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Brief report

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# Comparison of two methods skipping cell lysis and protein extraction for identification of bacteria from blood cultures by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry

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## ABSTRACT

**Objective.** Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) is widely used for fast identification of bacteria from blood cultures (BC). We compared the performance of two procedures, one including a pre-enrichment step in brain heart infusion and the other a direct method using vacutainer separator gel tubes (DI), for identification of bacteria from blood cultures by MALDI-TOF MS.

**Material and methods.** We first prepared a training set of 20 simulated bacteremia specimens, including 10 Gram-negative and 10 Gram-positive species. A total of 145 non-consecutive BCs flagged as positive (68 Gram-negative rods, and 77 Gram-positive cocci) were prospectively analyzed (validation set).

**Results.** A total of 82% and 49% of isolates were correctly identified to the species level by the respective methods.

**Conclusion.** The pre-enrichment method outperformed the DI method for identification of virtually all bacterial species included in the panels.

**Key words: MALDI-TOF M; blood culture; bacterial identification.**

## Comparación de dos métodos que eluden la lisis celular y la extracción de proteínas para la identificación de bacterias crecidas en hemocultivos mediante espectrometría de masas MALDI-TOF

## RESUMEN

**Objetivo.** La espectrometría de masas MALDI-TOF se utiliza comúnmente para la identificación rápida de bacterias crecidas en hemocultivos (HC). Hemos comparado el rendimiento de dos procedimientos, uno que incluye un paso previo del enriquecimiento en caldo corazón-cerebro y el otro un método directo que usa tubos vacutainer con gel separador (DI), para la identificación de bacterias a partir de hemocultivos mediante MALDI-TOF MS.

**Material y métodos.** Analizamos prospectivamente un total de 145 HC no consecutivos (68 con crecimiento de bacterias gramnegativas y 77 de cocos grampositivos).

**Resultados.** Un total de 82% y 49% de los aislamientos fueron identificados correctamente a nivel de especie por los dos métodos, respectivamente.

**Conclusión.** El rendimiento del método de pre-enriquecimiento en caldo corazón-cerebro fue mejor que el del método DI para la identificación de la práctica totalidad de las especies bacterianas incluidas en el panel de estudio.

**Palabras clave: MALDI-TOF MS; hemocultivo; identificación bacteriana.**

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## INTRODUCTION

Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) is widely used for fast identification of bacteria from blood cultures (BC) [1]. For the sake of simplicity, several methods skipping blood cell lysis and protein extraction have been developed for this purpose, using either bacteria directly pelleted by centrifugation from BCs - DI methods [2-5], or bacteria harvested following a pre-cultivation step in solid [6] or liquid [7] media.

We previously developed a simple method by which positive BCs were subjected to short-term enrichment in brain heart infusion broth (BHI) prior to MALDI-TOF MS analysis (ENR method), and showed that it outperformed pre-incubation in blood agar in terms of efficiency and speed in identifying a wide array of bacterial species, in particular Gram-negative bacteria [7]. Here, we compared the performance of our procedure with a DI method from positive BCs collected in vacutainer tubes with separator gels.

## MATERIAL AND METHODS

We first prepared a training set of 20 simulated bacteremia specimens. Bacteria, including 10 Gram-negative and 10 Gram-positive species (table 1), were selected from cryopreserved stocks of clinical isolates recovered during the preceding year, in accordance with a protocol approved by our institutional review board. Bacterial isolates were spiked into blood culture bottles (BACTEC™ Plus Aerobic/F and Plus Anaerobic/F medium bottles, Becton Dickinson and Company, New Jersey, USA) at $10^3$ CFU/ml and incubated in automated continuous-monitoring blood culturing instrument (BACTEC™ FX; BD). The isolates had been grown overnight on chocolate agar medium (BD) at 36 °C in air with 5% $CO_2$ using a Heracell 240i $CO_2$ incubator, (Thermo Fisher Scientific, Langenselbold, Germany). The bacterial inocula were prepared in sterile saline. The concentration of bacteria was determined by the quantitative plating method prior to inoculation into the blood culture bottles. Next, a total of 145 non-consecutive BCs flagged as positive and collected between September 2017 and May 2018 from unique adult (n=138) or pediatric (n=7) patients were prospectively analyzed (validation set). Only monomicrobial BCs were selected for this study. All coagulase-negative staphylococci (CNS) included in the panel were deemed be clinically significant. Positive BCs were processed for MALDI-TOF MS identification following two different protocols, performed in parallel: our pre-cultivation protocol [7] and a DI method. For the former method, a volume of 50 µl of blood culture was inoculated into a volume of 500 µl of BHI (Oxoid Limited, Hampshire, United Kingdom) in sterile vials and incubated at 36 °C in air with 5% $CO_2$ for 2-4 h. Then the vials were centrifuged at 13,000 rpm for 2 min, the supernatants discarded, and the pellets used for MALDI-TOF MS analyses, as previously described [7]; for the latter method, a volume of 8.0 ml of BCs was transferred with a syringe to a serum separator tube (Vacutainer 8.5 ml SST Plus; Beckton Dickinson-BD- New Jersey, USA) and centrifuged at 3,600 rpm for 15 minutes at room temperature. The supernatant was aspirated with caution to avoid disrupting the formed pellet of bacteria present at the surface of the polymeric gel.

