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Performances of VITEK 2 Colorimetric Cards for Identification of Gram-Positive and Gram-Negative Bacteria

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The purpose of this study was to evaluate the new VITEK 2 identification cards that use colorimetric reading to identify gram-positive and gram-negative bacteria (GP and GN cards, respectively) in comparison to fluorimetric cards (ID-GPC and ID-GNB, respectively). A total of 580 clinical isolates and stock collection strains belonging to 116 taxa were included in the study. Of the 249 gram-positive strains tested with both the ID-GPC and GP cards, 218 (87.5%) and 235 (94.4%) strains were correctly identified (to the genus and species level), respectively. Of the 331 gram-negative strains tested with the ID-GNB and GN cards, 295 (89.1%) and 321 (97%) strains were correctly identified, respectively. Another focus of the study was to apply the percentages of correct identifications obtained in this study to the list of bacteria isolated in our laboratory (32,739 isolates) in the year 2004. We obtained 97.9% correct identifications with the colorimetric cards and 93.9% with fluorescent cards.

The correct and rapid identification of gram-negative and gram-positive bacteria in clinical microbiology is the first step in the interpretation of antimicrobial susceptibility tests for correct treatment of patients (8). Although the VITEK 2 system (bioMérieux, Marcy l'Etoile, France) combined with the ID-GNB and ID-GPC cards allowed an identification within 3 h using fluorescence reading, the weakness of this system was the breadth of its identification database, especially for nonfermenting bacilli, such as *Pseudomonas* spp. and *Acinetobacter*, and for gram-positive cocci, such as *Streptococcaceae* (2, 5). New cards (GP and GN cards) will soon be available that use colorimetric reading. These cards are suited to the VITEK 2 system, improving the identification of nonfermenting bacteria and gram-positive cocci (3, 4). The aims of this study were (i) to evaluate the performances of the new colorimetric cards in comparison to fluorimetric cards (ID-GNB, ID-GPC) to identify 580 clinical isolates and stock collection strains belonging to 116 taxa and (ii) to determine the accuracy obtained with both readings by applying the percentages of correct identifications obtained in this study with the colorimetric and fluorescent cards to the list of bacteria isolated in our laboratory in the year 2004.

MATERIALS AND METHODS

Strains. A total of 580 strains were tested, consisting of 331 gram-negative bacilli and 249 gram-positive cocci belonging to 68 and 48 taxa, respectively. Clinical isolates were collected over 6 months from nonconsecutive patient cultures and selected either to obtain around 20 strains of the most frequently isolated species or to be in agreement with the distribution of isolates annually recovered in the laboratory. In order to have an idea of the performance of testing for the most rarely isolated species, a panel of 181 microorganisms was selected from the laboratory stock collection.

Identification protocol. All isolates were cultured onto Columbia agar with 5% horse blood (18 to 24 h at 35°C) to ensure purity and viability. Stock strains were subcultured twice. Microorganisms were tested separately with two VITEK 2 instruments: the first for fluorescence reading, the second for colorimetric reading. The new VITEK 2 cards and the upgraded VITEK 2 were previously described (3). Both systems were used according to the recommendations of the manufacturer. Bacterial suspensions were made in 0.45% sodium chloride solution and adjusted to a McFarland standard of 0.50 to 0.63 by using a Densicheck system (bioMérieux). Identical inocula of each strain were tested in parallel using fluorimetric cards (ID-GNB, ID-GPC) and colorimetric cards (GN, GP) according to the manufacturer's instructions. When the identification results were different between the fluorimetric and the colorimetric cards, the strain was retested with the both methods. In case of persistent discrepancy, the strain was identified with API strips (ID-32 Staph, Rapid ID-32 Strept, ID-32 E, ID-32 GN, API 20NE; bioMérieux) to resolve the identity of the strain. When there was a mismatch between identifications obtained with both the VITEK 2 cards and the API strips, isolate identification was determined by DNA sequencing of the 16S rRNA (Microseq 500; Applera, Foster City, Calif.) (1, 10) and/or *sodA* (9), and/or *rpoB* (7) gene.

