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Performances of the MALDI-TOF Mass Spectrometry system VITEK MS for the Rapid Identification of Bacteria in Routine Clinical Microbiology

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Running title: BACTERIAL IDENTIFICATION WITH MALDI-TOF VITEK MS

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ABSTRACT

Rapid and cost-effective MALDI-TOF MS-based systems will replace conventional phenotypic methods for routine identification of bacteria. We report here the first evaluation of the new MALDI-TOF MS-based VITEK MS system in a large clinical microbiology laboratory. This system used an original spectra classifier algorithm and a specific database designed for the identification of clinically relevant species.

We have tested 767 routine clinical isolates representative of 50 genera and 124 species. VITEK MS-based identifications were performed by means of a single deposit on MALDI disposable target, without any prior extraction step, and compared with reference identifications, mainly obtained with the VITEK2 phenotypic system; if discordant, molecular techniques provided reference identifications.

The VITEK MS system provided 96.2% of correct identifications: to the species level (86.7%), to the genus level (8.2%), or within a range of species belonging to different genera (1.3%). Conversely, 1.3% of isolates were misidentified and 2.5% unidentified, partly because the species was not included in the database; a second deposit provided a successful identification for 0.8% of isolates unidentified with the first deposit.

The VITEK MS system is a simple, convenient and accurate method for routine bacterial identification with a single deposit, considering the high bacterial diversity studied as evidence by the low prevalence of species without correct identification. In addition to a second deposit in uncommon cases, expanding the spectral database is expected to further enhance performances.

Keywords: MALDI-TOF MS, bacteria, identification

INTRODUCTION

Due to the dramatic increase of bacterial resistance and to the ecological cost of broad spectrum antimicrobial therapies, rapid and accurate identification (ID) of bacteria are essential for the appropriate management of infections. Conventional identification methods require at least 4 to 12 h and molecular methods are not suitable for large scale routine identification.

Nearly 40 years ago, chemists proposed to identify bacterial cultures via the detection of small organic molecules using mass spectrometry (2). More than ten years later, matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) allowed the detection of intact larger biomolecules such as proteins and was further developed for microbial ID in routine clinical laboratories (13). During the analysis process, proteins are ionized without fragmentation by the coordinated action of the laser and the small organic acids of the matrix, and separated on the basis of their mass-to-charge ratios, which results in a characteristic mass spectral profile. Microbial ID is based on the comparison of the protein spectrum generated from intact whole bacterial cells to a database of species-specific reference protein profiles using a particular algorithm.

In the mid-1990s, different groups have developed their own library of bacterial reference mass spectra and software for bacterial identification and taxonomic classification. After a decade of optimization of the method parameters, like the reproducibility of mass spectral profiles at different locations, the robustness to account for variations and variability in culture conditions, the application to the majority of clinically relevant bacteria, and an automated mass spectral analysis, the MALDI-TOF MS-based bacterial ID became suitable for a routine use in applied laboratories (11). Commercial user-friendly devices containing different algorithms for the classification of bacterial protein mass patterns, associated with

databases including several thousand bacterial reference entries for bacterial ID were available, mainly represented by the Biotyper (Bruker Daltonics, Germany), VITEK MS RUO (formerly SARAMIS) and VITEK MS (bioMérieux, France), and Andromas (Andromas SAS, France) systems (8, 19). Numerous studies reported the fast, easy-to-use, cost-effective and thus high-throughput performances of these MALDI-TOF MS systems for bacterial ID in clinical laboratories, using duplicate deposit on MALDI target (4, 5, 7, 9, 15, 20, 24).

The objective of the present study was to evaluate the performances and technical praticability of the VITEK MS system (bioMérieux), a recently commercialized MALDI-TOF-based method using an original spectra classifier algorithm (i.e comparison of the presence and the absence of specific peaks between the obtained spectrum and the typical spectrum of each claimed species, previously determined with 10 different reference strains, using an analysis mass range from 3,000 to 17,000 Da) and a database of 586 species (including 508 bacterial and 78 fungal taxa). In contrast to most of previous studies having analyzed the ID performances of other MALDI-TOF-based systems with two deposits or even protein extraction (5, 7, 15, 20, 24), the VITEK MS was assessed here using a single deposit without any prior extraction step from bacterial colonies.

MATERIALS AND METHODS

Bacterial isolates

In order to capture the broad bacterial diversity clinically significant encountered in our large medical laboratory's routine, bacterial isolates were prospectively recovered over a 6-week period from various clinical specimens (such as blood, urine, stool, pus, biopsy, cerebrospinal fluid, respiratory tract, wounds specimens, swabs from any site of the body), and different medical departments, including no more than 30 consecutive isolates per species (any surplus isolates tested for a given species were kept for performance analysis). Isolate duplicates (i.e. from the same patient) were discarded.

The isolates were recovered after laboratory's routine ID and purity control on appropriate agar plate (5% sheep blood agar, chocolate agar, or BCYE agar media – bioMerieux) and under appropriate atmosphere (aerobic, microaerophilic, or anaerobic incubation) after 24h to 72h of incubation at 35°C.

