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Critical Points of Direct Pathogens Identification by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Methods

Masafumi Seki[1*], **Maya Hariu**[2] **and Yuji Watanabe**[2]

[1]*Division of Infectious Diseases and Infection Control, Tohoku Medical and Pharmaceutical University Hospital, Sendai City, Miyagi, Japan*

[2]*Laboratory for Clinical Microbiology, Tohoku Medical and Pharmaceutical University Hospital, Sendai City, Miyagi, Japan*

[*]**Corresponding author:** Masafumi Seki, Division of Infectious Diseases and Infection Control, Tohoku Medical and Pharmaceutical University Hospital, Sendai City, Japan, Tel: +81-22-983-1221; E-mail: m-seki@tohoku-mpu.ac.jp

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Abstract

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (TOF MS) is now widely used to detect pathogens in clinical settings in the world. However, there are some critical points, including polymicrobial samples handling and the kinds of lysis buffer in the protocol of direct identification of specific pathogens from blood culture samples.

The infecting bacteria were not correctly identified in many polymicrobial samples although all monomicrobial samples were detected by TOF MS, however, if the culture ratio were changed, two pathogens were correctly detected.

Furthermore, in the effects of adding lysis buffer in the TOF MS method to directly detect bacteria from three blood culture systems, three types of blood culture broths showed similar detection efficiencies without lysis buffer use and most of gram negative rods were efficiently detected in all broths when lysis buffer was used. However, Streptococcus pneumoniae was not detected in BD broth when lysis buffer was added. Furthermore, Haemophilus influenzae and Bacteroides fragilis were not detected in all three systems when lysis buffer was used.

These results suggested that TOF-MS is a strong tool for the rapid and correct detection of pathogens from blood culture samples, although results need to be carefully checked when handling known or suspected polymicrobial samples, and optimization of blood culture system and lysis buffer dependent on the pathogens is necessary according to each pathogen for direct identification by TOF MS methods.

Keywords: Blood cultures broth; Lysis buffer; Polymicrobial samples

Introduction

Sepsis often presents as multiple-organ dysfunction and bacteremia is typically diagnosed by microbiological tests, including blood cultures (BCs). However, the pathogens in the blood are detected in only 4% – 12% of all BCs and identification of the pathogens by BC usually take 2 – 3 days [1,2]. Therefore, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (TOF MS) has been recently applied because it allows the identification of most pathogenic bacteria and fungus grown in BC bottle directly within a few minutes and has been proven efficiency and reproducibility [3-5].

However, there have been several problems, including decreasing the pathogen detection in the polymicrobial samples and the effects of adding lysis buffer in the process of direct pathogen detection and found some bacteria could not be detected in certain BC systems.

Handling of Polymicrobial Samples

It was reported that TOF MS analysis did not produce scores high enough for species identification in two *bacteremia* cases that presented with diverticular diseases; instead, the infecting bacteria were identified by the sequencing method [6] as we have previously reported [7-9].

We demonstrated the identification of bacteria from BCs using MALDI-TOF BioTyper, which allowed 95.5% correct, single-step identifications among a total of 20 microorganisms from 66 clinical blood samples, including 3 polymicrobial samples, starting from small volumes of BC. Monomicrobial samples were correctly identified at the species level in 100% of cases. All bacteria were identified within the first 2-3 h following BC positivity.

Therefore, for polymicrobial samples, the observed profile may represent the mixed profiles of two distinct bacteria, with both showing significant scores. Such a situation will require closer examination in the TOF-MS context. In these cases, the corresponding BCs will need to be carefully checked at the next isolation plate (typically grown for testing antimicrobial susceptibility), to distinguish the presence of additional bacterial isolates for subsequent identification, if necessary. This follow-up evaluation may help to validate the initial status of the blood samples, if not precluded earlier by Gram staining.

Christner et al. reported that BioTyper scores exceeding 1.5 were essential for the identification of 8% of the isolates, but that work did not consider the possibility of polymicrobial samples. Mossaoui et al. tested a new protocol for bacterial identification from BC broths, but only 10 of a total of 50 isolates from 21 polymicrobial samples were identified in that work. La Scola et al. reported the identification of only one of each mixture of species for 18 samples among 22 bacteria-

positive BC broths that contained two or more different species. No species was identified in two of those polymicrobial samples, and false species identifications were obtained in two cases. Using an in-house saponin lysis method (in place of the MALDI Sepsityper kit), Meex et al. were able to identify only one of each pair of isolates in six separate polymicrobial BCs. These results suggest that the identification by TOF-MS of two or more bacteria in polymicrobial samples is a challenge. Therefore, if the presence of more than one pathogen is suspected, it may be better to try to test the various mixture ratios as we previously reported (Table 1) [10].