Positive BCs were subcultured overnight on chocolate agar following our routine diagnostic protocol, and the growing biomass was used for MALDI-TOF MS identification. The

**Table 1** **Performance of two methods for identifying bacteria to the species level from blood cultures by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry: training set**

| Isolates in the panel (n) | Accurate identification following pre-enrichment in Brain Heart Infusion broth | Accurate identification by direct processing of blood cultures |
|---|---|---|
| | no. of isolates (%) | no. of isolates (%) |
| All (20) | 15 (75) | 9 (45) |
| ***Enterobacteriaceae*** (5) | 5 (100) | 5 (100) |
| *Escherichia coli* (2) | 2 (100) | 2 (100) |
| *Klebsiella pneumoniae* (2) | 2 (100) | 2 (100) |
| *Enterobacter cloacae* (1) | 1 (100) | 1 (100) |
| ***Pseudomonas aeruginosa*** (5) | 2 (40) | 0 (0) |
| ***Staphylococcus aureus*** (5) | 3 (60) | 1 (20) |
| ***Streptococcus/Enterococcus*** spp. (5) | 5 (100) | 3 (60) |
| *Enterococcus faecalis* (2) | 2 (100) | 1 (50) |
| *Enterococcus faecium* (1) | 1 (100) | 0 (0) |
| *Streptococcus pneumoniae* (1) | 1 (100) | 1 (100) |
| *Streptococcus agalactiae* (1) | 1 (100) | 1 (100) |

**Table 2** **Performance of two methods for identification to the species level of bacteria from blood cultures by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry: validation set**

| Isolates in the panel (n) | Time to positive blood culture after incubation (hours) | Accurate identification following pre-enrichment in Brain Heart Infusion broth no. of isolates (%) | Incubation time in Brain Heart Infusion broth (hours) | Accurate identification by direct processing of blood cultures no. of isolates (%) |
|---|---|---|---|---|
| **All** (145) | 12.3 | 119 (82) | 2.9 | 72 (49) |
| ***Enterobacteriaceae*** (58) | 11.5 | 55 (94.8) | 2.4 | 44 (75.8) |
| *Escherichia coli* (31) | 11.3 | 29 (93.5) | 2.1 | 25 (80.6) |
| *Klebsiella pneumoniae* (13) | 11.9 | 12 (92.3) | 2.2 | 12 (92.3) |
| *Enterobacter cloacae* (7) | 11.6 | 7 (100) | 2.8 | 3 (42.8) |
| *Proteus mirabilis* (4) | 13.9 | 4 (100) | 2.7 | 1 (25) |
| *Citrobacter koseri* (1) | 6.8 | 1 (100) | 2.5 | 1 (100) |
| *Enterobacter kobei* (1) | 10.2 | 1 (100) | 2.0 | 1 (100) |
| *Serratia marcescens* (1) | 6.9 | 1 (100) | 2.5 | 1 (100) |
| **Non-fermenting Gram-negative rods** (10) | 13.6 | 7 (70) | 3.8 | 6 (60) |
| *Pseudomonas aeruginosa* (9) | 13.9 | 7 (77.8) | 3.9 | 6 (66.7) |
| *Pseudomonas putida* (1) | 11.2 | 0 (0) | 3.0 | 0 (0) |
| ***Staphylococcus* spp.** (37) | 12.8 | 24 (64.8) | 3.4 | 5 (13.5) |
| *Staphylococcus aureus* (12) | 12.4 | 8 (66.7) | 3.4 | 2 (16.7) |
| *Staphylococcus hominis* (10) | 13.2 | 7 (70) | 3.6 | 1 (10) |
| *Staphylococcus epidermidis* (9) | 13.5 | 7 (77.7) | 3.2 | 1 (11.1) |
| *Staphylococcus haemolyticus* (4) | 12.0 | 2 (50) | 3.4 | 0 (0) |
| *Staphylococcus capitis* (2) | 12.7 | 0 (0) | 2.7 | 1 (50) |
| ***Streptococcus* spp./*Enterococcus* spp.** (38) | 12.6 | 33 (86.8) | 2.9 | 17 (44.7) |
| *Enterococcus faecium* (11) | 13.0 | 10 (90.9) | 2.7 | 5 (45.4) |
| *Enterococcus faecalis* (7) | 14.7 | 5 (71.4) | 3.5 | 3 (42.8) |
| *Streptococcus pneumoniae* (7) | 10.6 | 6 (85.7) | 2.9 | 4 (57.1) |
| *Streptococcus pyogenes* (5) | 11.3 | 5 (100) | 2.8 | 3 (69) |
| *Streptococcus anginosus* (3) | 13.3 | 3 (100) | 2.7 | 0 (0) |
| *Streptoptococcus* oralis (2) | 13.1 | 2 (100) | 2.0 | 0 (0) |
| *Enterococcus hirae* (1) | 11.0 | 1 (100) | 3.5 | 1 (100) |
| *Streptococcus agalactiae* (1) | 11.9 | 1 (100) | 3.0 | 1 (100) |
| *Streptococcus parasanguinis* (1) | 12.8 | 0 (0) | 2.5 | 0 (0) |
| ***Gemella morbillorum*** (2) | 13.7 | 0 (0) | 2.0 | 0 (0) |