The 767 isolates included in the study encompassed 282 *Enterobacteriaceae,* 94 nonfermentative Gram negative rods, 47 other Gram negative bacteria, 127 *Staphylococci* and related species, 177 *Streptococci* and related species, 30 anaerobes, and 10 other Gram positive rods (**Table 1**).

Reference identification and results management

Isolates were simultaneously identified by the VITEK MS system and, as reference methods, by the conventional VITEK2 system using the GP, GN, NH, or ANC cards (bioMérieux) if applicable, or by genomic methods otherwise. VITEK2 IDs were performed according to the recommendations of the manufacturer, including complementary tests if required.

When the VITEK MS system proposed as a single choice or in a multiple choice the VITEK2 IDs to the species level, no further investigation was performed. In case of discordant results between VITEK MS and VITEK2 methods, or low discrimination results with VITEK2 , or 'No ID' obtained with VITEK2 or VITEK MS methods, genomic IDs were performed and considered then as the reference ID.

Genomic IDs were performed using the sequencing-based Mastermix 16S Complete kit (Molzym GmbH) targeting the 5' partial 16S rRNA gene as first line. If inconclusive ID remained, sequencing-based IDs using *sodA* gene for coagulase-negative *Staphylococci*, *Streptococci*, and *Enterococci*, *recA* gene for *Burkholderia cepacia* complex, and 3' partial 16S rRNA gene for other taxa were implemented (16, 17, 21). Moreover, the PCR- and hybridization-based system GenoType® EHEC (Hain LifeScience) detecting the *ipaH* gene associated to serotyping method were performed to confirm *Shigella* isolates. An optochin susceptibility test for *Streptococcus pneumoniae* isolates and a species-specific PCR targeting *crgA* gene for *Neisseria meningitidis* isolates were also used (23).

MALDI-TOF MS

Technical training

Prior to the assessment initiation, the four operators involved were trained for sample and slide preparation by performing three slides of 48 deposits with duplicate deposits per isolate, during three independent days (one slide per day). Mucoid and rough isolates were only included in the third slide performed by each operator. A proficiency test was passed by each operator using 16 strains with single deposits.

Plate preparation

The disposable plate preparation was performed with the VITEK® MS Preparation Station software to link sample informations to VITEK® MS spectrophotometer, using the single-use FlexiMass MALDI target plates, supplied in a 48 well microscope slide format, divided in three acquisition groups of 16 spots, and inoculated by picking an overnight culture with a 1 µL disposable loop and by smearing the specimen directly onto the plate (mostly one deposit/colony). The preparations were overlaid with 1 µl of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, and 2.5% trifluoroacetic acid) and air-dehydrated 1–2 min at room temperature. As recommended by the manufacturer, the *Escherichia coli* ATCC 8739 strain, used as a calibrator and internal ID control, was inoculated on the calibration spots of each acquisition groups (small spot in the middle of each acquisition group). Each bacterial isolate had been tested with an unique deposit.

Generation of mass spectra

Mass spectra were generated with a VITEK® MS Axima® Assurance mass spectrometer (bioMérieux), in positive linear mode, at a laser frequency of 50 Hz, with an acceleration voltage of 20 kV and an extraction delay time of 200 ns. For each spectrum 500 shots in 5-shot steps from different positions of the target spot (automatic mode) were collected by the mass spectrometer operating in conjunction with the Acquisition Station software (VITEK® MS version 1.0.0). Measured mass spectra ranged from 2,000 to 20,000 Da.

MS identification

For each bacterial sample, mass fingerprints were processed by the compute engine and the Advanced Spectra Classifier (ASC) algorithm associated to the VITEK MS system which then automatically identifies the organism by comparing the characteristics of the

spectrum obtained (presence and absence of specific peaks) with the typical spectrum of each claimed species.

The ASC algorithm is a supervised learning methods that analyze spectral data and recognize patterns used to build the knowledge base. It can be classified in the "non-probabilistic linear classifier" family. In most cases, one pattern in the knowledge base corresponds to one species. Sometimes, when two (or more) species cannot be separated efficiently with MALDI-TOF technology, only one pattern is created by using all the spectra collected for both species. In the other hand, when spectra collected for one species are so variable due to strain variability or culture conditions like incubation time and culture media, several patterns are created for one species. For each species integrated in the knowledge base, the variability of the spectra is evaluated with clustering application and inter-spectra distance calculation.

The spectral database was built by the manufacturer as follow. Ten isolates belonging to the same species were carefully selected to take into account diversity in clinical specimen origins, geographic origin (different countries), and in year of isolation. All isolates were previously characterized by phenotypic and/or molecular methods. Spectral database was built through an experimental plan design including several culture media, several media suppliers, different incubation times ranging from 18h-24h up to 72h, and several mass spectrometers. Masses were collected from 2 to 20 kDa and the analysis focused on the 3-17 kDa mass range.

