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Evaluation of the New VITEK 2 Card for Identification of Clinically Relevant Gram-Negative Rods

Guido Funke* and Pascale Funke-Kissling

Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Weingarten, Germany

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The VITEK 2 card for gram-negative bacteria (bioMérieux, Marcy-l′Etoile, France) has been redesigned to improve the identification of fermenting and nonfermenting bacilli. Forty-seven biochemical tests, including 19 enzymatic tests, are present in the new card and interpreted in a kinetic mode. Final identification results are available within 10 h. The database allows the identification of 159 different taxa. Six hundred fifty-five gram-negative rods (GNR; 511 fermenters and 144 nonfermenters), representing 54 taxa, were tested. Strains were taken from fresh routine primary isolation plates ($n = 157$), from stored routine plates ($n = 301$), and from stock cultures ($n = 197$). Six hundred thirty-seven strains (97.3%) were correctly identified to the species level, 14 strains (2.1%) gave low discrimination results requiring additional tests, and 4 strains (0.6%) gave discordant results; not a single strain remained unidentified. Nearly 92% of all isolates were correctly identified within 7 h of incubation. The robustness of the system was demonstrated by the fact that strains were grown on four different agar media before testing. The system may also have the potential to be applied directly to primary isolation plates, since in this instance 96.2% of 157 GNR were correctly identified and 3.8% gave low discrimination results. The new VITEK 2 card for gram-negative bacteria seems to be a promising new tool for routine, rapid identification of GNR.

Highly automated identification systems have been introduced in many medium- to high-throughput clinical microbiology laboratories worldwide within the last 15 years. These systems, such as VITEK (bioMérieux, Marcy l'Etoile, France), MicroScan (Dade, West Sacramento, Calif.), and PHOENIX (BD, Sparks, Md.), have contributed to better and more-cost-effective management of patients by enabling clinical microbiologists to identify medically relevant bacteria more rapidly and accurately. In a previous article, it was emphasized that an important value for a highly standardized commercial identification system must be the capability of the manufacturer to maintain or even improve the performance of an identification system over time (2). The new VITEK 2 card (NGNC; bioMérieux) for identification of gram-negative rods (GNR) was recently created during the evolutionary process of research on and further development of the VITEK 2 instrument. The rationale for designing the NGNC was to broaden the database and to improve identification results. The NGNC contains 47 tests (26 that had been included in the previous card and 21 new tests), compared to 41 in the established VITEK 2 ID-GNB card (GNC), and 159 taxa are covered by the new database corresponding to the NGNC, compared to only 101 for the GNC database. While the GNC tests are based on fluorescence technology, the NGNC tests are based on colorimetric detection. Measurements are required every 15 min for both the GNC and NGNC tests, and the total incubation times are up to approximately 10 h for the NGNC and 3 h for the GNC. The aim of the present study was to evaluate the use of the NGNC in a routine clinical laboratory by a combination of a weighted laboratory profile and a stress test (7).

* Corresponding author. Mailing address: Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Hoyerstrasse 51, D-88250 Weingarten, Germany. Phone: 49-751-502-630. Fax: 49-751-502-385. E-mail: ldg.funke@t-online.de.

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MATERIALS AND METHODS

Strains, culture conditions, and identification. One hundred fifty-seven strains of GNR were taken from primary isolation plates (Columbia sheep blood agar [BD] [$n = 142$] or MacConkey agar [bioMérieux] [$n = 15$]) set up in our routine clinical laboratory for various materials from patients (e.g., urine specimens, wound swabs, respiratory specimens, etc.). Three hundred one strains came from primary isolation plates which had been stored at 4 to 8°C for less than 1 week. These strains were subcultured on Columbia sheep blood agar (BD) ($n = 143$), Columbia sheep blood agar (bioMérieux) ($n = 4$), MacConkey agar ($n = 77$), or Trypticase soy agar (bioMérieux) ($n = 77$) for 18 to 24 h at 37°C before they were subjected to the VITEK 2 analysis. All strains used from the primary isolation plates came from unrelated patients, and consecutive cultures from the same patient were excluded. One hundred ninety-seven strains (stored in Microbank tubes [Mast Diagnostica, Reinfeld, Germany] at −70°C) were taken from our culture collection and subcultured on Columbia sheep blood agar (bioMérieux) ($n = 53$), MacConkey agar ($n = 71$), or Trypticase soy agar ($n = 73$). The 655 strains used in this study were identified by conventional methods (8, 10), by ID 32 GN and API 20 NE strips (both from bioMérieux), and by VITEK 1 testing with the GNI card. Discrepancies between the laboratory identifications and the identifications provided by the VITEK 2 system with the NGNC were resolved by using API 50CHE and Biotype 100 galleries (both from bioMérieux), as well as by sequencing 16S rRNA genes as previously outlined (1).

