Journal of Clinical Microbiology

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J. Clin. Microbiol. **2013, 51(7):2225. DOI: 10.1128/JCM.00682-13.**
Published Ahead of Print 8 May 2013.

JCM
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Multicenter Evaluation of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of Gram-Positive Aerobic Bacteria

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) is gaining momentum as a tool for bacterial 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 multicenter study to evaluate the Vitek MS v2.0 system (bioMérieux, Inc.) for the identification of aerobic Gram-positive bacteria. A total of 1,146 unique isolates, representing 13 genera and 42 species, were analyzed, and results were compared to those obtained by nucleic acid sequence-based identification as the reference method. For 1,063 of 1,146 isolates (92.8%), the Vitek MS provided a single identification that was accurate to the species level. For an additional 31 isolates (2.7%), multiple possible identifications were provided, all correct at the genus level. Mixed-genus or single-choice incorrect identifications were provided for 18 isolates (1.6%). Although no identification was obtained for 33 isolates (2.9%), there was no specific bacterial species for which the Vitek MS consistently failed to provide identification. In a subset of 463 isolates representing commonly encountered important pathogens, 95% were accurately identified to the species level and there were no misidentifications. Also, in all but one instance, the Vitek MS correctly differentiated *Streptococcus pneumoniae* from other viridans group streptococci. The findings demonstrate that the Vitek MS system is highly accurate for the identification of Gram-positive aerobic bacteria in the clinical laboratory setting.

In the clinical microbiology laboratory, bacteria are typically identified using phenotypic and biochemical methods. These methods are generally reliable but have several drawbacks. In particular, they can be time-consuming, sometimes taking several days before a definitive identification can be reached. Furthermore, many are subjectively interpreted, rendering them susceptible to misinterpretation and requiring substantial training and experience on the part of the technologist to establish and maintain competency. In the case of some Gram-positive bacteria, such as the coagulase-negative staphylococci, the viridans group streptococci, and some enterococci, distinguishing one species from another using these methods can be unreliable or overly complicated (1). Thus, many laboratories do not attempt to distinguish among them, or do so only selectively.

Matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) has the potential to address these drawbacks. Proteomic analysis of bacteria by mass spectrometry can be performed as soon as colonies are isolated, often in primary culture, and the analysis itself takes just a few minutes (2–6). Thus, on average, routine bacterial identifications can be obtained more than 30 hours earlier than identification by conventional methods (7). MALDI-TOF MS also provides equal or superior accuracy compared with conventional phenotypic methods (8–10) and does not require subjective interpretation. Finally, it can readily differentiate bacterial species that have similar phenotypic characteristics (11–19).

There are only a few commercially available MALDI-TOF MS systems used for identification of bacteria. Numerous studies have described the performance of the two most common, the Bruker Biotyper and the bioMérieux Vitek MS RUO, primarily compared to phenotypic testing as the reference method (3, 4, 6, 8, 9, 13–23). There are currently no published reports available on the performance of the Vitek MS IVD system, with its new method of spectral analysis and updated database (version 2.0). Here, we report the findings of a large multicenter evaluation of the Vitek MS v2.0 system for the identification of Gram-positive aerobic bacteria in which DNA sequence-based identification served as the reference method.

Received 12 March 2013 Returned for modification 1 April 2013
Accepted 30 April 2013
Published ahead of print 8 May 2013

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.00682-13.

doi:10.1128/JCM.00682-13

MATERIALS AND METHODS

Study sites. The performance of the Vitek MS v2.0 system (bioMérieux, Inc., Durham, NC) was assessed at five sites within the United States (Boston, MA; St. Louis, MO; Los Angeles, CA; Lake Success, NY; and Cleveland, OH). Prior to initiation of the study, operators at each site were trained in target slide preparation, instrument use, and result review. Each operator was required to demonstrate proficiency by successfully analyzing a masked panel of organisms consisting of 10 isolates representing common aerobic and anaerobic Gram-positive and Gram-negative bacteria and yeasts. This study was approved by the human subject committees at the respective sites, when deemed necessary by their internal review boards.

