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Note

Evaluation of an Automated System for Identification of *Enterobacteriaceae* and Nonfermenting Bacilli

M.-F. Jossart, R.J. Courcol

Abstract The performance of the Vitek 2 (bioMérieux, France), a new fully automated system allowing rapid identification of microorganisms and susceptibility testing, and the Vitek 2 ID-GNB card (bioMérieux) was evaluated using 502 clinical isolates and stock collection strains of gram-negative rods belonging to 70 taxa. The number of isolates correctly identified to species and genus levels was 430 (85.7%) and 485 (96.6%), respectively. Clinical isolates of both *Enterobacteriaceae* and non-*Enterobacteriaceae* were better identified at the species level (95.3% and 74%, respectively) than stock collection strains (86.4% and 52.2%, respectively). The Vitek 2 ID-GNB card provides after 3 h a highly acceptable level of accuracy for identification of *Enterobacteriaceae* and non-*Enterobacteriaceae,* including most atypical strains encountered in clinical situations.

Introduction

For the last 20 years, a variety of automated systems for the identification of microorganisms have been developed for clinical microbiology laboratories. The goals for these systems were to be highly automated, cost-effective, accurate, reliable, flexible, and hands-off, with rapid turnaround time [1]. Some of the automated systems are no longer manufactured but are still in service. Others are still marketed and developed by industrial companies. This is the case of the Vitek system (bioMérieux, France), which originated in the 1960s and for which improvements have continued to increase the capability of the system to accurately identify microorganisms and provide susceptibility results.

M.-F. Jossart, R.J. Courcol (✉)
Centre Hospitalier Régional Universitaire,
Laboratoire de Bactériologie-Hygiène,
Boulevard Prof. J. Leclercq, F-59037 Lille, France
e-mail: rcourcol@chru-lille.fr

Currently, an evolution of the Vitek system, the Vitek 2 system, is being introduced. It is a fully automated system allowing rapid identification of microorganisms. Determining the performance of a new system represents the first step in its evaluation. Here, we report the results of a study carried out on 502 clinical isolates and stock collection strains of gram-negative bacilli (*Enterobacteriaceae* and non-*Enterobacteriaceae*, fermenters and nonfermenters) belonging to 70 taxa.

Materials and Methods

Five hundred two strains of gram-negative bacilli, consisting of 356 members of the family *Enterobacteriaceae* and 146 non-*Enterobacteriaceae* (fermenters and nonfermenters) belonging to 44 and 26 taxa, respectively, were tested by the Vitek 2, a new fully automated identification and susceptibility testing system developed by bioMérieux (France) [2]. Clinical isolates ($n=375$; 38 species) were collected over a 3-month period from nonconsecutive patient cultures and selected either to obtain approximately 20 strains of the most frequently recovered species or to be in agreement with the distribution of isolates recovered annually in the laboratory. In order to have an idea of the system's performance in identifying the most rarely isolated species (not well represented in the previous group), a panel of 127 microorganisms was selected from the laboratory stock collection (rare clinical isolates).

Prior to testing, all isolates were cultured onto Columbia agar with 5% sheep blood (16–24 h at 35 °C) to ensure purity and viability. Stock strains were subcultured twice. Organisms were tested separately with Vitek 2 for the ID-GNB card (bioMérieux) and with ATB Expression for ID 32 E (*Enterobacteriaceae* or oxidase-negative isolates) and ID 32 GN (non-*Enterobacteriaceae* or oxidase-positive isolates) (bioMérieux). For each method, inoculations, readings, and interpretations were performed according to the manufacturer's recommendations.

ID 32 E and ID 32 GN were used as the respective reference methods based on their wide acceptance [3, 4]. When discrepancies were observed between the reference identification and the Vitek 2 identification, isolates were identified either with Biotype 100 strips for *Enterobacteriaceae* (strip of 100 tubes for carbon assimilation tests; bioMérieux) [5, 6] or conventional biochemical tests (for non-*Enterobacteriaceae*) according to reference manuals [7, 8]. These additional tests were performed in the following cases: insufficient growth, known card/strip inoculation error, discrepancy between ID-GNB card and reference method, or lack

of identification. Isolates requiring repeat testing were retested only once. A few simple additional tests were performed, when required, by Vitek 2 in order to resolve results indicating low discrimination of oxidase, motility, hemolysis, indole, or pigmentation.

Results were separated into four groups: correct identification (either excellent, very good, good, or acceptable identification); low discrimination (either identification at the genus level or low discrimination between several species, including the correct species); misidentification (incorrect identification); or no identification (doubtful, unacceptable, or unreliable identification). Results were expressed in numbers and percentages. A supplemental analysis of data was also carried out to be representative of the 17 more frequent gram-negative rods recovered from blood cultures in the microbiology laboratories of 33 French university hospitals [9].

