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Evaluation of the Colorimetric VITEK 2 Card for Identification of Gram-Negative Nonfermentative Rods: Comparison to 16S rRNA Gene Sequencing▽

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Ninety strains of a collection of well-identified clinical isolates of gram-negative nonfermentative rods collected over a period of 5 years were evaluated using the new colorimetric VITEK 2 card. The VITEK 2 colorimetric system identified 53 (59%) of the isolates to the species level and 9 (10%) to the genus level; 28 (31%) isolates were misidentified. An algorithm combining the colorimetric VITEK 2 card and 16S rRNA gene sequencing for adequate identification of gram-negative nonfermentative rods was developed. According to this algorithm, any identification by the colorimetric VITEK 2 card other than *Achromobacter xylosoxidans*, *Acinetobacter* sp., *Burkholderia cepacia* complex, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* should be subjected to 16S rRNA gene sequencing when accurate identification of nonfermentative rods is of concern.

Accurate identification of gram-negative nonfermentative rods in the clinical laboratory mainly relies on biochemical characteristics. Some gram-negative nonfermentative rods such as *Pseudomonas aeruginosa* are readily identified by distinct phenotypic traits, i.e., production of diffusible pigments, positive oxidase reaction, growth at 42°C, metallic sheen, and typical odor. However, many nonfermentative rods cannot be identified by morphological characteristics alone. Currently, there are commercially available systems which determine biochemical characteristics in a miniaturized format for bacterial identification (6). Typical characteristics such as colony morphology, key biochemical reactions, and drug susceptibility pattern assist in establishing the correct identification of the microorganism. 16S rRNA gene sequence analysis has become the new gold standard for identification of bacteria in clinical microbiology (3).

Previous studies have compared 16S rRNA gene sequencing with two commercially available biochemical systems (API 20 NE and the VITEK 2 fluorescent card; both from bioMérieux, Marcy l'Etoile, France) for identification of clinically relevant isolates of nonfermentative gram-negative rods (except typical *Pseudomonas aeruginosa*). Compared to 16S rRNA gene sequencing, API 20 NE and the fluorescent VITEK 2 card identified only 43% of the isolates correctly to the species level (1); this is a low rate, considering that the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex (2) and the *Burkholderia cepacia* complex were accepted as representing one species each. An algorithm for proper identification of nonfermenting gram-negative rods in the diagnostic laboratory was proposed. According to this algorithm, excellent or very good species identification by API 20 NE or the VITEK 2 fluorescent card assays are to be reported; isolates with only good or acceptable identification to species level are to be subjected to 16S rRNA gene sequencing when accurate species assignment is of concern (1).

The new VITEK 2 colorimetric card (bioMérieux) has recently been introduced on the European market. This card contains 47 biochemical tests instead of the 41 tests provided

TABLE 1. Comparison of identification systems

Assay	% Assignment at taxonomic level			% Correct identification at taxonomic level		% Incorrect assignment at taxonomic level		
	Species	Genus	No identification	Species	Genus	Species	Genus	No identification
16S rRNA gene sequencing	90 ($n = 81$)	10 ($n = 9$)						
API 20 NE	56 ($n = 50$)	5 ($n = 5$)	39 ($n = 35$[a])	45.5 ($n = 41$)	10 ($n = 9$)	6.5 ($n = 6$)		38 ($n = 34$)
VITEK 2 fluorescent card	56 ($n = 50$)		44 ($n = 40$[b])	48 ($n = 43$)	2 ($n = 2$)	5.5 ($n = 5$)		44.5 ($n = 40$)
VITEK 2 colorimetric card	87 ($n = 78$)	3 ($n = 3$)	10 ($n = 9$[c])	59 ($n = 53$)	10 ($n = 9$)	20 ($n = 18$)	1 ($n = 1$)	10 ($n = 9$)

[a] Of the 35 isolates not identified, 11 belong to a species not included in the API 20 NE database.
[b] Of the 40 isolates not identified, 31 belong to a species not included in the VITEK 2 identification database.
[c] Of the 9 isolates not identified, 3 belong to a species not included in the VITEK 2 identification database.

