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Performance of the Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry System for Rapid Bacterial Identification in Two Diagnostic Centers in China.

Running Title: Vitek MS performance in China

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ABSTRACT

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems had not been officially launched for diagnostic use in clinical microbiology laboratories in China until 2012. Here, we reported the findings from the first large-scale evaluation study of Vitek MS for routine bacterial identification in two major diagnostic centers in Beijing and Hong Kong. A total of 2,266 unique isolates, representing 56 genera and 127 species were analyzed, and results were compared to those obtained by Vitek 2. Any discrepancies were resolved by 16S rRNA sequencing. Overall, Vitek MS provided correct identification for 2,246 (99.1%) isolates, including 2,193 (96.8%) isolates with correct species-level identifications and 53 (2.3%) isolates matched at genus level only. Vitek MS surpassed Vitek 2 consistently in species-level identification for the important pathogens, including non-*Enterobacteriaceae* Gram-negative bacilli (94.7%vs92%), Staphylococci (99.7%vs92.4%), Streptococci (92.6%vs79.4%), Enterococci (98.8%vs92.6%), and Clostridia (97.3%vs55.5). The findings demonstrated that the Vitek MS is highly accurate and reliable for routine bacterial identification in clinical settings in China.

Keywords: MALDI-TOF MS, Vitek MS, bacterial identification

1. INTRODUCTION

In an era of dramatic increase of drug resistance, rapid bacterial identification facilitates better management of antimicrobial therapies and infection control. Nowadays, semi-automated biochemical test platforms such as Vitek 2 (BioMerieux, Marcy l'Etoile, France), or PHOENIX (BD Diagnostics, Sparks, MD, USA) are commonly used in clinical microbiology laboratories to complement the conventional cultured based method for routine bacterial identification, reducing the average time-to-identification to about 10 hours (Chatzigeorgiou *et al.* 2011). However, the reagent cost is extremely high in the laboratory settings in densely populated countries, such as China.

The drawback can be potentially addressed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which was originally applied in biochemical industries for analysis of different macromolecules. The concept of microbial identification by measuring the unique bacterial proteomic fingerprints was firstly proposed about 30 years ago (Anhalt & Fenselau 1975). With the advent of automated algorithm in mass spectral acquisition and database-matching, bacterial identification can be performed in batchwise with an average reagent cost of less than 1 US dollar and the turnaround time of only a few minutes per sample. Numerous studies have described and compared the performance of two most common MALDI-TOF MS systems, the Bruker Biotyper (Bruker Daltonics, Germany) and the Vitek MS (BioMerieux, Marcy l'Etoile, France), in identification of aerobic bacteria, anaerobes, yeasts and mycobacteria isolated from primary cultures (Chen *et al.* 2013; Garner *et al.* 2013; Manji *et al.* 2013; Rychert *et al.* 2013; Westblade *et al.* 2013). The results consistently showed that MALDI-TOF MS offered equivalent or even superior accuracy in comparison to conventional phenotypic methods. Nevertheless, despite this promising data reported elsewhere, MALDI-TOF MS had not been officially launched for clinical use in China until the bioMerieux Vitek MS system was approved by the

China State Food and Drug Administration (SFDA) for in *vitro* diagnostic (IVD) purpose in 2012.

In the present study, we report the findings of a large scale evaluation of the Vitek MS IVD system for the identification of aerobic and anaerobic bacteria in two major diagnostic centers in Beijing and Hong Kong, two major cosmopolitan cities with good geographic representation of northern and southern China respectively. To the best of our knowledge, no similar studies of this sample size have been performed in developing countries including China.

2. MATERIALS AND METHODS

2.1 Study sites. The performance of the Vitek MS IVD system was evaluated at the Departments of Microbiology in Chinese People's Liberation Army General Hospital (PLA 301 Hospital) of Beijing and Queen Mary hospital (QMH) of Hong Kong. PLA 301 Hospital, which serves patients mostly from the North China regions, is the biggest military tertiary hospital in China with 3,500-beds and around 100,000 inpatients per year, whereas QMH is a tertiary referral university-affiliated acute hospital with 1,600 beds, serving a population of 0.53 million resident in southern area of China.

Prior to the initiation of the study, the operators in the two different study sites were trained by the same team of technical staff from BioMerieux so that the techniques used in Vitek MS target slide preparation, instrument operation and data interpretation were consistent with each other.

2.2 Bacterial isolates. During a three-month period (March 2013 – May 2013), all aerobic and anaerobic bacterial isolates recovered from various clinical specimens, such as blood,

urine, stool, cerebrospinal fluid, wound swabs, throat swabs, sputum and other lower respiratory specimens, were collected for this study prospectively.

Prior to MALDI-TOF MS analysis, bacterial isolates were recovered on appropriate agar media (Columbia horse blood agar for aerobes or facultative anaerobes and neomycin blood agar supplemented with hemin and vitamin K for anaerobes) under 35℃ incubation for 24h to 72h in aerobic, microaerophilic or anaerobic conditions as appropriate. Test results of the isolates from the same patients were deduplicated to ensure that a particular isolate was only tested once.

2.3 Conventional bacterial identification. In each diagnostic centre, after the Gram staining and determination of catalase and oxidase activities, bacterial identification relied on semi-automated biochemical test platforms, Vitek 2, using the GP, GN, NH or ANC cards (BioMerieux, Marcy l'Etoile, France) according to manufacturer's instructions. The criterion used for the acceptance of species-level identifications obtained from Vitek 2 was that the isolates were identified as the only choice with a confidence value of ≥85%. Genus-level identifications were reported when 2 to 3 species of the same genus were given. A no identification (No ID) result was denoted as (i) single identification result with confidence value less than 85%, (ii) multiple identification results including species of different genera or (iii) an "no ID" flag was provided by Vitek 2 owing to no identification present in the system database.

2.4 Vitek MS identification. Isolates were identified by Vitek MS system using a single deposit directly from bacterial colonies without any prior extraction step according to the manufacturer's guideline. In brief, a portion of a fresh colony was applied onto an individual spot of the 48-wells Vitek MS-DS disposable target slide, followed by overlaying with 1µl ready-to-use Vitek MS Matrix solution — saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid (BioMerieux, Marcy l'Etoile, France).

After drying, the target plate was loaded onto the Vitek MS mass spectrometer for target interrogation. The system reported the best identification match(es) along with confidence value(s) from 0% to 99.9%.

Samples returned with single identification results with any confidence values were considered as highly confidence at species level, whereas the result was only considered as genus level identification when the system proposed a split identification with any confidence values (low discrimination) that included species of the same genus. A “No ID” result was denoted if (i) no identification result was provided by the system or (ii) split identification result including species of different genera were given.

