

# Performance of the Vitek MS v2.0 System in Distinguishing *Streptococcus pneumoniae* from Nonpneumococcal Species of the *Streptococcus mitis* Group

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**The Vitek MS v2.0 matrix-assisted laser desorption ionization–time of flight mass spectrometry system accurately distinguished *Streptococcus pneumoniae* from nonpneumococcal *S. mitis* group species. Only 1 of 116 nonpneumococcal isolates (<1%) was misidentified as *S. pneumoniae*. None of 95 pneumococcal isolates was misidentified. This method provides a rapid, simple means of discriminating among these challenging organisms.**

Using conventional phenotypic identification methods, it has been challenging for clinical laboratories to distinguish accurately between bacterial species within certain groups, such as the coagulase-negative staphylococci or the nonfermenting Gram-negative bacilli. The *Streptococcus mitis* group is another set of closely related species between which conventional identification methods cannot reliably differentiate. The most important pathogen within the *S. mitis* group, *S. pneumoniae*, is conventionally distinguished from the others (*S. mitis*, *S. oralis*, *S. pseudopneumoniae*, *S. sanguinis*, *S. parasanguinis*, *S. gordonii*, *S. cristatus*, *S. infantis*, *S. peroris*, *S. australis*, *S. sinensis*, *S. orisratti*, *S. oligofermentans*, and *S. massiliensis*) on the basis of its susceptibility to optochin or its solubility in bile. However, both the sensitivity and the specificity of optochin susceptibility testing are suboptimal. Some *S. pneumoniae* strains are optochin resistant (1–3), and closely related species such as *S. pseudopneumoniae* or *S. mitis* can exhibit optochin susceptibility, particularly when incubated in ambient air rather than CO<sub>2</sub>-enriched air (4–8). Likewise, the most convenient method of bile solubility testing, the plate method, is relatively nonspecific (9) and some strains of *S. pneumoniae* are bile insoluble even by the tube method (10) or the disk method (11). Even when larger batteries of phenotypic tests are applied, such as the API rapid ID 32 Strep strip or the Vitek 2 GP card (bioMérieux, Marcy l’Etoile, France), discrimination among species within the *S. mitis* group is poor (12). In fact, *S. mitis* group species are so closely related that the AccuProbe *Streptococcus pneumoniae* assay (Hologic Gen-Probe, Inc., San Diego, CA), a commercially available DNA probe hybridization test, cannot differentiate between *S. pneumoniae* and *S. pseudopneumoniae* isolates (4, 6), and 16S rRNA gene sequencing cannot reliably distinguish among *S. pneumoniae*, *S. mitis*, and *S. oralis* (13, 14).

Recent investigations have demonstrated the ability of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) to distinguish between closely related bacterial species with a high degree of confidence (15–23). Yet with regard to the *S. mitis* group species, initial reports have been disappointing, inasmuch as one widely used, commercially available MALDI-TOF MS platform is prone to misidentify *S. mitis*, *S. oralis*, or *S. pseudopneumoniae* as *S. pneumoniae* (7, 11, 24–29). However, other commercial platforms may perform differently in this regard. In particular, a recent multicenter evaluation of the

bioMérieux Vitek MS v2.0 system demonstrated accurate separation of 51 *S. pneumoniae* strains and 71 nonpneumococcal strains from the *S. mitis* group, although for one *S. mitis* isolate the system did report a split identification that included *S. pneumoniae* among the alternatives (30). Here, we used a larger collection of *S. mitis* group clinical isolates to assess the performance of the bioMérieux Vitek MS v2.0 system in differentiating *S. pneumoniae* from other *S. mitis* group species.

The study included 211 *S. mitis* group clinical isolates selected from frozen archives at Massachusetts General Hospital. None of the study isolates overlapped with those entered into the recent multicenter evaluation of the bioMérieux Vitek MS v2.0 system (30). In our laboratory, all of the clinical isolates identified as *S. pneumoniae* by conventional phenotypic methods during the calendar year 2012 had been archived and 100 of these isolates were randomly selected for the present study by choosing every second unique isolate recovered between January and November 2012. Most of the isolates had been recovered from respiratory or blood specimens and had been identified prior to archiving as *S. pneumoniae* by examination of colonial and microscopic morphology and optochin susceptibility testing in CO<sub>2</sub>-enriched air. Also included in the present study was a convenience sample of 111 archived clinical isolates that had been identified prior to archiving as *S. mitis* on the basis of conventional phenotypic methods, which included examination of colonial and microscopic morphology, and characterization by the API 20 Strep strip (bioMérieux). Between approximately 1995 and 1998, all *S. mitis* isolates that required full identification to the species level for clinical purposes (most of which had been recovered from blood or deep tissue) were archived in our laboratory. For the present study, we selected the first 111 unique, viable isolates we could locate in the frozen archive.

