MALDI-TOF MS versus VITEK 2 ANC card for identification of anaerobic bacteria

Yang Li[1,2*], Bing Gu[1,2*], Genyan Liu[1,2], Wenying Xia[1,2], Kun Fan[1,2], Yaning Mei[1,2], Peijun Huang[1,2], Shiyang Pan[1,2]

[1]Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; [2]National Key Clinical Department of Laboratory Medicine, Nanjing 210029, China

*These authors contributed equally to this work.

Correspondence to: Genyan Liu. Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Guangzhou Road, No. 300, Nanjing 210029, China. Email: liugenyan@njmu.edu.cn.

Background: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an accurate, rapid and inexpensive technique that has initiated a revolution in the clinical microbiology laboratory for identification of pathogens. The Vitek 2 anaerobe and *Corynebacterium* (ANC) identification card is a newly developed method for identification of corynebacteria and anaerobic species. The aim of this study was to evaluate the effectiveness of the ANC card and MALDI-TOF MS techniques for identification of clinical anaerobic isolates.

Methods: Five reference strains and a total of 50 anaerobic bacteria clinical isolates comprising ten different genera and 14 species were identified and analyzed by the ANC card together with Vitek 2 identification system and Vitek MS together with version 2.0 database respectively. 16S rRNA gene sequencing was used as reference method for accuracy in the identification.

Results: Vitek 2 ANC card and Vitek MS provided comparable results at species level for the five reference strains. Of 50 clinical strains, the Vitek MS provided identification for 46 strains (92%) to the species level, 47 (94%) to genus level, one (2%) low discrimination, two (4%) no identification and one (2%) misidentification. The Vitek 2 ANC card provided identification for 43 strains (86%) correct to the species level, 47 (94%) correct to the genus level, three (6%) low discrimination, three (6%) no identification and one (2%) misidentification.

Conclusions: Both Vitek MS and Vitek 2 ANC card can be used for accurate routine clinical anaerobe identification. Comparing to the Vitek 2 ANC card, Vitek MS is easier, faster and more economic for each test. The databases currently available for both systems should be updated and further developed to enhance performance.

Keywords: Anaerobe; matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS); Vitek 2 anaerobe and *Corynebacterium* card (Vitek 2 ANC card); identification

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Introduction

Anaerobic bacteria are a significant component of human mucous membranes bacterial flora, and often are the causative agent of respiratory, gastrointestinal and female genital tract infections and bacteremia. Anaerobes that combine with aerobic bacteria can cause serious mixed infections, and they are frequently overlooked. Rapid and accurate identification of anaerobes play an important role in timely and appropriate treatments.

Conventional identification of anaerobes has long been

mainly based on the detection of phenotypic characteristics, such as Gram staining, colony morphology, microscopic examination, differential growth on selective media and various manual biochemical tests. Most of these conventional methods are laborious and time-consuming processes. While the development and popularization of automated and semi-automated systems continue for the identification of isolates, clinical application studies using these systems to identify anaerobes are limited. This is especially the case for the newly developed Vitek 2 anaerobe and *Corynebacterium* (ANC) card (bioMérieux, France) and the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) system.

To expand the capabilities for identification of corynebacteria and anaerobic species on the Vitek 2 system, bioMérieux, Inc. has developed the new ANC card. Previous studies have confirmed that the Vitek 2 ANC card is a simple, rapid, and satisfactory method for the identification of anaerobes in a clinical microbiology laboratory (1-3). However, less information is provided in evaluating the Vitek 2 ANC card comparing to other identification systems (4). The ANC card has a database that includes 49 taxa of anaerobic bacteria belonging to the genera *Actinomyces*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Collinsella*, *Eggerthella*, *Eubacterium*, *Finegoldia*, *Fusobacterium*, *Lactobacillus*, *Parabacteroides*, *Parvimonas*, *Peptoniphilus*, *Peptostreptococcus*, *Prevotella*, *Propionibacterium*, and *Veillonella*. *Bifidobacterium spp.* and *Veillonella spp.* are identified only at the genus level in this system. Identification is accomplished within approximately 6 h incubation time using a 64 microwell card that contains dehydrated biochemical substrates.

MALDI-TOF MS technology for the identification of bacteria is now gaining increased attention due to its accurate, inexpensive and rapid performance efficiencies (5-9). This technique is a soft ionization method, which allows desorption of peptides/proteins from both whole different cultured bacteria and crude bacterial extracts (10). Identification is based on the comparison of the tested isolate mass spectrum to a reference database. Bruker MS and Shimadzu MS, two types of MALDI-TOF MS systems, were frequently studied for anaerobic bacteria identification, proving varied rates of identification for anaerobes (11-14). The majority of previous studies have compared MALDI-TOF identification with conventional identification or reference standard methods simply for the identification of anaerobic bacteria (15-21).