NGNC and the VITEK 2 instrument. A bacterial suspension was adjusted to a McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution with a VITEK 2 DensiChek instrument (bioMérieux). The time between preparation of the inoculum and the filling of the card was always less than 30 min. The format of the NGNC, i.e., a 64-well plastic card, is the same as that of the GNC, but the NGNC contains 47 tests, while the GNC contains 41 tests (see above). The NGNC is a fully closed system to which no reagents have to be added. The card was put on the cassette designed for VITEK 2, placed in the instrument, automatically filled in a vacuum chamber, sealed, incubated at 35.5°C, and automatically subjected to a colorimetric measurement by use of a new optical reading head every 15 min for a maximum incubation period of 10 h. Data were analyzed using VITEK 2 database version 4.01, which allows for organism identification in the kinetic mode after 2 h of incubation.

TABLE 1. Performance of the NGNC by species

Species	No. of strains				
	Total tested	Correctly identified	With low discrimination	Not identified	Misidentified
Acinetobacter baumannii	23	23	0	0	0
Acinetobacter haemolyticus	5	5	0	0	0
Acinetobacter lwoffii	12	8	3	0	1
Aeromonas hydrophila	7	7	0	0	0
Alcaligenes faecalis subsp. *faecalis*	2	2	0	0	0
Chryseobacterium indologenes	3	3	0	0	0
Citrobacter amalonaticus	6	6	0	0	0
Citrobacter braakii	10	10	0	0	0
Citrobacter farmeri	2	2	0	0	0
Citrobacter freundii	22	22	0	0	0
Citrobacter koseri	18	18	0	0	0
Enterobacter aerogenes	9	9	0	0	0
Enterobacter amnigenus	1	0	0	0	1
Enterobacter asburiae	7	7	0	0	0
Enterobacter cancerogenus	1	1	0	0	0
Enterobacter cloacae	45	45	0	0	0
Enterobacter intermedius	7	7	0	0	0
Enterobacter sakazakii	5	5	0	0	0
Escherichia coli	58	58	0	0	0
Escherichia hermannii	10	10	0	0	0
Escherichia vulneris	3	3	0	0	0
Hafnia alvei	10	10	0	0	0
Klebsiella oxytoca	26	22	4	0	0
Klebsiella pneumoniae subsp. *pneumoniae*	36	35	1	0	0
Leclercia adecarboxylata	13	13	0	0	0
Morganella morganii subsp. *morganii*	14	14	0	0	0
Morganella morganii subsp. *sibonii*	4	4	0	0	0
Myroides spp.	1	1	0	0	0
Proteus mirabilis	54	54	0	0	0
Proteus penneri	2	2	0	0	0
Proteus vulgaris group	19	19	0	0	0
Providencia alcalifaciens	4	4	0	0	0
Providencia rettgeri	18	18	0	0	0
Providencia rustigianii	7	7	0	0	0
Providencia stuartii	6	6	0	0	0
Pseudomonas aeruginosa	40	37	3	0	0
Pseudomonas fluorescens	20	17	3	0	0
Pseudomonas oryzihabitans	1	1	0	0	0
Pseudomonas putida	12	12	0	0	0
Pseudomonas stutzeri	2	1	0	0	1
Rahnella aquatilis	9	8	0	0	1
Salmonella enterica serovar Enteritidis	9	9	0	0	0
Salmonella spp.	6	6	0	0	0
Salmonella enterica serovar Typhi	1	1	0	0	0
Salmonella enterica serovar Typhimurium	4	4	0	0	0
Serratia fonticola	9	9	0	0	0
Serratia liquefaciens	9	9	0	0	0
Serratia marcescens	18	18	0	0	0
Serratia odorifera	2	2	0	0	0
Serratia plymuthica	1	1	0	0	0
Shigella spp.	2	2	0	0	0
Shigella sonnei	7	7	0	0	0
Stenotrophomonas maltophilia	23	23	0	0	0
Yersinia enterocolitica	10	10	0	0	0
Total no. (%)	655	637 (97.3)	14 (2.1)	0 (0.0)	4 (0.6)

Quality control strains. During the 3-month evaluation period, the following quality control strains were checked at regular intervals: *Acinetobacter baumannii* ATCC BAA-747, *Aeromonas hydrophila* ATCC 35654, *Brevundimonas diminuta* ATCC 11568, *Chryseobacterium meningosepticum* ATCC 13253, *Citrobacter freundii* ATCC 14135, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 11775, *Klebsiella oxytoca* ATCC 700324, *Proteus vulgaris* ATCC 6380, and *Pseudomonas aeruginosa* ATCC 9721.

Reporting of results. The interpretations provided by the software were taken into account; the identification scores provided by the software (*t* index, probability, likelihood, and confidence) were not. The four result categories were as follows: (i) correct identification (unambiguous correct identification to the species level), (ii) low level of discrimination (either identification to the genus level or low level of discrimination between two or more species, including the correct species), (iii) no identification, and (iv) misidentification (the species identified with the NGNC was different from that identified by the reference method).

RESULTS

We did not encounter any major technical problems using the VITEK 2 instrument during the evaluation. Quality control strains were correctly identified to the species level in every instance, demonstrating the reliability of the NGNC as well as the reliability and reproducibility of the technique. The hands-on time was the same for the NGNC as for the GNC.

Table 1 shows the performance of the NGNC in evaluating the 54 individual taxa (representing 21 genera) tested. Five hundred eleven strains (42 taxa and 15 genera) were fermenting bacteria, and 144 strains (12 taxa and 6 genera) were gram-negative nonfermenters. Of the total of 655 strains, 637 (97.3%) were correctly identified to the species level, 14 strains (2.1%) were identified with low discrimination, no strain remained unidentified, and 4 strains (0.6%) were misidentified. Identification results were slightly better for fermenting bacteria, with 98.3% correctly identified, 1.2% identified with low discrimination, and 0.5% misidentified, than for nonfermenting isolates, with 92.4% correctly identified, 6.3% identified with low discrimination, and 1.4% misidentified. The numbers of strains of all taxa tested were not equal but rather were partly weighted to reflect the frequencies of different species seen in a routine clinical laboratory. The 10 most frequently isolated GNR, namely *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii*, represented 52.8% of all strains included in the present study. Of these, 97.7% were

TABLE 2. Strains identified with low discrimination and misidentified strains

Reference method identification (no. of strains)	NGNC identification
Strains identified with low discrimination	
Acinetobacter lwoffii (2)	Nonreactive biopattern, *Acinetobacter lwoffii*
Acinetobacter lwoffii (1)	*Acinetobacter lwoffii*, *Moraxella* group
Klebsiella oxytoca (3)	*Klebsiella oxytoca*, *Klebsiella pneumoniae*
Klebsiella oxytoca (1)	*Klebsiella pneumoniae*, *Klebsiella oxytoca*
Klebsiella pneumoniae (1)	*Klebsiella oxytoca*, *Klebsiella pneumoniae*
Pseudomonas aeruginosa (3)	*Pseudomonas aeruginosa*, *Pseudomonas putida*
Pseudomonas fluorescens (2)	*Pseudomonas fluorescens*, *Acinetobacter lwoffii*
Pseudomonas fluorescens (1)	*Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*
Misidentified strains	
Acinetobacter lwoffii (1)	*Moraxella* group
Enterobacter amnigenus (1)	*Enterobacter cloacae*
Pseudomonas stutzeri (1)	CDC group EO-2 or *Psychrobacter* sp.
Rahnella aquatilis (1)	*Pantoea* spp.

TABLE 3. Time of completed identifications using the NGNC

Species or group	No. of strains tested	% of strains identified at:								
		<3 h	<4 h	<5 h	<6 h	<7 h	<8 h	<9 h	<10 h	10 h
Acinetobacter baumannii	23			9	9	100	100	100	100	100
Acinetobacter haemolyticus	5				40	40	100	100	100	100
Acinetobacter lwoffii	12						17	33	100	100
Aeromonas hydrophila	7			57	86	86	86	86	100	100
Alcaligenes faecalis subsp. *faecalis*	2						100	100	100	100
Chryseobacterium indologenes	3			33	33	100	100	100	100	100
Citrobacter amalonaticus	6			67	83	100	100	100	100	100
Citrobacter braakii	10		10	70	80	90	90	90	100	100
Citrobacter farmeri	2		50	100	100	100	100	100	100	100
Citrobacter freundii	22		27	77	77	91	91	91	100	100
Citrobacter koseri	18		83	100	100	100	100	100	100	100
Enterobacter aerogenes	9		22	100	100	100	100	100	100	100
Enterobacter amnigenus	1			100	100	100	100	100	100	100
Enterobacter asburiae	7			86	100	100	100	100	100	100
Enterobacter cancerogenus	1			100	100	100	100	100	100	100
Enterobacter cloacae	45			80	89	96	96	96	98	100
Enterobacter intermedius	7			100	100	100	100	100	100	100
Enterobacter sakazakii	5		60	100	100	100	100	100	100	100
Escherichia coli	58	38	50	100	100	100	100	100	100	100
Escherichia hermannii	10			100	100	100	100	100	100	100
Escherichia vulneris	3			67	67	67	100	100	100	100
Hafnia alvei	10				20	90	90	90	100	100
Klebsiella oxytoca	26		12	69	85	85	85	85	96	100
Klebsiella pneumoniae subsp. *pneumoniae*	36			69	81	83	92	97	100	100
Leclercia adecarboxylata	13			62	85	85	100	100	100	100
Morganella morganii subsp. *morganii*	14				100	100	100	100	100	100
Morganella morganii subsp. *sibonii*	4			100	100	100	100	100	100	100
Myroides spp.	1						100	100	100	100
Proteus mirabilis	54	22	70	100	100	100	100	100	100	100
Proteus vulgaris group	19	42	89	100	100	100	100	100	100	100
Proteus penneri	2		50	100	100	100	100	100	100	100
Providencia alcalifaciens	4			100	100	100	100	100	100	100
Providencia rettgeri	18			100	100	100	100	100	100	100
Providencia rustigianii	7				100	100	100	100	100	100
Providencia stuartii	6		33	100	100	100	100	100	100	100
Pseudomonas aeruginosa	40			10	25	88	92	100	100	100
Pseudomonas fluorescens	20				75	85	90	90	100	100
Pseudomonas putida	12					67	88	100	100	100
Pseudomonas stutzeri	2			50	100	100	100	100	100	100
Pseudomonas oryzihabitans	1				100	100	100	100	100	100
Rahnella aquatilis	9			11	11	67	78	78	100	100
Salmonella enterica serovar Enteritidis	9		11	44	100	100	100	100	100	100
Salmonella enterica serovar Typhi	1						100	100	100	100
Salmonella enterica serovar Typhimurium	4			100	100	100	100	100	100	100
Salmonella spp.	6		17	50	100	100	100	100	100	100
Serratia fonticola	9	44	100	100	100	100	100	100	100	100
Serratia liquefaciens	9		11	100	100	100	100	100	100	100
Serratia marcescens	18		33	100	100	100	100	100	100	100
Serratia odorifera	2	50	100	100	100	100	100	100	100	100
Serratia plymuthica	1					100	100	100	100	100
Shigella spp.	2					50	100	100	100	100
Shigella sonnei	7			100	100	100	100	100	100	100
Stenotrophomonas maltophilia	23		4	65	78	100	100	100	100	100
Yersinia enterocolitica	10		10	90	90	100	100	100	100	100
Total	655	7.2	21.4	66.3	78.3	91.6	95.0	96.3	99.7	100.0
Fermenters	511	9.2	27.2	80.4	90.4	95.1	96.9	97.3	99.6	100.0
Nonfermenters	144	0.0	0.7	16.0	35.4	79.2	88.2	93.1	100.0	100.0

correctly identified, which is comparable to the result for all 655 strains tested.

No difference in identification results was observed when different culture media were used. Of the 285 strains tested on Columbia sheep blood agar from BD, 97.5% were correctly identified, 2.5% were identified with low discrimination, and no strain was misidentified. The rates for correct identification, identification with low discrimination, and misidentification were 96.4, 1.8, and 1.8%, respectively, for strains tested on Columbia sheep blood agar from bioMérieux; 96.9, 1.8, and 1.2, respectively, for strains tested on MacConkey agar; and 97.3, 2.0, and 0.7, respectively, for strains tested on Trypticase soy agar.

Table 2 lists the strains identified with low discrimination

TABLE 4. Performance of the NGNC with primary isolation plates

Species	No. of strains				
	Total tested	Correctly identified	With low discrimination	Not identified	Misidentified
Acinetobacter baumannii	2	2	0	0	0
Citrobacter braakii	3	3	0	0	0
Citrobacter freundii	5	5	0	0	0
Citrobacter koseri	3	3	0	0	0
Enterobacter cloacae	16	16	0	0	0
Escherichia coli	29	29	0	0	0
Klebsiella oxytoca	11	9	2	0	0
Klebsiella pneumoniae subsp. *pneumoniae*	16	15	1	0	0
Morganella morganii subsp. *morganii*	1	1	0	0	0
Morganella morganii subsp. *sibonii*	1	1	0	0	0
Proteus mirabilis	27	27	0	0	0
Proteus penneri	1	1	0	0	0
Proteus vulgaris group	7	7	0	0	0
Providencia rettgeri	2	2	0	0	0
Pseudomonas aeruginosa	17	16	1	0	0
Pseudomonas fluorescens	3	1	2	0	0
Pseudomonas putida	2	2	0	0	0
Serratia liquefaciens	2	2	0	0	0
Serratia marcescens	6	6	0	0	0
Serratia odorifera	1	1	0	0	0
Shigella spp.	1	1	0	0	0
Stenotrophomonas maltophilia	1	1	0	0	0
Total no. (%)	157	151 (96.2)	6 (3.8)	0 (0.0)	0 (0.0)

and the misidentified strains. Differentiation between *K. pneumoniae* (indole negative) and *K. oxytoca* (indole positive) was readily achieved. The same was true for differentiation between *Pseudomonas aeruginosa* (pyocyanin positive and with growth at 42°C) and *Pseudomonas putida* (no pyocyanin and no growth at 42°C) and between *Pseudomonas fluorescens* (oxidase positive) and *Acinetobacter lwoffii* (oxidase negative). *Acinetobacter lwoffii* can also be discerned from the *Moraxella* group by a negative oxidase reaction. The reasons for the misidentification of four strains were the following: one *Acinetobacter lwoffii* strain lacked tyrosine arylamidase activity, although there is 99% positivity for this activity for the *Acinetobacter lwoffii* strains in the database; one *Enterobacter amnigenus* strain lacked β-glucuronidase activity, for which there is 81 to 99% positivity for the *E. amnigenus* biotype strains in the database, but produced acid from D-sorbitol and sucrose (there is only 1% positivity for D-sorbitol production in *E. amnigenus* biotype 1 strains and 1% positivity for sucrose production in *E. amnigenus* biotype 2 strains); one *Pseudomonas stutzeri* strain expressed urease activity, for which there is only 1% positivity for the *Pseudomonas stutzeri* strains in the database; and one *Rahnella aquatilis* strain expressed *N*-acetyl-β-glucosaminidase and β-D-xylosidase, although there is only 5% positivity for each of these expressions for *Rahnella aquatilis* strains in the database.

Table 3 gives a detailed report on the exact times required for final identification of the strains tested. Nearly 92% of all strains were identified within 7 h. Fermenting bacterial strains were identified faster than nonfermenting ones.

Table 4 lists the identification results when GNR from primary plating media were tested. As in the overall study, a weighted distribution of isolates was tested. The results of testing primary isolation plates were similar to the results of the overall study.

Table 5 outlines the other relevant publications concerning the GNC run on the VITEK 2 instrument. Except for one study, all other evaluations tested both fermenting and nonfermenting bacteria. The reporting of the results in some stud-

TABLE 5. Evaluations of identification cards for GNR on the VITEK 2 instrument

Reference	Yr of publication	Card evaluated	No. of strains	Bacteria tested[a]		% of tests with:				
				Fermenters	Nonfermenters	Correct results	Correct results with additional tests	Indeterminate results	No identification	Misidentification
Present study	2004	NGNC	655	+	+	97.3	2.1		0.0	0.6
2	1998	GNC	845	+	+	84.7	3.8	9.5	1.2	0.8
4	1999	GNC	502	+	+	85.7		11.0	1.2	2.2
6	2001	GNC	281	+	+	95.0			2.8	2.1
5	2001	GNC	198	−	+	66.6		24.2	8.6	0.5
3	2002	GNC	858	+	+	86.1	9.2		2.9	1.7
9	2003	GNC	585	+	+	85.8		7.5	3.6	3.1

[a] +, tested; −, not tested.

ies differed from the reporting in our present evaluation, as an "indeterminate" category was defined in four of the other six studies, whereas only four result categories were defined in our study. The overall identification rates were similar (about 85% correct identifications) for the four major studies of the GNC. In contrast, the results of the present evaluation of the NGNC were significantly better (>97% correct identifications) than the results in the previous studies, and not a single strain remained unidentified.

DISCUSSION

To the best of our knowledge, this is the first study of the performance of the NGNC in a routine clinical laboratory. We were surprised by the overall performance of the system, since more than 97% of the isolates were correctly identified to the species level without the use of any additional tests. This performance is clearly better than the 90% accuracy level which has been demanded by some authorities in the field of evaluations of commercial clinical microbiology devices (9). The taxonomy used in the database was very up-to-date, which is not always the case for commercial GNR identification systems.

The present study is the third largest for VITEK 2 cards for GNR identification and the second largest from a single study center (Table 5). In general, the extended incubation and reading times as well as the larger database (for both reactions and number of taxa) led to significantly improved identification results (97.3% correct identifications to the species level) when the NGNC instead of the GNC was used (Table 5). It is interesting that the extension of the database did not lead to poorer identification results. Not a single strain remained unidentified, whereas in the previous studies of the GNC, 1.2 to 8.6% of the strains were unidentified (Table 5). VITEK 2 users will obtain more precise identification through the use of the NGNC, although the identification results provided by the GNC are regarded as acceptable by numerous authors (2, 3, 4, 6). If the kinetic mode is used, the overall majority of the results obtained with the NGNC are available only about 4 h later (Table 3) than those obtained with the GNC. However, this time frame might be a disadvantage for microbiology laboratories not providing full 24-h service. Overall, the NGNC seems to be a promising new tool for rapid identification of GNR in the routine clinical laboratory. We cannot presently comment on the cost-effectiveness of the NGNC, as the price of the NGNC was not known to us at the time this study was done.

It is recommended that other evaluations include a larger number of strains from primary isolation plates in order to study whether the VITEK 2 NGNC performs as acceptably as it did in our study, which had only a limited number of such strains. We also recommend a pure stress test evaluation that includes nearly all taxa present in the database or GNR with atypical reactions, since our evaluation, while covering the most frequently encountered and clinically relevant GNR, nevertheless covered only a part of the taxa in the database. We encourage the pursuit of other studies to evaluate the performance of the NGNC in different countries and under different laboratory conditions, as has been done with the GNC.

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