Results and Discussion

Of the 502 strains of gram-negative bacilli identified with the Vitek 2 ID-GNB card, 430 (85.7%) and 485 (96.6%) were identified to the species and genus levels, respectively (Tables 1 and 2). Misidentifications were observed for 11 strains (2.2%). No identification was generated for six isolates of *Enterobacteriaceae* (1.2%). Regardless of their origin (combined stock collection and clinical isolates), *Enterobacteriaceae* (93.3%) were better identified than non-*Enterobacteriaceae* (67.1%) to the species level. Identification to the genus level was 96.6% for both *Enterobacteriaceae* and non-*Enterobacteriaceae*. When the strain origin was considered, clinical isolates of both *Enterobacteriaceae* (95.3%) and non-*Enterobacteriaceae* (74%) were better identified to the species level than stock collection strains (86.4% and 52.2%, respectively). For bacteria with slow metabolism, such as stock collection strains or nonfermenting bacteria, it might be necessary, in a few cases, to identify them over 24 h using either commercial kits or conventional media. Funke et al. [2] tested 845 strains belonging to 70 taxa using the same automated system and obtained results similar to ours: 84.7% of all bacteria were identified correctly, 0.8% were misidentified, and 1.2% were not identified.

The database of the Vitek 2 allowed species identification of rare *Enterobacteriaceae* such as *Edwardsiella tarda, Enterobacter asburiae, Escherichia hermannii, Kluyvera ascorbata, Leclercia adecarboxylata, Moellerella wisconsensis, Proteus penneri,* and some other enteric bacilli selected from stock collections. However, the database evaluated was preliminary; misidentifications or nonidentifications of *Enterobacteriaceae* could be reduced by introducing some slight changes in the database. Likewise, Funke et al. [2] also emphasized the need to improve the database to allow a better discrimination between related taxa. Some microorganisms would then be better identified, such as *Citrobacter braakii*, previously part of the *Citrobacter freundii* species, or infrequently isolated bacteria such as *Pantoea agglomerans*, *Edwardsiella hoshinae, Serratia ficaria,* and *Serratia plymuthica*. Most of these rare bacteria are merely encountered in the environment or are infrequently associated with human disease. Consequently, the choice of microorganisms for the evaluation of automated systems intended for clinical bacteriology should be limited to species encountered in clinical situations only.

After reviewing the literature, it appears that the ratio of correct identifications obtained with the ID-GNB card was in the range of those obtained with similar systems [10–14]. Nevertheless, it must be noted that in most of the publications, correct identifications also include low-discrimination results that are resolved, or not, with conventional tests, sometimes after a few days. With the ID-GNB card, correct identifications are true identifications, as the supplemental tests recommended are very easy to perform and do not delay the results of the ID-GNB card. For *Enterobacteriaceae*, identifications at the species level with the Vitek 2 ID-GNB card were similar to those obtained with either the Vitek GNI card (range, 82.9–94.7%) [P. Colonna et al., 90th Annual Meeting of the American Society for Microbiology, 1990, Abstract no. C-157] or the GNI Plus card (92.7%) [P.P. Bourbeau et al., 97th Annual Meeting of the American Society for Microbiology, 1997, Abstract no. C-455], whereas they ranged from 78.6% [11] to 94.3% [15] for the WalkAway system (Dade Behring, USA) and were 52% [11] for the Biolog GN microplate (Biolog, USA). The misidentification percentages obtained previously with Vitek [P.P. Bourbeau et al., P. Colonna et al., 13] ranged from 1.1 to 2.1% and are almost equivalent to those found in this study; with other systems, these percentages ranged from 9.2% [P. Colonna et al.] to 1.3% [12].

All of the non-*Enterobacteriaceae* bacteria tested (fermenters and nonfermenters) were identified. The ID-GNB card allowed correct identification of 96.6% of these bacteria to the genus level, and 67.1% of them were identified to the species level. Although the rate of misidentification remained very low (3.4%) and comparable to that observed for *Enterobacteriaceae*, the ID-GNB card is less adapted to the identification of non-*Enterobacteriaceae* than *Enterobacteriaceae*, especially *Acinetobacter* spp., *Burkholderia* spp., *Alcaligenes* spp., and *Pseudomonas fluorescens*. With any identification system, the microbiologist should consider the balance between rapid results and accuracy, especially for bacteria with slow metabolism. It is important to point out that 74% of clinical isolates of non-*Enterobacteriaceae* were definitively identified (to species) with the ID-GNB card after 3 h, which is unusual with the currently available identification products and is especially interesting from a clinical and laboratory workflow standpoint.