Quality controls. Fifteen strains were used as quality controls every 2 months during the evaluation. For gram-positive bacteria, the strains were *Staphylococcus saprophyticus* ATCC BAA 750, *S. aureus* subsp. *aureus* ATCC 29213, *Kocuria kristinae* ATCC BAA 752, *Listeria monocytogenes* ATCC BAA 751, *Streptococcus thermophilus* ATCC 19258 T, *S. sciuri* ATCC 29061, *Enterococcus casseliflavus* ATCC 700327, and *S. equi* subsp. *zooepidemicus* ATCC 43079. For gram-negative quality control, the strains were *Klebsiella oxytoca* ATCC 700324, *Acinetobacter baumannii* BAA 747, *Enterobacter cloacae* ATCC 700323, *Ochrobactrum anthropi* ATCC BAA 749, *Proteus vulgaris* ATCC 6380, *Shigella sonnei* ATCC 25931, and *Stenotrophomonas maltophilia* ATCC 17666. Each quality control strain was tested with both the VITEK 2 fluorimetric system and the VITEK 2 colorimetric system.

Data analysis. Results were separated into four groups: first-choice identification (i.e., the determination of the genus-and-species level was the same with both systems and was given as excellent, very good, good, or acceptable); low discrimination (the determination resulted in a choice among two or four species with different values of T indexing needing a few supplemental procedures such as oxidase, motility, indole, pigmentation, or hemolysis testing to determine the correct identification); misidentification (the determination resulted in incorrect identification); and no identification (the determination resulted in doubtful, unacceptable, or unreliable identification). Correct identification was defined as the association of first-choice identification and low discrimination. Results were expressed in numbers and percentages.

Two supplemental analyses of the data were carried out. The first analysis was performed to determine the levels of accuracy obtained with the two systems by comparing the bacterial species identified in the year 2004 in our laboratory and isolated five times or more to the bacterial species tested in the study. The second analysis was to perform the same evaluation with the 17 gram-negative rods most frequently recovered from blood cultures in the microbiology laboratories of 33 French university hospitals (11).

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TABLE 1. Identification of gram-positive bacteria by fluorimetric and colorimetric VITEK 2 cards

Family and species or subspecies	No. (%) of strains								
	Tested	Identified as first choice		Identified with low discrimination		Misidentified		Not identified	
		ID-GPC	GP	ID-GPC	GP	ID-GPC	GP	ID-GPC	GP
Streptococcaceae									
Enterococcus avium	4	4	4	0	0	0	0	0	0
Enterococcus durans	3	3	2	0	1	0	0	0	0
Enterococcus faecalis	12	11	12	0	0	0	0	1	0
Enterococcus faecium	13	8	12	2	0	2	1	1	0
Enterococcus gallinarum	3	0	3	3	0	0	0	0	0
Enterococcus hirae	1	0	0	1	0	0	1	0	0
Gemella morbillorum	2	0	2	0	0	1	0	1	0
Gemella sanguinis	1	0	1	0	0	1	0	0	0
Granulicatella adiacens	1	0	1	0	0	1	0	0	0
Helcococcus kunzil	1	0	1	0	0	0	0	1	0
Pediococcus acidilactici	1	0	1	0	0	0	0	1	0
Pediococcus pentosaceus	1	0	0	0	0	0	1	1	0
Streptococcus dysgalactiae subsp. *dysgalactiae*/*Streptococcus dysgalactiae* subsp. *equisimilis*	11	11	11	0	0	0	0	0	0
Streptococcus equi subsp. *zooepidemicus*	1	1	1	0	0	0	0	0	0
Streptococcus infantarius subsp. *infantarius*	1	1	1	0	0	0	0	0	0
Streptococcus mitis/*Streptococcus oralis*	12	11	12	0	0	1	0	0	0
Streptococcus agalactiae	11	11	10	0	1	0	0	0	0
Streptococcus anginosus	7	7	7	0	0	0	0	0	
Streptococcus bovis	6	5	5	1	1	0	0	0	0
Streptococcus constellatus	8	3	1	2	2	1	3	2	2
Streptococcus gordonii	5	4	3	0	0	0	2	1	0
Streptococcus intermedius	3	3	2	0	1	0	0	0	0
Streptococcus mitis	1	0	0	1	0	0	1	0	0
Streptococcus mutans	1	1	1	0	0	0	0	0	0
Streptococcus oralis	2	0	0	2	1	0	1	0	0
Streptococcus pneumoniae	10	8	10	2	0	0	0	0	0
Streptococcus pyogenes	6	5	5	1	1	0	0	0	0
Streptococcus salivarius	2	2	2	0	0	0	0	0	0
Streptococcus sanguinis	2	2	2	0	0	0	0	0	0
Total	132	101 (76.5)	112 (84.8)	15 (11.4)	8 (6.1)	7 (5.3)	10 (7.6)	9 (6.8)	2 (1.5)
Micrococcaceae									
Aerococcus viridans	1	1	1	0	0	0	0	0	0
Micrococcus luteus	5	5	5	0	0	0	0	0	0
Rothia mucilaginosa	1	0	1	0	0	0	0	1	0
Staphylococcus arlettae	2	0	2	0	0	2	0	0	0
Staphylococcus aureus	21	21	20	0	1	0	0	0	0
Staphylococcus auricularis	1	1	1	0	0	0	0	0	0
Staphylococcus capitis	15	12	14	0	0	2	1	1	0
Staphylococcus cohnii subsp. *urealyticus*	1	1	1	0	0	0	0	0	0
Staphylococcus cohnii	1	0	0	1	0	0	1	0	0
Staphylococcus cohnii subsp. *cohnii*	2	2	2	0	0	0	0	0	0
Staphylococcus epidermidis	23	18	22	3	1	1	0	1	0
Staphylococcus haemolyticus	9	9	9	0	0	0	0	0	0
Staphylococcus hominis	14	3	14	6	0	3	0	2	0
Staphylococcus lugdunensis	4	4	4	0	0	0	0	0	0
Staphylococcus saprophyticus	4	4	4	0	0	0	0	0	0
Staphylococcus schleiferi	3	1	3	2	0	0	0	0	0
Staphylococcus sciuri	2	0	2	0	0	2	0	0	0
Staphylococcus simulans	3	3	3	0	0	0	0	0	0
Staphylococcus warneri	5	4	5	1	0	0	0	0	0
Total	117	89 (76.1)	113 (96.6)	13 (11.1)	2 (1.7)	10 (8.5)	2 (1.7)	5 (4.3)	0
All strains	249	190 (76.3)	225 (90.4)	28 (11.2)	10 (4)	17 (6.8)	12 (4.8)	14 (5.6)	2 (0.8)

