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CORRESPONDENCE

Comparison of Vitek MS (MALDI-TOF) to standard routine identification methods: an advance but no panacea

Sir,

In recent years, proteomic identification of microorganisms using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) has promised a revolution in medical microbiology in terms of enhanced accuracy, increased speed and reduced costs.[1] Although the technology has found widespread application, it has only recently been incorporated into a limited number of Australian diagnostic laboratories. Currently, two instruments are available on the market: Biotyper 2.0 (Bruker Daltonics, Germany) and Vitek MS (bioMérieux, France). A comparison between the Bruker and Shimadzu systems (the latter being an earlier version of the Vitek MS) for 720 isolates found the performance of both instruments to be accurate and broadly comparable, although a greater proportion of high confidence identifications were obtained by the Bruker system (94.4% versus 88.8%; $p < 0.0001$).[2] Although some published data have been provided for the performance of the Bruker system in the Australian context,[3,4] little diagnostic laboratory experience with the Vitek MS instrument has yet been published.

We conducted a parallel verification trial of the Vitek MS system in comparison to routine identification methods. A total of 750 pure isolates were tested, including over 150 different species of bacteria and yeasts. Of these, 695 (93%) were wild-type strains obtained from clinical specimens, 39 (5%) were stored external Quality Assurance Program (QAP) organisms from the Royal College of Pathologists of Australasia (RCPA) and 16 (2%) were American Type Culture Collection (ATCC) reference strains. All isolates were tested using α-cyano-4-hydroxycinnamic acid matrix solution with spectra generated by 100 laser shots. An *Escherichia coli* control strain was used for each run. If initial testing failed to provide an adequate spectrum, this was repeated up to three times. Re-testing using 25% formic acid extraction was performed if no identification was obtained. All suspected yeasts were also treated with 25% formic acid. Routine identification methods in our laboratory include the Vitek2 (bioMérieux), API (bioMérieux), BBL Crystal (Becton Dickinson, USA), RapID ANAII (Remel, USA) systems, rapid bench tests (e.g., indole, oxidase, catalase, latex agglutination tests) and chromogenic media, as well as supplementary biochemical testing as required. The comparative standard for organism identification against which the Vitek MS was compared was the routine laboratory method. If results of the Vitek MS and routine methods remained unresolved by supplementary testing, isolates were referred to a reference laboratory for definitive identification, including 16S rDNA sequencing. All identifications for *Clostridium difficile* and *Neiserria gonorrhoeae* isolates were routinely confirmed by molecular methods. Since large colony β-haemolytic streptococci with Lancefield group A, B, C or G antigens are not routinely identified to species level in our laboratory, identification by Vitek MS for species known to express these antigens (e.g., *Streptococcus agalactiae* and group B antigen) were considered concordant to species level. Identification by Vitek MS provides a percentage probability match to database spectra. Isolates with scores from 60 to 99.9% with a single organism choice were considered a good identification. For isolates with probability scores >60% and a choice of 2–4 organisms, a genus level identification was recorded if all choices were within the same genus. However, no valid identification was recorded if the organism choices were of multiple genera, despite formic acid extraction. Scores of <60% were considered to have no valid identification.

Overall, 707 of 750 isolates (94.3%) showed concordance between Vitek MS and standard identification methods to at least genus level, with 639 (85.2%) concordant to species level. No identification by Vitek MS, despite formic acid extraction, occurred in 36 (4.8%) isolates and a further 7 (0.9%) showed lack of concordance between the two comparison methods. The results are summarised by organism group in Table 1. Proportions of identifications achieved for each species are provided in Supplementary Table 1 (http://links.lww.com/PAT/A5).

