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Microbiological Evaluation of the New VITEK 2 *Neisseria-Haemophilus* Identification Card▽

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VITEK 2 is an automated identification system for diverse bacterial and fungal species. A new card (the *Neisseria-Haemophilus* [NH] card) for the identification of *Neisseria* spp., *Haemophilus* spp., and other fastidious gram-negative or gram-variable microorganisms has been developed, but its performance in a routine clinical laboratory has not yet been evaluated. In this study, a total of 188 bacterial strains belonging to the genera *Actinobacillus*, *Campylobacter*, *Capnocytophaga*, *Cardiobacterium*, *Eikenella*, *Gardnerella*, *Haemophilus*, *Kingella*, *Moraxella*, and *Neisseria* were investigated. The NH card was able to identify 171 strains (91%) correctly without the need for extra tests; one strain (0.5%) was misidentified, and five strains (2.7%) could not be classified. Eleven strains (5.8%) were identified with a low level of discrimination, and simple additional tests were required to increase the correct-identification rate to 96.8%. The results were available within 6 h. Based on these results, the new VITEK 2 NH card appears to be a good method for the identification of diverse groups of fastidious organisms, which would otherwise require testing with multiple systems. However, more work is needed to evaluate the performance of VITEK 2 with regard to *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella* bacteria because of the insufficient number of strains tested in this study. Moreover, further reduction of the detection time would be desirable.

Fastidious bacteria are frequently associated with severe infectious diseases. In particular, *Neisseria* and *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella* (HACEK) organisms are responsible for such serious infectious conditions as sepsis, meningitis, and endocarditis (5, 7, 11, 13, 14, 17, 22, 23, 26). These organisms are sometimes difficult to cultivate and identify. The VITEK 2 system (bioMérieux, Marcy L'Etoile, France) is used worldwide for the rapid and accurate identification of gram-positive cocci, gram-negative rods, and yeasts (1, 2, 16, 18, 24) in routine clinical microbiology tests. BioMérieux has recently developed a new *Neisseria-Haemophilus* (NH) identification card in order to identify fastidious gram-negative and gram-variable organisms (HACEK organisms), but its performance under the conditions of a routine laboratory has not yet been evaluated. The new VITEK 2 NH system allows for the identification of 28 taxa belonging to the genera *Actinobacillus*, *Campylobacter*, *Capnocytophaga*, *Cardiobacterium*, *Eikenella*, *Gardnerella*, *Haemophilus*, *Kingella*, *Moraxella*, *Neisseria*, *Oligella*, and *Suttonella.* The NH card is based on colorimetric technology and uses dehydrated media containing chromogenic substrates, which are detected by optical heads. A computer-assisted algorithm based on data collected from known strains of the claimed species is used to interpret the data for a final identification. In this study, 188 isolates belonging to the species included in the database of the VITEK 2 system's software were used to evaluate the ability of the new VITEK 2 NH card to identify fastidious gram-negative and gram-variable bacteria.

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MATERIALS AND METHODS

Bacterial strains. A total of 188 strains belonging to the species listed in the database of the VITEK 2 software were investigated. Twelve strains were reference strains from the American Type Culture Collection (ATCC): *Campylobacter coli* ATCC 43478; *Campylobacter jejuni* ATCC 33560; *Gardnerella vaginalis* ATCC 14018; *Haemophilus paraphrophilus* ATCC 49917; *Haemophilus influenzae* ATCC 10211, ATCC 49247, and ATCC 49766; *Haemophilus parainfluenzae* ATCC 33392; *Neisseria gonorrhoeae* ATCC 49759, ATCC 700825, and ATCC 49226; and *Neisseria meningitidis* ATCC 13090. Eight strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ): *Capnocytophaga ochracea* DSM 7271; *Cardiobacterium hominis* DSM 8339; *Haemophilus actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*) DSM 8324 and DSM 11122; *Neisseria cinerea* DSM 4630; *Neisseria elongata* DSM 17712; *Neisseria lactamica* DSM 4691; and *Neisseria sicca* DSM 17713. Fifty strains were selected from the strain collection of the German National Reference Center for Meningococci, i.e., five strains of *N. gonorrhoeae*, five strains of *N. lactamica* (3), and 40 genetically diverse strains of *N. meningitidis* (8, 9). Twenty-six strains of *N. meningitidis* were serotyped as serogroup B, six as serogroup C, four as serogroup Y, one as serogroup 29E, one as serogroup A, one as serogroup W135, and one as serogroup X. The remaining 118 strains were clinical isolates from unrelated patients belonging to the clinical strain collection of the Institute of Hygiene and Microbiology of the University of Würzburg. The sources of the isolates included blood, cerebrospinal fluid, urogenital, and respiratory samples. For the study, the strains were transferred from storage at −70°C, placed on chocolate agar supplemented with PolyVitex (bioMérieux, Nürtingen, Germany), and subcultured a second time in a CO_2 incubator for 24 h. The clinical isolates were previously identified to the species level by colony morphology, Gram stain, catalase and oxidase testing, and the API NH system (bioMérieux) in the case of *Haemophilus*, *Neisseria*, and *Moraxella* species. *G. vaginalis* was identified by growth and beta-hemolysis on human blood agar and by API Coryne or API Strep strips (both from bioMérieux). *C. jejuni* was identified by colony morphology, Gram stain (curved rods), and hydrolysis of hippurate-indoxyl-acetate in addition to susceptibility to nalidixic acid and cephalothin. *E. corrodens* was identified by growth pattern and by the API E system. *C. coli* and the HACEK organisms were identified by 16S rRNA gene sequencing. The standard identification results were compared with those obtained with the new VITEK 2 NH cards. In the case of low discrimination or misidentification by the VITEK 2 NH card, the 16S rRNA gene sequencing method was used for genetic identification.