The ASC algorithm compared the generated spectra to the expected spectrum of each organism, or organism group of the database to provide identification. A percent probability or confidence value which represents the similarity in terms of presence/absence of specific peaks between the generated spectrum and the database spectra was calculated by the algorithm. A perfect match between the spectrum and the unique spectrum of a single

organism, or organism group, provided a confidence value of 99.9% ('Good ID'). When a perfect match was not obtained, it was still possible for the spectrum to be sufficiently close to that of a reference spectrum, such that a clear decision was provided about the organism ID ('Good ID', confidence value >60-99.8%). If an unique ID pattern was not recognized, a list of possible organisms was given ('Low discrimination'- LD, confidence value >60%) , or the strain was determined to be outside the scope of the database ('No ID'). The range of percent probabilities in the single choice case was 60 to 99. Values closer to 99.9 indicate a closer match to the typical pattern for the given organism. When the confidence value obtained was under 60, the organism was considered as non-identified.

The overall correct ID was defined as including the followed levels: (i) correct ID to the species level, when the system proposed the reference species ID as a single choice or as a low discrimination to the sub-species level (with any level of confidence), (ii) correct ID to the genus level, when the system proposed the reference species ID among a low discrimination including species of the same genera, and (iii) correct ID above the genus level, when the system proposed the reference species ID among a low discrimination including species of different genera.

When a human error or a poor quality deposit occured (including the warning messages 'bad spectrum', 'not enough peaks', 'too many peaks', 'too much background noise' or in case of calibration/control failure), the incriminated isolates or all the isolates of the incriminated acquisition group had been retested with single deposit and the second result had been taken into account for the analysis. For informative purpose, samples with 'No ID' or 'misID' first spot result were secondarily retested with a single spot.

Calculation of global assessment indices

For the MALDI-TOF-based identification method, positive predictive values to the genus level and to the species level were calculated considering isolates with a correct ID to the genus and species level as true positives, and isolates with a correct ID to the species level as true positives, respectively. Isolates misidentified were considered as false positives. Negative predictive value was calculated considering isolates with an absence of ID and belonging to species not included in the database as true negatives, and isolates with an absence of ID and belonging to species included in the database as false negatives.

RESULTS

Technical praticability

During the study, one out of 48 spot calibrations performed failed and 2.2% of all generated spectra were uninterpretable. These latter corresponded to seven 'too many / not enough peaks' and ten 'bad spectrum' warning messages, and were not associated with any particular taxonomic group and colonial characteristics. Fifteen out of these 17 isolates were correctly identified to the species level with a second deposit. One *Staphylococcus epidermidis* isolate and one *Klebsiella Oxytoca* isolate generated again a bad spectrum, and then were discarded from the analysis.

Global identification performances

During the study period, 767 isolates were analyzed by the VITEK MS system and, in parallel, by conventional VITEK2 system (760 isolates, 99.1%), or directly by genotypic methods (7 isolates, 0.9%, for *Legionella pneumophila*, *Helicobacter pylori* and *Bacillus cereus/thuringiensis/mycoides* species). Implementation of DNA-based ID methods to manage discrepancies or to obtain a more accurate reference ID (to the species or sub-species level) was performed for 79 (10.3%) isolates. Reference IDs proposed by the VITEK MS as a single choice (SC) whatever the confidence value, or included in a multiple choice result (LD with up to four proposed species) were considered as overall correct ID. Among the 767 isolates including 124 species and 50 genera, 738 (96.2%) isolates were correctly identified by the MALDI-TOF MS system as defined previously (**Table 1**). No ID and discordant results (MisIDs) were obtained for 2.5% and 1.3% of the isolates, respectively.

244 **(i) Correct identifications.** A correct ID to the species or sub species was obtained for
245 86.7% (n=665) of the isolates with the following confidence values: 99.9, 90.0 to 99.8, and
246 80.0 to 89.9 for 97.6%, 2.0%, and 0.3% of these isolates, respectively. A correct ID to the
247 genus level only, that is the correct species ID included in a mutiple choice result of species
248 from a same genera was obtained for 8.2% (n=63) of the isolates. These LDs to the species
249 level proved to be recurrent in 79.3% of cases including species complexes as *Enterobacter*
250 *cloacae*/*asburiae* (n=31), *Proteus vulgaris*/*penneri* (n=8), *Achromobacter*
251 *xylosoxidans*/*denitrificans* (n=6), *Bacillus cereus/thuringiensis/mycoides* (n=2),
252 *Staphylococcus intermedius/pseudintermedius* (n=3). LD results above the genus level, that is
253 with the correct ID proposed among species of different genera were obtained for 1.3%
254 (n=10) of the isolates (**Table 2**), some of which seem also to be recurrent, like the
255 "*Staphylococcus warneri*/*Prevotella buccalis*" LD for some *S. warneri* isolates. An identical
256 and high confidence value was mostly obtained for each proposed species in case of LD to the
257 species level or above the genus level. In the few cases where a confidence value difference
258 occurred, it was either in favor of, or in disadvantage of the correct species ID.

