JOURNAL OF CLINICAL MICROBIOLOGY, June 2009, p. 1923–1926 Vol. 47, No. 6
0095-1137/09/$08.00+0 doi:10.1128/JCM.01778-08

Evaluation of the New Vitek 2 ANC Card for Identification of Medically Relevant Anaerobic Bacteria▽

Francine Mory,[1] Corentine Alauzet,[1] Céline Matuszeswski,[1] Philippe Riegel,[2] and Alain Lozniewski[1]*

Laboratoire de Bactériologie, Centre Hospitalier Universitaire, Nancy,[1] and Laboratoire de Bactériologie, Hôpitaux Universitaires, Strasbourg,[2] France

Received 15 September 2008/Returned for modification 25 November 2008/Accepted 14 April 2009

Of 261 anaerobic clinical isolates tested with the new Vitek 2 ANC card, 257 (98.5%) were correctly identified at the genus level. Among the 251 strains for which identification at the species level is possible with regard to the ANC database, 217 (86.5%) were correctly identified at the species level. Two strains (0.8%) were not identified, and eight were misidentified (3.1%). Of the 21 strains (8.1%) with low-level discrimination results, 14 were correctly identified at the species level by using the recommended additional tests. This system is a satisfactory new automated tool for the rapid identification of most anaerobic bacteria isolated in clinical laboratories.

Accurate identification of numerous bacterial species is nowadays possible with highly automated systems that are increasingly used in clinical laboratories because of their cost effectiveness, practicability, and ability to provide rapid turnaround time. It is now well established that anaerobes may be involved in numerous infections, including severe infections (8, 12). Until recently, however, identification of anaerobes in clinical laboratories relied mainly on the use of time-consuming and labor-intensive conventional methods or of manual commercial systems, the performances of which are quite variable at the species level (3, 5, 7, 10, 13, 14).

bioMérieux (Marcy, France) has recently developed a new colorimetric identification card (ANC card) which, in conjunction with the Vitek 2 system, permits this automated and widely distributed identification system to identify 63 taxa, including 49 taxa of anaerobic bacteria belonging to the genera *Actinomyces*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Collinsella*, *Eggerthella*, *Eubacterium*, *Finegoldia*, *Fusobacterium*, *Parabacteroides*, *Parvimonas*, *Peptoniphilus*, *Peptostreptococcus*, *Prevotella*, *Propionibacterium*, and *Veillonella*. It is noteworthy that this system identifies *Bifidobacterium* spp. and *Veillonella* spp. only at the genus level. In the present study, the ANC card was evaluated for the identification of anaerobes in a routine clinical laboratory.

A total of 261 nonconsecutive clinical isolates belonging to 43 medically relevant taxa included in the ANC database and collected over a 1-year period in our laboratory were used. Strains were selected to represent the distribution of anaerobic isolates recovered annually in our laboratory. These organisms have been previously identified using conventional reference identification methods (6). The sources of the isolates included blood (n = 102), central nervous system samples (n = 9), pleuropulmonary samples (n = 11), intra-abdominal samples (n = 54), soft tissue samples (n = 29), osteoarticular samples (n = 14), urogenital samples (n = 11), stool samples (n = 20), and various other samples (n = 11). *Actinomyces israelii* ATCC 12102, *Propionibacterium acnes* ATCC 6919, and *Clostridium difficile* ATCC 9689 were also investigated. *Bacteroides ovatus* ATCC BAA-1296, *Bacteroides vulgatus* ATCC 8482, *Parabacteroides distasonis* ATCC BAA-1295, *Clostridium septicum* ATCC 12464, and *Clostridium sordellii* ATCC 9714 were used as quality controls and checked every month during the evaluation. Isolates were stored frozen, except for the available clinical isolates that were recovered directly from clinical specimens. Prior to testing, strains were subcultured twice onto Columbia sheep blood agar (bioMérieux) in an anaerobic atmosphere at 35°C. Inoculum preparation, incubation (approximately 6 h), and reading of the test panels were performed according to the manufacturer's instructions. Data were analyzed using the Vitek 2 ANC system software, which permits categorization of the results into four groups: correct identification (i.e., unambiguous identification [given as excellent, very good, good, or acceptable] to the species level or to the genus level for *Bifidobacterium* spp. and *Veillonella* spp. strains), low level of discrimination (low level of discrimination between two or more species, including the correct species, requiring additional tests), misidentification (the genus or the species identified with the ANC card was different from that identified using the reference methods), and no identification (strains without results). In the case of discrepancy between the identification obtained with the routine method and that obtained with the Vitek 2 system, 16S rRNA gene sequencing was used for genetic identification (1).