VITEK 2 NH card. A bacterial suspension made in 0.45% aqueous NaCl was adjusted to a McFarland standard of 3 with a VITEK 2 DensiCheck instrument

TABLE 1. Discrepancies in species identification by the VITEK 2 NH system and reference methods

Species (reference identification)	No. of strains tested	No. (%) of strains with indicated result by reference methods			No. (%) of strains with indicated type of identification by the VITEK 2 NH system		
		No identification	Misidentification (species)	Genus identification	Correct	With low discrimination (possible species)	Correct after additional tests
Campylobacter coli	9	0	0	9 (100)	9 (100)	0	
Campylobacter jejuni	27	0	0	27 (100)	24 (88.8)	3 (*C. coli-C. jejuni*)	27 (100)
Gardnerella vaginalis	18	0	0	18 (100)	18 (100)	0	
Haemophilus influenzae	25	2	0	23 (92)	21 (84)	2 (*H. influenzae-H. haemolyticus*)	23 (92)
Haemophilus parainfluenzae	27	1	0	26 (96)	25 (92.5)	1 (*H. parainfluenzae-H. segnis*)	26 (96.2)
Moraxella catarrhalis	5	0	0	5 (100)	5 (100)	0	
Neisseria gonorrhoeae	12	0	1 (*N. cinerea*)	12 (100)	11 (91.6)	0	
Neisseria meningitidis	41	0	0	41 (100)	39 (95.1)	1 (*N. meningitidis-N. sicca*); 1 (*N. meningitidis-K. denitrificans*)	41 (100)
Neisseria cinerea	1	0	0	1	1		
Neisseria elongata subsp. *elongata*	1	0	0	1	0	1 (*N. elongata-E. corrodens*)	1
Neisseria lactamica	6	0	0	6 (100)	6 (100)		
Neisseria sicca	1	0	0	1	1		
HACEK group							
Capnocytophaga spp.	1	0	0	1	1		
Cardiobacterium hominis	2	1	0	1	1		
Haemophilus actinomycetemcomitans[a]	2		0	0	2	2	
Haemophilus aphrophilus or *H. paraphrophilus*	2	1	0	1	0	1 (*H. aphrophilu-H. segnis*)	1
Kingella denitrificans	1	0	0	1	0	1 (*K. denitrificans-N. cinerea*)	1
Eikenella corrodens	7	0	0	7 (100)	7 (100)	0	
Total	188	5 (2.7)	1 (0.5)	184 (97.8)	171 (91)	11 (5.8)	182 (96.8)

[a] Formerly *Actinobacillus actinomycetemcomitans*.

(bioMérieux). The time period from preparation of the inoculum to loading of the card was less than 30 min. The card, placed on the tray and applied to the VITEK 2 system, was automatically processed in a vacuum chamber and incubated at 35.5°C. The 64-well NH card contains 30 biochemical tests in the following categories: 11 glycosidase and peptidase tests, 10 acidification tests, 5 alkalinization tests, and 4 miscellaneous tests. An intermittent reading every 15 min allowed for identification after 6 h. The results were generated by a computer-assisted algorithm of the VITEK 2 system.

Interpretation of results. The interpretation was performed on the basis of the provided software. The four categories of results 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 a low level of discrimination between two or more species, including the correct species), (iii) no identification (strains without results), and (iv) misidentification (the species identified with the NH card was different from that identified using the reference method).