259

260 **(ii) Incorrect identifications.** Ten isolates were misidentified by the VITEK MS
261 system, six out of them with correct or closed genus (**Table 3**). Two *Shigella* isolates were
262 misidentified as *E. coli*, even after retest. Misidentified as *Haemophilus influenzae*, one
263 *Aggregatibacter segnis* isolate (species belonging formerly to the *Haemophilus* genus) was
264 correctly identified with a second deposit, whereas one *Neisseria mucosa* isolate was
265 misidentified as its close species *N. subflava* twice. Belonging to species absent from the
266 database, one *Streptococcus australis* isolate was misidentified as *S. parasanguinis*, even after
267 one retest, and one *Streptococcus canis* isolate gave a LD result between other species of the
268 "pyogenic" group, and a correct "No ID" result with a second deposit. One *Campylobacter*

jejuni isolate and one *Lactobacillus rhamnosus* isolate were misidentified as distant species *Citrobacter braakii* and *Propionibacterium avidum* respectively, but were correctly identified with a second deposit. With a LD result between species of distant genera, the misidentified *Haemophilus parainfluenzae* and *Ralstonia pickettii* isolates were correctly identified to the species level with a second deposit.

(iii) No identifications. The VITEK MS system gave an absence of ID for 19 (2.5%) isolates that were tested again using one deposit for informative purpose (**Table 1**). Nine isolates belonging to nine species were not included in the database of the MALDI-TOF MS system: one *Acinetobacter* sp. (unnamed species in public nucleotide databases), two *Staphylococci*, two *Corynebacteria*, and four anaerobes isolates, for which the system gave the same 'No ID' answer after reading a second deposit. With the analysis of an additional deposit, six out of ten isolates with species included into the database were correctly identified. One *Staphylococcus haemolyticus* among three tested in the study, and three *Helicobacter pylori* isolates gave again a 'No ID' result, despite an additional retest.

(iv) Global assessment indices. According to the criteria detailed in the "Materials and methods" section, the positive predictive values to the genus level and to the species level of the VITEK MS system were 98.6 and 98.5, respectively; and the negative predictive value was 47.4.

Analysis of *Streptococcaceae* group

Considering the organism groups largely tested, the VITEK MS gave a good ID overall to the genus level with a single deposit for 98.2% of the *Enterobacteriaceae,* 94.7% of the Nonfermentative Gram-negative rods, 94.5% of the *Staphylococci*, and 97.2% of the

294 *Streptococci* and related isolates (**Table 1**), some species of the latter group reported to be
295 difficult to discriminate using MALDI-TOF MS systems. Focusing on the 177 isolates tested
296 belonging to the *Streptococcaceae* family, 93.9% of the *Enterococci* (62 out of 66 isolates
297 including six species) and 98% of the pyogenic streptococci (49 out of 50 isolates including
298 four species) were correctly identified to the species level with a single deposit (**Table 1**). Of
299 note, the LDs to the subspecies level seem to be recurrent for *Streptococcus dysgalactiae* ssp
300 *dysgalactiae* and *S. dysgalactiae* ssp *equisimilis* (n=9), probably due to the resolution limit of
301 the settled system. Among the *'milleri'* group streptococci, 24 out of 25 isolates were correctly
302 identified to the species level and one LD result was obtained for a *S. constellatus* isolate with
303 *S. anginosus*, whereas five isolates were identified as other alpha- or non-hemolytic
304 streptococci by the VITEK2 system. The VITEK MS system correctly identified to the
305 species level all 19 *S. pneumoniae* isolates and 15 out of 17 alpha- or non-hemolytic
306 *Streptococcaceae* isolates, including nine *S. mitis/oralis* isolates. One *S. australis* isolate,
307 species not included into the VITEK MS database was misidentified as *S. parasanguinis*, and
308 one *S. vestibularis* isolate was not discriminated from the closed *S. salivarius* sp *salivarius*
309 species. In contrast, the VITEK2 system misidentified two alpha-hemolytic streptococci
310 isolates as ‘milleri’ group streptococci.
311

DISCUSSION

The introduction of the high tech MALDI-TOF MS technology in clinical laboratories is reducing the time required while improving the accuracy of bacterial identification. Without an intensive training background of the operators, the technical ownership of the VITEK MS system is straightforward and fast, as previously mentioned (5). However, the operator must remain vigilant in routine practice during sample preparation because of reduced interspot distance (especially for spots near the *E. coli* calibrant spot), that can mix two bacterial deposits, particularly during the matrix application step, as happened during the training period.

Many authors evaluating other MALDI-TOF MS-based systems have previously reported the use of a formic acid-based protein extraction using a bacterial lysis step, or directly onto the bacterial smear before matrix application to be needed, mainly for Gram-positive bacteria (1, 5, 9, 15, 24). In this study, the use of the VITEK MS system generated a low frequency of unusable spectra without formic acid use that is compatible and convenient for routine practice. Only one *Staphylococcus epidermidis* isolate and one *Klebsiella oxytoca* isolate generated twice a bad spectrum; in routine practice, these two isolates should have been managed then using a formic acid extraction step. These good performances of VITEK MS spectral acquisition for both Gram-negative and -positive isolates may be due to the efficient displacement raster of the laser onto the deposit. The laser scans the entire sample and the instrument acquires good quality sub-spectra from each 5-shot step. When 100 good quality sub-spectra are not reached during the first large screening of the deposit, the laser goes back onto sample areas giving good quality sub-spectra to obtain sufficient data (30 sub-spectra are the minimum acceptable), before the average spectrum data is analyzed.