Compared with other studies, the percentage of correct identifications of non-*Enterobacteriaceae* bacteria

Table 1 Identification of stock strains using the Vitek 2 ID-GNB system

	No. of strains tested	No. correctly identified	No. with low discrimination	No. misidentified	No. not identified
Enterobacteriaceae					
Edwardsiella hoshinae	3	2			1
Edwardsiella tarda	2	2			
Enterobacter amnigenus	6	5	1		
Enterobacter asburiae	4	4			
Enterobacter cloacae	3	3			
Enterobacter gergoviae	1	1			
Enterobacter sakazakii	2	1			1
Escherichia coli	2	2			
Escherichia hermannii	3	3			
Escherichia vulneris	2	1	1		
Hafnia alvei	2	2			
Klebsiella ornithinolytica	1	1			
Klebsiella pneumoniae	1	1			
Kluyvera ascorbata	4	4			
Leclercia adecarboxylata	3	3			
Moellerella wisconsensis	1	1			
Pantoea agglomerans	2	1			1
Proteus penneri	1	1			
Salmonella paratyphi A	3	3			
Serratia ficaria	2	1	1		
Serratia fonticola	1	1			
Serratia liquefaciens	2	2			
Serratia odorifera	1	1			
Serratia plymuthica	4	2	2		
Shigella sonnei	2	2			
Shigella spp.	3	3			
Yersinia enterocolitica	6	6			
Yersinia kristensenii	1	1			
Yersinia pseudotuberculosis	11	9	1		1
Yersinia ruckeri	2	1	1		
Total (%)	81	70 (86.4)	7 (8.6)	0	4 (4.9)
Nonfermenting GNB					
Acinetobacter lwoffii	1		1		
Burkholderia cepacia	9	3	5	1	
Burkholderia stutzeri	2		2		
Chryseomonas luteola	1	1			
Chryseobacterium indologenes	3	1	2		
Chryseobacterium meningosepticum	1	1			
Pseudomonas fluorescens	3		2	1	
Pseudomonas mendocina	1		1		
Shewanella putrefaciens	1		1		
Stenotrophomonas maltophilia	1	1			
Total (%)	23	7 (30.4)	14 (60.9)	2 (8.7)	0
Other GNB					
Aeromonas hydrophila	2	2			
Aeromonas sobria	6	5	1		
Myroides spp.	1		1		
Moraxella osloensis	2		2		
Pasteurella aerogenes	2	1	1		
Pasteurella multocida	7	6		1	
Vibrio alginolyticus	1	1			
Vibrio cholerae	1	1			
Vibrio parahaemolyticus	1	1			
Total (%)	23	17 (73.9)	5 (21.7)	1 (4.3)	0
All stock strains	127	94 (74.0)	26 (20.5)	3 (2.4)	4 (3.1)

GNB, gram-negative bacteria

obtained with the Vitek 2 ID-GNB card (67.1%) was lower than that obtained with either the Vitek GNI card (range, 79.6–86.8%) [P. Colonna et al., 13] or the WalkAway system (range, 74.6–92.3%) [P. Colonna et al., 13]. As described above, correct identifications also frequently include low-discrimination results that are resolved, or not, with conventional tests, sometimes after a few days. This was not the case with the ID-GNB card. The percentage of correct identifications was higher than that obtained with the Biolog GN