RESULTS

Identification results were obtained after a 6-hour incubation of the NH cards inside the VITEK 2 system. Table 1 lists all strains investigated. The new VITEK 2 NH card correctly identified 184 of 188 (97.8%) strains to the genus level and 171 of 188 (91%) strains to the species level without the need for extra tests (Table 1). Five strains (2.7%) could not be identified. A single *N. gonorrhoeae* isolate (0.5%) was misidentified as *N. cinerea*. Eleven strains (5.8%) were identified with low discrimination, but the correct species identification was among the listed choices (Table 1). In detail, the overall correct identification rates were 100% (nine of nine strains) for *C. coli* and 88.8% (24 of 27 strains) for *C. jejuni*. The remaining three strains of *C. jejuni* were identified as *C. coli-C. jejuni*, because the VITEK 2 NH system was not able to distinguish between the two species. *G. vaginalis* (18 strains) was identified at a rate of 100%. Furthermore, 21 of the 25 *H. influenzae* strains (84%) and 25 of the 27 *H. parainfluenzae* strains (92.5%) were correctly identified to the species level. Two strains of *H. influenzae* and one strain of *H. parainfluenzae* could not be identified, and another two strains of *H. influenzae* and one strain of *H. parainfluenzae* were identified as *H. influenzae-H. haemolyticus* and as *H. parainfluenzae-H. haemolyticus-H. segnis*, respectively. In addition, all strains of *M. catarrhalis* (five strains) were correctly identified; correct-identification rates were 91.6% (11 of 12 strains) for *N. gonorrhoeae*, 95.1% (39 of 41 strains) for *N. meningitidis*, and 73.3% (11 of 15 strains) for the HACEK organisms. As mentioned above, one strain of *N. gonorrhoeae* was misidentified as *N. cinerea*, and two strains of meningococci were identified as *N. meningitidis-N. sicca* and as *N. meningitidis-K. denitrificans*, respectively. Among the HACEK organisms, 11 isolates were correctly identified (all *Eikenella corrodens* strains, one *Capnocytophaga* strain, two *H. actinomycetemcomitans* strains, and one *C. hominis* strain), two strains were identified with low discrimination, and two were not detected (Table 1).

The identification results were improved when simple additional tests were applied to clarify the results for strains identified with low discrimination (Tables 1 and 2). The application

TABLE 2. Additional tests for correct identification of strains identified with low discrimination by VITEK 2 NH card

Reference identification	VITEK 2 NH results	Additional test(s) for correct identification to species level
C. jejuni subsp. *jejuni*	*C. coli-C. jejuni*	Hippurate hydrolysis: *C. jejuni* subsp. *jejuni* (positive), *C. coli* (negative)
H. influenzae	*H. influenzae-H. haemolyticus*	Hemolysis on horse blood agar: *H. haemolyticus* (positive), *H. influenzae* (negative)
H. parainfluenzae	*H. parainfluenzae-H. segnis-H. haemolyticus*	Oxidase testing: *H. parainfluenzae* (positive), *H. haemolyticus* (positive), *H. segnis* (negative) Hemolysis on horse blood agar: *H. haemolyticus* (positive), *H. parainfluenzae* (negative), *H. segnis* (negative) X-factor requirement: *H. haemolyticus* (positive), the other species (negative)
N. meningitidis	*N. meningitidis-N. sicca*	Growth on Martin-Lewis agar: *N. meningitidis* (positive), *N. sicca* (negative)
	N. meningitidis-K. denitrificans	Catalase testing: *N. meningitidis* (positive), *K. denitrificans* (negative) Gram stain: *N. meningitidis* (cocci), *K. denitrificans* (rods)
N. elongata subsp. *elongata*	*N. elongata-E. corrodens*	Colony morphology: typical for *E. corrodens* colonies after 48 h of incubation (clear center often surrounded by spreading growth, may pit the agar)
K. denitrificans	*K. denitrificans-N. cinerea*	Gram stain: *N. cinerea* (cocci), *Kingella* (rods); catalase testing: *N. cinerea* (positive), *K. denitrificans* (negative)
H. aphrophilus or *H. paraphrophilus*	*H. aphrophilus-H. paraphrophilus-H. segnis*	V-factor requirement: positive for *H. paraphrophilus*, *H. segnis*; negative for *H. aphrophilus* X-factor requirement: weakly positive for *H. aphrophilus* Oxidase testing: positive for *H. paraphrophilus*, negative for *H. segnis*