The quality control strains were always correctly identified, with the test results being reproducible and in accordance with those expected from the database previously established by bioMérieux. The other three reference strains tested were correctly identified at the species level. Among the 261 routine clinical isolates tested, 257 (including all *Bifidobacterium* spp. and *Veillonella* spp. strains [n = 10], which can only be identified at the genus level with the ANC card) of 261 (98.5%) were correctly identified at the genus level and 217 of 251

* Corresponding author. Mailing address: Laboratoire de Bactériologie, Hôpital Central, 29 Avenue du Maréchal de Lattre de Tassigny, 54035 Nancy Cedex, France. Phone: 33 3 83 85 18 14. Fax: 33 3 83 85 26 73. E-mail: a.lozniewski@chu-nancy.fr.

▽ Published ahead of print on 22 April 2009.

TABLE 1. Identification of 261 clinical anaerobic isolates with the Vitek 2 ANC card

Organisms	No. (%) of strains that were:					
	Tested	Correctly identified	Identified with low discrimination	Misidentified	Correctly identified after additional tests	Not identified
Bacteroides spp.	61	60 (98.4)	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)
Bacteroides fragilis group	55	54	0	1	0	0
Bacteroides caccae	3	3	0	0	0	0
Parabacteroides distasonis	5	5	0	0	0	0
Bacteroides fragilis	20	20	0	0	0	0
Bacteroides ovatus	5	5	0	0	0	0
Bacteroides thetaiotaomicron	14	13	0	1	0	0
Bacteroides uniformis	3	3	0	0	0	0
Bacteroides vulgatus	5	5	0	0	0	0
Bacteroides ureolyticus	6	6	0	0	0	0
Prevotella spp.	36	31 (86.1)	2 (5.6)	3 (8.3)	2 (5.6)	0 (0.0)
Prevotella bivia	10	7	2	1	2	0
Prevotella buccae	5	5	0	0	0	0
Prevotella disiens	4	4	0	0	0	0
Prevotella intermedia	5	4	0	1	0	0
Prevotella melaninogenica	6	5	0	1	0	0
Prevotella oralis	6	6	0	0	0	0
Fusobacterium spp.	19	18 (94.7)	1 (5.3)	0 (0.0)	1 (5.3)	0 (0.0)
Fusobacterium mortiferum	2	2	0	0	0	0
Fusobacterium necrophorum	3	3	0	0	0	0
Fusobacterium nucleatum	12	11	1	0	1	0
Fusobacterium varium	2	2	0	0	0	0
Veillonella spp.	5	5 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gram-positive cocci	24	24 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Finegoldia magna	5	5	0	0	0	0
Parvimonas micra	10	10	0	0	0	0
Peptinophilus asaccharolyticus	4	4	0	0	0	0
Peptostreptococcus anaerobius	5	5	0	0	0	0
Nonsporeforming gram-positive bacilli	46	44 (95.7)	0 (0.0)	2 (4.3)	0 (0.0)	0 (0.0)
Actinomyces israelii/gerencseriae	4	4	0	0	0	0
Actinomyces meyeri	1	1	0	0	0	0
Actinomyces naeslundii	1	1	0	0	0	0
Bifidobacterium spp.	5	5	0	0	0	0
Eggerthella lenta	11	11	0	0	0	0
Eubacterium limosum	1	1	0	0	0	0
Propionibacterium acnes	20	20	0	0	0	0
Propionibacterium granulosum	3	1	0	2	0	0
Sporeforming gram-positive bacilli	70	45 (64.3)	21 (30)	2 (2.9)	14 (20)	2 (2.9)
Clostridium bifermentans	2	0	0	0	0	2
Clostridium butyricum	5	0	5	0	0	0
Clostridium cadaveris	6	6	0	0	0	0
Clostridium clostridioforme	6	6	0	0	0	0
Clostridium difficile	20	7	12	1	10	0
Clostridium paraputrificum	3	3	0	0	0	0
Clostridium perfringens	10	10	0	0	0	0
Clostridium ramosum	5	4	1	0	1	0
Clostridium septicum	1	1	0	0	0	0
Clostridium sordellii	2	2	0	0	0	0
Clostridium sporogenes	4	1	3	0	3	0
Clostridium tertium	6	5	0	1	0	0
Total	261	227 (87)	24 (9.2)	8 (3.1)	17 (6.6)	2 (0.8)