Combinations	Ratio											
	1:9	1:8	1:7	1:6	1:5	1:4	1:3	1:2	1:1	2:1	3:1	1:4
1	b	b	b	b	b	both	both	both	both	both	both	a
2	Both	both	both	both	both	Both	both	both	a	a	a	a
3	d	d	d	d	d	both	both	both	a	a	a	a
4	e	both	both	both	both	both	both	both	a	a	a	a
5	f	f	f	f	f	f	both	both	both	a	a	a
6	both	both	both	both	both	both	both	both	both	both	both	b
7	d	both	both	both	both	both	both	both	both	both	both	b
8	e	e	e	e	both	both	both	both	both	b	b	b
9	f	f	f	both	both	both	both	both	both	both	both	b
10*	g	g	g	g	g	g	g#	both##	both	both	both	b
11	d	d	d	d	d	d	both	both	both	both	both	c
12	e	e	e	e	e	both	both	both	c	c	c	c
12**	f	f	f	f	f	f	both	both	both	both	both	c
14	e	e	e	e	e	both	both	both	d	d	d	d
15	f	both	both	both	both	both	both	both	both	both	d	d
16	f	f	f	f	f	f	both	both	both	both	e	e
17	g	g	g	g	g	g	g	g	g	both	both	F

a. E.coli; *b. P.aeruginosa*; *c. Ec.faecalis*; *d. S.aureu*; *e. S.pneumoniae*; *f. S.epidermidis*; *g. E.cloacae*; 1=a:b, 2=a:c, 3=a:d, 4=a:e, 5=a:f, 6=b:c, 7=b:d, 8=b:e, 9=b:f, 10=b:g, 11=c:d, 12=c:e, 13=c:f, 14=d:e, 15=d:f, 16=e:f, 17=f:g

Table 1: Detected bacteria in various combination ratios by TOF-MS.

The Effects of Lysis Buffer

To avoid any delay and misdiagnosis in bacterial identification during TOF MS analysis, specialized software such as Biotyper can be used, which has been shown to permit high-quality microbial identification, and some methods including RBC lysis have been performed [11,12].

We demonstrated the effects of adding lysis buffer in combination with several BC systems. BC broths from BD, bioMérieux, and Oxoid were prepared, and bacterial detection rates and MALDI-TOF MS scores were similar with and without lysis buffer for representative bacteria, such as E. coli, S. pneumoniae, and H. influenza (Table 2) [13].

	BD		BioMerieux		Oxoid	
	(LB+)	(LB-)	(LB+)	(LB-)	(LB+)	(LB-)
Escherichia coli	2.360 ± 0.215	2.012 ± 0.114	2.315 ± 0.157	1.779 ± 0.142	2.419 ± 0.257	2.319 ± 0.121
Klebsiella pneumoniae	2.488 ± 0.212	1.889 ± 0.256	2.578 ± 0.223	2.008 ± 0.114	2.368 ± 0.222	2.177 ± 0.165
Pseudomonas aeruginosa	2.344 ± 0.197	1.653 ± 0.196	2.268 ± 0.212	None	2.434 ± 0.275	2.225 ± 0.253

Streptococcus pneumoniae	None	None	2.295 ± 0.534	None	2.212 ± 0.266	2.006 ± 0.217
Haemophilus influenzae	None	1.621 ± 0.188	None	1.590 ± 0.171	None	1.977 ± 0.111
Bacteroides fragilis	None	1.789 ± 0.175	None	1.921 ± 0.178	None	2.280 ± 0.197
The number indicated the mean ± SD score of each case						

Table 2: Detection efficiency of bacteria from blood culture broth by TOF-MS with or without lysis buffer.

For E coli, addition of lysis buffer led to clearer detection of *E. coli* (i.e., increased MALDI-TOF MS scores) compared with analysis without the RBC lysis step. This finding may be because lysis buffer inhibits the effects of RBC mixing. In fact, the composition of BC broth, incubation atmosphere, and bacterial extraction method play a key role in the quality of further direct identification [5,14] and an in house procedure for bacterial separation from BC broth using saponin has been developed [15]. Similar results were observed for *K. pneumoniae*, and more effective detection of *P. aeruginosa* was found in our study when we used lysis buffer in MALDI-TOF MS analysis directly from BC.

However, *S. pneumoniae* was not detected by MALDI-TOF MS using the BD BC system when lysis buffer was added, and we could not detect any bacilli in this BC broth. However, regardless of the extraction method used in MALDI-TOF MS analysis, Gram-positive cocci are generally more difficult to detect than Gram-negative rods. Lysis buffer may destroy not only RBCs but also Gram-positive cocci, although Gram-positive cocci contain capsular polysaccharides and peptidoglycans. Moreover, Gram-positive cocci may be more permeable than Gram-negative rods that containing lipopolysaccharides and outer membranes. In addition, we could not detect *S. pneumoniae* without lysis buffer, suggesting that the lysis protocol may need to be optimized when using the BD BC system. Furthermore, *S. pneumoniae* were not identified without lysis buffer in the bioMérieux BC system, indicating the necessity of the lysis step in *S. pneumoniae* detection, similar to *P. aeruginosa*.

H. influenzae and *B. fragilis* were not detected in all three BC systems when lysis buffer was added, although we used Oxoid broth, which was the only system that could detect both *S. pneumoniae* and *P. aeruginosa*. Therefore, it may be better to use the pellet without resuspension as TOF MS samples to avoid the risk of lysis buffer and RBC effects as we previously reported [13].

Conclusion

TOF-MS technique yielded valid identification for greater than 95% bacteria derived from monomicrobial and polymicrobial BC samples. Even in polymicrobial cases, analysis of corrected mixture ratios of combinations of the candidate infecting bacteria may facilitate detection of individual species.

Moreover, detection of some bacteria in particular BC broths may be improved by adding a lysis step especially for the gram negative pathogens, including E coli, *K. pneumoniae*, and *P. aeruginosa*. However, the caution may be needed for the detection process of *S. pneumoniae, H. influenza* and *B. fragilis*. Protocols for individual species should be optimized to improve pathogen identification by MALDI-TOF MS analysis.

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