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# Evaluation of the Vitek 2 ANC Card for Identification of Clinical Isolates of Anaerobic Bacteria[▽]

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**An evaluation of the Vitek 2 ANC card (bioMérieux, Marcy l'Etoile, France) was performed with 301 anaerobic isolates. Each strain was identified by 16S rRNA gene sequencing, which is considered to be the reference method. The Vitek 2 ANC card correctly identified 239 (79.4%) of the 301 clinical isolates to the genus level, including 100 species that were not represented in the database. Correct species identification was obtained for 60.1% (181/301) of the clinical isolates. For the isolates not identified to the species level, a correct genus identification was obtained for 47.0% of them (47/100), and 16 were accurately designated not identified. Although the Vitek 2 ANC card allows the rapid and acceptable identification of the most common clinically important anaerobic bacteria within 6 h, improvement is required for the identification of members of the genera *Fusobacterium*, *Prevotella*, and *Actinomyces* and certain Gram-positive anaerobic cocci (GPAC).**

The involvement of anaerobes in numerous and severe clinical infections has been reported (5, 13). The differences in antimicrobial susceptibility (9, 16) and the development of resistance to antimicrobial drugs (16) among anaerobic bacteria have been documented. Traditional methods for the identification of anaerobic pathogens are not always available in clinical bacteriology laboratories and are often laborious and time-consuming (12). Therefore, the need for a rapid and accurate method for the identification of anaerobic pathogens is highly desirable for appropriate treatment.

In the last decades, different commercial enzyme kits for the identification of clinically relevant anaerobe isolates have been developed and evaluated, e.g., the RapID-ANA II panel, the Minitek systems, the Vitek ANI card, the BBL Crystal ANR ID kit, the API rapid ID 32 A system, and the API 20 A system (6–8, 10, 14, 18, 19).

The new Vitek 2 ANC card (bioMérieux, Marcy l'Etoile, France) is designed to provide clinical laboratories with the capability for the rapid and accurate identification of clinically relevant anaerobic bacteria and *Corynebacterium* species. The card contains 64 microwells with 36 colorimetric enzymatic tests. The ANC database comprises 63 taxa of anaerobic bacteria and corynebacteria. Twenty genera are listed in the Vitek 2 ANC database: *Actinomyces*, *Arcanobacterium*, *Bacteroides* (*Parabacteroides*), *Bifidobacterium*, *Clostridium*, *Collinsella*, *Corynebacterium*, *Eggerthella*, *Eubacterium*, *Finegoldia*, *Fusobacterium*, *Lactobacillus*, *Microbacterium*, *Parvimonas* (formerly *Micromonas*), *Peptoniphilus*, *Peptostreptococcus*, *Prevotella*, *Propionibacterium*, *Staphylococcus*, and *Veillonella*. The system provides only a genus-level identification for *Bifidobacterium* sp. and *Veillonella* sp. Of the 36 biochemical profiles, 13 are fermentation tests, 17 are glycosidase and arylamidase tests, 2 are alkaline reactions, and 4 are other biochemical tests. Additional simple off-line tests, including cell morphology, Gram stain characteristic, and aerotolerance testing, are required to complete the identifications.

In order to assess the accuracy of the ANC card in a "real-life" setting, unknown clinical isolates of anaerobic bacteria were used, regardless of whether the species are present in the Vitek database. This is in contrast to the methods used in two previously reported studies (15, 17). The identification obtained by use of the Vitek 2 ANC system was compared with that obtained by use of 16S rRNA gene sequencing.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** A total of 301 anaerobic clinical isolates comprising 129 Gram-negative bacilli, 3 Gram-negative cocci, 64 Gram-positive bacilli, and 105 Gram-positive anaerobic cocci were included in the study. All isolates were collected from patients treated at the University Medical Center Groningen, Groningen, Netherlands, and were chosen randomly. Phenotypic characterization was performed by use of conventional methods (12). Strains were stored at −80°C and were subcultured at least twice on *Brucella* blood agar (BBA; Oxoid) to ensure viability prior to inoculation for the Vitek system. All culture handlings were performed in an anaerobic cabinet at 37°C. Fast-growing strains were incubated for 24 h and slow-growing strains were incubated for 48 h to 72 h before inoculation in the Vitek 2 ANC system.