TABLE 2. Identification of gram-negative bacteria by fluorimetric and colorimetric VITEK 2 cards

Family and species or subspecies	Tested	No. (%) of strains							
		Identified as first choice		Identified with low discrimination		Misidentified		Not identified	
		ID-GPC	GP	ID-GPC	GP	ID-GPC	GP	ID-GPC	GP
Fermenting									
Aeromonas hydrophila/Aeromonas caviae	5	3	5	2	0	0	0	0	0
Citrobacter amalonaticus	1	1	1	0	0	0	0	0	0
Citrobacter braakii	2	0	2	2	0	0	0	0	0
Citrobacter freundii	6	6	6	0	0	0	0	0	0
Citrobacter koseri	11	11	11	0	0	0	0	0	0
Citrobacter youngae	1	1	1	0	0	0	0	0	0
Enterobacter aerogenes	18	17	17	1	0	0	0	0	1
Enterobacter amnigenus	1	0	1	1	0	0	0	0	0
Enterobacter asburiae	2	2	2	0	0	0	0	0	0
Enterobacter cloacae	15	10	14	4	0	1	1	0	0
Enterobacter gergoviae	1	1	1	0	0	0	0	0	0
Enterobacter sakazakii	1	1	1	0	0	0	0	0	0
Escherchia coli	26	24	25	1	1	1	0	0	0
Escherichia hermannii	2	2	2	0	0	0	0	0	0
Escherichia vulneris	1	0	1	0	0	1	0	0	0
Hafnia alvei	7	7	7	0	0	0	0	0	0
Klebsiella oxytoca	11	11	10	0	1	0	0	0	0
Klebsiella pneumoniae subsp. *pneumoniae*	12	10	11	2	1	0	0	0	0
Klebsiella pneumoniae subsp. *ozaenae*	1	1	1	0	0	0	0	0	0
Klebsiella spp.	1	0	1	1	0	0	0	0	0
Leclercia adecarboxylata	1	1	1	0	0	0	0	0	0
Moellerella wisconsensis	1	1	1	0	0	0	0	0	0
Morganella morganii	11	11	11	0	0	0	0	0	0
Pantoea agglomerans	3	3	3	0	0	0	0	1	0
Pantoea spp.	2	0	2	1	0	0	0	0	0
Pasteurella aerogenes	1	1	1	0	0	0	0	0	0
Pasteurella multocida	6	4	6	1	0	1	0	0	0
Pasteurella pneumotropica	2	2	2	0	0	0	0	0	0
Proteus mirabilis	17	17	17	0	0	0	0	0	0
Proteus vulgaris group/*Proteus penneri*	7	7	7	0	0	0	0	0	0
Providencia rettgeri	1	1	1	0	0	0	0	0	0
Providencia stuartii	11	11	11	0	0	0	0	0	0
Rahnella aquatilis	1	1	1	0	0	0	0	0	0
Salmonella group	12	9	12	3	0	0	0	0	0
Serratia liquefaciens group	1	1	1	0	0	0	0	0	0
Serratia marcescens	15	15	15	0	0	0	0	0	0
Serratia proteamaculans	1	0	1	1	0	0	0	0	0
Shigella group	3	1	3	2	0	0	0	0	0
Shigella sonnei	5	5	5	0	0	0	0	0	0
Vibrio alginolyticus	1	1	1	0	0	0	0	0	0
Yersinia enterocolitica group	13	11	13	1	0	0	0	1	0
Yersinia pseudotuberculosis	3	2	2	0	0	0	0	1	1
Total	243	213 (87.7)	237 (97.5)	23 (9.5)	3 (1.2)	4 (1.6)	1 (0.4)	3 (1.2)	2 (0.8)
Nonfermenting									
Achromobacter xylosoxidans subsp. *denitrificans*	1	0	0	0	1	0	0	1	0
Achromobacter xylosoxidans subsp. *xylosoxidans*	7	0	6	0	1	5	0	2	0
Acinetobacter baumannii	8	6	8	2	0	0	0	0	0
Acinetobacter haemolyticus	4	0	4	0	0	3	0	1	0
Acinetobacter lwoffii	2	0	2	2	0	0	0	0	0
Acinetobacter spp.	5	0	1	2	1	0	3	3	0
Bordetella bronchiseptica	4	0	3	3	0	0	1	1	0
Bordetella trematum	1	0	0	0	1	1	0	0	0
Burkholderia cepacia	8	7	8	1	0	0	0	0	0
Chryseobacterium indologenes	2	2	2	0	0	0	0	0	0
Chryseobacterium meningosepticum	1	1	1	0	0	0	0	0	0
Comamonas testosteroni	1	0	1	0	0	0	0	1	0
Delftia acidovorans	1	0	1	1	0	0	0	0	0
Moraxella osloensis	1	0	0	1	1	0	0	0	0
Ochrobactrum anthropi	1	1	1	0	0	0	0	0	0
Oligella ureolytica	2	0	2	0	0	0	0	2	0
Pseudomonas aeruginosa	13	12	13	0	0	0	0	1	0