It is clear that the Vitek MS system is accurate in identifying the most common pathogens encountered in routine work, such as staphylococci, *Escherichia coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, enterococci and most streptococci. A previous study of 1019 isolates from the 13 most commonly encountered genera of bacteria showed that the Shimadzu MALDI-TOF MS system, upon which the Vitek MS is based, identified 94.7% of isolates in concordance to API and BD Phoenix systems, which increased to 98% once discrepancies were resolved by 16S rDNA sequencing.[2] In our current study, the Vitek MS was reliable in discriminating critical pathogens such as *Neiserria gonorrhoeae* or *N. meningitidis* from non-pathogenic *Neiserria* species, although confirmatory testing for *N. gonorrhoeae* is still recommended by the manufacturer. Furthermore, Vitek MS provides much improved identification of some uncommon organisms that may be difficult to identify, such as *Abiotrophia defectiva*, *Kingella kingae*, *Aggregatibacter aphrophilus* or *Fusobacterium necrophorum*. For some isolates the lack of specificity to species level arose because of the inability of Vitek MS to discriminate between closely related species (e.g., *Enterobacter cloacae/asburiae*, *Corynebacterium xerosis/amycolatum* or *Aeromonas hydrophila/caviae*). However, these can usually be differentiated by simple additional bench tests if required and species distinction may not be critical for clinical management in many situations.

Similar to other commercial systems, it is not reliable in identifying some unusual organisms. This occurs because either reference spectra for rare species are not yet included in the current database (such as *Varibaculum cambriense*) or are not sufficiently differentiated from closely related species (e.g., *Cryptococcus gattii*, which identified as *C. neoformans*). In others, failure to obtain an adequate spectrum presumably arose from difficulties in extraction or crystallisation of cellular proteins. Organisms that proved difficult to identify by Vitek MS included *Actinomyces* spp., mucoid strains of *Streptococcus pneumoniae*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*, some corynebacteria and a small number of Gram positive anaerobes. A high proportion (8/38, 21%) of non-*Pseudomonas* Gram negative environmental bacteria, such as *Brevundimonas* or *Cupriavidus*, failed to be identified by the Vitek MS (with two returning incorrect identifications and six with no identification). In some areas the current database

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Table 1 Summary of Vitek MS MALDI-TOF identification by organism group

Organism groups	Total tested	(%)	Concordant to species	(%)	Concordant to genus only	(%)	Non-concordant	(%)	No MALDI identification	(%)
Staphylococci	103	(14)	103	(100)	0	(0)	0	(0)	0	(0)
Streptococci	87	(12)	77	(89)	8	(9)	0	(0)	2	(2)
Enterococci	55	(7)	55	(100)	0	(0)	0	(0)	0	(0)
Enterobacteriaceae	199	(27)	154	(77)	36[†]	(18)	4[‡]	(2)	5	(3)
Pseudomonas spp.	47	(6)	46	(98)	0	(0)	0	(0)	1	(2)
Non-fermenting Gram negative bacilli	38	(5)	24	(63)	6	(16)	2	(5)	6	(16)
Oxidase positive fermenting Gram negative bacilli	14	(2)	6	(43)	8[†]	(57)	0	(0)	0	(0)
Gram negative anaerobes	6	(1)	5	(83)	0	(0)	0	(0)	1	(17)
Campylobacter spp.	5	(1)	5	(100)	0	(0)	0	(0)	0	(0)
Gram positive anaerobes	27	(4)	23	(85)	0	(0)	1	(4)	3	(11)
Corynebacteria	15	(2)	8	(53)	5[†]	(33)	0	(0)	2	(13)
Neisseria spp.*	31	(4)	29	(94)	2	(6)	0	(0)	0	(0)
Haemophilus spp.	38	(5)	37	(97)	1	(3)	0	(0)	0	(0)
Candida spp.	32	(4)	27	(84)	1	(3)	0	(0)	4	(13)
Others	53	(7)	40	(75)	1	(2)	0	(0)	12	(23)
Totals	750	-	639	(85)	68	(9)	7	(1)	36	(5)