Bacterial isolates. Fresh isolates obtained in the course of routine clinical work during the study period were collected and tested at each of the study sites. Each site was responsible for analyzing a minimum of 10 isolates per species for a predetermined set of frequently encountered organisms (*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*) and six isolates per species for a predetermined set of less frequently encountered organisms (including *Enterococcus avium*, *Enterococcus casseliflavus*, *Enterococcus durans*, *Enterococcus gallinarum*, *Gardnerella vaginalis*, *Listeria monocytogenes*, *Micrococcus luteus/lylae*, *Staphylococcus capitis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* subsp. *hominis*, *Staphylococcus lugdunensis*, *Staphylococcus saprophyticus*, *Staphylococcus simulans*, *Staphylococcus warneri*, *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus dysgalactiae* subsp. *equisimilis*, *Streptococcus mitis/Streptococcus oralis*, and *Streptococcus sanguinis*). In the event that a rarer organism, not included in the list above, was tested, it was included in the study. If a site was unable to obtain the minimum number of preselected species, additional testing of frozen isolates obtained from their culture collections or provided by the study sponsor was permitted. In all cases, cultures were incubated under standard conditions for a minimum of 18 h at 35 to 37°C in ambient air or with CO_2 enrichment. Each isolate was analyzed on the Vitek MS system within 72 h of visible growth either from the primary culture or from a subculture. Subculturing was performed when colonies on the primary culture plate were insufficiently isolated or for convenience to allow batching. All subculturing was performed using tryptic soy agar with 5% sheep blood (Remel, Lenexa, KS). More than 95% of the isolates were tested after growth on this medium. Of the remaining isolates, 53 were tested after growth on brucella agar supplemented with 5% horse blood (Remel, Lenexa, KS), two after growth on chocolate agar (Remel, Lenexa, KS), and one after growth on human blood-Tween bilayer (BD, Franklin Lakes, NJ). Frozen (archived) isolates were serially subcultured twice on tryptic soy agar with 5% sheep blood prior to analysis. Duplicate isolates of the same species from the same patient were not included in the study.

Sample preparation for MALDI-TOF MS analysis. Isolated bacterial colonies were applied to a single well of a disposable, barcode-labeled target slide (Vitek MS-DS; bioMérieux, Inc.) using a 1.0-μl loop, overlaid with 1.0 μl of a saturated solution of alpha-cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile and 2.5% trifluoroacetic acid (Vitek MS-CHCA; bioMérieux, Inc.), and air dried. The same bacterial culture was also used to set up a slide for Gram stain and prepare the organism for shipment to a centralized laboratory for reference testing.

For instrument calibration, an *Escherichia coli* reference strain (ATCC 8739) was transferred to designated wells on the target slide using the procedure described above. For quality control purposes, positive and negative controls were analyzed on each day of testing. The positive control consisted of at least one of four quality control organisms, including *Staphylococcus aureus* (ATCC 29213), *Klebsiella oxytoca* (ATCC 13182), *Pseudomonas aeruginosa* (ATCC 10145), and *Enterobacter aerogenes* (ATCC 13048). The negative control was matrix alone.

Organism identification using the Vitek MS v2.0 system. The Vitek MS v2.0 system includes an OEM (original equipment manufacturer)-labeled Shimadzu AXIMA Assurance mass spectrometer linked to a reference database, referred to as Knowledge Base. During target interrogation, mass spectra within a range of 2,000 to 20,000 Da are recorded in linear positive mode at a laser frequency of 50 Hz. For each interrogation, laser shots at different positions within the target well produce up to 100 mass profiles that are summed into a single, raw mass spectrum. The spectrum is then processed by baseline correction, denoising, and peak detection to identify well-defined peaks. The list of these significant peaks is subjected to a proprietary process called "mass binning" (see the supplemental material). The processed (binned) data are used to query Knowledge Base to determine the unknown's taxonomic identity. These results are then provided in the form of a single species-level (and sometimes subspecies-level) identification, a split (low discrimination) identification with up to four species-level alternatives displayed, or no identification. For the present study, when a report of "no identification" was provided, or when analysis of an isolate yielded absent or poor-quality spectra, the organism was reanalyzed a single time on a new target slide. Isolates yielding split identifications were not reanalyzed. Additionally, isolates that yielded a reference identification that is not included in the database were excluded from analysis.

Reference method for bacterial identification. All study isolates were sent to a centralized laboratory (MIDI Labs, Inc., Newark, DE) for nucleic acid sequence-based identification. Sequencing of a 527-bp region within the 16S rRNA gene was performed using universal 16S primers at positions 0005F and 0531R (MicroSeq system; Applied Biosystems, Carlsbad, CA). The resulting sequence was run through the MicroSeq, GenBank, and BiBi databases to determine the identity. In the event of a discrepancy or low-discrimination result, or when no match was obtained using this method, supplemental sequencing of a different gene target (*sodA* or *rpoB*) and/or phenotypic testing was performed by bioMérieux, Inc. (Durham, NC).