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TABLE 2—*Continued*

Family and species or subspecies	Tested	No. (%) of strains							
		Identified as first choice		Identified with low discrimination		Misidentified		Not identified	
		ID-GPC	GP	ID-GPC	GP	ID-GPC	GP	ID-GPC	GP
Pseudomonas alcaligenes	1	0	0	0	0	0	1	1	0
Pseudomonas fluorescens	3	0	1	2	1	1	0	0	1
Pseudomonas mendocina	1	0	1	0	0	0	0	1	0
Pseudomonas putida	3	0	2	2	0	1	1	0	0
Pseudomonas stutzeri	6	0	6	3	0	1	0	2	0
Ralstonia mannitolilytica	1	0	1	0	0	1	0	0	0
Rhizobium radiobacter	1	0	1	1	0	0	0	0	0
Sphingomonas paucimobilis	1	1	1	0	0	0	0	0	0
Stenotrophomonas maltophilia	9	8	8	1	1	0	0	0	0
Total	88	38 (43.2)	74 (84.1)	21 (23.8)	7 (8.0)	13 (14.8)	6 (6.8)	16 (18.2)	1 (1.1)
All strains (%)	331	251 (75.8)	311 (94)	44 (13.3)	10 (3)	17 (5.1)	7 (2.1)	19 (5.7)	3 (0.9)