* *N. gonorrhoeae* and *N. meningitidis* correctly identified in all cases.
† Inability to reliably discriminate in all cases between *A. caviae/hydrophila, Citrobacter freundii/braakii, Enterobacter cloacae/asburiae, Corynebacterium xerosis/amycolatum* and *Salmonella* spp. accounted for the lower proportions of agreement to species level in these groups.
‡ Significant errors included failure to identify *Shigella flexneri/boydii/sonnei.*

seems limited; for instance only one species of *Actinomyces* (*A. europaeus*) is currently included. Rarely encountered species, such as *Leifsonia acquatica, Roseomonas gilardii* or *Capnocytophaga canimorsus,* are also missing. The manufacturer advises that certain species are poorly identified by the Vitek MS, including *Aerococcus urinae, Cardiobacterium hominis, Haemophilus haemolyticus, Helicobacter pylori* and *Peptinophilus asaccharolyticus.* These deficiencies can be disappointing as laboratories often have difficulties reliably identifying such organisms and this should be where MALDI-TOF MS can provide greatest advantage. In addition, the Vitek MS is presently unable to discriminate *E. coli* from *Shigella* spp. This was confirmed by testing *S. flexneri, S. dysenteriae* and *S. sonnei* isolates, all of which were identified as *E. coli.* However, this problem is found with all MALDI-TOF MS systems[5] and the identification software and product specification provides alerts to this effect. In our experience, the system also cannot reliably separate *Salmonella paratyphi* from non-typhoidal salmonellae. Potential users of this instrument need to be aware of these deficiencies when considering the system for routine use, especially for faecal isolates.

Although no specialised techniques are required to use the instrument, and the training time is minimal, there remains a 'learning curve' in terms of applying the correct concentration of inoculum to the slide and gaining experience in deciding which types of organism to select for MALDI-TOF MS identification. As a system, it performs best for the routine identification of common pathogens, and is presently less reliable when challenged with exotic or rarely encountered species, particularly if these are generally considered environmental or of low pathogenicity. This limitation, however, may improve as databases are refined and expanded. A study of 123 external QAP specimens tested by Vitek MS found 78% identified to genus (72% to species) level and in 18% no identification was obtained.[6] The higher identification failure rate probably reflects the proportion of unusual isolates encountered in the QAP samples, which might explain the comparatively better performance seen in our study (only 4.8% unidentified).

Limitations of this study are acknowledged. We aimed to compare Vitek MS to our current laboratory identification methods and, where necessary, discrepant results were resolved by additional biochemical or molecular testing. However, it is possible that identification by routine methods for some wild-type strains may have been inaccurate and, if in agreement with MALDI-TOF MS, may overestimate its accuracy. However, for the purposes of replacing commonly employed identification methods, Vitek MS seems to be largely reliable.

The major advantages of introducing MALDI-TOF MS has been both rapidity with which results can be obtained, and the dramatically reduced consumable costs. In our laboratory, the current cost of a Vitek 2 identification card is approximately AU$7.60–10.75, and API panels range from AU$14.80–18.50, depending upon the test organism. This can be compared to approximately 50 cents for identification by MALDI-TOF. The ease and reliability of MALDI-TOF MS allows less reliance on several additional tests such as latex agglutination or chromogenic media, providing further savings. While the capital investment required to purchase a MALDI-TOF MS instrument is considerable (currently in the region of AU$200 000–225 000), estimates from one Australian laboratory have suggested that ongoing savings would offset the initial purchase cost within 3 years of use.[5] In the future, the technology holds promise to enhance detection of pathogens by direct testing of clinical specimens such as positive blood cultures[7] and our limited experience to date with such techniques appears promising. However, as with all automated systems, there is a requirement to maintain basic microbiological skills and knowledge, not only for when the MALDI-TOF MS fails to identify an organism but also to appreciate erroneous identifications. Occasional major identification errors occur; the laboratory must be vigilant that such results are not released.

In summary, we found the Vitek MS system to be reliable and accurate for routine microbial identification in most instances. It has the potential to significantly reduce turnaround times, improve clinical care by directing targeted and timely antibiotic therapy, enhance the identification of some

challenging organisms and provide long-term cost savings, despite a large initial capital investment. However, the limitations of the system need to be recognised and such new techniques do not obviate maintaining basic microbiological bench skills. Defining the optimal manner in which to integrate these methods into the routine laboratory workflow requires careful consideration. Further studies to directly compare the performance of different systems would also be welcome.

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