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TABLE 2. Isolates tested by the VITEK 2 colorimetric card assay

Well-characterized strain (no. of isolates)	VITEK 2 gram-negative (colorimetric) card result and/or quality of identification (no. of isolates)
Achromobacter xylosoxidans (9)	*A. xylosoxidans* subsp. *xylosoxidans*, excellent (3); *A. xylosoxidans* subsp. *denitrificans*, excellent (1); *A. xylosoxidans* subsp. *denitrificans* or *A. xylosoxidans* subsp. *xylosoxidans*, low selectivity (2); *Aeromonas salmonicida* or *Oligella ureolytica*, low selectivity (1); *Burkholderia cepacia* group, good (1); no identification (1)
Achromobacter sp. (1)	*A. xylosoxidans* subsp. *denitrificans*, excellent (1)
Acidovorax[a] *temperans* (3)	*Comamonas testosteroni*, excellent (2); *Delftia acidovorans*; excellent (1)
Acinetobacter calcoaceticus[a] subsp. *anitratus* (1)	*A. baumannii*, excellent (1)
Acinetobacter baumannii (1)	*A. baumannii*, excellent (1)
Acinetobacter baumannii or *A. calcoaceticus*[a] subsp. *anitratus* (3)	*A. baumannii*, excellent (3)
Acinetobacter calcoaceticus[a] (6)	*A. baumannii*, excellent (5); *Alcaligenes faecalis* subsp. *faecalis* or *Pseudomonas pseudoalcaligenes*, low selectivity (1)
Acinetobacter calcoaceticus[a] or *A. haemolyticus* (4)	*A. baumannii*, excellent (4)
Acinetobacter junii (1)	*Bordetella bronchiseptica*, excellent (1)
Acinetobacter lwoffii (1)	*A. lwoffii*, excellent (1)
Acinetobacter sp. (1)	*Bordetella bronchiseptica*, excellent (1)
Acinetobacter ursingii[a] (1)	*Bordetella bronchiseptica*, excellent (1)
Agrobacterium larrymoorei[a] (1)	*Rhizobium radiobacter*, excellent (1)
Alcaligenes faecalis (1)	*Acinetobacter baumannii*, excellent (1)
Alcaligenes sp. (1)	*Ralstonia pickettii*, very good (1)
Bordetella petrii[a] (1)	*Moraxella* group, very good (1)
Bordetella sp. (1)	No identification (1)
Burkholderia cepacia group (13)	*Burkholderia cepacia* group (12), excellent (7), very good (4), good (1); *Pseudomonas aeruginosa*, acceptable (1)
Chryseobacterium sp. (1)	*Myroides* sp.[b], very good (1)
Herbaspirillum[a] huttiense (1)	*Achromobacter xylosoxidans* subsp. *denitrificans*, very good (1)
Oligella urethralis[a] (2)	*Francisella tularensis*, excellent (1); *Oligella ureolytica* or *Francisella tularensis*, low selectivity (1)
Pseudomonas aeruginosa (6)	*P. aeruginosa* (5), excellent (3), very good (1), good (1); *P. fluorescens*, excellent (1)
Pseudomonas fluorescens/jessenii[a] (1)	No identification (1)
Pseudomonas sp. (1)	*P. putida*, excellent (1)
Pseudomonas mendocina (1)	*P. mendocina*, excellent (1)
Pseudomonas monteilii[a] (1)	*P. putida*, excellent (1)
Pseudomonas putida/parafulva[a] (1)	*P. putida*, excellent (1)
Pseudomonas pseudoalcaligenes or *P. oleovorans*[a] (1)	*P. pseudoalcaligenes* or *Alcaligenes faecalis* subsp. *faecalis* or *Comamonas testosteroni*, low selectivity (1)
Pseudomonas sp. (2)	*P. stutzeri*, excellent (1); *P. fluorescens* or *P. aeruginosa*, low selectivity (1)

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TABLE 2—*Continued*

Well-characterized strain (no. of isolates)	VITEK 2 gram-negative (colorimetric) card result and/or quality of identification (no. of isolates)
Pseudomonas stutzeri (1)	*P. stutzeri*, very good (1)
Pseudoxanthomonas[a] *mexicana* (2)	*Chryseobacterium meningosepticum*, excellent (1); *Sphingomonas paucimobilis* or *Brevundimonas diminuta/vesicularis*, low selectivity (1)
Ralstonia pickettii (2)	*R. pickettii*, excellent (1); *R. paucula* or *R. pickettii*, low selectivity (1)
Ralstonia sp. (1)	*R. mannitolilytica*, good (1)
Stenotrophomonas maltophilia (14)	*S. maltophilia* (11), excellent (10), very good (1); *Sphingomonas paucimobilis*, good (1); *Aeromonas salmonicida*, excellent (1); no identification (1)
Wautersia[a] *gilardii* (1)	*Comamonas testosteroni*, excellent (1)
Wautersia[a] sp. (1)	*Comamonas testosteroni*, excellent (1)

