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## ORIGINAL ARTICLE

# Performance Evaluation of an In-House Blood Culture Pretreatment Kit and MALDI-TOF Mass Spectrometry for Rapid Identification of Microorganisms

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**SUMMARY**

***Background:*** **The challenge in the use of MALDI-TOF MS for detection of microorganisms in culture-positive blood bottles is sample preparation. This study aimed to evaluate the accuracy of an in-house blood culture pretreatment kit coupled with MALDI-TOF MS system for directly identifying pathogens.**
***Methods:*** **A total of 114 blood samples were pre-enriched and identified using an in-house blood culture pretreatment kit and MALDI-TOF MS. The performance of the methods was compared to that of the conventional bacterial culture plus VITEK® 2 Compact.**
***Results:*** **The kit showed high identification rates for both Gram-negative and Gram-positive bacteria, compared to that of conventional bacterial culture ($p > 0.05$). The identification rate of Gram-negative bacteria using the serum separator tube method was significantly higher than that of Gram-positive bacteria and yeasts ($p < 0.05$).**
***Conclusions:*** **The kit can be used for the pretreatment of blood culture bottles, which can significantly shorten the identification and reporting times.**
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**KEYWORDS**

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## INTRODUCTION

Bloodstream infection is one of the most serious infections associated with high morbidity and mortality [1,2]. Rapid identification of pathogenic microorganisms and their associated resistance patterns is a prerequisite for rational antibiotic treatment and can reduce mortality from bloodstream infections [3,4]. At present, blood culture remains the gold standard for diagnosing bloodstream infections, and rapid and accurate identification of pathogens and correct initial antibiotic treatment are particularly important for sepsis [5,6].
In recent years, a new mass spectrometry technology for

the rapid detection of pathogenic bacteria has rapidly developed in clinical applications, characterized by rapidity and low cost. One of the key challenges in utilizing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the direct detection of microorganisms in culture-positive blood samples lies in the optimization of sample preparation techniques [7,8].

The Skyray MicroTyper MS (Jiangsu Skyray Biotechnology Co., Ltd., Jiangsu, China) is a novel MALDI-TOF MS instrument developed in 2015 that received community European-marked *in vitro* diagnostic (IVD) clearance in August 2020 [9]. Recently, the MicroTyper MS was evaluated for clinical isolates locally in China, and it showed comparable identification performance to the two main commercial MALDI-TOF MS systems available for routine use: V-K MS (VITEK, bioMerieux, Marcy I'Etoile, France) and MALDI Biotyper systems (Bruker Daltonics, Bremen, Germany) [10,11]. Kangmei blood culture preparation kit (Kangmei Biotechnology Co., Ltd., Shenzhen, China) is a recently developed pretreatment method to improve the performance of direct MicroTyper MS identification from positive blood culture, which was produced locally in China, with the aim of increasing sensitivity. However, the analytical performance of this assay has not been studied in large prospective clinical samples. Additionally, the performance of MicroTyper MS for the routine identification of microorganisms has not been extensively evaluated in clinical microbiology laboratories in China.

This study aimed to compare the diagnostic performance of in-house MicroTyper MS with that of V-K MS combined with a serum separator tube® (INSEPACK, ST535CG, Division of China, Sekisui MEDICAL Co., Ltd., Tokyo, Japan) and a blood culture pretreatment kit.

## MATERIALS AND METHODS

A total of 114 positive blood culture bottles were collected from our Clinical Microbiology Laboratory from April 2022 through December 2024, including 47 aerobic bottles, 37 anaerobic bottles, and 30 yeast samples. After positive cultures were obtained, routine microscopy was performed, followed by routine mass spectrometry and fully automated bacterial identification according to the reference [6,7]. A flowchart of the process is shown in Figure 1.

