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Original Article

# A simple method for rapid microbial identification from positive monomicrobial blood culture bottles through matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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**Abstract** *Background and purpose:* Rapid identification of microbes in the bloodstream is crucial in managing septicemia because of its high disease severity, and direct identification from positive blood culture bottles through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can shorten the turnaround time. Therefore, we developed a simple method for rapid microbiological identification from positive blood cultures by using MALDI-TOF MS.
*Methods:* We modified previously developed methods to propose a faster, simpler and more economical method, which includes centrifugation and hemolysis. Specifically, our method comprises two-stage centrifugation with gravitational acceleration (*g*) at 600*g* and 3000*g*, followed by the addition of a lysis buffer and another 3000*g* centrifugation.
*Results:* In total, 324 monomicrobial bacterial cultures were identified. The success rate of species identification was 81.8%, which is comparable with other complex methods.

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The identification success rate was the highest for Gram-negative aerobes (85%), followed by Gram-positive aerobes (78.2%) and anaerobes (67%). The proposed method requires less than 10 min, costs less than US$0.2 per usage, and facilitates batch processing.
*Conclusion:* We conclude that this method is feasible for clinical use in microbiology laboratories, and can serve as a reference for treatments or further complementary diagnostic testing.


## Introduction

Patients who acquire bloodstream infections experience severe diseases. Orsi and Noah (2002)[1] reported a mortality rate of 35.2%—40.9% for hospital-acquired bloodstream infections. However, rapid microbial identification is helpful for treating septic patients; and also helps to minimize the length of hospital stays. As Beekmann (2003)[2] discovered in a study investigating the effect of positive blood culture identification turnaround time on hospital stay and cost, the average hospital stay was 18.1, 22.2, and 26.6 days when the turnaround time of positive blood culture identification was 24, 48, and 72 h, respectively. Shorter hospital stays are also more cost-effective.

Confirmation and treatment of bloodstream infections depend on blood culture results. New microbial identification techniques, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), have been demonstrated to effectively reduce the turnaround time of positive blood cultures.[3] In our hospital, determining whether a blood culture sample is infected takes an average of 17.1 h when using the BACTEC FX. Subsequently, 2.4 h are required for a Gram stain report, and then another 18 h is necessary for subcultures and identification to be made through MALDI-TOF MS. Although MALDI-TOF MS decreases the turnaround time by 24 h compared with traditional biochemical methods,[4] it still requires 37.5 h for completion. By contrast, direct microbial identification from positive blood culture bottles can shorten the turnaround time to less than 24 h.

Direct identification from positive blood cultures must be simple, fast, inexpensive, and can be easily adopted by clinical microbiology laboratories. Numerous protocols for direct microbial identification from positive blood culture broths, including commercial kits, have been developed.[5—11] However, these protocols are usually complex and entail either complicated preprocessing methods or subcultures to increase their accuracy,[12,13] which render these methods time- and cost-intensive.[5,6,8,7,14] In this study, we developed a simple, fast, economical and accurate method that is suitable for clinical microbiology laboratories. The identification results obtained using this simple method indicate that it is highly consistent with single colony identification. Moreover, Gram stain and preliminary strain identification can be reported within 24 h of placing the blood culture bottles in the culture system. We suggest that the results of this study can serve as a treatment reference for clinicians.

## Materials and methods

### Blood cultures

Three hundred and twenty four monomicrobial blood cultures were selected for this study from Chang Gung Memorial Hospital in Linkou, a 3715-bed tertiary medical center in northern Taiwan, between August 2013 to May 2014. All blood culture bottles were incubated in BACTEC FX (Becton Dickinson, Heidelberg, Germany), an automated continuous blood culture monitoring system. The blood cultures were collected using standard procedures, and positive samples were processed further.

### Processing positive blood cultures using MALDI-TOF MS

By using MALDI-TOF MS, pathogens were identified from positive blood cultures through a two-part process that comprises direct and lytic steps (Fig. 1). In Step 1, positive blood culture bottles were first shaken vigorously to ensure homogeneous mixing. Subsequently, each 1.5-mL sample was dispensed into two Eppendorf tubes (Fig. 2A) and centrifuged at 600 gravitational acceleration (*g*) for 10 s using a 45° fixed-angle rotor (Eppendorf 5415D) (Fig. 2B). Approximately 1.5 mL of each supernatant was then aspirated into a new Eppendorf tube and centrifuged at 3000*g* for 60 s (Fig. 2C). Next, the supernatant was discarded and the white layer of one of the Eppendorf tubes was picked using a toothpick and placed on a 96-spot polished steel target plate (Bruker Daltonik GmbH, Leipzig, Germany).

In Step 2, the white layer of the other Eppendorf tube was added on 1.5 mL of a lysis buffer (ratio = 8.29 g of $NH_4Cl$:0.037 g of Na:EDTA:1 g of $KHCO_3$: 1 L of water) and mixed homogeneously. After incubation for 3 min at room temperature, the mixture was centrifuged at 3000*g* for 60 s. Then, the supernatant was discarded, and the white layer (Fig. 2D) was picked up using a toothpick and placed on a 96-spot polished steel target plate (Bruker Daltonik GmbH, Leipzig, Germany).

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