(86.5%) at the species level without performing additional tests (Table 1). Two strains (0.8%) were not identified, and eight (3.1%) were misidentified (Table 2). Of the 121 gram-negative strains, 114 (94.3%) were correctly identified without further testing and 3 (2.5%), which gave low-level discrimination results, were identified with additional tests (Table 3). Among the 140 gram-positive isolates, 80.7% (100% of the cocci, 95.7% of the nonsporeforming bacilli, and 64.3% of the

TABLE 2. Strains misidentified by the Vitek 2 ANC system[a]

Organism identified by conventional methods (no. of strains)	Identification of isolate by	
	Vitek 2 ANC card (level)	DNA sequencing
B. thetaiotaomicron (1)	*B. ovatus* (good)	*B. thetaiotaomicron*
P. bivia (1)	*P. melaninogenica* (very good)	*P. bivia*
P. intermedia (1)	*P. disiens* (excellent)	*P. intermedia*
P. melaninogenica (1)	*P. bivia* (excellent)	*P. melaninogenica*
P. granulosum (2)	*C. difficile* (excellent to good)	*P. granulosum*
C. difficile (1)	*C. sporogenes* (excellent)	*C. difficile*
C. tertium (1)	*Clostridium baratii* (acceptable)	*C. tertium*

[a] Full Latin binomials are in Table 1.

clostridia) were correctly identified at the species level without further testing. Of the 21 *Clostridium* species that gave low-level-discrimination identifications, 14 were correctly identified by using recommended additional tests, while 7 (5 *C. butyricum* and 2 *C. difficile*) could be easily identified by using other supplementary tests (Table 3). Indeed, a simple Gram stain permitted us to differentiate *C. butyricum* (gram-positive straight rod with subterminal spore) from *C. clostridioforme* (cigar-shaped gram-negative rod), while *C. difficile* could be differentiated from *C. subterminale* by determining the fermentation of glucose and mannitol. Thus, the use of additional tests other than those recommended by the manufacturer permitted an increase in the rate of correct identification of clostridia from 84.3% to 94.3%.

Identification systems should be able to correctly identify, overall, 90% of the organisms isolated in routine laboratories, while commonly isolated organisms should be identified with at least 95% accuracy (2). This cutoff was achieved at the genus level without the need for additional tests for all strains tested. With regard to the species level, an accuracy rate of at least 95% was achieved without the need for additional tests for gram-positive cocci, nonsporeforming bacilli, and species belonging to the genus *Bacteroides*. Satisfactory results were also achieved for the identification of *Fusobacterium* spp., considering that 94.7% of the strains tested were correctly identified at the species level without further testing and that the only strain which was identified with a low level of discrimination could be correctly identified after performing a simple Gram stain as recommended by the manufacturer. Slightly less satisfactory results were observed for the identification of *Prevotella* species. Indeed, for these latter, a correct identification rate of 91.7% was achieved after the application of additional tests. Overall, these results are comparable to those recently reported by other authors evaluating the Vitek 2 ANC system (11, 15). As in those studies, difficulties were encountered in the present study in identifying clostridia species, except for *C. perfringens*.