Samples with “No ID” were retested with a single deposit and the repeated result was considered as the final result for that specimen.

In addition to the *Escherichia coli* ATCC 8739, which served as the calibration control and internal identification control, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145 and *Clostridium perfringens* ATCC 13124 were used as the external positive control for Gram positive, Gram negative and anaerobic bacteria respectively. For quality control purpose, these external positive controls and the bacteria-free Vitek MS Matrix solution, which served as a negative control, were analyzed on each day of testing.

2.5 Final reference identification and Discrepancy resolution. When the Vitek MS system proposed a species-level identification that was completely matched with that provided by Vitek 2, the result was considered as the final reference identification and no further investigation was performed.

In case species-level identification was not available from either systems, or if there was mismatch between the two systems, bacterial DNA was extracted and mailed to Hong Kong for 16S rRNA sequencing. The resulting sequence was run through the Ridom and

16SpathDB databases to determine the final reference identification (Janda & Abbott 2007; Woo *et al.* 2011).

A correct identification was defined as any result from Vitek MS and Vitek 2 concordant with the final reference identification at the species and/or genus level, whereas a misidentification (mis-ID) result was denoted when the bacterial identifications obtained from Vitek MS and Vitek 2 did not match with the final reference bacterial identification.

2.6 Statistical analysis. Pearson's Chi-square test or Fisher's Exact test, where appropriate, were used to compare the results obtained by Vitek MS and Vitek II with the same specimen. Cohen's kappa coefficients (κ) were also calculated to determine the level of agreement between two methods.

3. RESULTS

3.1 Overall results. A total of 2,266 bacterial isolates were analyzed in this study. This included 1,581 aerobic Gram negative bacteria, 535 aerobic Gram positive bacteria and 150 anaerobes, representing 56 genera and 127 species. Overall, Vitek MS correctly identified 99.1% (2246/2266) of the isolates, including 96.8% (2193/2266) to the species level and an additional 2.3% (53/2266) to the genus level. The remaining 0.9% (20/2266) isolates were either misidentified (n=4) or denoted as no identification (n=16).

3.2 Aerobic Gram-Negative Bacteria. The 1,581 aerobic Gram negative isolates collected in the study were categorized into the *Enterobacteriaceae* family (n=1,182) and non-*Enterobacteriaceae* Gram negative organisms (n=399) (Table S1).

Vitek MS results agreed with the reference final identification for 99.4% (1175/1182) of the *Enterobacteriaceae* isolates, with 97.1% (1148/1182) identified to species level and 2.3% (28/1182) corrected to the genus level only (Table S1). Correct species-level identification was consistently obtained for all isolates among 12 species (*Enterobacter aerogenes*, *Enterobacter gergoviae*, *Enterobacter sakazakii*, *E. coli*, *Escherichia hermannii*, *Klebsiella oxytoca*, *Morganella morganii*, *Providencia rettgeri*, *Providencia stuartii*, *Serratia liquefaciens*, *Serratia marcescens* and *Salmonella typhi*) whereas four species were more likely to have a correct genus-level ID rather than species level by using Vitek MS *: Proteus vulgaris* (n=17, 0% species, 100% genus), *Raoultella ornithinolytica* (n=4, 0% species, 75% genus, 25% no ID), *Salmonella enterica ssp arizonae* (n=1, 0% species, 100% genus) and *Salmonella paratyphi A* (n=1, 0% species, 100% genus).

Vitek MS misidentified four isolates (two *Shigella spp*. isolates were misidentified as *E. coli*, one *Raoultella ornithinolytica* was misidentified as *Enterobacter aerogenes* and one *Proteus mirabilis* was misidentified as *Proteus vulgaris* / *Proteus penneri*) and failed to identify three

isolates ("no ID" for one each of *Citrobacter freundii* complex, *Citrobacter koseri* and *Klebsiella pneumoniae*).

Vitek MS correctly reported 99.2% (396/399) of non-*Enterobacteriaceae* Gram negative organisms, which encompassed 38 species and 25 genera, to species level (94.7%, 378/399) or to genus but not species level (4.5%, 18/399) (Table S1). A total of 29 species of organisms, including those commonly encountered important pathogens, such as *Acinetobacter baumannii* complex (n=44), *Pseudomonas aeruginosa* (n=212) and *Stenotrophomonas maltophilia* (n=67), showed concordant species-level identification with the reference method for over 90% of their isolates. Some species, including *Aeromonas caviae* (n=4), *Burkholderia pseudomallei* (n=2), *Ochrobacturm intermedium* (n=2), *Pseudomonas alcaligenes* (n=1) and *Pseudomonas otitidis* (n=1), were only limited to genus-level identification for all the isolates (0% species, 100% genus). The three isolates that could not be identified with Vitek MS (i.e. "No ID" flaggings) included one each of *Acinetobacter radioresistens*, *Campylobacter coli* and *Pasteurella dagmatis.*

3.3 Aerobic Gram-positive Bacteria. Among the 535 Gram-positive bacterial isolates (Table S2), the Staphylococci (n=357), Streptococci (n=68) and Enterococci (n=81) are the most commonly encountered pathogens in the clinical laboratories. In addition, 29 isolates of other Gram-positive cocci (n=9) and Gram-positive rods (n=20) were also collected during the study period (Table S2).

Of the 357 staphylococci, all but one was correctly identified to species level by Vitek MS. The missing isolate was *Staphylococcus lugdunensis*, which was only corrected to genus level as multiple choices of species-level identification were given. Similarly, with the exception of one *Enterococcus durans* isolate, which was returned with "No ID" from the system, Vitek MS provided correct species-level identification for all the Enterococci. The species-level identification for the Streptococci appeared to be more challenging than Staphylococci and

Enterococci, with 63/68 (92.6%) of the isolates being correctly identified to the species level. In particular, only 75% of the *Streptococcus bovis* group, 77.8% of the *Streptococcus dysgalactiae* and 85.7% of the *Streptococcus anginosus* group were correctly identified to species level (Table S2). Nevertheless, the Vitek MS correctly differentiated *Streptococcus pneumoniae* from *Streptococcus mitis* group for all but one case. The final reference identification for this case was *S. pneumoniae*, the Vitek MS, however, reported a split identification between *S. pneumoniae* and *S. mitis* group.

The remaining 29 Gram-positive organisms represented 9 additional genera. Vitek MS can readily provide 28 (96.6%) accurate identifications, with 27 (93.1%) corrected to species-level. Only one isolate of *Nocardia brasiliensis* was reported as no identification (Table S2).