LDs between species of different genera, accounting for 14 isolates (1.8%) of the tested isolates were not reported in the literature for other MALDI-TOF MS-based ID systems. The basis of this phenomenon may lie in the spectra classifier algorithm that takes into account the absence and the presence of species-specific peaks. Although most of the correct species ID included in such LD results can be found out with growth conditions, or by simple and immediate tests (catalase, Gram staining, pigmentation), the correct species was only proposed for 11 out of 14 isolates with these LDs; a correct result (species ID or 'No ID') was obtained for the three other isolates using a second spot. As a consequence and according to our subsequent experience, these few LD results should not be removed by complementary tests but the isolates should be retested in order to obtain a single choice. Moreover, whatever the level of LDs, confidence values do not appear to be reliable to determinate the right species. Nevertheless, recurrent LDs to the species level have mostly no impact on isolate management and bio-clinical interpretation, since species show a similar pathogenicity and antibiotics susceptibility pattern (for example, *E. cloacae*/*asburiae*) and/or can be discriminated by simple and immediate tests (for example, indole test for *P. vulgaris*/*penneri*). For other LDs to the species level, an additional deposit should also be performed as it mostly provided the correct species ID during our subsequent experience.

The misID of *Shigella* isolates as *E. coli* was previously reported with other MALDI-TOF MS-based ID systems (4-6, 15, 20). These results are not surprising since the genus *Shigella* belonged genetically to the *E. coli* species and was kept in this way to differentiate these 'specific *E coli'* with a particular virulence towards humans (12). This point reflects the resolution limits of the MALDI-TOF MS-based ID method currently used for routine bacterial ID. These misIDs are a major drawback from a clinical point of view, particularly for stool sample analysis, that needs to be overcome by conventional phenotypic testing.

Regarding the other major misIDs (*Campylobacter jejuni, Lactobacillus rhamnosus, and Aggregatibacter segnis* isolates), one can not rule out a technical mistake or an undetected mixed culture generating two superimposed spectra wrongly interpreted as an unique spectra and thus as a third species. These major incorrect results could have been amended in routine practice according to Gram staining, growth conditions, colonial features and oxydase/catalase tests. These few but nonetheless critical misIDs confirmed that, like any identification system, experienced laboratory personnel have to manage VITEK MS results and take into account bacterial and clinical data, as highlighted by other authors using different MALDI-TOF MS-based systems (4, 15, 22).

Nine isolates belonging to nine species not included in the VITEK MS database obtained twice the same 'No ID' answer, highlighting the specificity of the algorithm used in these bacterial groups. Two out of four *Corynebacterium* species and four out of 15 Anaerobes species collected in our routine practice were not identified. The lack of species diversity of *Corynebacteria* and Anaerobes in the currently available database should be adressed for overall routine use. As noted by other authors, our results emphasize the widespread ignorance and failure to correctly identify Anaerobes species by biochemical methods in medical bacteriology (10, 14, 25, 26). Moreover, from microbiologist views and clinical purpose, the ID to the genus level of many Anaerobes species is usually sufficient, that is unfortunately not achievable with the original algorithm of tested system.

With the analysis of an additional deposit, six out of ten isolates with species included into the database were correctly identified. As mentionned for another MALDI-TOF MS-based bacterial ID system, these results may indicate that deposition of an excessive amount of bacteria during sample spot preparation can lead to loss of accuracy of the Vitek MS system, providing quality spectrum warning messages or 'No ID' results (5). As a

consequence, all isolates with a 'No ID' result given by the Vitek MS using one deposit should be retested in routine practice. The absence of ID for one *S. haemolyticus* isolate and all three *H. pylori* isolates tested twice may be due to a representation lack of the species diversity among the ten reference spectra embedded into the database, or the need for a prior protein extraction.

The good ID performances for the largely tested *Enterobacteriaceae*, Nonfermentative Gram-negative rods, *Staphylococci*, and *Streptococci* groups using only one deposit and no extraction step were reported previously in only one study (4). In addition to the spectral acquisition step, the good results obtained with the VITEK MS system may be due to the database building using 10 different reference strains for each species claimed into the database and generating different spectra for each strain under different culture conditions, in order to set their typical spectrum. Previous report has noted that including at least ten strains by species into the database, with many replicates per strain is a prerequisite to obtain an accurate MALDI-TOF MS ID (4, 20). Allowing quick and reliable ID of *Streptococcaceae* isolates, the VITEK MS showed better ID performances for *'milleri'* group streptococci and other alpha- or non-hemolytic *Streptococcaceae* than the VITEK2 system. In contrast to previous studies using other MALDI-TOF MS systems and although our results need to be confirmed on a larger number of isolates, the VITEK MS appears particularly to discriminate *S. pneumoniae*, an undeniable pathogen species from other alpha-hemolytic streptococci (3, 4, 6, 7, 15, 18, 20, 22, 24).