**Vitek analyses.** Additional testing consisted of Gram characteristic, cell morphology, and aerotolerance testing. Each isolate was inoculated onto a blood agar (BA; Oxoid) plate and incubated in a 5% $CO_2$ atmosphere for aerotolerance testing. Inoculum suspensions were prepared with 0.45% aqueous NaCl until a turbidity of between 2.70 and 3.30 McFarland standards was reached by using a calibrated Vitek 2 Densichek instrument (bioMérieux, Marcy l'Etoile, France).

**Quality control.** The quality of each batch ANC card was determined by using three control strains, *Bacteroides vulgatus* ATCC 8482, *Clostridium perfringens* ATCC 13124, and *Corynebacterium striatum* ATCC BAA-1293. The anaerobic control strains were subcultured onto BBA agar and incubated for 24 h at 37°C. *C. striatum* ATCC BAA-1293 was subcultured onto BA and incubated for 24 h at 37°C in a 5% $CO_2$ atmosphere.

**Sequencing.** DNA of the clinical strains was extracted (4) and amplified by using universal primers (11). The obtained sequences were compared to the sequences present in the GenBank database by using BLASTn (www.ncbi.nlm.nih.gov/blast).

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TABLE 1. Comparison of the Vitek 2 ANC card with 16S rRNA gene sequencing as a reference for the identification of 201 clinical isolates of species included in the database

| Reference organism (total no. of isolates) | No. (%) of isolates | | | | |
|---|---|---|---|---|---|
| | Correct identification to the species level | Correct identification to the genus level | Low discrimination | No identification | Misidentification at the species level |
| Gram-negative strains (95) | | | | | |
| *Bacteroides fragilis* sp. (76) | 68 (89.5) | 75 (98.7) | 1 | 0 | 9 |
| *B. fragilis* (42) | 38 (90.5) | 41 (97.6) | 0 | 0 | 4 |
| *B. uniformis* (4) | 2 | 4 | 0 | 0 | 2 |
| *B. ovatus* (8) | 8 | 8 | 0 | 0 | 0 |
| *B. vulgatus* (5) | 5 | 5 | 0 | 0 | 0 |
| *B. thetaiotaomicron* (16) | 14 (87.5) | 16 (100.0) | 1 | 0 | 3 |
| *B. caccae* (1) | 1 | 1 | 0 | 0 | 0 |
| *Parabacteroides* sp. (4) | 3 | 3 | 0 | 1 | 1 |
| *P. distasonis* (4) | 3 | 3 | 0 | 1 | 1 |
| *Campylobacter* sp. (2) | 1 | 1 | 0 | 1 | 1 |
| *C. ureolyticus* (2) | 1 | 1 | 0 | 1 | 1 |
| *Prevotella* sp. (6) | 5 | 6 | 0 | 0 | 1 |
| *P. buccae* (2) | 1 | 2 | 0 | 0 | 1 |
| *P. disiens* (1) | 1 | 1 | 0 | 0 | 0 |
| *P. bivia* (1) | 1 | 1 | 0 | 0 | 0 |
| *P. melaninogenica* (2) | 2 | 2 | 0 | 0 | 0 |
| *Fusobacterium* sp. (7) | 5 | 7 | 1 | 0 | 2 |
| *F. nucleatum* (4) | 4 | 4 | 0 | 0 | 0 |
| *F. necrophorum* (2) | 1 | 2 | 1 | 0 | 1 |
| *Fusobacterium* sp. (1) | 0 | 1 | 0 | 0 | 1 |
| Gram-positive strains (106) | | | | | |
| Gram-positive anaerobic cocci (55) | 54 (98.2) | 54 (98.2) | 1 | 1 | 1 |
| *Finegoldia magna* (33) | 33 (100.0) | 33 (100.0) | 0 | 0 | 0 |
| *Parvimonas micra* (18) | 17 (94.4) | 17 (94.4) | 1 | 1 | 1 |
| *Peptostreptococcus anaerobius* (4) | 4 | 4 | 0 | 0 | 0 |
| *Propionibacterium* sp. (16) | 13 (81.3) | 13 (81.3) | 3 | 0 | 3 |
| *P. acnes* (15) | 13 (86.7) | 13 (86.7) | 3 | 0 | 2 |
| *P. granulosum* (1) | 0 | 0 | 0 | 0 | 1 |
| *Actinomyces* sp. (5) | 3 | 3 | 0 | 1 | 2 |
| *A. meyeri* (1) | 1 | 1 | 0 | 0 | 0 |
| *A. israelii* (3) | 2 | 2 | 0 | 1 | 1 |
| *A. naeslundii* (1) | 0 | 0 | 0 | 0 | 1 |
| *Clostridium* sp. (27) | 27 (100.0) | 27 (100.0) | 7 | 0 | 0 |
| *C. difficile* (15) | 15 (100.0) | 15 (100.0) | 3 | 0 | 0 |
| *C. perfringens* (7) | 7 | 7 | 0 | 0 | 0 |
| *C. septicum* (1) | 1 | 1 | 0 | 0 | 0 |
| *C. baratii* (1) | 1 | 1 | 1 | 0 | 0 |
| *C. butyricum* (3) | 3 | 3 | 3 | 0 | 0 |
| *Eggerthella* sp. (2) | 2 | 2 | 0 | 0 | 0 |
| *E. lenta* (2) | 2 | 2 | 0 | 0 | 0 |
| *Bifidobacterium* sp. (1) | 0 | 1 | 0 | 0 | 0 |

