Multi-Site Validation of the Vitek MS MALDI-TOF Platform for the Identification of Gram-Positive Aerobes

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Abstract

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is gaining momentum as a tool for bacterial and fungal identification in the clinical microbiology laboratory. Compared with conventional methods, this technology can more readily and conveniently identify a wide range of organisms. Here, we report the findings from a multi-center study to evaluate the Vitek MS v2.0 system (bioMerieux) for the identification of aerobic gram-positive bacteria. A total of 1146 unique isolates, representing 13 genera and 42 species, were analyzed at five clinical microbiology laboratories in the United States. The accuracy of results obtained using the Vitek MS was determined by comparison to nucleic acid sequence-based identification. The Vitek MS instrument reports results either as a single identification, multiple possible identifications, or no identification. Overall, a single choice, correct identification to the species level was obtained for 1063 isolates (92.8%), with an additional 31 isolates (2.7%) correctly identified to the genus level. For 463 isolates representing commonly occurring, important pathogens, the accuracy was 95%. Also, in contrast to previous reports, the Vitek MS correctly differentiated the viridans streptococci from S. pneumoniae in all but one case. In this case, a S. mitis isolate was identified as a split between S. mitis/oralis and S. pneumoniae. In 18 cases, either a single incorrect choice, multiple choices from the same incorrect genus, or multiple alternatives of mixed genera were provided. In most of the latter cases, the correct identification was included among the alternatives. No identification was obtained for 33 isolates (2.9%); however, there was no specific bacterial species for which the method consistently failed. These findings demonstrate that when compared to sequence-based identification, the Vitek MS v2.0 systemis highly accurate for identifying aerobic gram-positive bacteria.

Background

- Identification of some gram-positive bacteria, such as the coagulase-negative staphylococci, the viridans group streptococci, and some enterococci, can be unreliable or overly complicated using phenotypic and biochemical methods
- MALDI-TOF mass spectrometry may be a good alternative for identifying gram positive aerobic bacteria as it is easy to perform, relatively quick, and not subjective
- There are currently no published reports on the performance of the VITEK MS v2.0 system (bioMerieux) with its new method of spectral analysis and updated database

Methods

- Fresh and frozen archived bacterial isolates were obtained during the normal clinical workflow at five sites within the USA
- Cultures were incubated under standard conditions for a minimum of 18 hours and analyzed within 72 hours of visible growth either from the primary or subculture
- Isolated bacterial colonies were applied to a single well of a target slide, overlaid with 1.0 µl of a saturated solution of CHCA matrix, and air-dried
- 16s rRNA sequencing using the MicroSeq system was used as the reference method. In the event of a low discrimination result or when no match was obtained, supplemental sequencing (sodA or rpoB) and/or phenotypic testing was performed
- Analysis:

✓ **Correct to the species level** - a single identification was given and it matched that obtained by the reference method

✓ **Correct to the genus level** - multiple alternative species all from the same genus were reported and this matched the genus obtained by the reference method

✓ **Incorrect** - a single identification was given that did not match the reference method, when multiple identifications of different genera were reported, or when multiple identifications of the same genera were reported but did match the genus of the reference method