[a] Species and/or genus not included in the VITEK 2 database.
[b] *Myroides* sp. was accepted as a correct genus identification; some *Flavobacterium* spp. had been transferred earlier to the genus *Chryseobacterium* and to the genus *Myroides* (7).

with the fluorescent VITEK 2 card; the database of the colorimetric card was extended to include 159 taxa in comparison to the 101 taxa included in the database for the fluorescent VITEK 2 card (1, 4, 5). The first published evaluation of a large collection of 655 gram-negative rods, including 144 nonfermentative rods, gave encouraging results: 92.4% of nonfermentative bacteria were correctly identified to the species level (4). However, in this study, the nonfermentative rods included were representatives of only 12 taxa almost exclusively belonging to taxa which are frequently analyzed in the microbiological laboratory. A similar collection had already been investigated earlier with the fluorescent VITEK 2 card; 73.3% of the nonenteric bacilli were identified to the species level (5). The aim of the present study was to evaluate the colorimetric VITEK 2 card for identification of clinical isolates of gram-negative nonfermentative rods other than typical *P. aeruginosa*.

A total of 90 of 107 strains from the previous study (1) were included in the present investigation (13 of the 107 strains could not be subcultured; 4 of the 107 strains were excluded because their final identification by discrepancy analysis of biochemical and molecular results remained unresolved). For the 90 strains included in the present study, the outcome of the previous discrepancy analysis ($n = 12$; see reference 1) was accepted as representing the final identification. The frozen strains were subcultured twice on sheep blood agar and identified by the colorimetric VITEK 2 card (ID-GN; BioMérieux) according to the instructions of the manufacturer (McFarland standard of 0.5 to 0.62). Strain identification at the species level was divided into four groups based upon the probability of accurate identification as follows: excellent (probability of accurate identification, ≥96%), very good (93 to 95%), good (89 to 92%), and acceptable (85 to 88%). We considered identification with low selectivity between two subspecies of the same species, e.g., *Achromobacter xylosoxidans* subsp. *xylosoxidans* and *denitrificans*, to represent identification to the species level. Identification with low selectivity between two or more species of the same genus was regarded as identification to the genus level; low selectivity between species belonging to different genera was classified as "not identified." For comparison with the colorimetric VITEK 2 card results, the interpretation of the results obtained with fluorescent VITEK 2 and API 20 NE assays was determined as follows: (i) fluorescent VITEK 2 results were similar to colorimetric VITEK 2 results, with all identifications with low discrimination categorized as "not identified" because the two proposed species were never in the same genus; and (ii) API 20 NE (version 6.0) results were as described earlier (1), with all identifications with low discrimination classified as "not identified" because two or

TABLE 3. Comparison of commercially available phenotypic identification systems

Assay and result	No. (%) of isolates correctly identified on the indicated level		No. (%) of isolates with erroneous identification or not identified
	Species	Genus	
API 20 NE			
Excellent ($n = 19$)	17 (90)	1 (5)	1 (5)
Very good ($n = 16$)	13 (81)	1 (6)	2 (13)
Good ($n = 15$)	9 (60)	5 (33)	1 (7)
Acceptable ($n = 6$)	2 (33)	1 (17)	3 (50)
Low discrimination ($n = 25$)		1 (4)	24 (96)
Unacceptable profile ($n = 4$)			4 (100)
Doubtful profile ($n = 3$)			3 (100)
No identification ($n = 2$)			2 (100)
VITEK 2 fluorescent card			
Excellent ($n = 33$)	31 (94)	1 (3)	1 (3)
Very good ($n = 10$)	6 (60)	1 (10)	3 (30)
Good ($n = 4$)	3 (75)		1 (25)
Acceptable ($n = 3$)	3 (100)		
Low discrimination ($n = 13$)			13 (100)
Not identified ($n = 27$)			27 (100)
VITEK 2 colorimetric card			
Excellent ($n = 60$)	42 (70)	5 (8)	13 (22)
Very good ($n = 11$)	7 (64)	1 (9)	3 (27)
Good ($n = 5$)	2 (40)	1 (20)	2 (40)
Acceptable ($n = 1$)			1 (100)
Low selectivity ($n = 9$)	2[a] (22)	2 (22)	5 (56)
Not identified ($n = 4$)			4 (100)

[a] Low selectivity on the subspecies level.