3.4 Anaerobes. A total of 150 anaerobic bacterial isolates (8 genera and 19 species) were analyzed in this study. The Vitek MS provided correct identification for 95.3% (143/150) of the isolates, including 94.0% (141/150) down to species-level and 1.3% (2/150) achieved genus-level. The remaining 4.7% (7/150) isolates were provided with "no ID" results by the system (Table S3).

Vitek MS correctly identified 100% (28/28) and 97.3% (107/110) of the *Bacteroides spp.* and *Clostridia* spp., respectively. All the *Clostridium difficile* (n=85) and the 17 *Clostridium perfringens* (n=17) isolates were correctly identified to species level by Vitek MS. However, only 6 correct species-level identifications were obtained from those 12 non-Clostridia and non-Bacteroides anaerobes (Table S3).

3.5 Vitek MS versus Vitek 2. The Vitek 2 system provided 2,237 (98.7%) correct identification to the species level (n=2096; 92.5%) and genus level (n=141; 6.2%) respectively. Comparison of performance in species-level identification between Vitek MS and Vitek 2 were summarized in Table 1. In brief, with the exception of *Enterobacteriaceae*,

non-Enterobacteriaceae Gram-negative organisms and *Bacteroides*, for which species-level identifications were performed equally well by both system, Vitek MS surpassed the performance of Vitek 2 for those clinically important pathogens, including the Staphylococci, Streptococci, Enterococci and Clostridia. Conversely, Vitek 2 system demonstrated better performance in identifying the species of those non-Clostridia and non-Bacteroides anaerobes.

The final reference identifications for the 20 organisms with "mis ID" and "no ID" obtained from Vitek MS were listed in Table 2.

4 DISCUSSION

MALDI-TOF MS systems have been demonstrated to be a fast, accurate and reliable technique for identification of clinical relevant bacteria in many countries. This was the first large-scale evaluation study of Vitek MS performed in China. The major strength of this study is the extensive breadth of tested clinical isolates from good geographic representation of northern and southern region of China.

In this study, an almost perfect agreement between the identifications inferred by Vitek MS and those provided by phenotypic and genotypic reference methods was observed, with the overall concordance of 99.1%, which is better than those reported in Switzerland (94.7%) (Benagli *et al.* 2011), Netherlands (95.1%) (van Veen *et al.* 2010), France (96.2%) (Dubois *et al.* 2012).

Among the isolates collected during the study period, more than 50% belonged to the *Enterobacteriaceae* family. In accordance with a previous study that evaluated Vitek MS performance for identification of *Enterobacteriaceae* (Richter *et al.* 2013), our study showed accurate genus- and species-level identifications for majority of isolates (99.4 %) with only a small number of misidentifications. There was no particular members of *Enterobacteriaceae*

for which the Vitek MS consistently failed except *Shigella*. It has been well-documented that *Shigella* spp. and *E. coli* are indistinguishable from each other using 16S rRNA sequencing or MALDI-TOF MS systems, which is attributed to the taxonomic similarity of these two organisms (Johnson 2000; van den Beld & Reubsaet 2012). The misidentification, however, is considered as a major drawback from clinical point of view, particularly for diagnosis of acute gastrointestinal infections that necessitates to be resolved by conventional biochemical tests, such as lactose fermentation and indole production or motility.

Richter *et al* reported the inability of Vitek MS to discriminate the members of *Citrobacter*, such as *C. freundii*, *C. youngae*, *C. braakii* and *C. werkmanii*, and also the members of *Enterobacter*, such as *E. asburiae*, *E. cloacae*, *E. hormaechei*, and *E. kobei* (Richter, Sercia et al. 2013). Fortunately, as the clinical significances and the drug susceptibilities among the members are similar, the impact of misidentification by Vitek MS on patient care is minimal (Janda *et al.* 1994; Paauw *et al.* 2008). In our study, these species were interpreted as "*Citrobacter freundii* complex" and "*Enterobacter cloacae* complex" with the accuracy of 89.3% and 97.4% respectively, showing that this is probably the best approach to report MALDI-TOF MS results for these strains.

For non-*Enterobacteriaceae* Gram-negative bacilli, our study showed that the Vitek MS IVD system showed correct genus and species identification for 99.2% of the tested isolates. The result was in consistent with a multicenter evaluation study performed in US (Manji, Bythrow et al. 2013). Manji *et al* evaluated the performance of Vitek MS v2.0 for identification of 558 unique non-*Enterobacteriaceae* Gram-negative bacilli in US and was returned with overall accurate species and genus identification of 90.9% (Manji, Bythrow et al. 2013). Likewise, Van Veen *et al*, using the Microflex LT instrument, showed that the system could achieve the genus and species identification to 94.3% with similar variety of tested strains in Europe (van Veen, Claas et al. 2010). It should be noted that incorrect

identification of non-*Enterobacteriaceae* Gram-negative bacilli always results in significant negative clinical impact, especially for species with high drug resistant rate, such as *Acinetobacter baumannii* complex, or for bacteria with limited therapeutic choices, such as *Stenotrophomonas maltophilia* (Dortet *et al.* 2006; Howard *et al.* 2012; Vila & Pachon 2012). No false identification was obtained in this study, indicating that Vitek MS is a reliable diagnostic tool for enhancing the management of infections associated with non-*Enterobacteriaceae* Gram-negative bacilli in China.

In clinical microbiology laboratories, distinguishing one species from another among some Gram positive bacteria, especially the coagulase-negative Staphylococci, the viridians group Streptococci and some Enterococci, is often unreliable and overly complicated using conventional biochemical methods (Ruoff 2011). It has been previously demonstrated that MALDI-TOF MS system provides accurate and reliable platform for identification of these Gram-positive aerobic bacteria (Moon *et al.* 2013; Rychert, Burnham et al. 2013). Consistent with the findings of those studies, we also experienced the significant better performance of Vitek MS than Vitek 2 in species identification of Gram-positive bacteria, especially Staphylococci (99.7%), Enterococci (98.8%) and Streptococci (92.6%). This is particularly important for diagnosis of sepsis and meningitis, in which identification to species level is necessary. Additionally, the Vitek MS was shown to readily differentiate *S. pneumoniae* from *S. mitis* group species in our study. This was recognized as a strength of Vitek MS over MALDI Biotyper given that misidentifications of *S. mitis* group strains as *S. pneumoniae* by MALDI Biotyper were reported in several studies (Bizzini *et al.* 2010; Cherkaoui *et al.* 2010; Neville *et al.* 2011; McElvania Tekippe *et al.* 2013). However, this problem appears to have been overcome in the latest release of the Bruker Biotyper software (version 3.1; MBT-BDAL-5627 MSP library, Bruker Daltonics) (Harju *et al*. 2014).