Results

Accuracy of organism identification using the VITEK MS v2.0

Reference Identification	Number of Isolates (fresh/archive/sponsor) [a]	VITEK MS Identification			
		Single Result Correct to Species (%)	Multiple Results All Correct to Genus (%)	Single/Multiple Incorrect Results (%)	No Identification (%)
Common Pathogens	**463**	**442 (95)**	**4 (<1)**	**-**	**17 (4)**
Enterococcus faecalis	62/6/0	66 (97)	-	-	2 (3)
Enterococcus faecium	41/16/0	57 (100)	-	-	-
Staphylococcus aureus	59/2/0	60 (98)	-	-	1 (2)
Staphylococcus lugdunensis	25/8/0	33 (100)	-	-	-
Staphylococcus saprophyticus	26/9/0	32 (91)	-	-	3 (9)
Streptococcus pneumoniae	45/6/0	49 (96)	-	-	2 (4)
Streptococcus pyogenes	45/10/0	53 (96)	-	-	2 (4)
Streptococcus agalactiae	53/5/0	58 (100)	-	-	-
Listeria monocytogenes	12/33/0	34 (76)	4 (9)	-	7 (15)
Other Enterococci	**134**	**130 (97)**	**1 (<1)**	**1 (<1)**	**2 (1)**
Enterococcus avium	16/15/2	30 (91)	1 (3)	-	2 (6)
Enterococcus casseliflavus	13/22/2	37 (100)	-	-	-
Enterococcus durans	2/7/21	29 (97)	-	1 (3)	-
Enterococcus gallinarum	10/19/5	34 (100)	-	-	-
Other Coagulase-Negative Staphylococci	**249**	**240 (96)**	**-**	**8 (3)**	**1 (<1)**
Staphylococcus capitis	26/8/0	32 (94)	-	2 (6)	-
Staphylococcus cohnii	2/0/0	2 (100)	-	-	-
Staphylococcus epidermidis	78/10/0	86 (98)	-	2 (2)	-
Staphylococcus haemolyticus	23/15/0	38 (100)	-	-	-
Staphylococcus hominis	17/3/1	21 (100)	-	-	-
Staphylococcus schleiferi	1/1/0	2 (100)	-	-	-
Staphylococcus simulans	11/14/6	31 (100)	-	-	-
Staphylococcus warneri	14/7/12	28 (85)	-	4 (12)	1 (3)
Other Streptococci	**218**	**178 (82)**	**25 (11)**	**4 (2)**	**11 (5)**
Streptococcus anginosus	18/22/7	45 (96)	-	-	2 (4)
Streptococcus constellatus	7/19/4	26 (86)	2 (7)	-	2 (7)
Streptococcus dysgalactiae	18/25/4	24 (51)	20 (43)	-	3 (6)
Streptococcus gallolyticus	3/0/0	3 (100)	-	-	-
Streptococcus infantarius	5/0/0	4 (100)	-	-	-
Streptococcus intermedius	6/7/0	11 (85)	2 (15)	-	-
Streptococcus mitis/ oralis	29/7/0	32 (86)	1 (3)	1 (3)	3 (8)
Streptococcus mutans	1/0/0	-	-	-	1 (100)
Streptococcus salivarius	2/0/0	2 (100)	-	-	-
Streptococcus sanguinis	9/8/17	31 (91)	-	3 (9)	-
Other Genera	**81**	**73 (90)**	**1 (1)**	**5 (6)**	**2 (2)**
Abiotrophia defectiva	2/0/0	2 (100)	-	-	-
Aerococcus viridans	6/0/0	6 (100)	-	-	-
Corynebacterium jeikeium	1/0/0	1 (100)	-	-	-
Gardnerella vaginalis	11/0/16	24 (89)	-	3 (11)	-
Gemella haemolysans	3/0/0	3 (100)	-	-	-
Granulicatella adiacens	1/0/0	1 (100)	-	-	-
Lactococcus garvieae	1/0/0	1 (100)	-	-	-
Lactococcus lactis	0/1/0	1 (100)	-	-	-
Leuconostoc mesenteroides	1/0/0	-	-	-	1 (100)
Micrococcus luteus/lylae	16/8/11	33 (94)	-	1 (3)	1 (3)
Rothia mucilaginosa	3/0/0	1 (33)	1 (33)	1 (33)	-
Total	**1146**	**1063 (92.8)**	**31 (2.7)**	**18 (1.6)**	**33 (2.9)**

[a] Fresh - isolates were obtained during the normal clinical workflow, archive – isolates were obtained from frozen stocks at one of the trial sites, sponsor – isolates were obtained from the sponsors frozen stocks.

Split identifications reported by the VITEK MS v2.0 that were accurate to the genus level

Reference Identification	Number of isolates	VITEK MS Identification
Listeria monocytogenes	3	*L. ivanovii, L. monocytogenes*
Listeria monocytogenes	1	*L. ivanovii, L. monocytogenes, L. welshimeri*
Enterococcus avium	1	*E. raffinosus, E. avium*
Streptococcus constellatus	2	*S. anginosus, S. constellatus*
Streptococcus dysgalactiae subsp. equisimilis	1	*S. equi subsp. zooepidemicus, S. equi subsp. equi*
Streptococcus dysgalactiae subsp. equisimilis	19	*S. pyogenes, S. dysgalactiae subsp. equisimilis, S. dysgalactiae subsp. dysgalactiae*
Streptococcus intermedius	2	*S.constellatus, S. intermedius*
Streptococcus mitis	1	*S. mitis/oralis, S. pneumoniae*
Rothia mucilaginosa	1	*R. dentocariosa, R. mucilaginosa*
Total	**31**	

Inaccurate results reported by the VITEK MS v2.0

Reference Result	Number of Isolates	VITEK MS Identification
Single Choice, Incorrect to Species	**7**	
Enterococcus durans	1	*E. faecium*
Staphylococcus epidermidis	1	*S. hominis subsp. hominis*
Staphylococcus epidermidis	1	*S. caprae*
Staphylococcus warneri	1	*S. pasteuri*
Streptococcus sanguinis	1	*S. anginosus*
Streptococcus sanguinis	2	*S. mitis/oralis*
Multiple Choices, Incorrect to Genus	**2**	
Staphylococcus capitis	1	*Corynebacterium coyleae, Riemerella anatipestifer*
Staphylococcus capitis	1	*S. vestibularis, S. salivarius subsp. salivarius*
Multiple Choices, Mixed Genera	**9**	
Gardnerella vaginalis	3	*Bifidobacterium spp, G. vaginalis*
Micrococcus lylae	1	*M. luteus/lylae, Kocuria rosea*
Rothia mucilaginosa	1	*Alcaligenes faecalis subsp. faecalis, R. mucilaginosa*
Staphylococcus warneri	3	*Prevotella buccalis, S. warneri*
Streptococcus oralis	1	*S. mitis/oralis, S. parasanguinis, Gemella morbillorum*
Total	**18**	

Conclusions

The VITEK MS v2.0 was able to:

- accurately identify gram-positive aerobic bacteria in the clinical laboratory
- successfully differentiate S. pneumoniae from other members of the S. mitis group
- provide species-level identification for the coagulase-negative staphylococci and the less common enterococci and streptococci