The kit consists of six parts: lysis solutions A and B, extraction solution, substrate solution, 75% ethanol, and sterile water. In summary, the procedure for preparing a positive blood culture specimen (1 mL) involves the following steps: First, the specimen is transferred to a 1.5 mL EP tube, followed by the addition of 200 µL of lysis solution A. The mixture is then vortexed for 10 seconds and incubated at room temperature for 3 minutes. Subsequently, it is centrifuged at 13,000 g for 1 minute, and the supernatant is carefully discarded. Next, the EP tube is centrifuged again at 13,000 g for 1 minute, and any remaining supernatant is removed. Lysis solution B is added to the samples, which are subsequently mixed thoroughly using a pipette and allowed to stand at room temperature for 2 minutes. An equal volume of extraction solution is added to the mixture and combined using a pipette. Using a micropipette, 1 µL of the super-natant is transferred to the sample spot on the MicroTyper MS target plate in the instrument for identification. Next, the following process was conducted using a published method [9]: V-K MS analysis was performed using V-K MS MALDI-TOF with MylaLab software (v. 4.8.2-0) and a database (v. 3.2). The MicroTyper MS test was performed to analyze the results using the MicroTyper with Acquirer version 3.0. The database contains the mass spectra of 3, 782 strains and 375 species. MicroTyper MS recommended cutoffs for identification are "acceptable species" if score $\geq$ 2.0, and "acceptable genus" if score $\geq$ 1.7. Meanwhile, we performed the serum separator tube® as a control [10]. If the pretreatment methods or device results do not match at the species level, sequence analysis was performed in consultation with a reference [11]. Statistical analysis was conducted using SPSS software version 20.0. The $\chi^2$-test, Fisher's exact probability test, or nonparametric tests were applied as appropriate. A p-value of less than 0.05 was considered to indicate a statistically significant difference.

## RESULTS AND DISCUSSION

The coincidence rate of the kit for Gram-negative bacteria was 100%, while it was 98.2% for the tube®; the coincidence rate of the kit for the Gram-positive bacteria was 96.42%, while it was 75% for the tube®; and the yeast identification in the tube® was 13.3% (4/30) (Table 1). The identification rate of yeasts using the tube® was lower. Two pretreatment methods were used to identify the detection rates of aerobic and anaerobic blood culture bottles: the detection rate of the kit for aerobic bottles was 100% (47/47) and that of anaerobic bottles was 97.29% (36/37), the detection rate of the tube® was 89.36% (42/47) in aerobic bottles and 86.48% (32/37) in anaerobic bottles ($p > 0.05$). The positive rate of the tube® was 13.3% in yeast bottles. Additionally, the overall identification rates of V-K MS and MicroTyper MS were 98.2% (112/114) and 97.36% (111/114), respectively, compared with the different pretreatment methods. The identification rates of V-K MS and MicroTyper MS were 91.2% (104/114) and 92.98% (106/114), respectively, after the pretreatment by the tube® ($p > 0.05$), as shown in Table 2. Both mass spectra were consistent with V-K 2 Compact data. There were 6 misidentifications produced by MicroTyper MS, and seven by VITEK MS using the tube®. Both instruments correctly identified four strains from all strains of yeasts at the genus level. Except for those

**Table 1. Comparison of strains directly identified by in-house MALDI-TOF MS system MicroTyper MS and Vitek MS (bioMeriux) with the kit method (%).**

| Species | MicroTyper MS | | Vitek MS | |
|---|---|---|---|---|
| | species | no detection | species | no detection |
| **Gram-negative bacteria (n = 56)** | **56** | **0** | **56** | **0** |
| ***Escherichia coli* (n = 29)** | **29** | **0** | **29** | **0** |
| ***Klebsiella pneumoniae* (n = 15)** | **15** | **0** | **15** | **0** |
| ***Klebsiella aerogenes* (n = 2)** | **2** | **0** | **2** | **0** |
| ***Enterobacter cloacae* (n = 4)** | **4** | **0** | **4** | **0** |
| ***Acinetobacter baumannii* (n = 2)** | **2** | **0** | **2** | **0** |
| ***Citrate bacteria* (n = 1)** | **1** | **0** | **1** | **0** |
| ***Pseudomonas aeruginosa* (n = 3)** | **3** | **0** | **3** | **0** |
| **Gram positive bacteria (n = 28)** | **25** | **2** | **27** | **1** |
| ***Staphylococcus hominis* (n = 9)** | **9** | **1** | **8** | **1** |
| ***Staphylococcus aureus* (n = 8)** | **8** | **0** | **8** | **0** |
| ***Staphylococcus epidermidis* (n = 5)** | **4** | **1** | **5** | **0** |
| ***Streptococcus agalactiae* (n = 2)** | **2** | **0** | **2** | **0** |
| ***Enterococcus* (n = 4)** | **4** | **0** | **4** | **0** |