Anaerobic bacteria represent a group of major infectious agents identified in clinical microbiology laboratories. However, owing to the low doubling time and relatively inert biochemical reactivity, phenotypic identification methods, including the commercially available biochemical kits, can be both time-consuming and laborious, hindering the selection of appropriate therapy. The development of MALDI-TOF MS systems provides the opportunity for rapid and accurate identification of anaerobe (Nagy *et al.* 2009; Garner, Mochon et al. 2013). Importantly, Vitek MS correctly identified all of the 85 tested *C. difficile* isolates to species level, which facilitate the timely initiation of appropriate infection control measures to limit the spread of disease. Nevertheless, MALDI-TOF MS showed varied rates of identification for other anaerobes. Asides from *Clostridia* and *Bacteroides*, Vitek MS only correctly identified 2/3 of anaerobes to genus or species level. The finding was similar to other previous investigations, which only resulted in up to 61% correct species-level identifications for similar variety of strains (Seng *et al.* 2009; La Scola *et al.* 2011). This highlights the importance of extending the coverage of database with the mass spectra of additional reference strains to improve the identification capacity for anaerobes using MALDI-TOF MS. Nevertheless, since there were only a small number of isolates were analyzed for several rarely encountered species, the study findings may underestimate the system's true capabilities in identification of anaerobes.

In conclusion, Vitek MS offered equivalent or even superior accuracy in comparison to Vitek 2 in identification of bacterial species among different clinically important pathogens. The introduction of Vitek MS system in our clinical laboratories would therefore facilitate shorter turnaround time with improved diagnostic accuracy for routine bacterial identification. With the current mass spectral reference database, Vitek MS is accurate and reliable for identification of nearly all aerobic bacteria, *Clostridia spp.* and *Bacteroides spp.* in our region. However, this technology still cannot completely supersede the biochemical

test panels, which are essential to supplement the areas of weakness of Vitek MS, such as the inability to differentiate *Shigella* species from *E. coli* and the suboptimal performance in identification of non-Clostridia and non-Bacteroides anaerobic bacteria. On the other hand, continuous expansion of Vitek MS spectral database is needed, particularly for anaerobic bacteria, in order to improve both the efficiency and the accuracy of Vitek MS in routine diagnostic microbiology.

5. ACKNOWLEDGEMENT

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6. REFERENCE

Anhalt, J. P. & C. Fenselau (1975). "Identification of bacteria using mass-spectrometry." *Anal. Chem.* **47**, 219-225.

Benagli, C., V. Rossi, M. Dolina, M. Tonolla & O. Petrini (2011). "Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of clinically relevant bacteria." *PLoS One* **6**(1), e16424.

Bizzini, A., C. Durussel, J. Bille, G. Greub & G. Prod'hom (2010). "Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory." *J Clin Microbiol* **48**(5), 1549-1554.

Chatzigeorgiou, K. S., T. N. Sergentanis, S. Tsiodras, S. J. Hamodrakas & P. G. Bagos (2011). "Phoenix 100 versus Vitek 2 in the identification of gram-positive and gram-negative bacteria: a comprehensive meta-analysis." *J Clin Microbiol* **49**(9), 3284-3291.

Chen, J. H., W. C. Yam, A. H. Ngan, A. M. Fung, W. L. Woo, M. K. Yan, G. K. Choi, P. L. Ho, V. C. Cheng & K. Y. Yuen (2013). "Advantages of using matrix-assisted laser desorption ionization-time of flight mass spectrometry as a rapid diagnostic tool for identification of yeasts and mycobacteria in the clinical microbiological laboratory." *J Clin Microbiol* **51**(12), 3981-3987.

Cherkaoui, A., J. Hibbs, S. Emonet, M. Tangomo, M. Girard, P. Francois & J. Schrenzel (2010). "Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level." *J Clin Microbiol* **48**(4), 1169-1175.

Dortet, L., P. Legrand, C. J. Soussy & V. Cattoir (2006). "Bacterial identification, clinical significance, and antimicrobial susceptibilities of Acinetobacter ursingii and Acinetobacter schindleri, two frequently misidentified opportunistic pathogens." *J Clin Microbiol* **44**(12), 4471-4478.

Dubois, D., M. Grare, M. F. Prere, C. Segonds, N. Marty & E. Oswald (2012). "Performances of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology." *J Clin Microbiol* **50**(8), 2568-2576.

Garner, O., A. Mochon, J. Branda, C. A. Burnham, M. Bythrow, M. Ferraro, C. Ginocchio, R. Jennemann, R. Manji, G. W. Procop other authors (2013). "Multi-centre evaluation of mass spectrometric identification of anaerobic bacteria using the VITEK MS system." *Clin Microbiol Infect*.

Harju, I., C. Lange, M. Kostrzewa, T. Maier & K. Rantakokko-Jalava (2014). Differentiation of Streptococcus pneumoniae And S.mitis-group Streptococci by MALDI Biotyper Using an Improved Database And Algorithm. *ASM 2014 114th General Meeting*. Boston, Massachusetts, US, American Society for Microbiology.

Howard, A., M. O'Donoghue, A. Feeney & R. D. Sleator (2012). "Acinetobacter baumannii: an emerging opportunistic pathogen." *Virulence* **3**(3), 243-250.

Janda, J. M. & S. L. Abbott (2007). "16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls." *J Clin Microbiol* **45**(9), 2761-2764.

Janda, J. M., S. L. Abbott, W. K. Cheung & D. F. Hanson (1994). "Biochemical identification of citrobacteria in the clinical laboratory." *J Clin Microbiol* **32**(8), 1850-1854.

Johnson, J. R. (2000). "Shigella and Escherichia coli at the crossroads: machiavellian masqueraders or taxonomic treachery?" *J Med Microbiol* **49**(7), 583-585.

La Scola, B., P. E. Fournier & D. Raoult (2011). "Burden of emerging anaerobes in the MALDI-TOF and 16S rRNA gene sequencing era." *Anaerobe* **17**(3), 106-112.

Manji, R., M. Bythrow, J. A. Branda, C. A. Burnham, M. J. Ferraro, O. B. Garner, R. Jennemann, M. A. Lewinski, A. B. Mochon, G. W. Procop other authors (2013). "Multi-center evaluation of the VITEK(R) MS system for mass spectrometric identification of non-Enterobacteriaceae Gram-negative bacilli." *Eur J Clin Microbiol Infect Dis*.

