101

Multi-Center Evaluation of the VITEK MS for the Mass Spectrometric Identification of Anaerobic Bacteria in the Clinical Microbiology Laboratory

Omai B Garner[1], A. Brian Mochon[1], John A. Branda[2], Carey-Ann D. Burnham[3] ,Maureen Bythrow[4], Mary Jane Ferraro[2], Christine C. Ginocchio[4,5], Rebecca Jennemann[6], Ryhana Manji[4], Gary W. Procop[7], Sandra S. Richter[7], Jenna A. Rychert[2], Linda Sercia[7], Lars F. Westblade[3,5] and Michael A. Lewinski[1]

[1]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [2]Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; [3]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA; [4]Department of Pathology and Laboratory Medicine, North Shore-LIJ Health System Laboratories, Lake Success, NY, USA; [5]Department of Pathology and Laboratory Medicine, Hofstra North Shore-LIJ School of Medicine, Hempstead, NY, USA; [6]Barnes Jewish Hospital, St. Louis, MO, USA; [7]Department of Clinical Pathology, Cleveland Clinic, Cleveland, OH, USA

UCLA Health System

Abstract

Accurate and timely identification of anaerobic bacteria is critical to successful treatment. Classic phenotypic methods for identification require long turn-around times and can exhibit poor species level identification. Matrix-assisted laser desorption ionization time-of –flight (MALDI-TOF) mass spectrometry is an identification system that can provide rapid identification of anaerobes. We present a multi-center study assessing the clinical performance of the Vitek MS in the identification of relevant anaerobic bacteria.

5 different test sites analyzed a collection of 651 unique anaerobic isolates comprising 11 different relevant genera. Multiple species types were included for several of the genera. (*Actinomyces*, 3 species; *Bacteroides*, 6 species; *Clostridium*, 4 species; *Finegoldia magna*; *Fusobacterium*, 2 species; *Mobiluncus curtisii*; *Parvimonas micra*; *Peptoniphilus asaccharolyticus*; *Peptostreptococcus anaerobius*; *Prevotella*, 5 species; and *Propionibacterium acnes*). Briefly, anaerobic isolates were applied to a well of a target plate. Matrix solution (α-cyano-4-hydroxycinnamic acid) was added and allowed to dry. Mass spectra results were generated with the VITEK MS, and the comparative spectral analysis and organism identification was determined by the VITEK MS database 2.0. Identification results were confirmed by 16s rRNA gene sequencing.

Of the 651 isolates analyzed, 91.2% (594/651) exhibited the correct species identification. An additional 7 isolates were correctly identified only to the genus level. The correct to genus only isolates included *Bacteroides* and *Prevotella* species. In total, 5.5% (36/651) of isolates gave no identification (No ID) and 2% (13/651) gave mixed genera results. *Fusobacterium nucleatum* (42.9% No ID, n=7), *Actinomyces neuii* (25% No ID, n=12), and *Bacteroides uniformis* (23.3% No ID, n=30) were notable for an increased percentage of no identification results compared to the other tested species.

Vitek MS identification of clinically relevant anaerobes is highly accurate and represents a dramatic improvement over phenotypic methods in accuracy and turn-around time. An overall correct species identification of 91.2% for anaerobes represents a significant increase in accuracy from other published MALDI-TOF analyses.

Introduction

Anaerobic bacteria constitute a major component of the microbial flora found within the oral cavity and the gastrointestinal tract.
Most anaerobic infections/intoxications develop in areas of devitalized tissue secondary to surgical and traumatic wounds, bites, and ischemic extremities
Anaerobic infections are often polymicrobic, contributing to difficulty in medical management.
Rapid species identification of anaerobes is critical to successful treatment.

The identification of anaerobes has classically relied upon phenotypic assays such as Gram staining, growth characteristics and biochemical reactivity patterns.
Anaerobes typically exhibit very slow doubling times contributing to the extended length of time required for correct identification with phenotypic-based methods.
Nucleic acid sequencing provides a more reliable identification, but is currently too expensive, technically complex, and labor intensive for routine identification of all clinically isolated anaerobic bacteria.

The implementation of Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry in the routine clinical laboratory provides the opportunity for inexpensive, rapid and accurate identification of anaerobes.
Mass spectrometry uses an ionization source to charge and separate ionized particles according to their mass-to-charge ratio (m/z). A detector and mass analyzer then determine the relative abundance of each molecular fragment by their m/z ratio and generate a mass spectrum.
MALDI-TOF MS produces and detects unfragmented large proteins/peptides from whole cells that generate spectral profiles that are reproducible and specific to bacterial species.
These stable mass spectral fingerprints are then compared with reference mass spectra of well characterized strains to produce a reliable identification.

The aim of this multi-center study was to evaluate the performance of the VITEK® MS MALDI-TOF and VITEK® MS v2.0 strain database in the identification of clinically relevant anaerobic bacteria in a clinical microbiology laboratory.