Analysis. The Vitek MS result was considered accurate to the species level if a single identification was given and it matched that obtained by the reference method. The Vitek MS result was considered correct to the genus level if multiple alternative identifications, all from the same genus, were reported and this matched the genus obtained by the reference method. The Vitek MS result was considered incorrect if a single identification was given that did not match (at some taxonomic level) the result obtained with 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

Overall results. A total of 1,146 unique, aerobic, Gram-positive isolates was analyzed, representing 16 genera and 42 species. As shown in Table 1, for 1,063 isolates (92.8%), the Vitek MS provided a single identification that matched at the species level the identification obtained by the reference method. An additional 31 isolates (2.7%) were correctly identified to the genus level, meaning that the Vitek MS result included a split identification with multiple alternative species all from the same genus, and the genus in all cases matched that obtained by the reference method (Tables 1 and 2). For all but one of these isolates, the correct species was listed among the alternate identifications provided (Table 2). A total of 18 isolates were not accurately identified (1.6%) (Tables 1 and 3). This included seven isolates for which a single identification was provided that was accurate to the genus level but not the species level, two isolates for which multiple identifications were provided, all of which were incorrect to the genus level, and nine isolates where multiple different genera were reported. In the later

TABLE 1 Accuracy of organism identification using the Vitek MS v2.0 system

Reference identification	No. of isolates (fresh/archive/sponsor)[a]	No. (%) with Vitek MS result			
		Single result correct to the species level[b]	Multiple results all correct to the genus level[c]	Single/multiple incorrect results[d]	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 defective	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	1,146	1,063 (92.8)	31 (2.7)	18 (1.6)	33 (2.9)

[a] Fresh isolates were those obtained during the normal clinical workflow; archived isolates were obtained from frozen stocks at one of the trial sites; sponsor isolates were obtained from the sponsor's frozen stocks. Sponsor-derived isolates were not used in the development of Knowledge Base.
[b] The result was a single identification that matched the reference method to the species level.
[c] The result was multiple possible identifications, all within the same genus, which matched (at the genus level) the identification obtained by the reference method.
[d] The result was either a single identification that did not match the identification obtained by the reference method or multiple possible identifications that included more than one genus.

TABLE 2 Split identifications reported by the Vitek MS v2.0 system that were accurate to the genus level

Reference identification	No. 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	

cases, the correct species-level identification was included among the alternatives. Finally, no identification was obtained for 33 isolates, all of which were represented in the database (2.9%) (Table 1). There was no specific bacterial species for which the Vitek MS consistently failed.

Commonly encountered important pathogens. Given the breadth of species analyzed in this study, some of which are rarely encountered in the clinical microbiology laboratory, we considered the performance of the Vitek MS system for the identification of a subset of commonly encountered and clinically important Gram-positive bacteria (Table 1). This set of organisms was chosen prior to data analysis on the basis of being pathogenic and commonly isolated from clinical specimens. Among the 463 isolates included in this group, 95% were accurately identified. The level of accuracy was similar to that for all isolates combined ($P = 0.0557$, Fisher exact test). Although 17 isolates (4%) within this subset were not identified, there were no organisms within this subgroup that were misidentified. *Listeria monocytogenes* was the only organism within this group that was correctly identified to the species level less than 90% of the time (Table 1). Seven of the 45 *L. monocytogenes* isolates (15%) were not identified, and an additional four (9%) were correctly identified to the genus but not the species level (listed in Table 2).

TABLE 3 Inaccurate results reported by the Vitek MS v2.0 system

Reference result	No. of isolates	Vitek MS results
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	

Other enterococci, staphylococci, and streptococci. Among the Gram-positive bacteria, the enterococci, staphylococci, and streptococci are the most commonly encountered in the clinical laboratory. Aside from the organisms included above, identification of these bacteria to the species level may not be feasible using conventional methods due to the poor specificity and technical challenges of these methods. With this in mind, we determined the performance of the Vitek MS in accurately identifying these groups of organisms (Table 1). Of the 134 enterococci, 130 (97%) were correctly identified to the species level. Similarly, 96% of the coagulase-negative staphylococci were correctly identified to the species level (240 of 249). The accuracy was lower for the streptococci, with 82% being identified to the species level (178 of 218). In particular, only 51% of the *S. dysgalactiae* isolates were correctly identified to the species level (24 of 47). The remaining were either not identified or correctly identified only to the genus level. Importantly, the Vitek MS correctly distinguished *S. pneumoniae* from other *S. mitis* group streptococci in all but one case. In this case, the reference method identified an isolate as *S. mitis*, but the Vitek MS reported a split identification between *S. mitis/oralis* and *S. pneumoniae*. These data indicate that the Vitek MS can readily provide species-level identifications for this challenging group of organisms.

Other genera. The remaining isolates included in the study represent an additional 10 genera. As shown in Table 1, the majority of these isolates were correctly identified to either the species level (90%) or the genus level (1%). Six percent of the isolates in this group were misidentified, including three *G. vaginalis*, one *M. luteus/lylae*, and one *Rothia mucilaginosa* isolate. No identification

was obtained for 2% of these isolates, including one isolate each of *Leuconostoc mesenteroides* and *M. luteus/lylae*.