In conclusion, the VITEK MS system allows with only one deposit of crude bacteria and without any extraction step, a fast and reliable acquisition of bacterial ID for most bacterial species isolated routinely in a medical laboratory. The remarkable performance of

411 the VITEK MS system may be due to its novel laser displacement mode, original algorithm,
412 and quality of the database building. It is worth noting that the presence and the absence of
413 specific peaks analyzed by the spectra classifier algorithm of the VITEK MS is double-edged.
414 When the tested species is missing from the database, the algorithm gives a clear absence of
415 ID in most cases, that is overall what is expected for an ID system. However, due to the
416 limited species diversity of the database for some taxa groups, an ID result to the genus level
417 would be interesting, as for some anaerobe taxa. For routine purpose, in addition to the
418 isolates that generated a spectrum of poor quality, we suggested a retest with one to two
419 deposits for isolates that give 'no ID' and 'no reccurent' LD results with a first deposit, as
420 false 'no ID' results can be obtained with deposits of poor quality and the confidence values
421 are indicators with weak usefulness in cases of LD, respectively. Considering the higher
422 bacterial diversity included in this study than in routine practice, and the low prevalence of
423 species without correct identification, the performances should be even better for routine
424 activity in clinical laboratories. However, expanding the spectral database is warranted,
425 particularly for anaerobic, coryneform and some highly pathogenic bacteria, in ordrer to use
426 almost exclusively this system for isolates ID in routine medical practice.
427

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429

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526 **Table 1.** Valid VITEK MS results of 767 bacterial isolates using a single deposit and no
527 protein extraction step.

Reference identification	No. of isolates	No. (%) of isolates with results:				
		Correct ID			No ID	MisID
		Species	Genus	Above genus		
Enterobacteriaceae	**282**	**231 (82.2)**	**45 (16.0)**	**2 (0.7)**	**2 (0.7)**	**2 (0.7)**
Citrobacter braakii	1	1 (100)				
Citrobacter freundii	12	9 (75)	2 (17)	1 (8)		
Citrobacter koseri	14	14 (100)				
Citrobacter youngae	2	1 (50)	1 (50)			
Enterobacter aerogenes	13	13 (100)				
Enterobacter asburiae	1		1 (100)			
Enterobacter cloacae	31		30 (97)		1 (3)	
Escherichia coli	31	30 (100)	1 (3)			
Escherichia fergusonii	2	1 (50)	1 (50)			
Escherichia vulneris	1	1 (100)				
Hafnia alvei	13	13 (100)				
Klebsiella oxytoca	27	27 (100)				
Klebsiella pneumoniae	33	33 (100)				
Morganella morganii	23	23 (100)				
Pantoea agglomerans	1	1 (100)				
Proteus mirabilis	30	28 (93)		1 (3)	1 (3)	
Proteus vulgaris	8		8 (100)			
Providencia rettgeri	1	1 (100)				
Providencia stuartii	4	4 (100)				
Raoultella ornithinolytica	2	2 (100)				
Raoultella planticola	1		1 (100)			
Serratia liquefaciens	1	1 (100)				
Salmonella group[2]	7	7 (100)				
Serratia marcescens	21	21 (100)				
Shigella flexneri	2					2 (100)
Nonfermentative Gram negative rods	**94**	**81 (86.2)**	**8 (8.5)**	**2(2.1)**	**2 (2.1)**	**1 (1.1)**
Achromobacter denitrificans	1		1 (100)			
Achromobacter xylosoxidans	5		5 (100)			
Acinetobacter baumannii complex[2]	5	5 (100)				
Acinetobacter lwoffii	2	1 (50)		1 (50)		
Acinetobacter radioresistens	1	1 (100)				
Acinetobacter ursingii	2	2 (100)				
Acinetobacter sp[1]	1				1 (100)	
Aeromonas caviae[3]	1	1 (100)				
Aeromonas sobria	1		1 (100)			
Alcaligenes faecalis ssp *faecalis*	4	3 (75)			1 (25)	
Burkholderia cepacia	1	1 (100)				
Burkholderia vietnamiensis	1		1 (100)			
Burkholderia stabilis	1	1 (100)				
Chryseobacterium indologenes	2	2 (100)				
Elizabethkingia meningoseptica	1	1 (100)				
Pseudomonas aeruginosa	36	35 (97)		1 (3)		
Pseudomonas putida	6	6 (100)				
Psychrobacter sp.[5]	1	1 (100)				
Ralstonia pickettii	1					1* (100)
Stenotrophomonas maltophilia	21	21 (100)				

