composition of gram-positive bacteria conferring increased resistance to lysis, and (iv) partially to the possible presence of some residual blood proteins. For staphylococci, the major goal is to differentiate *S. aureus* from coagulase-negative staphylococci, and this may be accurately done with blood culture bacterial pellets by MALDI-TOF MS. In routine practice, the difficulty in distinguishing *S. pneumoniae* from other species of the *S. mitis* group is much more clinically relevant and represents a current limitation of MALDI-TOF MS. The presence of a capsule may also partially explain the low identification rate of *S. pneumoniae*, *H. influenzae*, and *K. pneumoniae*. Improved extraction protocols specifically designed for encapsulated bacteria are thus warranted.

The use of ammonium chloride-driven hemolysis before analyzing positive blood cultures by MALDI-TOF MS is a very promising new method allowing fast, accurate, and inexpensive identification of the etiological agents of life-threatening bloodstream infections. An alternative approach for rapid MALDI-TOF-based bacterial identification starting from a short culture on agar might yield sufficient bacterial growth in 4 to 6 h (data not shown). Given the importance of positive blood cultures, this delay may be clinically relevant compared to the 30 to 45 min needed for the ammonium chloride erythrocyte-lysing procedure. Further work is needed to confirm our results with a larger diversity of strains and to assess the clinical impact of this new approach.

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