TABLE 4. VITEK 2 identification results

VITEK 2 colorimetric card identification[a] (no. of isolates [n = 54 of a total of 90 isolates])	Reference identification[d] (no. of isolates)
A. xylosoxidans (8)	*A. xylosoxidans* (7); *Herbaspirillum huttiense* (1)
Acinetobacter sp. (16)	*Acinetobacter* sp. (15); *Alcaligenes faecalis* (1)
Burkholderia cepacia group (13)	*Burkholderia cepacia* group (11 plus 1 additional isolate[b]); *Achromobacter xylosoxidans* (1)[b]
P. aeruginosa (6)	*P. aeruginosa* (4 plus 1 additional isolate[b]); *Burkholderia cepacia* group (1)[c]
S. maltophilia (11)	*S. maltophilia* (11)

[a] Excellent or very good identification as defined by the manufacturer.
[b] Good identification by VITEK 2 colorimetric card assay.
[c] Acceptable identification by VITEK 2 colorimetric card assay.
[d] Identification by 16S rRNA gene sequencing and/or discrepancy analysis (1).

more species belonging to different genera were proposed, and unacceptable and doubtful profiles were categorized as "not identified." For all three biochemical identification systems, *A. calcoaceticus* and *A. baumannii* were accepted as members of the *A. calcoaceticus-A. baumannii* complex (2); *B. cepacia* complex was accepted as one species.

For 50 (56%) of the 90 isolates, API 20 NE and VITEK 2 fluorescent card assays yielded assignments to the species level compared to 78 (87%) of the 90 isolates assigned by the colorimetric VITEK 2 card assay (Table 1). Detailed results obtained with the colorimetric VITEK 2 card are given in Table 2. The study collection of 90 isolates included a number of strains (n = 14) representative of taxa not included in the database of the VITEK 2 colorimetric card. In addition, six of seven *A. calcoaceticus* strains were categorized as *A. baumannii* by the colorimetric VITEK 2 card assay; these results were regarded as identification to the species level because only *A. baumannii* of the *A. calcoaceticus-A. baumannii* complex is included in the VITEK 2 database. The outcome of the assays using the different identification systems is summarized in Table 1. This analysis revealed that correct identification to the species level was obtained for 45.5%, 48%, and 59% of the strains by the API 20 NE, VITEK 2 fluorescent card, and VITEK 2 colorimetric card assays, respectively. Although 78 (87%) of all strains were assigned to the species level by the VITEK 2 colorimetric card assay, only 53 (59%) of these were correct identifications (Table 1). However, the rates of correct identification were lower for the two other systems, i.e., 48% (n = 43) for the VITEK 2 fluorescent card and 45.5% (n = 41) for the API 20 NE assay.

In the diagnostic workflow it is important to know whether a correlation exists between the quality of biochemical identification, e.g., excellent or very good identification (as defined by the manufacturer), and the accuracy of the outcome, i.e., the correctness of species assignment. Such correlation was the case for API 20 NE and the fluorescent VITEK 2 card assays (1) (see Table 3). Compared to API 20 NE and fluorescent VITEK 2, the colorimetric VITEK 2 card identified more isolates to the species level. The most important aspect of this study is that even excellent identification by the VITEK 2 colorimetric card assay allows no prediction of the correctness of the results (Table 3). Nonetheless, there is a correlation between correct identification as assigned by the colorimetric VITEK 2 card and the nature of the species. We found that almost all identifications of *A. xylosoxidans*, *B. cepacia* group, *P. aeruginosa*, and *S. maltophilia* were correct (Table 4), as was the identification of *Acinetobacter* sp. to the genus level. The numbers of representatives of other taxa classified as representing excellent or very good identification were too small to allow for substantial analysis.

According to our data, the colorimetric VITEK 2 card assay for identification of gram-negative nonfermentative rods can be implemented in a diagnostic algorithm as follows: (i) as with API 20 NE and VITEK 2 fluorescent card assays, identifications not categorized as excellent or very good according to the instructions of the manufacturer are doubtful; and (ii) any result showing excellent or very good identification of species other than *A. xylosoxidans*, *Acinetobacter* sp., *B. cepacia* group, *P. aeruginosa*, and *S. maltophilia* should be subjected to 16S rRNA gene sequencing when accurate identification is of concern.

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