Materials and Methods

Clinical Evaluation Sites:
Five clinical sites within the United States participated in the evaluation of the VITEK® MS system including the UCLA Health System (Los Angeles CA), North Shore LIJ Hospital (Lake Success, NY), Barnes Jewish Hospital (St. Louis MO), Cleveland Clinic (Cleveland OH), and Massachusetts General Hospital (Boston, MA).

Bacterial Strains:
Anaerobic strains were derived from clinical specimens collected from the five clinical sites. A limited number of frozen isolates were provided by bioMérieux in order to expand the analysis to include additional organisms. A total of 651 clinically relevant anaerobic isolates were tested. The collection included multiple isolates of six different species within the genus *Bacteroides*, five different species of the genus *Prevotella,* four different species of the genus *Clostridium*, three different species of the genus *Actinomyces*, two different species of the genus *Fusobacterium*, and one species per genus represented by *Finegoldia magna, Mobiluncus curtisii, Parvimonas micra, Peptoniphilus asaccharolyticus, Peptostreptococcus anaerobius,* and *Propionibacterium acnes* (Table 1). All cultures were incubated under anaerobic conditions for a minimum of 24 h and a maximum of 72 h after visible growth at 35°C. Frozen isolates were subcultured twice before analysis. Anaerobic isolates were all cultivated on Brucella blood agar plates (BBL™, Sparks, MD).

Sample Preparation:
Isolated anaerobic bacterial colonies were applied to the VITEK® MS-DS target slide using a 1µl loop. Typical application involved more than one isolated colony due to the small size of anaerobic isolates. A thin layer of organism was applied to the center of the well. One microliter of VITEK® MS CHCA (bioMérieux) matrix solution (α-cyano-4-hydroxycinnamic acid) was overlaid and allowed to air dry completely. Isolates from the same plate were selected for Gram stain analysis and subcultured for shipment to the reference testing facility (MIDI, Inc., Newark, DE) for 16S rRNA gene sequencing analysis.

Materials and Methods

VITEK® MS Organism Identification:
For each target well, 100 mass spectra profiles were generated within a range of 2 to 20 kilodaltons. The laser frequency was 50Hz and was recorded in a linear positive mode. The mass profiles were averaged to produce a single, composite mass spectrum.

Analysis of a composite mass spectrum for accurate identification used the VITEK® MS v2.0 database. This database is not a library of spectra, but uses a bin matrix. The mass peaks between 2 and 20 kilodaltons are placed into 1300 separately analyzed bins. The bin matrix consists of a table of specificity values for mass peaks per bin for each species that is present in the database. The mass peaks found in the composite spectra are compared to the bin matrix, and the peak intensity of each mass signal allows for the calculation of a composite score and probability for each species.

A probability score between 60% to 100% represents a high discrimination value and a reliable identification. A probability score that is lower than 60% is found in a low discrimination identification that consists of a list of two to four choices for an identification match (Mixed Genera result). A report of no identification is produced when either no match is found for the composite spectra, or not enough spectral peaks were obtained in the analysis. Isolates that yielded no identification results were redeposited to the target plate and reanalyzed.