**Table 2. Comparison of strains directly identified by in-house MicroTyper MS and Vitek MS (bioMeriux) with blood separation tube method (%).**

| Species | MicroTyper MS | | Vitek MS | |
|---|---|---|---|---|
| | species | no detection | species | no detection |
| **Gram-negative bacteria (n = 56)** | **55** | **0** | **56** | **0** |
| ***Escherichia coli* (n = 29)** | **29** | **0** | **29** | **0** |
| ***Klebsiella pneumoniae* (n = 15)** | **15** | **0** | **15** | **0** |
| ***Pneumogenic klebsiella* (n = 2)** | **2** | **0** | **2** | **0** |
| ***Enterobacter cloacae* (n = 4)** | **4** | **0** | **4** | **0** |
| ***Citrate bacteria baumanii* (n = 2)** | **2** | **0** | **2** | **0** |
| ***Citrate bacteria* (n = 1)** | **1** | **0** | **1** | **0** |
| ***Pseudomonas aeruginosa* (n = 3)** | **3** | **0** | **3** | **0** |
| **Gram-positive bacteria (n = 28)** | **22** | **6** | **21** | **7** |
| ***Staphylococcus hominis* (n = 9)** | **6** | **3** | **5** | **4** |
| ***Staphylococcus aureus* (n = 8)** | **7** | **1** | **7** | **1** |
| ***Staphylococcus epidermidis* (n = 5)** | **3** | **2** | **3** | **2** |
| ***Streptococcus agalactiae* (n = 2)** | **2** | **0** | **2** | **0** |
| ***Enterococcus* (n = 4)** | **4** | **4** | **4** | **0** |
| **Yeasts (n = 30)** | **4** | **26** | **4** | **26** |
| ***Candida albicans* (n = 10)** | **1** | **9** | **1** | **9** |
| ***Candida glabrata* (n = 10)** | **2** | **8** | **2** | **8** |
| ***Candida tropicalis* (n = 10)** | **1** | **9** | **1** | **9** |