Table 2 Identification of clinical isolates using the Vitek 2 system

	No. of strains tested	No. correctly identified	No. with low discrimination	No. misidentified	No. not identified
Enterobacteriaceae					
Citrobacter braakii	7	1	2	4	
Citrobacter freundii	15	15			
Citrobacter koseri (diversus)	12	12			
Citrobacter youngae	1			1	
Enterobacter aerogenes	21	21			
Enterobacter asburiae	4	4			
Enterobacter cloacae	24	23	1		
Escherichia coli	22	20	1	1	
Hafnia alvei	9	9			
Klebsiella oxytoca	16	15			1
Klebsiella pneumoniae ozaenae	1	1			
Klebsiella pneumoniae	27	27			
Morganella morganii	19	19			
Proteus mirabilis	30	30			
Proteus penneri	1	1			
Proteus vulgaris	11	11			
Providencia rettgeri	2	2			
Providencia stuartii	11	11			
Salmonella spp.	13	13			
Serratia liquefaciens	2	2			
Serratia marcescens	25	23	1		1
Shigella sonnei	1	1			
Shigella spp.	1	1			
Total (%)	275	262 (95.3)	5 (1.8)	6 (2.2)	2 (0.7)
Nonfermenting GNB					
Acinetobacter baumannii	27	24	3		
Acinetobacter haemolyticus	8		7	1	
Acinetobacter lwoffii	1		1		
Alcaligenes faecalis	3		3		
Alcaligenes xylosoxidans	2		2		
Brevundimonas vesicularis	1		1		
Burkholderia cepacia	1	1			
Oligella spp.	1		1		
Pseudomonas aeruginosa	29	25	4		
Pseudomonas fluorescens	1		1		
Stenotrophomonas maltophilia	18	17		1	
Total (%)	92	67 (72.8)	23 (25.0)	2 (2.2)	0
Other GNB					
Aeromonas hydrophila	3	3			
Aeromonas sobria	1		1		
Pasteurella multocida	3	3			
Vibrio parahaemolyticus	1	1			
Total (%)	8	7 (87.5)	1 (12.5)	0	0
All clinical isolates					
Total (%)	375	336 (89.6)	29 (7.7)	8 (2.1)	2 (0.5)

GNB, gram-negative bacteria

microplate (38.6%) [2]. For non-*Enterobacteriaceae* bacilli, the percentage of misidentifications obtained with the ID-GNB card (3.4% for stock and clinical isolates, 2% for clinical isolates only) was rather low compared to that obtained with the other systems, ranging from 2.2% [13] to 15% [10].

The rate of misidentifications of non-*Enterobacteriaceae* remains comparable to that observed for *Enterobacteriaceae*. In this study, 25% of non-*Enterobacteriaceae* were identified to the genus or group level and required additional tests (leading to delayed results) for identification to the species level. Although some improvements could be made to reduce this rate, the ID-GNB card can be considered a routine identification tool for gram-negative bacilli, providing correct identification to the species level after 3 h for 90% of clinical isolates.

Table 3 reports the results obtained from a selection of 17 more frequent gram-negative taxa isolated in 33 French university hospitals [9]. Identification with the ID-GNB card gave an overall identification to the species level of 95.2%, comprised as follows: 97.2% for *Enterobacteriaceae* and 89.3% for non-*Enterobacteriaceae* (fermenters and nonfermenters). One strain each

Table 3 Data on clinical isolates, extracted according to the frequency of recovery from blood cultures in 33 French microbiology laboratories

	No. of strains tested	No. correctly identified	No. with low discrimination	No. misidentified	No. not identified
Enterobacteriaceae					
Citrobacter freundii	15	15			
Enterobacter cloacae	24	23	1		
Escherichia coli	22	20	1	1	
Klebsiella oxytoca	16	15			1
Klebsiella pneumoniae	27	27			
Morganella morganii	19	19			
Proteus mirabilis	30	30			
Proteus vulgaris	11	11			
Providencia rettgeri	2	2			
Providencia stuartii	11	11			
Salmonella spp.	13	13			
Serratia liquefaciens	2	2			
Serratia marcescens	25	23	1		1
Total (%)	217	211 (97.2)	3 (1.4)	1 (0.5)	2 (0.9)
Nonfermenting GNB					
Acinetobacter baumannii	27	24	3		
Burkholderia cepacia	1	1			
Pseudomonas aeruginosa	29	25	4		
Stenotrophomonas maltophilia	18	17		1	
Total (%)	75	67 (89.3)	7 (9.3)	1 (1.3)	0
All microorganisms					
Total (%)	292	278 (95.2)	10 (3.4)	2 (0.7)	2 (0.7)

GNB, gram-negative bacteria

of *Klebsiella oxytoca* and *Serratia marcescens* did not match with the database (i.e., 0.7% unidentified), and one strain each of *Escherichia coli* and *Stenotrophomonas maltophilia* were misidentified (0.7%).

Even with this first version of the database, we consider the automated Vitek 2 system to have potential for use as a routine method because it is accurate, hands-off, efficient, and time-saving. Like Funke et al. [2], we encountered no major technical problems with the Vitek 2 system. Analysis of the identification results shows that the ID-GNB card provides a highly acceptable level of identification accuracy for *Enterobacteriaceae* and non-*Enterobacteriaceae*, including most atypical strains encountered in clinical situations. With the ID-GNB card, results are available after 3 h of incubation. In conclusion, the Vitek 2 system should improve the quality of laboratory test results and make them available sooner. However, its routine use implies that the laboratory workflow should be reorganized in order to optimize the main features of this system and to provide the clinician with the most accurate and rapid identification result.

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