When taking into account taxa included in the databases of all identification systems, including that of the Vitek 2 ANC system, as well as taxonomic changes, the performance obtained with this system still compares favorably overall to those previously reported for other commercialized identification systems. Indeed, among these latter, the API 20 A system, which necessitates an anaerobic incubation of up to 48 h, is best suited for the identification of only saccharolytic, rapidly growing organisms, such as those belonging to the *Bacteroides fragilis* group (2, 6). Among rapid identification systems, accuracies as high as 95% were only reported, with regard to the species level and without the application of additional tests, with the Rapid ID 32A system for the identification of gram-positive cocci, with the Rapid ANA II system for the identification of these organisms and *Prevotella* spp., and with the BBL Crystal ANR system for the identification of *Fusobacterium* spp. (3, 4, 7). Moreover, even if the use of additional tests has been shown to increase the rates of correct identification with these systems, the performances achieved are variable, depending on the species tested and the study (5, 7, 9, 10).

Thus, our results indicate that the Vitek 2 ANC system is a simple, rapid, and satisfactory method for the identification of anaerobes in a clinical microbiology laboratory. This system is not yet perfect, particularly with regard to the identification of clostridia at the species level, but represents, overall, an improvement over other available systems used for the identification of the most frequently encountered anaerobes. In the present study, strains were subcultured twice prior to testing. Considering that this step is not performed routinely, further studies are needed to evaluate whether the Vitek 2 ANC system performs as well as in the present study when strains from primary isolation plates are analyzed.

TABLE 3. Strains identified with a low level of discrimination by the Vitek 2 ANC system[a]

Vitek 2 ANC result (no. of strains)	Additional test(s) proposed by the manufacturer	Result[c] after additional test	
		Expected	Obtained
P. bivia[b] or *P. melaninogenica* (2)	Saccharose	*P. bivia* (−), *P. melaninogenica* (+)	−
F. nucleatum[b] or *F. varium* (1)	Gram stain	Pointed ends: *F. nucleatum* (+), *F. varium* (−)	Pointed ends, +
C. butyricum[b] or *C. clostridioforme* (4)	Nitrate reductase	*C. butyricum* (−), *C. clostridioforme* (+⁻)	−
C. butyricum,[b] *C. clostridioforme*, or *C. bifermentans* (1)	Lecithinase	*C. bifermentans* (+), other species (−)	−
C. difficile,[b] *C. bifermentans*, or *C. sporogenes* (10)	Indole	*C. bifermentans* (+), other species (−)	−
	Lipase	*C. sporogenes* (+), other species (−)	−
C. difficile,[b] *C. subterminale*, or *C. sporogenes* (2)	Lipase	*C sporogenes* (+), other species (−)	−
C. ramosum[b] or *C. paraputrificum* (1)	Mannitol	*C. ramosum* (+⁻), *C. paraputrificum* (−)	+
C. sporogenes[b] or *C. bifermentans* (2)	Lipase	*C. sporogenes* (+), *C. bifermentans* (−)	+
C. sporogenes[b] or *C. subterminale* (1)	Lipase	*C. sporogenes* (+), *C. subterminale* (−)	+

[a] Full Latin binomials are in Table 1.
[b] Reference identification.
[c] +, positive; −, negative; +⁻, most strains positive, some negative.

bioMérieux provided the study materials.

We thank Monique Chaon, Céline Guichou and Laurence Genet for their technical assistance and Sonia Chatellier and Romuald Courtade for their support during the evaluation.