McElvania Tekippe, E., S. Shuey, D. W. Winkler, M. A. Butler & C. A. Burnham (2013). "Optimizing identification of clinically relevant Gram-positive organisms by use of the Bruker Biotyper

matrix-assisted laser desorption ionization-time of flight mass spectrometry system." *J Clin Microbiol* **51**(5), 1421-1427.

Moon, H. W., S. H. Lee, H. S. Chung, M. Lee & K. Lee (2013). "Performance of the Vitek MS matrix-assisted laser desorption ionization time-of-flight mass spectrometry system for identification of Gram-positive cocci routinely isolated in clinical microbiology laboratories." *J Med Microbiol* **62**(Pt 9), 1301-1306.

Nagy, E., T. Maier, E. Urban, G. Terhes, M. Kostrzewa & E. S. G. o. A. R. i. A. Bacteria (2009). "Species identification of clinical isolates of Bacteroides by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry." *Clin Microbiol Infect* **15**(8), 796-802.

Neville, S. A., A. Lecordier, H. Ziochos, M. J. Chater, I. B. Gosbell, M. W. Maley & S. J. van Hal (2011). "Utility of matrix-assisted laser desorption ionization-time of flight mass spectrometry following introduction for routine laboratory bacterial identification." *J Clin Microbiol* **49**(8), 2980-2984.

Paauw, A., M. P. Caspers, F. H. Schuren, M. A. Leverstein-van Hall, A. Deletoile, R. C. Montijn, J. Verhoef & A. C. Fluit (2008). "Genomic diversity within the Enterobacter cloacae complex." *PLoS One* **3**(8), e3018.

Richter, S. S., L. Sercia, J. A. Branda, C. A. Burnham, M. Bythrow, M. J. Ferraro, O. B. Garner, C. C. Ginocchio, R. Jennemann, M. A. Lewinski other authors (2013). "Identification of Enterobacteriaceae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system." *Eur J Clin Microbiol Infect Dis* **32**(12), 1571-1578.

Ruoff, K. (2011). General approaches to identification of aerobic gram-positive cocci. *Manual of clinical microbiology*. J. Versalovic, K. C. Carroll, G. Funkeet al. Washington DC, ASM Press. **1:** 304-307.

Rychert, J., C. A. Burnham, M. Bythrow, O. B. Garner, C. C. Ginocchio, R. Jennemann, M. A. Lewinski, R. Manji, A. B. Mochon, G. W. Procop other authors (2013). "Multicenter evaluation of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of Gram-positive aerobic bacteria." *J Clin Microbiol* **51**(7), 2225-2231.

Seng, P., M. Drancourt, F. Gouriet, B. La Scola, P. E. Fournier, J. M. Rolain & D. Raoult (2009). "Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry." *Clin Infect Dis* **49**(4), 543-551.

van den Beld, M. J. & F. A. Reubsaet (2012). "Differentiation between Shigella, enteroinvasive Escherichia coli (EIEC) and noninvasive Escherichia coli." *Eur J Clin Microbiol Infect Dis* **31**(6), 899-904.

van Veen, S. Q., E. C. Claas & E. J. Kuijper (2010). "High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories." *J Clin Microbiol* **48**(3), 900-907.

Vila, J. & J. Pachon (2012). "Therapeutic options for Acinetobacter baumannii infections: an update." *Expert Opin Pharmacother* **13**(16), 2319-2336.

Westblade, L. F., R. Jennemann, J. A. Branda, M. Bythrow, M. J. Ferraro, O. B. Garner, C. C. Ginocchio, M. A. Lewinski, R. Manji, A. B. Mochon other authors (2013). "Multicenter study evaluating the Vitek MS system for identification of medically important yeasts." *J Clin Microbiol* **51**(7), 2267-2272.

Woo, P. C., J. L. Teng, J. M. Yeung, H. Tse, S. K. Lau & K. Y. Yuen (2011). "Automated identification of medically important bacteria by 16S rRNA gene sequencing using a novel comprehensive database, 16SpathDB." *J Clin Microbiol* **49**(5), 1799-1809.

Table S1: Identification results from Gram-negative aerobic bacteria obtained from Vitek MS and Vitek 2.