RESULTS AND DISCUSSION

Of the 249 gram-positive strains tested with both the ID-GPC and GP cards and the 331 gram-negative strains tested with both the ID-GNB and GN cards, 218 (87.5%), 235 (94.4%), 295 (89.1%), and 321 (97%) were correctly identified (to the genus or species level), respectively (Tables 1 and 2). A total of 33 bacteria remained unidentified with the fluorimetric cards, whereas the colorimetric cards did not give an identification for five strains. Regardless of their origin (combined stock collection and clinical isolates), fermenting gram-negative bacteria were correctly identified with both ID-GN and GN cards (97.2% and 98.7%, respectively). In contrast, nonfermenting gram-negative bacteria were better identified with GN cards (92.1%) than with ID-GN cards (67%). Gram-positive bacteria were better identified with colorimetric cards than with fluorimetric cards. For *Streptococcaceae*, readings of fluorimetric and colorimetric cards gave 87.9% and 90.9% correct identifications, respectively. For *Micrococcaceae*, these readings were 87.2% and 98.3%, respectively. Our results obtained with gram-negative bacteria were for the most part in agreement with those reported by Funke and Funke-Kissling (3). Testing 511 fermenting and 144 nonfermenting gram-negative bacilli, these authors (3) obtained slightly better results with the GN cards than we did in the present study (99.5% and 98.7%, respectively). Recently, in another study focusing on gram-positive bacteria, Funke and Funke-Kissling (4) obtained correct identification of *Streptococcaceae* (217 strains) and *Micrococcaceae* (147 strains) for 99.1% and 99.3% of these bacteria, respectively, whereas the results were 90.9% and 98.3% in our study. It should be noticed that the results reported by Funke and Funke-Kissling (3, 4) appeared better but that the numbers of taxa tested (13, 18, and 12 for *Micrococcaceae*, *Streptococcaceae*, and nonfermenting bacilli, respectively) were lower than in our study, except for fermenting bacteria (42 taxa in both studies). In fact, as Funke and Funke-Kissling have previously claimed to have done (3, 4), we have selected rare bacteria isolated in routine practice and some of them, such as *S. constellatus* or *S. gordonii*, while infrequently isolated in routine testing, were isolated in large numbers in our study (for example, eight strains of *S. constellatus* instead of one in Funke's study).

The GP and GN identification cards contain new tests (16 and 21 tests for GP and GN, respectively) allowing an improvement of the VITEK 2 databases; in fact, 57 and 38 new species of gram-positive cocci and gram-negative bacilli were added to the database. Of these, seven gram-positive species (eight strains) were tested and only one strain of *Pediococcus pentosaceus* was not correctly identified. Of the 17 new gram-negative species tested (36 strains), only four species (four strains) were not correctly identified: *Bordetella bronchiseptica*, *Pseudomonas alcaligenes*, *and P. putida* were misidentified (one strain each), and one strain of *P. fluorescens* was not identified. Thus, the misidentification percentages obtained with the colorimetric cards ranged from 2.1% to 4.8%, results which were slightly better than those obtained with the fluorescent cards (5.1% to 6.8%). These misidentified bacteria were observed only with *Streptococcus* spp. such as *S. constellatus* (three strains) or *S. gordonii* (two strains) or nonfermenting gram-negative bacilli such as *Acinetobacter* spp. belonging to genomospecies 1, 2, 3, or 13 (*A. calcoaceticus*/*A. baumannii* complex). Compared to previous study results (2, 5, 6), the database was enlarged and improved, especially for nonfermenting bacilli and *Micrococcaceae*. The database was also enlarged to include some gram-positive bacilli such as *Erysipelothrix rhusiopathiae* and six species of the *Listeria* genus.

The second aim of this study was to evaluate the performance of the new colorimetric cards in routine practice. Thus, we applied the percentages of correct identifications obtained in this study with the colorimetric and fluorescent cards to the list of bacteria isolated in our laboratory in the year 2004. From the species included in the database of the VITEK 2, 71 species were selected, representing 32,739 bacteria isolated five times or more in 2004. An overall correlation of 97.9% correct identifications for gram-positive and gram-negative bacteria was obtained, whereas it was equal to 93.9% with fluorescent cards. The same determination was performed with a selection of 17 gram-negative taxa isolated more frequently in 33 French university hospitals (11). Identification with colorimetric cards

gave an overall identification to the species level of 99.7%, whereas it was equal to 95.9% with fluorescent cards.

The VITEK 2 system, equipped for colorimetric reading of the new GP and GN cards, keeps the advantages of the VITEK 2 (2, 5, 6, 8), i.e., reliable identification, fully automated incubation and interpretation, and minimal supplemental testing required. The results provided by the colorimetric VITEK 2 may be considered accurate due to the improvement and the extension of its database, mainly for nonfermenting bacteria and *Streptococcaceae*. In this study, the identifications of bacteria were provided between 5.2 h (fermenting bacteria) and 6.7 h (nonfermenting bacteria), which was slightly greater than the time required for reading with fluorescent cards. However, the results were always provided within a day. In conclusion, the results obtained in this study demonstrate the good performances of the new VITEK 2 cards, allowing their use in routine practice with a highly acceptable level of identification accuracy.

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