**Data analysis.** The results from the Vitek system were classified into four categories: (i) correct identification to the species level; (ii) low discrimination, with additional testing required, e.g., catalase, oxidase, beta-hemolysis, pigment, motility, and indole production, etc.; (iii) no identification; and (iv) misidentification. Species not included in the database that could not be identified by the ANC card were considered to be correctly classified.

## RESULTS

**Clinical isolates.** The set of 301 anaerobic clinical isolates contained 100 strains that were not included in the database. Correct species- and genus-level identifications were obtained for 60.1% (181/301) and 79.4% (239/301) of the isolates, respectively, with the Vitek 2 ANC card. Of the 102 misidentified isolates, 6.6% (20/301) were present in the database and 27.2% (82/301) were not.

**Species present in the database.** Table 1 summarizes the identification of 201 anaerobic clinical isolates of species included in the database. Correct species- and genus-level identifications were obtained for 90.0% (181/201) and 95.5% (192/201) of isolates, respectively, including strains identified with low discrimination but resolved by additional tests. Twenty strains (10.0%) were misidentified.

**Species not present in the database.** Of the species not present in the database, 84.0% (84/100) were misidentified to the species level. However, 47.0% (47/100) were correctly identified to the genus level, and 16 could not be identified by using the ANC card. These were considered to be correct results.

A total of five *Bacteroides dorei* isolates were correctly identified to the genus level. Of these, one was identified with low discrimination, and four were misidentified as *Bacteroides vulgatus* isolates. All five strains of *Bilophila wadsworthia* were

misidentified as *Campylobacter ureolyticus* isolates. Four of five isolates of *Prevotella nigrescens* and both isolates of *Fusobacterium naviforme* were misidentified as *Prevotella intermedia* and *Fusobacterium nucleatum*, respectively. Seventeen strains of *Peptoniphilus harei* were included in the study, 16 of which were misidentified as *Peptoniphilus asaccharolyticus* and 1 of which was misidentified as *Finegoldia magna*. *Clostridium citroniae* was misidentified as *F. nucleatum*. Two strains of *Clostridium hathewayi* were misidentified as *Clostridium clostridioforme*.

## DISCUSSION

In this study, we assessed the reliability of the Vitek ANC card system for the identification of anaerobic bacteria isolated from clinical materials. *B. dorei* isolates not included in the Vitek 2 ANC database were misidentified as *B. vulgatus*. The enzymatic activities of the Vitek 2 ANC card showed that all *B. dorei* and *B. vulgatus* isolates were esculin positive and esculin negative, respectively. However, this biochemical reaction was in contrast to data reported previously by Bakir et al. (2), who described that *B. dorei* is esculin negative. If the species *B. dorei* had been included in the database, the Vitek 2 ANC card could have distinguished between the two species by interpreting the esculin hydrolysis reaction. So far, the full potential of the Vitek 2 ANC card is not being used. Validation of more strains is required to determine whether this feature can be used to distinguish *B. dorei* from *B. vulgatus*.