DISCUSSION

The purpose of this study was to evaluate the new Vitek MS v2.0 MALDI-TOF mass spectrometry system for the identification of Gram-positive aerobic bacteria in the clinical laboratory setting. Compared to the reference method, the Vitek MS system provided an accurate, species-level identification for more than 92% of the isolates tested. This level of accuracy is similar to that obtained using modern biochemical testing platforms (24–28) and other MALDI-TOF MS systems (4, 6, 8, 12, 21, 23, 29, 30–33). The advantage of this and other MALDI-TOF MS systems over conventional methods is the potential improvement in turnaround time and reduction in reagent and labor costs (5, 7, 23, 34). This is especially true for the less common species of *Enterococcus*, *Staphylococcus*, and *Streptococcus*, which can be difficult to identify at the species level using conventional methods. Given the similar level of accuracy among the commercially available MALDI-TOF MS systems, factors including cost and ease of use will likely influence the choice of system.

Among the major commercial MALDI-TOF platforms, the instrument, sample preparation, and basic approach to signal acquisition are similar, but the approach to database building and analysis differs significantly (35). One approach uses a database of reference mass spectra and a pattern matching algorithm (36). In this approach, a "mass fingerprint" or peak list with mass-to-charge values and intensities is generated from an unknown sample. The mass fingerprint is then compared to a database of reference mass fingerprints, the mass signals and signal intensity (peak height) are compared, and the closest matches are identified (35–37). In contrast, the Vitek MS v2.0 uses a "bin matrix" approach to determine the identity of an unknown organism in comparison to the reference database (see the supplemental material). This analysis assigns added weight to species-specific peaks (35), a method that has been shown to aid in differentiating between very similar mass spectra derived from closely related species or subspecies (36). Additional studies are required to determine whether this difference in spectral analysis leads to improvements in the identification of bacterial species encountered in the clinical microbiology laboratory.

The Vitek MS v2.0 was able to reliably distinguish between *S. pneumoniae* and other *S. mitis* group species. This appears to be an advantage of this system in comparison to other MALDI-TOF MS systems, which have been reported to misidentify *S. mitis* group species (especially *S. mitis*, *S. oralis*, and *S. pseudopneumoniae*) as *S. pneumoniae* (2, 8, 21, 23, 38–42). It was previously suggested that this difficulty may stem from the extremely close relationship of these species (35). However, it has been demonstrated that the mass spectra of these organisms do contain characteristic peaks that clearly distinguish them (43), and in a head-to-head comparison, an earlier version of the Vitek MS system was better able to discriminate between these organisms than another commercial MALDI-TOF MS platform (6). Follow up studies are necessary to corroborate these findings.

This study has several strengths. First, it was a large multicenter investigation with good geographic representation using primarily freshly obtained clinical isolates. Second, the study relied upon nucleic acid sequence-based identification as the reference method, which is especially unusual for a study of this size, due to the cost associated with sequencing. Finally, very strict rules were applied when isolates were tested. In particular, a single deposit rather than multiple deposits of each isolate was spotted on the target plate, and protein extraction was not performed. This is a more stringent approach than has been adopted in other studies (6, 11, 44). Further, repeated testing was not allowed when split identifications were obtained. In this regard, the study findings may underestimate the system's true capabilities, as it is possible to obtain a single, species-level identification by repeating the analysis after initially obtaining multiple identifications. Although the breadth of organisms tested in this study was extensive, one limitation of the study was that there were several species for which only a small number of isolates were analyzed, and for some of these it was necessary to supplement the freshly collected isolates with archived isolates.

In summary, the Vitek MS v2.0 is an accurate and user-friendly system for identifying Gram-positive aerobic bacteria in the clinical laboratory. For some clinical microbiology laboratories, adoption of this technology is likely to improve service by providing species-level identification for the coagulase-negative staphylococci and the less common enterococci and streptococci. It should be noted, however, that this technology does not supersede the expertise of a trained microbiologist; Gram staining and colony morphology are still a valuable part of bacterial identification and can be used to identify errors that may be overlooked by relying solely on the identification result provided by the Vitek MS.

ACKNOWLEDGMENTS

This study was performed as part of an FDA trial of the Vitek MS v2.0 system and was funded by the device manufacturer (bioMérieux, Inc., Durham, NC). M.J.F., J.A.B., and J.R. have received research funding from bioMérieux and Becton, Dickinson. C.-A.D.B has received research funding from bioMérieux, Accler8, Cepheid, and T2 Biosystems. C.C.G. has received research funding and consultant fees from bioMérieux and Becton Dickenson. G.W.P. has received research funding from bioMérieux, Bruker, CDC, and Luminex. S.S.R. has received research funding from bioMérieux, Nanosphere, and Forest Laboratories.

We thank Marc van Nuenen and Maud Arsac for assistance in preparing the supplemental information.

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