Each of the 211 isolates included in this study was identified

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**TABLE 1** Performance of the Vitek MS v2.0 system in distinguishing *S. pneumoniae* from nonpneumococcal *S. mitis* group species

Vitek MS identification	No. of isolates identified by reference methods as:	
	<i>S. pneumoniae</i>	Nonpneumococcal species
<i>S. pneumoniae</i>	94	1
Nonpneumococcal species	0	108
Split identification <sup>a</sup>	0	7
No identification	1	0
Total	95	116

<sup>a</sup> For these isolates, more than one possible identification was reported by the Vitek MS instrument.

with the Vitek MS v2.0 system (bioMérieux, Marcy l'Etoile, France) after overnight growth on tryptic soy agar with 5% sheep blood (Remel, Lenexa, KS). Isolated bacterial colonies were applied (without prior extraction) to a single well of a disposable target slide, overlaid with a matrix solution, and then air dried prior to analysis as described previously (30). If the Vitek MS method provided a split identification or no identification, the isolate was reanalyzed once. If a single, species-level identification was provided upon repeat analysis, this identification was considered to be the final Vitek MS result; if a split identification or no identification was provided upon repeat analysis, no further analysis was performed.

The outcome of identification with the Vitek MS was compared with the original (prearchiving) phenotypic identification. When the Vitek MS identification matched the original phenotypic identification, no further testing was performed. When there were discrepancies ( $n = 32$ ), supplementary methods were used to arrive at a definitive identification. These included bile solubility

testing by the tube method; parallel optochin susceptibility testing in ambient and CO<sub>2</sub>-enriched air; analysis with the Vitek 2 GP card (bioMérieux); application of the AccuProbe *Streptococcus pneumoniae* hybridization probe (Gen-Probe); and/or sequence analysis of the 16S rRNA gene (31, 32), the *sodA* gene (33), the *groEL* gene (34), and/or the *recA* gene (35). All gene sequences were edited with ChromasPro software (Technelysium, South Brisbane, Australia) and analyzed with NCBI BLASTn and leBIBI V5 (36). Gene sequencing and analysis were performed by a scientist (C.D.G.) at bioMérieux who was blinded to the Vitek MS results. All other methods were performed by independent investigators at Massachusetts General Hospital. By this approach, it was determined that the present study included 95 *S. pneumoniae* isolates and 116 nonpneumococcal isolates from within the *S. mitis* group (93 *S. mitis/oralis*, 12 *S. parasanguinis*, 2 *S. australis*, 2 probable *S. australis*, 3 *S. pseudopneumoniae*, 2 probable *S. infantis*, 1 *S. cristatus*, and 1 *S. sanguinis*).

Among 95 *S. pneumoniae* isolates, 94 (99%) were identified as *S. pneumoniae* by the Vitek MS v2.0 system; the remaining *S. pneumoniae* isolate was not identified by the Vitek MS system (Table 1). Among 116 nonpneumococcal *S. mitis* group isolates, 102 (88%) were correctly identified to the species level by the Vitek MS v2.0 system. Only 1 of these 116 isolates (<1%), a probable *S. infantis* isolate according to sequence analysis, was misidentified as *S. pneumoniae* (Table 2). Six additional nonpneumococcal isolates were assigned to the correct genus but an incorrect species by the Vitek MS v2.0 system; in each case, however, the incorrect identification placed the isolate within the *S. mitis* group and did not classify it as *S. pneumoniae* (Table 2). Seven nonpneumococcal isolates were assigned a split identification by the Vitek MS system, but *S. pneumoniae* was never included among the alternatives (Table 2). Notably, six of the seven misidentified isolates and two of the seven isolates assigned a split identification