	Vitek MS				Vitek 2			
	No. (%) of isolates with correct ID to the level of				No. (%) of isolates with correct ID to the level of			
Reference identification (no. of isolates)	Species*	Genus†	No ID‡	Mis ID§	Species	Genus	No ID	Mis ID
Enterobacteriaceae (1,182)	**1,148 (97.1)**	**27 (2.3)**	**3 (0.3)**	**4 (0.3)**	**1,156 (97.8)**	**26 (2.2)**	**0**	**0**
Citrobacter freundii complex‖ (28)	25 (89.3)	2 (7.1)	1 (3.6)	0	28 (100)	0	0	0
Citrobacter koseri (11)	10 (91)	0	1 (9)	0	11 (100)	0	0	0
Enterobacter aerogenes (35)	35 (100)	0	0	0	35 (100)	0	0	0
Enterobacter cloacae complex¶ (76)	74 (97.4)	2 (2.6)	0	0	74 (97.4)	2 (2.6)	0	0
Enterobacter gergoviae (1)	1 (100)	0	0	0	1 (100)	0	0	0
Enterobacter sakazakii (2)	2 (100)	0	0	0	2 (100)	0	0	0
Escherichia coli (595)	595 (100)	0	0	0	595 (100)	0	0	0
Escherichia hermannii (1)	1 (100)	0	0	0	1 (100)	0	0	0
Klebsiella oxytoca (48)	48 (100)	0	0	0	48 (100)	0	0	0
Klebsiella pneumoniae (135)	134 (99.3)	1 (0.7)	0	0	134 (99.3)	1 (0.7)	0	0
Klebsiella ozaenae (1)	0	0	1 (100)	0	1 (100)	0	0	0
Morganella morganii (24)	24 (100)	0	0	0	24 (100)	0	0	0
Proteus mirabilis (146)	145 (99.3)	0	0	1 (0.7)	146 (100)	0	0	0
Proteus vulgaris (17)	0	17 (100)	0	0	0	17 (100)	0	0
Providencia rettgeri (3)	3 (100)	0	0	0	3 (100)	0	0	0
Providencia stuartii (3)	3 (100)	0	0	0	3 (100)	0	0	0
Raoultella ornithinolytica (4)	0	3 (75)	0	1 (25)	0	4 (100)	0	0
Serratia liquefaciens (1)	1 (100)	0	0	0	1 (100)	0	0	0
Serratia marcescens (46)	46 (100)	0	0	0	46 (100)	0	0	0
Salmonella enterica ssp arizonae (1)	0	1 (100)	0	0	1 (100)	0	0	0
Salmonella paratyphi A (1)	0	1 (100)	0	0	1 (100)	0	0	0
Salmonella typhi (1)	1 (100)	0	0	0	1 (100)	0	0	0
Shigella flexneri (1)	0	0	0	1 (100)	0	1 (100)	0	0
Shigella sonneii (1)	0	0	0	1 (100)	0	1 (100)	0	0
Non-*Enterobacteriaceae* Gram-Negative organisms (399)	**378 (94.7)**	**18 (4.5)**	**3 (0.8)**	**0**	**367 (92.0)**	**20 (5.0)**	**12 (3.0)**	**0**
Achromobacter xylosoxidans (5)	5 (100)	0	0	0	5 (100)	0	0	0
Acinetobacter baumannii complex (44)	40 (91.0)	4 (9.0)	0	0	42 (95.4)	1 (2.3)	1 (2.3)	0
*Acinetobacter jonnosonii (*1)	1 (100)	0	0	0	0	0	1 (100)	0
Acinetobacter radioresistens (1)	0	0	1 (100)	0	1 (100)	0	0	0
Acinetobacter ursingii (3)	3 (100)	0	0	0	3 (100)	0	0	0
Actinobacillus ureae (1)	1 (100)	0	0	0	0	0	1 (100)	
Aeromonas caviae (4)	0	4 (100)	0	0	0	4 (100)	0	0
Bergeyella zoohelcum (1)	1 (100)	0	0	0	0	0	1 (100)	0
Bordetella pertussis (1)	1 (100)	0	0	0	0	0	1 (100)	0
Burkholderia cepacia complex# *(4)*	4 (100)	0	0	0	4 (100)	0	0	0
Burkholderia pseudomallei (2)	0	2 (100)	0	0	2 (100)	0	0	0
Campylobacter jejuni (2)	2 (100)	0	0	0	0	0	2 (100)	0
Campylobacter coli (1)	0	0	1 (100)	0	0	0	1 (100)	0
Chromobacterium violaceum (1)	1 (100)	0	0	0	1 (100)	0	0	0
Chryseobacterium indologenes (4)	4 (100)	0	0	0	4 (100)	0	0	0
Cupriavidus pauculus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Delftia acidovorans (3)	3 (100)	0	0	0	1 (33.3)	0	2 (66.6)	0
Eikenella corrodens (1)	1 (100)	0	0	0	1 (100)	0	0	0
Elizabethkingia meningoseptica (3)	3 (100)	0	0	0	3 (100)	0	0	0
Haemophilus influenzae (2)	2 (100)	0	0	0	2 (100)	0	0	0
Haemophilus parahaemolyticus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Moraxella osloensis (1)	1 (100)	0	0	0	0	0	1 (100)	0
Neisseria gonorrhoeae (2)	2 (100)	0	0	0	1 (50)	1 (50)	0	0
Ochrobacturm intermedium (2)	0	2 (100)	0	0	0	1 (50)	1 (50)	0
Pasteurella dagmatis (1)	0	0	1 (100)	0	0	1 (100)	0	0
Pasteurella multocida (4)	4 (100)	0	0	0	0	4 (100)	0	0
Plesiomonas shigelloides (1)	1 (100)	0	0	0	1 (100)	0	0	0
Pseudomonas aeruginosa (212)	209 (98.6)	3 (1.4)	0	0	209 (98.6)	3 (1,4)	0	0
Pseudomonas alcaligenes (1)	0	1 (100)	0	0	0	1 (100)	0	0
Pseudomonas otitidis (1)	0	1 (100)	0	0	0	1 (100)	0	0
Pseudomonas putida (8)	8 (100)	0	0	0	6 (75)	2 (25)	0	0
Ralstonia mannitolilytica (2)	1 (50)	1 (50)	0	0	2 (100)	0	0	0
Ralstonia pickettii (1)	1 (100)	0	0	0	1 (100)	0	0	0
Shewanella algae (6)	6 (100)	0	0	0	6 (100)	0	0	0
Stenotrophomonas maltophilia (67)	67 (100)	0	0	0	66 (98.5)	1 (1.5)	0	0
Vibrio cholerae (1)	1 (100)	0	0	0	1 (100)	0	0	0
Vibrio parahaemolyticus (2)	2 (100)	0	0	0	2 (100)	0	0	0
Vibrio vulnificus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Total (1,581)	**1,526 (96.6)**	**45 (2.8)**	**6 (0.4)**	**4 (0.2)**	**1,523 (96.3)**	**46 (2.9)**	**12 (0.8)**	**0**

* Species-level identification was denoted when only one identification result was obtained from Vitek MS.
† Genus-level identification was denoted when a split identification (low discrimination) that included species of the same genus was obtained from Vitek MS.
‡ No ID was denoted when (i) multiple identifications that included species of different genera, or (ii) "no ID" flagging was obtained from Vitek MS.
§ Mis-ID was denoted when the bacterial identifications obtained from Vitek MS did not match with the final reference bacterial identification.
‖ *Citrobacter freundii complex includes Citrobacter freundii, C. braakii, C. gilenii, C. murliniae, C. werkmanii and youngae.*
¶ *Enterobacter cloacae complex includes Enterobacter cloacae, Enterobacter anigenus, Enterobacter intermedium and Enterobacter kobei.*
# *Burkholderia cepacia complex includes B. cepacia, B. multivorans, B. stabilis, B. vietnamiensis, B. ambifaria, B. anthina, B. pyrrocinia and genomovar III and VI.*

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Table S2: Identification results from Gram-positive aerobic bacteria obtained from Vitek MS and Vitek 2.