In this study, we evaluated the Vitek MS and the Vitek 2 ANC card for the identification of most common clinical anaerobic isolates. 16S rRNA gene sequencing was used as a reference method (22).

Materials and methods

Bacterial strains and culture condition

A total of 50 fresh and frozen anaerobic clinical isolates comprising ten different genera and 14 species were included in the study. All isolates were recovered from routine examination of clinical specimens submitted to the First Affiliated Hospital of Nanjing Medical University. The five reference strains, *Bacteroides fragilis* ATCC 25285, *Clostridium difficile* ATCC 43255, *Propionibacterium acnes* ATCC 11827, *Propionibacterium acnes* ATCC 6919 and *Lactobacillus acidophilus* ATCC 4356, were tested routinely. Prior to testing, all strains were subcultured twice onto Columbia blood agar (bioMérieux, Shanghai, China) and incubated in an anaerobic atmosphere produced by GENbag (bioMérieux, Shanghai, China) for 48 h at 35 ℃.

Vitek 2 ANC card

Bacterial colonies were suspended in 0.45% sodium chloride with a turbidity of 2.7-3.3 McFarland. Inoculums were then introduced into an ANC card in the Vitek 2 Compact automated identification system and incubated for approximately 6 h. Through the three additional tests of Gram staining, cell morphology, and aerotolerance testing, the Vitek 2 system deduced interpretations for final identifications. Isolates initially resulting in no identification were retested.

MALDI-TOF MS system

Vitek MS is an automated microbial identification system based on MALDI-TOF technology. Each isolate was directly smeared onto a disposable target slide and then covered by a small drop of matrix solution (Vitek MS-CHCA) and air dried. The loaded slide was then inserted into the Vitek MS system. The quality standard performed on each group was a spot of *E. coli* ATCC 8739. Microbial identification is achieved by obtaining a composite mass spectrum using MALDI-TOF technology and comparing the sample spectra to the reference spectra contained within the Vitek MS version 2.0 database.

Table 1 Reference strains identified by Vitek MS and Vitek 2 ANC card

Reference strains	Identification of isolates by	
	Vitek 2 ANC card	Vitek MS
ATCC 25285 *Bacteroides fragilis*	*B. fragilis*	*B. fragilis*
ATCC 43255 *Clostridium difficile*	*C. difficile*	*C. difficile*
ATCC 4356 *Lactobacillus acidophilus*	*L. acidophilus*	*L. acidophilus/L. gasseri*
ATCC 11827 *Propionibacterium acnes*	*P. acnes*	*P. acnes*
ATCC 6919 *Propionibacterium acnes*	*P. acnes*	*P. acnes*

Vitek MS, Vitek mass spectrometry; Vitek 2 ANC card, Vitek 2 anaerobe and *Corynebacterium* card.

16S rRNA gene sequencing

When discrepancies in identification were observed between the VITEK 2 ANC card and the VITEK MS system, or no identification achieved in both, 16S rRNA gene sequencing was used to confirm the result. Bacterial DNA was extracted using the TIANamp Bacteria DNA kit (TIANGEN, Beijing, China), and the sequencing reactions were performed with a 16S rDNA Bacterial Identification PCR Kit (Takara, Dalian, China) according to the manufacturer's instructions. PCR products were purified and sequenced by Genscript Corporation. The obtained 16S rRNA gene sequences were subjected to BLAST analysis against the NCBI nucleotide database.

Data analysis

Data obtained were classified into the following categories: (I) correct identification to the species level; (II) correct identification to the genus level, including a multiple choice within the same genus; (III) low discrimination, between two or more species, including the correct species requiring additional tests; (IV) no identification; and (V) misidentification, the final identification in which the genus or species were incorrect compared to that of the reference 16S rRNA gene sequence.

Results

In this study, five ATCC reference strains were selected for evaluating the accuracy of Vitek MS and Vitek 2 ANC card. All of these five reference strains were identified routinely and showed consistent results except for ATCC 4356 *Lactobacillus acidophilus* (*Table 1*). The Vitek MS cannot provide the accurate result of *Lactobacillus acidophilus* at the species level, but rather only provides a selection of low discrimination identification between *Lactobacillus acidophilus* and *Lactobacillus gasseri*, while ANC card can identify the *Lactobacillus acidophilus* accurately.