Other Gram negative bacteria	**47**	**38 (80.9)**	**1 (2.1)**	**0**	**4 (8.5)**	**4 (8.5)**
Aggregatibacter segnis	1					1 (100)
Haemophilus influenzae	21	19 (90)	1 (5)		1 (5)	
Haemophilus parainfluenzae	3	2 (67)				1* (33)
Pasteurella canis	1	1 (100)				
Pasteurella multocida	3	3 (100)				
Eikenella corrodens	1	1 (100)				
Moraxella catarrhalis	3	3 (100)				
Neisseria gonorrhoeae	1	1 (100)				
Neisseria meningitidis	2	2 (100)				
Neisseria mucosa	1					1 (100)
Neisseria subflava/flavescens/perflava	1	1 (100)				
Campylobacter fetus ssp. *fetus*[4]	1	1 (100)				
Campylobacter jejuni ssp. *jejuni*	3	2 (67)				1 (33)
Helicobacter pylori	3				3 (100)	
Legionella pneumophila	2	2 (100)				
***Staphylococci* and related**	**127**	**117 (92.1)**	**3 (2.4)**	**3 (2.4)**	**4 (3.1)**	**0**
Staphylococcus aureus	36	35 (97)			1 (3)	
Staphylococcus capitis	9	9 (100)				
Staphylococcus caprae	2	2 (100)				
Staphylococcus carnosus ssp. *carnosus*	1	1 (100)				
Staphylococcus cohnii ssp. *cohnii*[4]	1	1 (100)				
Staphylococcus epidermidis	36	35 (97)		1 (3)		
Staphylococcus haemolyticus	3	2 (67)			1 (33)	
Staphylococcus hominis ssp. *hominis*	5	5 (100)				
Staphylococcus intermedius	3		3 (100)			
Staphylococcus lugdunensis	9	9 (100)				
Staphylococcus pseudintermedius	1	1 (100)				
Staphylococcus saprophyticus	5	5 (100)				
Staphylococcus schleiferi	1	1 (100)				
Staphylococcus sciuri	1	1 (100)				
Staphylococcus simulans	4	4 (100)				
Staphylococcus warneri	6	4 (67)		2 (33)		
Staphylococcus xylosus	1	1 (100)				
Staphylococcus condimenti[1]	1				1 (100)	
Staphylococcus pasteuri[1]	1				1 (100)	
Micrococcus luteus/lylae	1	1 (100)				
***Streptococci* and related**	**177**	**169 (95.5)**	**3 (1.7)**	**2 (1.1)**	**1 (0.6)**	**2 (1.1)**
Enterococcus avium	8	5 (63)	1 (13)	2 (25)		
Enterococcus casseliflavus	1	1 (100)				
Enterococcus durans	1	1 (100)				
Enterococcus faecalis	38	38 (100)				
Enterococcus faecium	17	16 (94)			1 (6)	
Enterococcus gallinarum	1	1 (100)				
Streptococcus agalactiae	32	32 (100)				
Streptococcus pyogenes	8	8 (100)				
Streptococcus dys. equisimilis[4]	8	8 (100)				
Streptococcus dys. dysgalactiae[4]	1	1 (100)				
Streptococcus canis[1]	1					1* (100)
Streptococcus anginosus	14	14 (100)				
Streptococcus constellatus	9	8 (89)	1 (11)			
Streptococcus intermedius	2	2 (100)				
Streptococcus pneumoniae	19	19 (100)				
Streptococcus mitis/oralis	9	9 (100)				
Streptococcus gallolyticus sp *pasteurianus*[4]	2	2 (100)				
Streptococcus parasanguinis	2	2 (100)				
Streptococcus vestibularis	1		1 (100)			
Streptococcus australis	1					1 (100)
Granulicatella adiacens	1	1 (100)				
Vagococcus fluvialis	1	1 (100)				

Anaerobes	**30**	**25 (83)**	**0**	**1 (3)**	**4 (13)**	**0**
Anaerococcus hydrogenalis[1]	1				1 (100)	
Bacteroides vulgatus	1	1 (100)				
Bacteroides fragilis	8	8 (100)				
Bacteroides ovatus	1	1 (100)				
Bacteroides uniformis	2	2 (100)				
Clostridium difficile	5	5 (100)				
Clostridium perfringens[6]	2	2 (100)				
Clostridium celerecrescens[1]	1				1 (100)	
Finegoldia magna	1	1 (100)				
Prevotella intermedia	2	2 (100)				
Prevotella nanceiensis[1]	1				1 (100)	
Prevotella nigrescens[1]	1				1 (100)	
Propionibacterium acnes	2	2 (100)				
Propionibacterium avidum	1			1 (100)		
Veillonella parvula	1	1 (100)				
Other Gram positive rods	**10**	**4 (40)**	**3 (30)**	**0**	**2 (20)**	**1 (10)**
Corynebacterium amycolatum	1		1 (100)			
Corynebacterium striatum	4	4 (100)				
Corynebacterium fastidiosum/segmentosum[1]	1				1 (100)	
Corynebacterium macginleyi[1]	1				1 (100)	
Lactobacillus rhamnosus	1					1 (100)
Bacillus cereus/thuringiensis/ mycoides[2]	2		2 (100)			
Total	**767**	**665 (86.7)**	**63 (8.2)**	**10 (1.3)**	**19 (2.5)**	**10 (1.3)**

528

529 ID, identification; MisID, misidentification

530 Species, correct identification at the species level (Single choice or Low discrimination at the

531 sub-species level)

532 Genus, correct identification at the genus level (Low discrimination at the species level)

533 Above genus, correct identification proposed among a low discrimination including species of

534 different genera

535 *LD Discrepancies

536 [1]Species absent from the VITEK MS database. Using sequencing-based genomic methods and

537 according to nucleotide public databases, the *Acinetobacter* sp. isolate corresponded to an

538 unnamed *Acinetobacter* species.