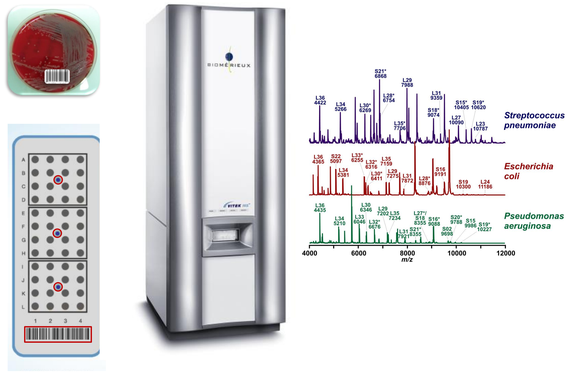

Table 1. Identification of Clinically Relevant Anaerobes by the VITEK® MS

Bacteria (Number of Isolates)	Genus-level (%)	Species-level (%)	Mixed Genera (%)	No ID (%)
Anaerobe isolates (651)	**602 (92.5)**	**594 (91.2)**	**13 (2)**	**36 (5.5)**
Gram-positive species (265)	**245 (92.5)**	**243 (91.7)**	**5 (1.9)**	**15 (5.7)**
Actinomyces meyeri (8)	7 (87.5)	6 (75)	0	1 (12.5)
Actinomyces neuii (12)	8 (66.7)	8 (66.7)	1 (8.3)	3 (25)
Actinomyces odontolyticus (7)	6 (85.7)	6 (85.7)	0	1 (14.3)
Clostridium clostridioforme (7)	7 (100)	7 (100)	0	0
Clostridium difficile (30)	27 (90)	27 (90)	0	3 (10)
Clostridium perfringens (61)	60 (98.4)	60 (98.4)	1 (1.6)	0
Clostridium ramosum (10)	10 (100)	10 (100)	0	0
Finegoldia magna (24)	23 (95.8)	23 (95.8)	0	1 (4.2)
Mobiluncus curtisii (4)	3 (75)	3 (75)	1 (25)	0
Parvimonas micra (10)	10 (100)	10 (100)	0	0
Peptoniphilus asaccharolyticus (4)	4 (100)	4 (100)	0	0
Peptostreptococcus anaerobius (36)	36 (100)	36 (100)	0	0
Propionibacterium acnes (52)	44 (84.6)	43 (82.7)	2 (3.8)	6 (11.5)
Gram-negative species (386)	**357 (92.5)**	**351 (90.9)**	**8 (2.1)**	**21 (5.4)**
Bacteroides caccae (30)	29 (96.7)	28 (93.3)	0	1 (3.3)
Bacteroides fragilis (71)	70 (98.6)	70 (98.6)	1 (1.4)	0
Bacteroides ovatus (40)	35 (87.5)	34 (85)	2 (5)	3 (7.5)
Bacteroides thetaiotaomicron (51)	49 (96.1)	48 (94.1)	0	2 (3.9)
Bacteroides uniformis (30)	22 (73.3)	22 (73.3)	1 (3.3)	7 (23.3)
Bacteroides vulgatus (41)	40 (97.6)	40 (97.6)	1 (2.4)	0
Fusobacterium necrophorum (26)	24 (92.3)	24 (92.3)	0	2 (7.7)
Fusobacterium nucleatum (7)	3 (42.9)	3 (42.9)	1 (14.3)	3 (42.9)
Prevotella bivia (34)	34 (100)	34 (100)	0	0
Prevotella buccae (23)	23 (100)	23 (100)	0	0
Prevotella denticola (6)	6 (100)	6 (100)	0	0
Prevotella intermedia (16)	14 (87.5)	13 (81.3)	0	2 (12.5)
Prevotella melaninogenica (11)	8 (72.7)	6 (54.5)	2 (18.2)	1 (9.1)

Table 2. Mixed Genera Results for Anaerobic Bacteria from the VITEK® MS

16S rRNA Gene Sequencing Identification (n)	VITEK® MS - Mixed Genera Results
***Actinomyces neuii* (1)**	***Actinomyces neuii***, *Haemophilus influenzae*
***Bacteroides fragilis* (1)**	***Bacteroides fragilis***, *Shewanella algae*
***Bacteroides ovatus* (2)**	***Bacteroides ovatus***, *Citrobacter amalonaticus, Staphylococcus auricularis, Bacteroides vulgatus, Microbacterium paraoxydans, Bacteroides thetaiotaomicron*
***Bacteroides uniformis* (1)**	***Bacteroides uniformis***, *Trueperella bernardiae, Bacteroides caccae*
***Bacteroides vulgatus* (1)**	***Bacteroides vulgatus***, *Staphylococcus cohnii spp. urealyticus, Enterococcus casseliflavus*
***Clostridium perfringens* (1)**	***Clostridium perfringens***, *Aeromonas hydrophila/caviae*
***Fusobacterium nucleatum* (1)**	***Fusobacterium nucleatum***, *Aerococcus viridans*
***Mobiluncus curtisii* (1)**	***Mobiluncus curtisii***, *Bifidobacterium spp, Vibrio alginolyticus*
***Prevotella melaninogenica* (2)**	***Prevotella melaninogenica***, *Pediococcus acidilactici Streptococcus gallolyticus spp. gallolyticus Corynebacterium pseudodiphtheriticum, Streptococcus constellatus*
***Propionibacterium acnes* (1)**	*Clostridium bifermentans*, ***Propionibacterium acnes***, *Parvimonas micra*

Conclusions

In this study, we evaluated the performance of the VITEK® MS v2.0 MALDI-TOF system in the identification of clinically relevant anaerobic bacteria.

Overall, the performance of this system was very accurate (91.2% correct to species-level identification compared to 16S rRNA gene sequencing.)

Less than 8% of the total tested isolates were not identified or gave mixed genera identification results.

The implementation of this technology in the clinical microbiology lab will lead to decreased turn-around times for identification from weeks to days.

This represents a significant shift in the laboratory diagnosis of anaerobic bacterial infections and will allow for improved patient care.

Acknowledgements
Dave Pincus
UCLA Clinical Microbiology Laboratory

Contact Information
Omai Garner, PhD, D(ABMM)
Health Sciences Assistant Clinical Professor
Department of Pathology and Lab Medicine,
UCLA School of Medicine
Phone: (310) 794-8748
Email: ogarner@mednet.ucla.edu