Reference identification (no. of isolates)	Vitek MS				Vitek 2			
	No. (%) of isolates with correct ID to the level of				No. (%) of isolates with correct ID to the level of			
	Species[*]	Genus[†]	No ID[‡]	Mis ID[§]	Species	Genus	No ID	Mis ID
***Staphylococcus* (357)**	**356 (99.7)**	**1 (0.3)**	**0**	**0**	**330 (92.4)**	**26 (7.3)**	**0**	**1 (0.3)**
Staphylococcus aureus (213)	213 (100)	0	0	0	195 (91.5)	18 (18.5)	0	0
Staphylococcus caprae (1)	1 (100)	0	0	0	1 (100)	0	0	0
Staphylococcus capitis (12)	12 (100)	0	0	0	12 (100)	0	0	0
Staphylococcus cohnii (3)	3 (100)	0	0	0	3 (100)	0	0	0
Staphylococcus epidermidis (55)	55 (100)	0	0	0	54 (98.2)	1 (1.8)	0	0
Staphylococcus haemolyticus (16)	16 (100)	0	0	0	15 (93.8)	1 (6.2)	0	0
Staphylococcus hominis (41)	41 (100)	0	0	0	37 (90.2)	4 (9.8)	0	0
Staphylococcus lugdunensis (9)	8 (88.9)	1 (11.1)	0	0	8 (88.9)	0	0	1 (11.1)
Staphylococcus saprophyticus (3)	3 (100)	0	0	0	2 (66.7)	1 (33.3)	0	0
Staphylococcus sciuri (2)	2 (100)	0	0	0	2 (100)	0	0	0
Staphylococcus simulans (1)	1 (100)	0	0	0	1 (100)	0	0	0
Staphylococcus schleiferi (1)	1 (100)	0	0	0	0	1 (100)	0	0
Enterococcus (81)	**80 (98.8)**	**0**	**1 (1.2)**	**0**	**75 (92.6)**	**4 (4.9)**	**0**	**2 (2.5)**
Enterococcus avium (4)	4 (100)	0	0	0	4 (100)	0	0	0
Enterococcus casseliflavus (4)	4 (100)	0	0	0	3 (75)	1 (25)	0	0
Enterococcus faecalis (27)	27 (100)	0	0	0	27 (100)	0	0	0
Enterococcus faecium (38)	38 (100)	0	0	0	35 (92.1)	2 (5.3)	0	1 (2.6)
Enterococcus gallinarum (4)	4 (100)	0	0	0	3 (75)	1 (25)	0	0
Enterococcus durans (4)	3 (75)	0	1 (25)	0	3 (75)	0	0	1 (25)
***Streptococcus* (68)**	**63 (92.6)**	**4 (5.9)**	**1 (1.5)**	**0**	**54 (79.3)**	**10 (14.7)**	**2 (3)**	**2 (3)**
Streptococcus agalactiae (12)	12 (100)	0	0	0	10 (83.3)	2 (16.7)	0	0
Streptococcus bovis group[‖] (4)	3 (75)	0	1 (25)	0	3 (75)	1 (25)	0	0
Streptococcus dysgalactiae (9)	7 (77.8)	2 (22.2)	0	0	9 (100)	0	0	0
Streptococcus anginosus group[¶] (7)	6 (85.7)	1 (14.3)	0	0	3 (42.9)	3 (42.9)	0	1 (14.2)
Steptococcus gallolyticus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Steptococcus gordonii(1)	1 (100)	0	0	0	1 (100)	0	0	0
Streptococcus mitis group[#] (11)	11 (100)	0	0	0	8 (72.7)	2 (18.2)	0	1 (9.1)
Steptococcus parasanguinis (1)	1 (100)	0	0	0	1 (100)	0	0	0
Streptococcus pneumoniae (10)	9 (90)	1 (10)	0	0	9 (90)	1 (10)	0	0
Streptococcus pyogenes (5)	5 (100)	0	0	0	5 (100)	0	0	0
Streptococcus salivarius (4)	4 (100)	0	0	0	3 (75)	1 (25)	0	0
Streptococcus sanguinis (1)	1 (100)	0	0	0	1 (100)	0	0	0
Streptococcus suis (2)	2 (100)	0	0	0	0	0	2 (100)	0
Other Gram-positive cocci (9)	**9 (100)**	**0**	**0**	**0**	**7 (77.8)**	**2 (22.2)**	**0**	**0**
Granulicatella adiacens (1)	1 (100)	0	0	0	1 (100)	0	0	0
Lactococcus garvieae (1)	1 (100)	0	0	0	0	1 (100)	0	0
Micrococcus luteus (6)	6 (100)	0	0	0	6 (100)	0	0	0
Pediococcus acidilactici (1)	1 (100)	0	0	0	0	1 (100)	0	0
Gram-positive rods (20)	**18 (90)**	**1 (5)**	**1 (5)**	**0**	**9 (45)**	**3 (15)**	**8 (40)**	**0**
Arcanobacterium haemolyticum (2)	2 (100)	0	0	0	2 (100)	0	0	0
Arcanobacterium pyogenes (1)	1 (100)	0	0	0	1 (100)	0	0	0
Bacillus cereus group[¤] *(4)*	4 (100)	0	0	0	0	0	4 (100)	0
Bacillus circulans(1)	1 (100)	0	0	0	0	0	1 (100)	0
Bacillus megaterium (1)	1 (100)	0	0	0	0	0	1 (100)	0
Corynebacterium jerkerium(1)	1(100)	0	0	0	1 (100)	0	0	0
Corynebacterium striatum (3)	3 (100)	0	0	0	1 (33.3)	1 (33.3)	1 (33.3)	0
Corynebacterium urealyticum (2)	2 (100)	0	0	0	2 (100)	0	0	0
Listeria monocytogenes (3)	3 (100)	0	0	0	1 (33.3)	2 (66.7)	0	0
Listeria ivanovii (1)	0	1 (100)	0	0	1 (100)	0	0	0
Nocardia brasiliensis (1)	0	0	1 (100)	0	0	0	1 (100)	0
Total (535)	**526 (98.3)**	**6 (1.1)**	**3 (0.6)**	**0**	**475 (88.8)**	**45 (8.4)**	**10 (1.9)**	**5 (0.9)**

[*] Species-level identification was denoted when only one identification result was obtained from Vitek MS.

[†] Genus-level identification was denoted when a split identification (low discrimination) that included species of the same genus was obtained from Vitek MS.

[‡] No ID was denoted when (i) multiple identifications that included species of different genera, or (ii) "no ID" flagging was obtained from Vitek MS.

[§] Mis-ID was denoted when the bacterial identifications obtained from Vitek MS did not match with the final reference bacterial identification.

[‖] *Streptococcus bovis group* includes *S. bovis, S. alactolyticus, S. infantarius and S. gallolyticus.*

[¶] *Streptococcus anginosus group includes S. anginosus, S. constellatus and S. intermedius.*

[#] *Streptococcus mitis group includes S. sanguis, S. parasanguis, S. gordonii, S. crista, S. oralis, S. mitis, S. peroris and S. infantis.*

[¤] *Bacillus cereus group includes Bacillus cereus, Bacillus thuringiensis and Bacillus mycoides.*

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Table S3: Identification results from anaerobic bacteria obtained from Vitek MS and Vitek 2.