spectra were acquired using the Microflex LT system (Bruker Daltonics, Bremen, Germany) and analyzed on MALDI BIOTYPER 3.3 (Bruker Daltonics) software. MALDI-TOF MS analyses were performed in triplicate on the same target slide. Criteria for successful identification were met when the spectral score of at least one of the three spots was ≥ 2.0 (species level) and ≥ 1.7 (genus level), as recommended by the manufacturer.

Discordant results were resolved using commercially available phenotypic methods (Vitek2, or API test strips, both from Biomeriuex, L'Etoile, France or BD Phoenix BD system, BD) or 16S rRNA gene sequencing.

## RESULTS

As shown in table 1, bacterial species in the training set were successfully identified in 75% of cases after pre-cultivation in BHI and 45% of cases by direct BC processing. ($P$=<0.001 by a chi-square test). Species accounting for this

difference were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus* spp. These results were largely reproduced in the validation set (table 2). In fact, the ENR method identified 82% of isolates to the species level, whereas only 49% could be identified by the DI method ($P$=<0.001 by a chi-square test). Both methods performed better with Gram-negative rods, particularly *Enterobacteriaceae*, than with Gram-positive cocci. The ENR method was more successful with all bacterial species included in the panel, except for *Staphylococcus capitis* (n=1). The DI method's performance was hampered by its failure to accurately identify a large number of Gram-positive cocci (*Staphylococcus* spp., 86.5%, *Enterococcus* spp., 52%, and *Streptococcus* spp., 57%).

All Gram-negative rods except *Pseudomonas putida* were successfully identified to the genus level by both methods. As for Gram-positive cocci, 5 (2 *Staphylococcus capitis*, 2 *Gemella morbillorum* and 1 *Streptococcus parasanguinis*) and 12 (4 *Staphylococcus haemolyticus*, 3 *Streptococcus anginosus,* 2 *Streptococcus oralis,* 2 *Gemella morbillorum* and 1 *Streptococcus parasanguinis*) were not accurately named to the genus level by the ENR and DI methods, respectively.

## DISCUSSION

A number of studies have evaluated DI methods avoiding blood cell lysis and protein extraction [2-5], and to our knowledge, only one of these used vacutainer separator gels tubes [5]. Overall, the rate of accurate identification to the species level varied widely across these studies (between 50% and 98%), although all consistently showed a better performance for Gram-negative than for Gram-positive bacteria. The disagreement among these studies may be attributable to the use of different starting volumes of blood for pelleting bacteria (1 to 6 ml), the speed of the centrifugation steps to remove blood cells, the spectrum of bacteria subjected to analysis, the platform used for MALDI-TOF MS analysis, and the stringency of the cut-off spectral scores for species identification.

Our study further emphasizes that DI methods struggle to identify Gram-positive bacterial species correctly (streptococci in particular), even when employing large volumes of blood; consequently, cell lysis and protein extraction prior to MALDI-TOF MS are warranted to enhance efficiency [8]. This does not appear to be the case for Gram-negative bacteria, in particular for those belonging to *Enterobacteriaceae*, for which the DI method performed reasonably well, in line with a previous study also using vacutainer separator gels tubes but perform reasonably well for *Enterobacteriaceae*, in line with a previous study also using vacutainer separator gels tubes [7]. Nevertheless, the DI method evaluated herein was clearly outperformed by our pre-enrichment method for identification of virtually all bacterial species included in the panels. The manipulation that is inherent to the pre-enrichment method we propose entails the risk of contamination, although this was negligible in our series, perhaps due to the training of the personnel in charge. In this sense, whether the simple incubation/agitation of positive blood culture bottles during a comparable time period, this practically minimizing the risk of contaminations, would have resulted in a bacterial identification rate comparable to that achieved by our method merits further investigation. Work addressing this issue is currently underway. Advantages of DI procedures in comparison with pre-enrichment methods include faster bacterial identification and lower risk of contamination by saprophytic bacteria. Whether or not the delay in bacterial identification has a tangible impact on patient outcomes remains to be investigated. Given the good performance of the DI method evaluated herein for the identification of *Enterobacteriaceae* and its rather limited success rate for identification of non-fermenting Gram-negative bacilli and Gram-positive cocci when compared to that of the pre-enrichment method, a mixed strategy, by which one method or another would be used according the bacterial morphotype observed on Gram stain smear preparations may find its place in the work flow for positive blood cultures.

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None to declare

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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