To further evaluate the capability of Vitek MS and Vitek 2 ANC card, we selected 50 clinical isolates from our clinical anaerobe bank. Each strain was identified with the two systems respectively. For the 50 isolates belonging to ten genera and 14 different species (*Table 2*), Vitek MS provided correct identification for 46 (92%) isolates to species level, 47 (94%) isolates to the genus level, but one (2%) with low discrimination, two (4%) with no identification, and one (2%) with misidentification. In comparison, the Vitek 2 ANC card achieved 43 (86%) correct identification to the species level, 47 (94%) correct identification to the genus level, three (6%) low discrimination, three (6%) no identification, and one (2%) misidentification.

As seen in *Table 3*, there are eight discrepant results among the 50 pairs of identifications produced by Vitek MS and Vitek 2 ANC card. All of these discrepant strains were confirmed by 16S rRNA gene sequencing. One minor error by Vitek ANC card was an identification of *Bacteroides stercoris* instead of *Bacteroides thetaiotaomicron*. *Bacteroides vulgatus* displayed mixed genera identification of *Bacteroides eggerthii* and *Bacteroides vulgatus* by the Vitek MS system. The Vitek MS produced superior accurate results for identification of *Clostridium difficile* while the Vitek 2 ANC card performed low discrimination consisting of *Clostridium spp.* of three isolates of *Clostridium difficile*. *C. difficile* requires further tests of indole and lipase for distinction from *C. bifermentans* and *C. sporogenes*. Our results also revealed that *Anaerococcus tetradius* was not included in the Vitek MS database and was misidentified as *Brevibacillus spp*. The Vitek 2 ANC card cannot identify this species too. Moreover, two strains of *Parabacteroides goldsteinii* were not identified by either system.

Table 2 Clinical isolates identified by Vitek MS and Vitek 2 ANC card

Organisms [No. of isolates]	No. [%] of isolates identified by									
	Vitek 2 ANC Card					Vitek MS				
	Correct identification to		Low discrimination	No identification	Mis-identification	Correct identification to		Low discrimination	No identification	Mis-identification
	Species level	Genus level				Species level	Genus level			
Gram-negative bacilli [35]	32	33	0	2	1	32	33	1	2	0
Bacteroides spp. [31]	30	31	0	0	1	30	31	1	0	0
B. Fragilis [26]	26	26	0	0	0	26	26	0	0	0
B. Thetaiotaomicron [3]	3	3	0	0	0	3	3	0	0	0
B. Stercoris [1]	0	1	0	0	1	1	1	0	0	0
B. Vulgatus [1]	1	1	0	0	0	0	1	1	0	0
Parabacteroides spp. [3]	1	1	0	2	0	1	1	0	2	0
P. Distasonis [1]	1	1	0	0	0	1	1	0	0	0
P. goldsteinii [2]	0	0	0	2	0	0	0	0	2	0
Fusobacterium spp. [1]										
F. Nucleatum [1]	1	1	0	0	0	1	1	0	0	0
Gram-positive cocci [6]	5	5	0	1	0	5	5	0	0	1
Peptostreptococcus anaerobius [2]	2	2	0	0	0	2	2	0	0	0
Parvimonas micra [2]	2	2	0	0	0	2	2	0	0	0
Finegoldia magna [1]	1	1	0	0	0	1	1	0	0	0
Anaerococcus tetradius [1]	0	0	0	1	0	0	0	0	0	1
Non-spore forming Gram-positive bacilli [2]	2	2	0	0	0	2	2	0	0	0
Collinsella aerofaciens [1]	1	1	0	0	0	1	1	0	0	0
Propionibacterium acnes [1]	1	1	0	0	0	1	1	0	0	0
Spore forming Gram-positive bacilli [7]										
Clostridium difficile [7]	4	7	3	0	0	7	7	0	0	0
Cumulative totals [50]	43 [86]	47 [94]	3 [6]	3 [6]	1 [2]	46 [92]	47 [94]	1 [2]	2 [4]	1 [2]

Vitek MS, Vitek mass spectrometry; Vitek 2 ANC card, Vitek 2 anaerobe and *Corynebacterium* card.