**TABLE 2** Resolution of discrepancies between original conventional identification and Vitek MS identification

Original conventional identification	Vitek MS identification	Identification based on reference method(s)	Reference method(s) used <sup>a</sup>	No. of isolates
<i>S. pneumoniae</i>	<i>S. pseudopneumoniae</i>	<i>S. pseudopneumoniae</i>	BS, Opt, HProbe, 16S, <i>sodA</i> , <i>groEL</i> <sup>b</sup>	3
<i>S. pneumoniae</i>	<i>S. mitis/oralis</i>	<i>S. mitis</i>	BS, HProbe, 16S, <i>sodA</i> <sup>c</sup>	2
<i>S. mitis/oralis</i>	<i>S. parasanguinis</i>	<i>S. australis</i>	16S, <i>sodA</i>	2
<i>S. mitis/oralis</i>	<i>S. parasanguinis</i>	Probable <i>S. australis</i>	16S, <i>sodA</i> , <i>groEL</i> , <i>recA</i>	2
<i>S. mitis/oralis</i>	<i>S. parasanguinis</i>	<i>S. parasanguinis</i>	VGP	12
<i>S. mitis/oralis</i>	<i>S. pneumoniae</i>	Probable <i>S. infantis</i>	BS, Opt, HProbe, 16S, <i>sodA</i> , <i>groEL</i> , <i>recA</i>	1
<i>S. mitis/oralis</i>	<i>S. cristatus</i>	<i>S. cristatus</i>	16S, <i>sodA</i>	1
<i>S. mitis/oralis</i>	<i>S. cristatus</i>	<i>S. mitis</i>	16S, <i>sodA</i>	1
<i>S. mitis/oralis</i>	<i>S. pseudopneumoniae</i>	<i>S. mitis</i>	BS, Opt, VGP, HProbe, 16S, <i>sodA</i>	1
<i>S. mitis/oralis</i>	<i>S. mitis/oralis-S. parasanguinis</i> split	Probable <i>S. infantis</i>	16S, <i>sodA</i> , <i>groEL</i> , <i>recA</i>	1
<i>S. mitis/oralis</i>	<i>S. anginosus-Vibrio cholerae-Lactobacillus paracasei-Lactobacillus casei</i> split	<i>S. mitis</i>	16S, <i>sodA</i>	1
<i>S. mitis/oralis</i>	<i>S. mitis/oralis-S. sanguinis</i> split	<i>S. mitis/oralis</i>	VGP	1
<i>S. mitis/oralis</i>	<i>S. parasanguinis-Finegoldia magna</i> split	<i>S. mitis/oralis</i>	VGP	1
<i>S. mitis/oralis</i>	<i>Prevotella denticola-Parvimonas micra-S. parasanguinis</i> split	<i>S. mitis/oralis</i>	VGP	1
<i>S. mitis/oralis</i>	<i>S. mitis/oralis-S. intermedius</i> split	<i>S. mitis/oralis</i>	VGP	1
<i>S. mitis/oralis</i>	<i>S. parasanguinis-Bifidobacterium</i> sp. split	<i>S. sanguinis</i>	VGP	1

<sup>a</sup> BS, bile solubility testing using the tube method; Opt, optochin susceptibility testing in parallel using CO<sub>2</sub>-enriched air and ambient air; HProbe, AccuProbe *Streptococcus pneumoniae* DNA hybridization probe; 16S, DNA sequencing of the 16S rRNA gene; *sodA*, DNA sequencing of the *sodA* gene; *groEL*, DNA sequencing of the *groEL* gene; VGP, Vitek 2 GP card; *recA*, DNA sequencing of the *recA* gene.

<sup>b</sup> One of these three isolates was also analyzed by sequencing of the *recA* gene.

<sup>c</sup> One of these two isolates was also analyzed by sequencing of the *groEL* gene.

could not be definitively identified by conventional phenotypic methods. Rather, these isolates required nucleic acid sequence-based analysis of multiple gene targets for confident identification, demonstrating the challenging nature of these particular isolates. Also, five of the seven misidentified isolates and one of the seven isolates assigned a split identification were *S. australis* or *S. infantis* isolates according to the results of DNA sequence analysis; these species are not represented in the Vitek MS v2.0 system database.

A limitation of this study is the fact that all clinical isolates were collected at a single site (Massachusetts General Hospital), and thus there was not a broad geographic representation. However, the present study's findings are similar to those of a recent multicenter study in which the Vitek MS v2.0 system's performance was determined at five geographically diverse trial sites (30). Compared with the multicenter study, the present study included a larger number of *S. mitis* group clinical strains, none of which had been included in the multicenter study. A second limitation of the present study is the potential for selection bias. The nonpneumococcal isolates, unlike the *S. pneumoniae* isolates, were chosen by convenience rather than by a truly random selection process. And although the *S. pneumoniae* isolates were chosen randomly and were unique isolates (only one isolate from an individual patient was included), it is possible that a clone (identical strain) was circulating among some of the patients from whom the isolates were derived. Finally, in this study, we avoided performing a protein extraction step prior to analysis with the Vitek MS system, even when the Vitek MS system provided no identification or a split identification. Although this was done in order to challenge the system in the most stringent fashion, the addition of an extraction step is known to improve MALDI-TOF MS performance (37), and had it been applied, it might have influenced our findings.

In summary, MALDI-TOF MS with the Vitek MS v2.0 system provides an accurate, fast, inexpensive, and technically non-demanding means of discriminating between *S. pneumoniae* and other *S. mitis* group species. Adoption of this method in the clinical laboratory may improve the ability to make this clinically relevant distinction.

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