	Vitek MS				Vitek 2			
	No. (%) of isolates with correct ID to the level of				No. (%) of isolates with correct ID to the level of			
Reference identification (no. of isolates)	Species*	Genus†	No ID‡	Mis ID§	Species	Genus	No ID	Mis-ID
Clostridia (110)	**107 (97.3)**	**0**	**3 (2.7)**	**0**	**61 (55.5)**	**49 (44.5)**	**0**	**0**
Clostridium bifermentans (2)	2 (100)	0	0	0	0	2 (100)	0	0
Clostridium butyricum (1)	1 (100)	0	0	0	0	1 (100)	0	0
Clostridium difficile (85)	85 (100)	0	0	0	42 (49.4)	43 (50.6)	0	0
Clostridium innocuum (2)	0	0	2 (100)	0	0	2 (100)	0	0
Clostridium novyi (1)	0	0	1 (100)	0	0	1 (100)	0	0
Clostridium perfringens (17)	17 (100)	0	0	0	17 (100)	0	0	0
Clostridium ramosum (1)	1 (100)	0	0	0	1 (100)	0	0	0
Clostridium septicum (1)	1 (100)	0	0	0	1 (100)	0	0	0
Bacteroides (28)	**28 (100)**	**0**	**0**	**0**	**28 (100)**	**0**	**0**	**0**
Bacteroides vulgatus (4)	4 (100)	0	0	0	4 (100)	0	0	0
Bacteroides thetaiotaomicron (3)	3 (100)	0	0	0	3 (100)	0	0	0
Bacteroides ovatus (6)	6 (100)	0	0	0	6 (100)	0	0	0
Bacteroides fragilis (15)	15 (100)	0	0	0	15 (100)	0	0	0
Other anaerobes (12)	**6 (50)**	**2 (16.7)**	**4 (33.3)**	**0**	**9 (75)**	**1 (8.3)**	**2 (16.7)**	**0**
Actinomyces europaeus (1)	1 (100)	0	0	0	0	0	1 (100)	0
Atopobium parvulum (1)	0	0	1 (100)	0	0	0	1 (100)	0
Eggerthella lenta (4)	0	2 (50)	2 (50)	0	4 (100)	0	0	0
Propionibacterium avidum (1)	0	0	1 (100)	0	0	1 (100)	0	0
Propionibacterium acnes (1)	1 (100)	0	0	0	1 (100)	0	0	0
Finegoldia magna (1)	1 (100)	0	0	0	1 (100)	0	0	0
Parvimonas micra (3)	3 (100)	0	0	0	3 (100)	0	0	0
Anaerobes (150)	**141 (94.0)**	**2 (1.3)**	**7 (4.7)**	**0**	**98 (65.4)**	**50 (33.3)**	**2 (1.3)**	**0**

* Species-level identification was denoted when only one identification result was obtained from Vitek MS.

† Genus-level identification was denoted when a split identification (low discrimination) that included species of the same genus was obtained from Vitek MS.

‡ No ID was denoted when (i) multiple identifications that included species of different genera, or (ii) "no ID" flagging was obtained from Vitek MS.

§ Mis-ID was denoted when the bacterial identifications obtained from Vitek MS did not match with the final reference bacterial identification.

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Table 1: Comparison of performance in species-level identification between Vitek MS and Vitek 2

Bacterial group	No. of isolates	Percentage (%) of species-level identification		kappa / *p* value
		Vitek MS	Vitek 2	
Enterobacteriaceae	1,182	97.1	97.8	0.863 / 0.598
Non-Enterobacteriaceae Gram-negative organism	399	94.7	92	0.484 / 0.074
Staphylococcus	357	99.7	92.4	0.066 / <0.01
Streptococcus	68	92.6	79.4	0.134 / <0.01
Enterococcus	81	98.8	92.6	0.270 / 0.043
Gram-positive rods	20	90	45	0.031 / <0.01
Clostridia	110	97.3	55.5	0.067 / <0.01
Bacteroides	28	100	100	n.a.*
Non-Clostridia and non-Bacteroides anaerobes	12	50	75	0.100 / 0.023
Anaerobes (Overall)	150	94	65.4	0.037 / 0.011

* Cohen's kappa coefficients (κ) cannot be calculated as the results from both methods are not variable.

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Table 2: Final reference identifications for the 20 isolates with mis ID or no ID obtained from Vitek MS

Final reference ID	No. of isolates	Identities given by		
		Vitek MS (Confidence value)	Vitek 2 (Confidence value)	16S rRNA
Citrobacter freundii complex	1	No ID	*Citrobacter braakii (97%)*	*Citrobacter murliniae*
Citrobacter koseri	1	No ID	*Citrobacter koseri (99%)*	*Citrobacter koseri*
Klebsiella ozaenae[*]	1	No ID	no ID	*Klebsiella ozaenae*
Proteus mirabilis	1	*Proteus penneri* (50.0%); *Proteus vulgaris* (50.0%)	*Proteus mirabilis* (99%)	*Proteus mirabilis*
Raoultella ornithinolytica	1	*Enterobacter aerogenes* (99.9%)	*Raoultella ornithinolytica (99%); Raoultella planticola (97%)*	*Raoultella ornithinolytica*
Shigella flexneri	1	*E. coli* (99.9%)	*Shigella flexneri (99.9%)*	*Shigella flexneri*
Shigella sonneii	1	*E. coli* (99.9%)	*Shigella sonneii (99.9%)*	*Shigella sonneii*
Acinetobacter radioresistens	1	No ID	*Acinetobacter radioresistens(99.9%)*	*Acinetobacter radioresistens*
Campylobacter coli	1	No ID	no ID	*Campylobacter coli*
*Pasteurella dagmatis**	1	No ID	*Pasteurella dagmatis* (99%); *Pasteurella stomatis*(97%)	*Pasteurella dagmatis*
Streptococcus bovis group	1	No ID	*Streptococcus bovis (96%); Streptococcus equinus(91%)*	*Streptococcus bovis/Streptococcus gallolyticus*
Enterococcus durans	1	No ID	*Enterococcus gallinarum* (88%)	*Enterococcus durans*
Nocardia brasiliensis[*]	1	No ID	No ID	*Nocardia brasiliensis*
Clostridium innocuum[*]	2	No ID	No ID	*Clostridium innocuum*
Clostridium novyi[*]	1	No ID	No ID	*Clostridium novyi*
Atopobium parvulum[*]	1	No ID	No ID	*Atopobium parvulum*
Eggerthella lenta	2	No ID	*Eggerthella lenta*	*Eggerthella lenta*
Propionibacterium avidum	1	No ID	*Propionibacterium granulosum* (91%); *Propionibacterium propionicus* (88%)	*Propionibacterium avidum*

[*] *Species absent from database*

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