Table 3 Discrepant results between Vitek MS and Vitek 2 ANC card confirmed by 16s rRNA gene sequencing

Organism identified by DNA sequencing	GenBank accession number	Identification of isolates by	
		VITEK 2 ANC card	VITEK MS
Parabacteroides goldsteinii	KJ130487	No identification	No identification
	KJ130488	No identification	No identification
Anaerococcus tetradius	KJ130489	No identification	*Brevibacillus spp.*
Bacteroides stercoris	KJ130490	*B. thetaiotaomicron*	*B. stercoris*
Bacteroides vulgatus	KJ101610	*B. vulgatus*	*B. eggerthii, B.vulgatus*
Clostridium difficile	KJ130491	*C. difficile, C. bifermentans, or C. sporogenes*	*C. difficile*
	KJ130492	*C. difficile, C. bifermentans, or C. sporogenes*	*C. difficile*
	KJ130493	*C. difficile, C. bifermentans, or C. sporogenes*	*C. difficile*

Vitek MS, Vitek mass spectrometry; Vitek 2 ANC card, Vitek 2 anaerobe and *Corynebacterium* card.

Discussion

Up to now, clinical anaerobe identification is still a time consuming and skilled process. There are a few automated systems or rapid identification reagents presently available for clinical laboratory use. The Vitek 2 ANC card and the Vitek MS are two new developed methods for anaerobe identification. Whether if they can improve clinical anaerobe identification? Which system should be better for clinical use? No study has answered these questions till now. In this study, five reference strains and 50 clinical isolates were selected to evaluate the two commercial automated systems in identifying anaerobic bacteria. From the results contained in *Tables 1*,*2*, we can see that both the ANC card and the Vitek MS can identify most of the reference strains and clinical isolates accurately. For the 50 clinical anaerobe strains, the two identification systems achieved the same percentage (94%) of correct identification at the genus level, which meets the requirements of clinical routine anaerobe identification. However, at the species level, Vitek MS got a considerably higher rate of identification accuracy of 92% compared to the Vitek ANC card of 86%. This data revealed that the Vitek MS is superior to the ANC card for species identification. Our results using Vitek MS are similar to a recent multi-centre evaluation of anaerobic bacteria, which exhibited 92.5% of correct identification at the genus level and 91.2% at the species level (15). Comparable results of Vitek ANC card were presented with 98.5% accuracy rate at genus level and 86.5% at species level by Francine Mory *et al.* (2). Moreover, both systems in our study generated excellent results in achieving 100% correct identification to the species level for *Bacteroides fragilis*.

However, three of seven of *Clostridium difficile* gave low-discrimination identification results at the species level when using ANC card. These findings are consistent with the previous work stating that the identification of *Clostridia* at the species level using the ANC card should be enhanced (1,2). But by contrast, 100% of *Clostridium difficile* isolates were identified correctly to the species level using Vitek MS. Among the 8 sequenced strains, there are four sequence results supporting the Vitek MS identification results, and only one isolate matches Vitek 2 ANC card identification result at species level, as shown in *Table 3*. Unsuccessful identification of *Anaerococcus tetradius* and *Parabacteroides goldsteinii* with both of the identification systems is due to the species not being included in the database. The reliability of the identification depends on the quality and composition of the reference spectra present in the database (3,11,23,24). The databases currently available for both systems need to be optimized with more spectra for certain genera and species and the very rare species need to be included to increase the identification capability of both automated systems.

As we known, only one anaerobe colony is required for identification using Vitek MS while bacterial turbidity need to be 2.7-3.3 McFarland using Vitek 2 ANC card. For slow growing of most anaerobic bacteria, the Vitek ANC card needs at least 24 h longer incubation time than the Vitek MS. On the other hand, all the isolates we studied can be identified on the first attempt by Vitek MS while more than 20% of the isolates had to be identified on a second or third attempt for discrepancies or no identification results were obtained by Vitek 2 ANC card. All these may increase the costs and extend the turnaround time of each test on the Vitek 2 ANC card additionally. A recent cost assessment study also demonstrated that the MALDI protocol provided

identifications 1.45 days earlier on average and can reduce reagent and labor costs of identification by $102,424, or 56.9% less when compared with standard protocols (25). Besides the advantages mentioned before, Vitek MS is easy to perform even for a relatively inexperienced technician.

Overall, both the Vitek 2 ANC card and the Vitek MS can provide accurate identification of anaerobic bacteria and meet routine clinical requirements. However, the Vitek MS system is an easier, faster and cheaper method than the Vitek 2 ANC card for identification of most clinically important anaerobic bacteria, except for the initial cost of the Vitek MS instrument is significantly higher than the Vitek 2 instrument. Both our study and others found that there are still many bacteria not included in the databases currently available for both systems. Future developments of the databases to include an expanded number of new species and more robust mass spectra profiles for current species will greatly improve the performance and utility of these automated systems for bacterial identification.

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