of additional tests increased the number of correctly identified strains to 182 (96.8%) (Table 1). Table 2 lists the strains identified with low discrimination and the simple additional tests used for their correct identification. The discrimination between *C. jejuni* (hippurate positive) and *C. coli* (hippurate negative) was readily achieved. Concerning the differentiation between *H. influenzae* and *H. haemolyticus*, lack of beta-hemolysis on horse agar excluded the identification of *H. haemolyticus* and confirmed the identification of *H. influenzae*. The discrimination between *H. parainfluenzae* (oxidase positive) and *H. segnis* (oxidase negative) was also easily obtained. Simple Gram stain and catalase tests led to the differentiation between *N. meningitidis* (cocci, catalase positive) and *K. denitrificans* (rods, catalase negative). All other differentiation tests are shown in Table 2. The performance of further tests increased the identification rates of *C. jejuni* from 88.8% to 100%, of *H. influenzae* from 84% to 92%, of *H. parainfluenzae* from 92.5% to 96.2%, of *N. meningitidis* from 95.1% to 100%, and of the HACEK organisms from 73.2% to 86.6%. Major differences in the identification results were observed when different culture media were used. For unknown reasons, testing on chocolate agar supplemented with PolyVitex from bioMérieux was associated with a high number of correct-identification rates. In contrast, testing on chocolate agar plates from Becton Dickinson led to significantly impaired identification results (data not shown).

DISCUSSION

In this study, the new VITEK 2 NH card was evaluated for its ability to identify fastidious gram-negative bacteria and gram-variable microorganisms. Our data show that the NH card identifies diverse groups of fastidious bacteria that would otherwise require testing with multiple products. Of the 188 strains tested, 91% were correctly identified to the species level without additional tests. Five of 70 reference strains and 6 of 118 clinical isolates were identified with low discrimination (Table 2). Therefore, no significant differences in the performance of the VITEK 2 NH card could be shown between the reference strains and clinical isolates (Fisher's exact test, $P > 0.5$). The use of additional test procedures led to a better identification rate for the bacteria identified with low discrimination and increased the rate of correctly identified strains to 96.8%. Only five strains (2.7%) could not be identified, and a single strain (0.5%) was misidentified. These results confirm the findings recently presented at the General Meeting of the

American Society for Microbiology by others: a correct-identification rate between 96% and 98% (8% to 15% with low discrimination), a misidentification rate between 1% and 2.5%, and a rate of unidentified organisms between 0% and 1.2% (6, 10, 19, 20).

In this study, no problems were observed concerning the identification of *C. coli* (100% identification rate). However, the number of strains tested (nine) was too small for a definitive conclusion, particularly when taking into account that the identification rate of other studies has been lower (between 60% and 89%) (6, 10, 20). On the other hand, the reason for a correct-identification rate of only 88.8% for *C. jejuni* is that the NH card was not able to distinguish *C. jejuni* from *C. coli* in 3 of 27 cases. These results are in accordance with those achieved by others (6, 10, 20). In the study of Funke and coworkers (10), 5 of 67 *C. jejuni* strains were identified as *C. coli-C. jejuni*. This may be explained by the fact that except for hippuricase activity, which is lacking in *C. coli*, *C. coli* and *C. jejuni* are biochemically similar. Since the NH card does not contain hippuricase, differentiation between *C. coli* and *C. jejuni* may be more difficult. A number of commercial systems have been developed as an additional help to identify *Campylobacter* species; all systems tested had identification rates of 74% to 100% (12, 21). However, discrimination between the two species can be readily achieved through hippurate hydrolysis, so there is no real advantage to using the NH card for the identification of *C. jejuni.*

Satisfactory results were achieved in the identification of the *Neisseria* species. Of the 41 genetically diverse *N. meningitidis* strains from patients and healthy carriers that were tested, 95% were correctly identified. Two strains were detected with low discrimination, one of them as *N. meningitidis-K. denitrificans.* This isolate was maltose negative and, therefore, difficult to identify. The other isolate was interpreted as *N. meningitidis-N. sicca*. Since the correct species was among the listed choices, the final identification was possible by simple additional tests, such as the catalase test (positive for *N. meningitidis*), the Gram stain (cocci versus rods), or growth on a Martin-Lewis medium. Except for one glucose-negative strain, which was misidentified as *N. cinerea*, the VITEK 2 NH card correctly classified all gonococci tested. Mills et al. (19) had also observed that 3 of 45 gonococci were misidentified as *N. cinerea*. This may be explained by the fact that, as in our case, atypical glucose-negative strains might be difficult to identify because *N. gonorrhoeae* (glucose positive) and *N. cinerea* (glucose negative) are biochemically similar and differ only in acid production from glucose. For such strains, colistin susceptibility and nitrite reduction assays are suggested. With the exception of a single strain (*N. elongata*), the NH card correctly identified all other *Neisseria* species, including the five strains of *M. catarrhalis*.