REFERENCES

1. **Alauzet, C., F. Mory, J. P. Carlier, H. Marchandin, E. Jumas-Bilak, and A. Lozniewski.** 2007. *Prevotella nanceiensis* sp. nov., isolated from human clinical samples. Int. J. Syst. Evol. Microbiol. **57:**2216–2220.
2. **Carroll, K. C., and M. P. Weinstein.** 2007. Manual and automated systems for detection and identification of microorganisms, p. 192–217. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
3. **Cavallaro, J. J., L. S. Wiggs, and J. M. Miller.** 1997. Evaluation of the BBL Crystal Anaerobe identification system. J. Clin. Microbiol. **35:**3186–3191.
4. **Celig, D. M., and P. C. Schreckenberger.** 1991. Clinical evaluation of the RapID-ANA II panel for identification of anaerobic bacteria. J. Clin. Microbiol. **29:**457–462.
5. **Downes, J., A. King, J. Hardy, and I. Phillips.** 1999. Evaluation of the Rapid ID 32A system for the identification of anaerobic gram-negative bacilli, excluding *Bacteroides fragilis* group. Clin. Microbiol. Infect. **5:**319–326.
6. **Jousimies-Somer, H. R., P. Summanen, E. J. Baron, D. M. Citron, H. M. Wexler, and S. M. Finegold (ed.).** 2002. Wadsworth-KTL anaerobic bacteriology manual, 6th ed. Star Publishing Company, Belmont, CA.
7. **Kitch, T. T., and P. C. Appelbaum.** 1989. Accuracy and reproducibility of the 4-hour ATB 32A method for anaerobe identification. J. Clin. Microbiol. **27:**2509–2513.
8. **Lassmann, B., D. R. Gustafson, C. M. Wood, and J. E. Rosenblatt.** 2007. Reemergence of anaerobic bacteremia. Clin. Infect. Dis. **44:**895–900.
9. **Looney, W. J., A. J. C. Gallusser, and H. K. Modde.** 1990. Evaluation of the ATB 32 A system for identification of anaerobic bacteria isolated from clinical specimens. J. Clin. Microbiol. **28:**1519–1524.
10. **Marler, L. M., J. A. Siders, L. C. Wolters, Y. Petttigrew, B. L. Skitt, and S. D. Allen.** 1991. Evaluation of the new RapID-ANA II system for the identification of clinical anaerobic isolates. J. Clin. Microbiol. **29:**874–878.
11. **Rennie, R. P., C. Brosnikoff, L. A. Turnbull, L. B. Reller, S. Mirrett, W. Janda, K. Ristow, and A. Krilcich.** 2008. Multicenter evaluation of the Vitek 2 anaerobe and *Corynebacterium* identification card. J. Clin. Microbiol. **46:** 2646–2651.
12. **Rosenblatt, J. E.** 1997. Can we afford to do anaerobic cultures and identification? A positive point of view. Clin. Infect. Dis. **25**(Suppl. 2)**:**S127–S131.
13. **Schreckenberger, P. C., D. M. Celig, and W. M. Janda.** 1988. Clinical evaluation of the Vitek ANI card for identification of anaerobic bacteria. J. Clin. Microbiol. **26:**225–230.
14. **Summanen, P., and H. Jousimies-Somer.** 1988. Comparative evaluation of Rapid ANA and API-20A for identification of anaerobic bacteria. Eur. J. Clin. Microbiol. Infect. Dis. **7:**771–775.
15. **Wilkey, K., J. Brinkmeyer, J. Colon-Reveles, N. Moss, J. Mills, E. Mathias, E. Fossman, D. Pincus, J. Clarridge III, C. Jitjai, A. McLaughlin, and S. Chatellier.** 2006. Rapid automated identification of anaerobic and coryneform species with the new Vitek 2 ANC card, abstr. C-013. Abstr. 106th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC.