Eur J Clin Microbiol Infect Dis (2005) 24: 671–676
DOI 10.1007/s10096-005-0026-6

ARTICLE

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Evaluation of the new Vitek 2 GN card for the identification of gram-negative bacilli frequently encountered in clinical laboratories

Published online: 27 October 2005

Abstract The new Vitek 2 GN card (bioMérieux, Marcy-l'Etoile, France) was developed for better identification of fermenting and nonfermenting bacilli. This new card allows the identification of 159 taxa. A total of 426 isolates (331 fermenting and 95 nonfermenting gram-negative bacilli) belonging to 70 taxa covered by the database were evaluated. All isolates were identified in parallel with the ID 32 GN, the API 20E, and the API 20NE methods. The system correctly identified 97.4% (n=415) of the strains. Only 2.1% (n=9) needed additional testing. One strain (0.25%) was misidentified (*Klebsiella pneumoniae* subsp. *pneumoniae*), and another one (0.25%) was not identified (*Morganella morganii* subsp. *morganii*). The new GN card gives more accurate identifications overall for gram-negative bacilli when compared to the systems described in other similar studies.

This study was presented in part at the 104th General Meeting of the American Society for Microbiology, New Orleans, Louisiana, 2004, Abstract C-180.

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Introduction

The fully automated Vitek 2 system (bioMérieux, Marcy-l'Etoile, France) has evolved to improve the performance of identification products and to increase the breadth of the databases. Different evaluations of this identification system have been published previously [1–7]. One of these studies [7] reported recently on the performance of the new Vitek 2 Advanced Colorimetry GN card (GN card). The GN card contains 47 colorimetric tests compared to 41 fluorometric tests in the previous ID-GNB card. The GN database includes 159 taxa, whereas the ID-GNB is limited to 101 taxa. In this study, we evaluated the performance of the GN card for identification of the most clinically relevant fermenting and nonfermenting gram-negative rods.

Materials and methods

Vitek 2 system

The GN card was used according to the instructions of the manufacturer (bioMérieux, Marcy-l'Etoile, France). The card contains 47 colorimetric tests that measure utilization of carbon sources, enzymatic activities, and resistance to inhibitory substances. The GN card allows identification of 159 gram-negative taxa. Test reactions are read automatically every 15 min until a maximum incubation time of 10 h. A specific kinetic algorithm calculation allows for a final identification result after 2–10 h of incubation. The GN card employs the use of some novel colorimetric substrates, and the Vitek 2 instrument has been modified with a new optical system.

Strains

A total of 426 unique clinical isolates (95 non-fermenting and 331 fermenting gram-negative bacilli) representing 70 taxa were tested (Table 1). Two hundred forty isolates came from the medical microbiology laboratories (fresh isolates)

Table 1 Performance of the new GN card

Species	No. of strains	One choice	Low discricimation	No ID	Mistaken ID
Nonfermenting gram-negative rods					
Achromobacter xylosoxidans subsp. *denitrificans*	1	0	1	0	0
Achromobacter xylosoxidans subsp. *xylosoxidans*	4	3	1	0	0
Acinetobacter baumannii	17	17	0	0	0
Acinetobacter haemolyticus	5	5	0	0	0
Acinetobacter junii	1	1	0	0	0
Acinetobacter lwoffii	2	1	1	0	0
Alcaligenes faecalis ssp. *faecalis*	1	1	0	0	0
Chryseobacterium indologenes	1	1	0	0	0
Chryseobacterium meningosepticum	1	1	0	0	0
Comamonas testosteroni	1	0	1	0	0
Pseudomonas aeruginosa	28	28	0	0	0
Pseudomonas fluorescens	4	2	2	0	0
Pseudomonas mendocina	1	1	0	0	0
Pseudomonas oryzihabitans	1	1	0	0	0
Pseudomonas pseudoalcaligenes	1	1	0	0	0
Pseudomonas putida	3	3	0	0	0
Pseudomonas stutzeri	4	4	0	0	0
Sphingomonas paucimobilis	2	2	0	0	0
Stenotrophomonas maltophilia	17	17	0	0	0
Total nonfermentative strains (%)	95	89 (93.7)	6 (6.3)	0 (0)	0 (0)
Fermenting gram-negative rods					
Aeromonas caviae	2	2	0	0	0
Aeromonas hydrophila	4	4	0	0	0
Citrobacter braakii	1	1	0	0	0
Citrobacter freundii	17	16	1	0	0
Citrobacter koseri	17	17	0	0	0
Enterobacter aerogenes	14	14	0	0	0
Enterobacter amnigenus[a]	1	0	0	0	1
Enterobacter asburiae	1	1	0	0	0
Enterobacter cloacae	17	17	0	0	0
Enterobacter gergoviae	2	2	0	0	0
Enterobacter sakazakii	4	4	0	0	0
Escherichia coli	46	46	0	0	0
Escherichia coli O157	1	1	0	0	0
Escherichia hermannii	4	4	0	0	0
Hafnia alvei	6	6	0	0	0
Klebsiella oxytoca	13	12	1	0	0
Klebsiella pneumoniae subsp. *ozaenae*	2	2	0	0	0
Klebsiella pneumoniae subsp. *pneumoniae*	24	24	0	0	0
Klebsiella pneumoniae subsp. *rhinoscleromatis*	1	1	0	0	0
Kluyvera ascorbata	1	1	0	0	0
Morganella morganii subsp. *morganii*	15	14	0	1	0
Morganella morganii subsp. *sibonii*	1	1	0	0	0
Moraxella osloensis[a]	1	0	1	0	0
Pasteurella aerogenes	1	1	0	0	0
Pasteurella-like EF4	2	2	0	0	0
Pasteurella multocida	2	2	0	0	0
Pasteurella pneumotropica	1	1	0	0	0
Plesiomonas shigelloides	2	2	0	0	0
Proteus mirabilis	29	29	0	0	0
Proteus penneri	1	1	0	0	0
Proteus vulgaris group	13	13	0	0	0
Providencia alcalifaciens	1	1	0	0	0

Table 1 (*continued*)

Species	No. of strains	One choice	Low discricimation	No ID	Mistaken ID
Providencia rettgeri	7	7	0	0	0
Providencia stuartii	6	6	0	0	0
Raoultella ornithinolytica	4	4	0	0	0
Salmonella choleraesuis subsp. *arizonae*	1	1	0	0	0
Salmonella spp.	20	20	0	0	0
Salmonella Typhi	1	1	0	0	0
Serratia liquefaciens	3	3	0	0	0
Serratia marcescens	19	19	0	0	0
Serratia odorifera	1	1	0	0	0
Serratia rubidaea	2	2	0	0	0
Shigella boydii	1	1	0	0	0
Shigella flexneri	1	1	0	0	0
Shigella sonnei	5	5	0	0	0
Shigella spp.[a]	3	3	0	0	0
Vibrio alginolyticus	1	1	0	0	0
Yersinia enterocolitica	6	6	0	0	0
Yersinia frederiksenii[a]	1	1	0	0	0
Yersinia kristensenii[a]	1	1	0	0	0
Yersinia pseudotuberculosis	1	1	0	0	0
Total fermentative strains (%)	331	326 (98.5)	3 (0.9)	1 (0.3)	1 (0.3)
Total strains (%)	426	415 (97.4)	9 (2,1)	1 (0.25)	1 (0.25)

[a]Name in the ID-GNB database

and 186 from our stock collection. Stock isolates were stored in lyophilized form. Forty-one of the fresh isolates were tested directly from the primary isolation culture on Columbia sheep blood agar. The age of the others was not higher than 1 month, and none had ever been frozen or lyophilized. All other isolates were subcultured on Columbia agar with 5% sheep blood, MacConkey agar, or trypticase soy agar (bioMérieux) prior to testing to ensure purity and viability. The isolates were identified by using at least two different identification kits, including API 20E and/or API 20NE and/or ID 32 GN (bioMérieux), which served as the reference method. Discrepancies were resolved by using additional API galleries (Biotype 100, API 50 CHE, bioMérieux) or by sequencing of the 16S ribosomal RNA gene.

Reporting of results

Identifications obtained with the new GN card were compared with the results of the reference method and summarized in four categories: (i) one choice, which corresponded to unambiguous correct identification at the species level, (ii) low-level discrimination between several species, including the correct species (low discrimination), where simple additional tests can be used to resolve the identification, (iii) misidentification (mistaken ID), when the species identified with the GN card was/were different from that/those identified by the reference method, and (iv) no identification (no ID), when the GN card was not able to give an identification result. The three strains tested that belonged to species not included in the database were excluded from the summary reports.

Results

The identification results obtained with the Vitek 2 GN system are presented in Table 1. The taxonomy evolutions applied to the GN card are reported in Table 2. Of the 426 strains belonging to the 70 taxa studied, 415 (97.4%) were correctly identified (1 choice), and nine (2.1%) required additional tests (low discrimination). Only one strain (0.25%) was not identified, and another strain (0.25%) was misidentified. The fermenting gram-negative bacilli were better identified (98.5% correct) than the nonfermenting gram-negative bacilli (93.7% correct).

Table 2 Nomenclature equivalency of species included in the GN card database

Species in ID-GNB database	Species in new GN database
Enterobacter amnigenus	*Enterobacter amnigenus* 1
	Enterobacter amnigenus 2
Shigella spp.	
	Shigella group (*Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*)
Yersinia frederikenii	*Yersinia enterocolitica* group
Yersinia kristensenii	
Moraxella osloensis	*Moraxella* group

The nine isolates for which low discrimination results were obtained (Table 3) were three fermentative bacilli, (*Citrobacter freundii*, *Klebsiella oxytoca*, and *Moraxella osloensis*) and six non-fermentative bacilli (*Acinetobacter lwoffii*, *Achromobacter xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, *Comamonas testosteroni* and 2 *Pseudomonas fluorescens*). To allow the differentiation of the discrepancies, supplemental tests such as nitrate reduction, production of indole, fermentation of carbohydrates such as D-melibiose or D-glucose, pigment production, gelatin hydrolysis, oxidase, and assimilation tests were used (see Table 3).

Only one isolate (*Morganella morganii* subsp. *morganii*) remained unidentified by the system. In addition, one strain of *Enterobacter amnigenus* was misidentified as *Enterobacter cloacae* by the system.

All isolates tested directly from the primary isolation medium were identified correctly except for one unidentified strain (*Morganella morganii* subsp. *morganii*). No difference in identification results was observed between fresh isolates and stock collection strains.

Table 4 lists for each species the average time required for a final identification. The mean time to identification of fermentative strains was 4.3 h and to identification of non-fermentative strains 6.1 h.

Discussion

Several investigators have described the usefulness of the previous Vitek 2 ID-GNB card for identification of gram-negative rods [1–7]. In our study, conducted with 426 clinical gram-negative bacilli, we showed correct identification for 97.4% of the strains without supplemental tests and for 99.5% after performing very simple additional tests (NO_3, pyocyanin, gelatin, indole, fermentation of sugars, and oxidase). Compared to the ID-GNB card, the GN card performed better. Table 5 details the performance of the ID-GNB card and the new GN card. Fermenting and nonfermenting strains are differentiated. In addition, the ID-GNB card identifies most of the relatively inert non-fermenting gram-negative bacilli as a large heterogeneous group ("various nonfermenting gram-negative bacilli", VNFGNB) that includes clinically important taxa such as *Acinetobacter lwoffii*, *Moraxella* spp., *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas stutzeri*. The VNFGNB "taxon" has been suppressed

Table 3 Bacterial isolates identified with low discrimination on the GN card

	Reference identification	New GN card identification result	Additional tests		(expected results)					
Fermentative bacilli	*Citrobacter freundii*	*C. freundii*	dMelibiose	+						
		C. youngae		−						
	Klebsiella oxytoca	*K. oxytoca*	Indole	+						
		K. pneumoniae subsp. *pneumoniae*		−						
	Moraxella osloensis	*A. lwoffi*	Oxidase	−						
		Moraxella group		+						
Nonfermentative bacilli	*Achromobacter xylosoxidans* subsp. *denitrificans*	*A. xylosoxidans* subsp. *denitrificans*	NO_3	+						
		Alcaligenes faecalis subsp. *faecalis*		−						
	Achromobacter xylosoxidans subsp. *xylosoxidans*	*A. xylosoxidans* subsp. *xylosoxidans*	dGlucose	+						
		A. xylosoxidans subsp. *denitrificans*		−						
	Acinetobacter lwoffi	*Metyhylobacterium* spp.	Oxidase	+						
		A. lwoffi		−						
	Pseudomonas fluorescens	*P. putida*	Gelatin	−	Pyocyanine	−				
		P. aeruginosa		+		+				
		P. fluorescens		+		−				
	Pseudomonas fluorescens	*P. fluorescens*	Gelatin	+						
		P. putida		−						
	Comamonas testoteroni	*C. testoteroni*	D-fructose	−	D-mannitol	−	Nitrate	+	Urea	−
		Delftia acidovorans	assimilation	+	assimilation	+	reduction	+		−
		Ralstonia paucula		ND		−		−		+

ND not determined

Table 4 Time-to-call results obtained with the new GN card

Species	Average time to call (h)	No. of strains
Non-fermenting gram-negative rods		
Pseudomonas aeruginosa	5.52	28
Acinetobacter baumannii	6.28	17
Stenotrophomonas maltophilia	4.57	17
Acinetobacter haemolyticus	6.50	5
Pseudomonas fluorescens	9.31	4
Achromobacter xylosoxidans subsp. *xylosoxidans*	8.75	4
Pseudomonas stutzeri	6.08	4
Pseudomonas putida	6.75	3
Acinetobacter lwoffii	9.00	2
Sphingomonas paucimobilis	5.25	2
Achromobacter xylosoxidans subsp. *denitrificans*	9.75	1
Comamonas testosteroni	9.75	1
Pseudomonas pseudoalcaligenes	9.75	1
Acinetobacter junii	7.75	1
Alcaligenes faecalis subsp. *faecalis*	7.00	1
Chryseobacterium indologenes	6.00	1
Pseudomonas oryzihabitans	5.75	1
Pseudomonas mendocina	4.75	1
Chryseobacterium meningosepticum	4.25	1
Mean time for nonfermenting strains	6.1	95
Fermenting gram-negative rods		
Escherichia coli	3.46	46
Proteus mirabilis	3.65	29
Klebsiella pneumoniae subsp. *pneumoniae*	4.65	24
Salmonella spp.	4.64	20
Serratia marcescens	3.83	19
Citrobacter freundii	5.25	17
Enterobacter cloacae	5.16	17
Citrobacter koseri	3.91	17
Morganella morganii subsp. *morganii*	4.75	15
Enterobacter aerogenes	4.11	14
Klebsiella oxytoca	4.13	13
Proteus vulgaris group	3.00	13
Providencia rettgeri	4.25	7
Hafnia alvei	6.20	6
Providencia stuartii	4.75	6
Yersinia enterocolitica	3.92	6
Shigella sonnei	4.75	5
Escherichia hermannii	4.75	4
Aeromonas hydrophila	4.38	4
Enterobacter sakazakii	3.94	4
Raoultella ornithinolytica	2.75	4
Shigella spp.[a]	6.25	3
Serratia liquefaciens	4.17	3
Pasteurella multocida	8.25	2
Pasteurella-like EF4	7.25	2
Aeromonas caviae	5.25	2
Klebsiella pneumoniae subsp. *ozaenae*	5.25	2
Plesiomonas shigelloides	4.75	2
Enterobacter gergoviae	4.25	2
Serratia rubidaea	3.38	2
Moraxella osloensis[a]	9.75	1
Enterobacter amnigenus[a]	9.50	1
Yersinia pseudotuberculosis	7.50	1
Klebsiella pneumoniae subsp. *rhinoscleromatis*	6.75	1
Citrobacter braakii	6.00	1
Shigella flexneri	6.00	1
Enterobacter asburiae	5.75	1
Morganella morganii subsp. *sibonii*	5.75	1
Pasteurella pneumotropica	5.75	1
Shigella boydii	5.50	1
Escherichia coli O157	4.75	1
Pasteurella aerogenes	4.75	1
Providencia alcalifaciens	4.75	1
Salmonella Typhi	4.75	1
Vibrio alginolyticus	4.50	1
Kluyvera ascorbata	4.00	1
Yersinia frederiksenii[a]	3.75	1
Yersinia kristensenii[a]	3.75	1
Proteus penneri	3.00	1
Serratia odorifera	2.75	1
Salmonella choleraesuis subsp. *arizonae*	2.50	1
Mean time for fermenting strains	4.30	331