539 [2]Species group is the final VITEK MS identification. The subspecies or species included in

540 each species group are for:

Salmonella group: *S. enterica* ssp *enterica*, *S.* ser. Enteritidis, *S.* ser. Paratyphi B, *S.* ser. Paratyphi C, *S.* ser. Typhimurium, *Salmonella* spp; *Acinetobacter baumannii* complex: *A. baumannii*, *A. calcoaceticus*, *A.* genomospecies 3, *A.* genomospecies TU13.

[3]*Aeromonas hydrophila/caviae s*pecies group is displayed as a species group result by the VITEK MS.

[4]The VITEK MS did not differenciate the subspecies *S. cohnii* ssp. *cohnii* and *S. cohnii* ssp. u*realyticum*, *S. dysgalactiae* ssp. *dysgalactiae* and *S. dysgalactiae* ssp. *equisimilis, S. gallolyticus* ssp. *pasteurianus* and *S. gallolyticus* ssp. *gallolyticus,* and *C. fetus* ssp *fetus* and *C. fetus* ssp *venerealis*.

[5]This isolate was identified as *Psychrobacter phenylpyruvicus* by the VITEK MS system, but the *Psychrobacter* species was undeterminable by sequencing.

[6]These isolates do not possess the epsilon toxin gene.

553 **Table 2.** Correct identifications proposed by the VITEK MS among a multiple choice including species of different genera.

Reference ID	Proposed ID results as multiple choice			
	1st choice	2nd choice	3rd choice	4th choice
Staphylococcus warneri*	*Staphylococcus warneri (99.9)*	*Prevotella buccalis (99.9)*	-	-
Staphylococcus epidermidis	*Staphylococcus epidermidis (99.9)*	*Leuconostoc pseudomesenteroides (99.7)*	-	-
Micrococcus luteus	*Micrococcus luteus/lylae (99.9)*	*Bacillus thuringiensis (99.9)*	*Bacillus mycoides (79.1)*	*Bacillus cereus (79.1)*
Enterococcus avium	*Enterococcus avium (99.9)*	*Clostridium butyricum (96.6)*	-	-
Enterococcus avium	*Enterococcus avium (99.9)*	*Clostridium butyricum (85.9)*	*Bacillus atrophaeus (78.8)*	-
Proteus mirabilis	*Proteus mirabilis (99.9)*	*Streptococcus constellatus (71.3)*	-	-
Citrobacter freundii	*Citrobacter freundii (99.0)*	*Citrobacter braakii (99.9)*	*Citrobacter koseri (92.4)*	*Haemophilus influenzae (71.5)*
Acinetobacter lwoffii	*Acinetobacter lwoffii (99.9)*	*Mycobacterium tuberculosis (99.9)*	*Mycobacterium bovis (99.9)*	-
Pseudomonas aeruginosa	*Pseudomonas oryzihabitans (99.4)*	*Peptoniphilus indolicus (99.2)*	*Pseudomonas aeruginosa (82.0)*	-
Propionibacterium avidum	*Propionibacterium avidum (96.8)*	*Clostridium butyricum (87.7)*	-	-

554

555 ID, identification; LD, Low discrimination

556 Numbers in brackets correpond to confidence value percentages.

557 *2 isolates

558

559 **Table 3.** Misidentifications of the VITEK MS system.

Reference ID	First spot proposed IDs as single or multiple choice			Second spot ID result
	1st choice	2nd choice	3rd choice	
Shigella flexneri*	*Escherichia coli* (99.9)	-	-	*E. coli* (99.9)
Aggregatibacter segnis	*Haemophilus influenzae* (99.4)	-	-	*Aggregatibacter segnis* (99.9)
Haemophilus parainfluenzae	*Haemophilus haemolyticus* (98.8)	*Enterobacter aerogenes* (93.5)	-	*Haemophilus parainfluenzae* (99.9)
Campylobacter jejuni	*Citrobacter braakii* (84.4)	-	-	*Campylobacter jejuni* (99.9)
Ralstonia pickettii	*Prevotella melaninogenica* (99.9)	*Staphylococcus saprophyticus* (99.9)	*Chryseobacterium gleum* (96.2)	*Ralstonia pickettii* (99.9)
Neisseria mucosa	*Neisseria subflava* (99.9)	-	-	*Neisseria subflava* (99.9)
Streptococcus australis	*Streptococcus parasanguinis* (99.9)	-	-	*Streptococcus parasanguinis* (99.9)
Streptococcus canis	*Streptococcus dysgalactiae dysgalactiae* (99.9)	*Streptococcus dysgalactiae equisimilis* (99.9)	*Streptococcus equi equi* (99.9)	No ID
Lactobacillus rhamnosus	*Propionibacterium avidum* (99.3)	-	-	*Lactobacillus rhamnosus* (99.9)

560

561 ID, identification

562 Numbers in brackets correspond to confidence value percentages.

563 *2 isolates