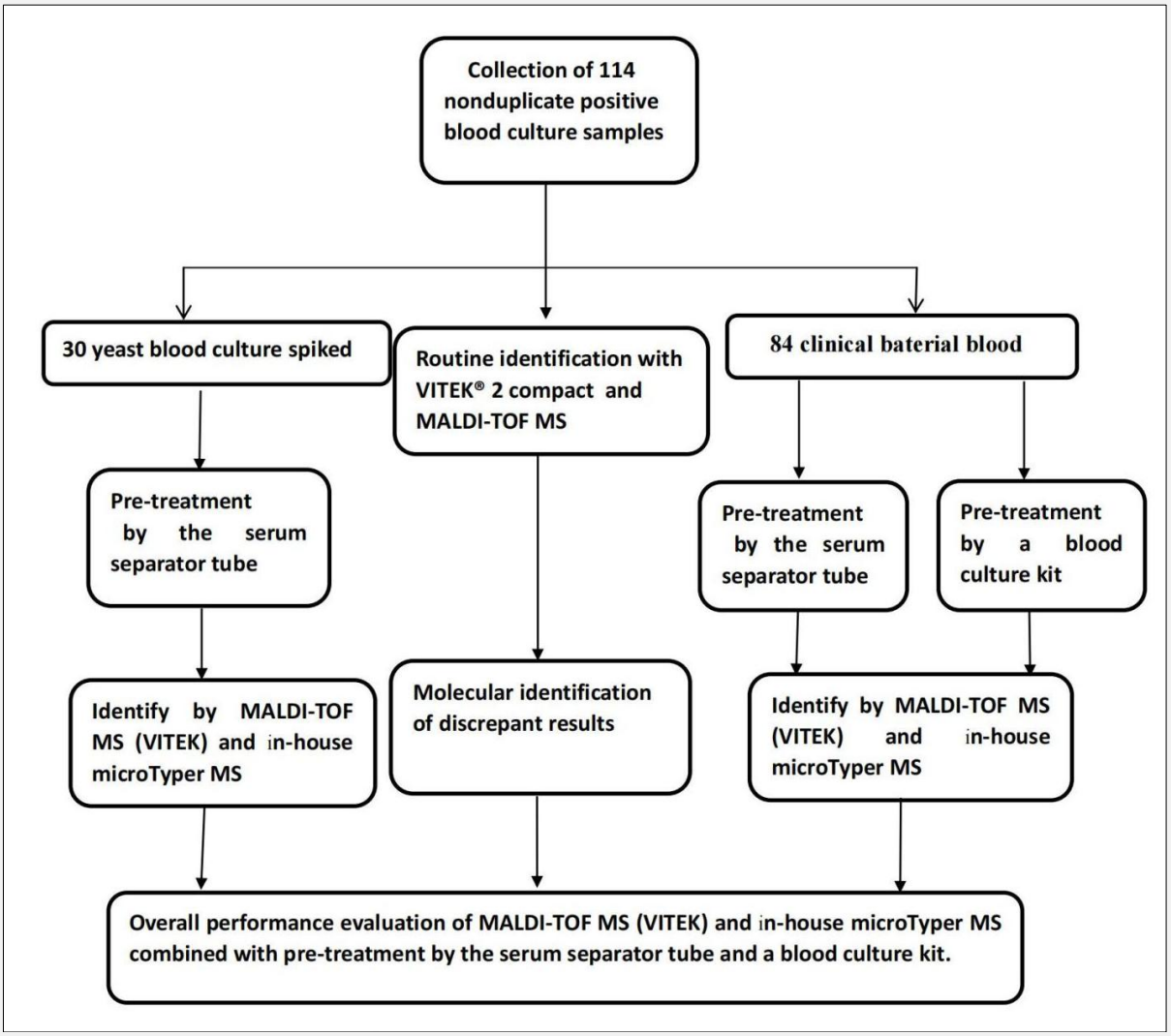


**Figure 1. Flowchart of the study design.**

strains, genus-level findings were consistent with the species results.

There were 2 misidentifications produced by MicroTyper MS and one by V-K MS with a blood culture pretreatment kit. The strain incorrectly identified was *Staphylococcus hominis* and *Staphylococcus epidermidis* by MicroTyper MS, and one strain of *Staphylococcus hominis* by V-K MS. There were 6 misidentifications produced by MicroTyper MS, and seven by VITEK MS using a blood separation tube method. Three strains of *Staphylococcus hominis,* one strain of *Staphylococcus aureus,* and two strains of *Staphylococcus epidermidis*. In addition, the instrument correctly identified all the *Enterococcus* at the genus level. Four strains of *Staphylococcus hominis,* one strain of *Staphylococcus aureus,* and two strains of *Staphylococcus epidermidis* were identified by V-K MS. Both instruments correctly identified four strains from all strains of yeasts at the genus level. Except for those strains, genus-level findings were consistent with the species results.

We conducted a side-by-side comparative performance evaluation of two blood culture pretreatment methods and commercial MALDI-TOF MS systems. The average identification rate using the kit was higher than that achieved using the tube®. The kit showed a high identification rate for both the Gram-negative and -positive bacteria. The identification rate of Gram-negative bacteria was significantly higher than that of Gram-positive bacteria using the tube®. The low identification rate of

the tube® for Gram-positive cocci indicated that the kit was superior. The tube® is based on centrifugal precipitation to enrich the bacterial liquid [12]; the enriched bacteria are then treated without blood cell fragmentation. This indicates that the quality and performance of MicroTyper MS can meet clinical requirements in China [13,14].

In general, our findings demonstrate that both V-K MS and MicroTyper MS perform comparably in the identification of common microorganisms encountered in clinical laboratories. They possess similar methodologies and no statistical differences in the number of isolates identified, misidentified, or not identified by either system [15,16]. The kit has several advantages, including low cost and strong practicality, and it requires only a low-speed centrifuge. The detection was fast, and the turnover time was short (15 minutes) for extracting pathogenic bacteria from blood culture bottles, and the extracted purity was high. In addition, without excessive transfer, washing, and other processes, it is not easy to cause biological safety risks such as aerosols. However, the shortcoming was that the extracted purity with the tube was not high [17].

## CONCLUSION

In-house pretreatment kit of blood culture kit was found to be equivalent to VITEK® MS kit and superior to the serum separator tube® in direct identification of microbes from positive blood cultures by MicroTyper MS analysis. The kit can be used for the pretreatment of positive blood culture bottles. It has a higher identification rate, shorter identification cycle, and is simpler to operate than the serum separator tube®. As a result of this study, we have integrated direct MicroTyper MS-based identification from positive blood cultures into our routine diagnostic workflow.

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**Data Availability Statement:**
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Ethical Approval Statement:**
This study, the collection of the clinical samples, was approved by the institutional ethics committees of the participating hospitals (protocol number 2022-04), and written informed consent was obtained from all participants of the study beforehand.

**Declaration of Interest:**
The authors declare that they have no competing interests.

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