*B. wadsworthia*, not present in the database, was consistently misidentified as *C. ureolyticus*. The Vitek 2 ANC card could have differentiated *B. wadsworthia* from *C. ureolyticus*. The biochemical profiles obtained by the Vitek 2 ANC system revealed that *C. ureolyticus* was positive for urease and leucine arylamidase, while *B. wadsworthia* was urease positive and leucine arylamidase negative. The positive leucine arylamidase reaction separates *C. ureolyticus* (1) from *B. wadsworthia*. We recommend that *B. wadsworthia* be included in the database, and this should be validated in practice.

Another species not included in the database, *C. clostridioforme*, cannot be differentiated from *C. hathewayi* by using the Vitek 2 ANC card. Therefore, we recommend that the Vitek 2 ANC system should give identification with low-level discrimination involving both species and that additional biochemical features should be determined (20). In our study, *C. citroniae* was incorrectly identified as *F. nucleatum*. This incorrect identification was due to the Gram characteristics, which were determined with the off-line tests. Certain clostridia, e.g., *C. clostridioforme*, *C. hathewayi*, and *C. citroniae*, tend to stain Gram negative. Determination of susceptibility to special-potency antibiotic disks of vancomycin, kanamycin, and colistin is recommended to aid in determining the Gram characteristics (12).

As expected, the Vitek system cannot differentiate between species that are difficult to distinguish from each other phenotypically, e.g., *P. nigrescens* from *P. intermedia*, *F. naviforme* from *F. nucleatum*, and *P. harei* from *P. asaccharolyticus* (12). Therefore, the result should be given as a low-level discrimination result by including both species instead of one and, if possible, with a recommendation for the need for additional testing, e.g., cell morphology differentiation for *F. naviforme* and *F. nucleatum*. Cells of *F. nucleatum* are slender, with pointed ends, while cells of *F. naviforme* are boat shaped (12).

Generally, the ANC card is unreliable for identifying species belonging to the genus *Actinomyces* and certain Gram-positive anaerobic coccal (GPAC) species (Table 2). For those isolates included in the database, a reliable identification was obtained for *Bacteroides fragilis*, *Clostridium*, *Prevotella*, *Fusobacterium*, *Parvimonas*, *Finegoldia*, and *Peptostreptococcus* species and the species *Eggerthella lenta*. However, additional improvement of the database will be necessary for the identification of members of the genus *Prevotella*, some fusobacteria, and certain GPAC species (Table 2).

In a recent study by Blairon et al. (3), 196 clinical isolates were tested, some of which were not included in the Vitek ANC database. Those authors reported correct species- and genus-level identifications for 51.5% and 70.9% of isolates, respectively. In our study, the Vitek ANC card provided better results, with correct species- and genus-level identifications for 60.1% and 79.4% of isolates, respectively. This may be explained by the fact that the collection of strains in that study was not based on sequenced species. Those authors compared the identification obtained by the Vitek 2 ANC card with those obtained with the Microbial Identification System (MIS) complemented with necessary biochemical tests. Also, species not included in the database that were correctly classified as having no identification were not assigned as a correct result. Therefore, their interpretation of the results cannot be fully compared to those of our study.

In contrast to other validations of the ANC card, Mory et al. (15) and Rennie et al. (17) previously reported correct species-level identifications for 86.5% and 95.1% of isolates, respectively. However, species and genera not present in the database had been eliminated from their study isolates (15, 17). The lower level of correct identification obtained in our clinical study can be explained by the inclusion of test isolates that are not present in the database but are encountered in clinical material. With our recommendations, the correct species- and genus-level identifications would increase from 60.1% (181/301) and 79.4% (239/301) to 70.1% (211/301) and 81.1% (244/301) of isolates, respectively. These percentages of correct identification are still lower than those reported previously (15, 17). However, including the previously reported 169 excluded isolates (17), which were not included in the system, correct genus- and species-level identifications of 90.0% and 67.0% of isolates, respectively, would be obtained. These numbers are comparable to those from our study.