Concerning the 15 HACEK organisms, 11 strains were correctly identified, and two strains which were identified with low discrimination could be correctly identified by further testing, resulting in an identification rate of 86.6%. Similar results were obtained by Mills et al. (20), who demonstrated an identification rate of 86.9% for the 434 investigated HACEK organisms using the VITEK 2 NH or reference methods. Overall, the number of HACEK strains tested in this study (i.e., 15) is too low for a definitive conclusion. Moreover, reliable results were achieved for *G. vaginalis* and most strains of *Haemophilus* species. Despite two strains of *H. influenzae* and one strain of *H. parainfluenzae* being identified with low discrimination as *H. influenzae-H. haemolyticus* and *H. parainfluenzae-H. segnis*, respectively, final discrimination of these species could be accomplished with a few simple test procedures, such as oxidase testing or observation of hemolysis on a horse blood medium. The difficulty with the correct identification of *H. influenzae* has also been observed by others (10, 20). *H. influenzae* and *H. haemolyticus* show identical biochemical test reactions (oxidase, catalase, glucose, sucrose, lactose, mannose, and xylose), so that biochemical differentiation might be difficult or even impossible without extra tests.

Compared with published reports on other systems already available for the identification of *Neisseria*, *Moraxella*, *Gardnerella*, and *Haemophilus* species, the VITEK 2 NH system performed well in our hands. Janda et al. (14) found that the HN ID kit (MicroScan Division, Baxter Diagnostics, Inc., CA) provides reliable results for *N. gonorrhoeae* and *M. catarrhalis* but is less efficient for *N. meningitidis*. Among the automated systems mentioned by Stager and Davis (25), very few were suitable for the identification of these three species. Barb é and coworkers (5) evaluated the API NH system and achieved an identification rate of 90.3% by performing additional tests for *H. influenzae* and *H. parainfluenzae*. Furthermore, the identification rate for gonococci achieved by Alexander and Ison (4) with various commercial kits ranged between 64% and 100%. Lien and Hillier (15), investigating the enhanced rapid identification method (Austin Biological Laboratories, TX) for the detection of *G. vaginalis*, had an identification rate of 91.4% compared with standard biochemical methods. An interesting study was performed by Janda et al. (13), who investigated the identification rate for fastidious microorganisms by a former VITEK NHI card (Vitek Systems, Inc., Hazelwood, MO). The authors found satisfactory results for the identification of *N. gonorrhoeae*, *N. meningitidis*, and rarely isolated fastidious microorganisms. The identification rates for *H. influenzae* and *H. parainfluenzae* were 94% and 95.6%, respectively. With regard to the identification of *M. catarrhalis*, the system was less satisfactory, as no strain could be identified correctly.

The mean time until the identification results for the fastidious organisms were provided was about 6 h. On the one hand, this clearly represents a disadvantage of the VITEK NH card versus the API NH system, which requires only 2 h for the identification of *Haemophilus*, *Moraxella*, and *Neisseria* species. On the other hand, this represents an advantage regarding the identification times required for *G. vaginalis* by API Coryne strips and for HACEK organisms by API Strep strips, both of which take 16 to 24 h. Nevertheless, a significant reduction in the detection time for *Haemophilus* and *Neisseria* species would be desirable.

In conclusion, the data demonstrate that the new VITEK 2 NH card was able to identify *Haemophilus* and *Neisseria* species and diverse groups of fastidious gram-negative organisms that otherwise would require testing with multiple products in the routine clinical laboratory. Convincing identification results were obtained for most of the strains tested, and even in cases of low discrimination, final identification of the species could be accomplished with a few simple test procedures. Therefore, the VITEK 2 NH system seems to be an improve-

ment over those conventional methods that are often time-consuming. The versatility of the NH card was evident in its ability to identify most of the HACEK organisms tested. In particular, this is beneficial since these bacteria often present difficulties in recognition and identification in a clinical laboratory. Nevertheless, the identification rate for these organisms is not yet perfect, and more work is needed to finally evaluate the performance of VITEK 2 with regard to HACEK bacteria because of the insufficient number of strains tested in this study. Finally, it is recommended that other studies investigate a larger number of strains to evaluate whether the VITEK 2 NH system provides acceptable results as in the present study.

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