[a]Name in the ID-GNB database

from the GN card, and the different species belonging to this group have been included in the new database, allowing accurate and discriminate identification of non-fermentative gram-negative bacilli.

Overall, the identification of non-fermenting strains has been substantially improved (from 62.7% [2] to 92.4% [4] correctly identified in previous published reports to up to 93.7% in the present study); moreover, identification of fermenting strains improved as well (from 83.4% [1] to 97.2% correctly identified [2] to up to 98.5%).

The results of our study and those of Funke et al. [7] were very similar: one-choice identification for 92.4% (non-fermenting) and 98.6% (fermenting) in the Funke et al. study, and 93.7% (non-fermenting) and 98.5% (fermenting) in our study. The new GN card system identified 99.5% [97.4% (1 choice)+2.1% (simple additional tests)] of the 426 strains evaluated.

Table 5 Published evaluations of identification cards for fermenting and nonfermenting organisms on the Vitek 2 instrument

Reference	No. of strains		No. of taxa		One choice (%)		Correct identification with additional test(s) (%)		Indeterminate result (%)		No identification (%)		Misidentification (%)	
	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
ID-GNB														
O'Hara and Miller [6]	72	513	20	64	83.3	86.1	–	–	11.1	7	2.8	3.7	2.8	3.1
Ling et al. [4]	92	188	15	25	92.4	96.8	–	–	–	–	3.3	2.1	4.3	1.1
Gavin et al. [5]	172	686	7	17	90.1	85.1	2.9	10.8	–	–	6.4	2	0.6	2
Funke et al. [1]	116	729	8	62	93.1	83.4	3.4	3.8	2.6	10.6	0	1.4	0.9	0.8
Jossart and Courcol [2]	118	384	19	51	62.7	97.2	–	–	33.9	3.9	0	0.5	3.4	2.9
Joyanes et al. [3]	198	–	3	–	66.7	–	–	–	24.2	–	8.6	–	0.5	–
GN														
Funke and Funke-Kissling [7]	144	511	12	42	92.4	98.6	6.2	1	–	–	0	0	1.4	0.4
Present study	95	331	19	51	93.7	98.5	6.3	0.9	–	–	0	0.3	0	0.3

F fermenting, *NF* nonfermenting

The misidentification and non-identification rates have also been dramatically reduced with the GN card: only one of the 426 strains in our study and four of the 655 strains in the study of Funke et al. [7] were misidentified or not identified.

Testing the strains directly from primary isolation plates did not appear to impact the performance of the GN card, since the collection strains and the fresh isolates were identified with the same level of accuracy. Moreover, testing performed from chromogenic media did not interfere with the automatic reading because of the low level of the inoculum (0.5 McFarland standard).

Furthermore, the time-to-call results provided 80% of the identifications between 3 h and 6.5 h in our study (Table 4). We consider that the new Vitek 2 Advanced Colorimetry GN card may be a very high-performing tool for the identification of gram-negative bacilli. In addition to our study and that of Funke et al. [7], which together included 83 species frequently encountered in a clinical setting, it would be of interest to carry out additional studies to test other taxa in the Vitek 2 GN database that are less frequently encountered in routine clinical microbiology laboratories and have not been covered by our study.

Acknowledgements We thank Sandrine Pétré, Bernadette Blanc, Béatrice Celliere, Geneviève Bossy, Daniel Monget, and Dave Pincus for their useful discussions about this study.

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