A high inoculum density is required for the inoculation of the Vitek 2 ANC card. Fast-growing bacteria are good candidates for Vitek 2 ANC identification. However, fastidious anaerobes such as *C. ureolyticus* and *B. wadsworthia* require several agar plates to obtain a sufficiently large inoculum. Cell morphology and Gram stain characteristics provide useful information to avoid a misidentification of the microorganism. Especially, the Gram stain characteristics of Gram-negative-staining isolates should be confirmed by use of special-potency disks (12).

The Vitek 2 ANC card provides a reliable identification for a limited number of relevant anaerobic bacterial species in a routine-diagnostic setting. The system performs inadequately

TABLE 2. Comparison of the Vitek 2 ANC card with 16S rRNA gene sequencing as a reference for the identification of 100 clinical isolates of species not included in the database

| Reference organism (total no. of isolates) | No. (%) of isolates | | | | |
|---|---|---|---|---|---|
| | Correct identification to the species level | Correct identification to the genus level | Low discrimination | No identification (%) | Misidentification at the species level |
| Gram-negative strains (37) | | | | | |
| *Bacteroides fragilis* sp. (9) | 0 | 7 | 1 | 0 | 9 |
| *Bacteroides* sp. (1) | 0 | 1 | 0 | 0 | 1 |
| *B. suis* (1) | 0 | 0 | 0 | 0 | 1 |
| *B. tectus* (1) | 0 | 0 | 0 | 0 | 1 |
| *B. salyersiae* (1) | 0 | 1 | 0 | 0 | 1 |
| *B. dorei* (5) | 0 | 5 | 1 | 0 | 5 |
| *Odoribacter* sp. (1) | 0 | 0 | 0 | 1 | 0 |
| *O. splanchnicus* (1) | 0 | 0 | 0 | 1 | 0 |
| *Parabacteroides* sp. (2) | 0 | 0 | 0 | 1 | 1 |
| *P. goldsteinii* (1) | 0 | 0 | 0 | 0 | 1 |
| *P. merdae* (1) | 0 | 0 | 0 | 1 | 0 |
| *Bilophila* sp. (5) | 0 | 0 | 0 | 0 | 5 |
| *B. wadsworthia* (5) | 0 | 0 | 0 | 0 | 5 |
| *Prevotella* sp. (8) | 0 | 8 | 0 | 0 | 8 |
| *P. nigrescens* (5) | 0 | 5 | 0 | 0 | 5 |
| *P. nanceiensis* (1) | 0 | 1 | 0 | 0 | 1 |
| *P. oris* (1) | 0 | 1 | 0 | 0 | 1 |
| *P. denticola* (1) | 0 | 1 | 0 | 0 | 1 |
| *Fusobacterium* sp. (2) | 0 | 2 | 0 | 0 | 2 |
| *F. naviforme* (2) | 0 | 2 | 0 | 0 | 2 |
| *Porphyromonas* sp. (2) | 0 | 0 | 0 | 1 | 1 |
| *P. somerae* (1) | 0 | 0 | 0 | 0 | 1 |
| *P. catoniae* (1) | 0 | 0 | 0 | 1 | 0 |
| *Campylobacter* sp. (3) | 0 | 0 | 1 | 1 | 2 |
| *C. rectus* (2) | 0 | 0 | 0 | 1 | 1 |
| *C. gracilis* (1) | 0 | 0 | 1 | 0 | 1 |
| *Dialister* sp. (1) | 0 | 0 | 0 | 0 | 1 |
| *D. pneumosintes* (1) | 0 | 0 | 0 | 0 | 1 |
| *Sutterella* sp. (1) | 0 | 0 | 0 | 1 | 0 |
| *S. stercoricanis* (1) | 0 | 0 | 0 | 1 | 0 |
| *Veillonella* sp. (3) | 0 | 2 | 1 | 0 | 3 |
| *V. parvula* (2) | 0 | 2 | 0 | 0 | 2 |
| *V. atypica* (1) | 0 | 0 | 1 | 0 | 1 |
| Gram-positive strains (63) | | | | | |
| Gram-positive anaerobic cocci (49) | 0 | 18 (36.7) | 7 | 11 (22.4) | 38 (77.6) |
| *Peptoniphilus octavius* (1) | 0 | 0 | 0 | 1 | 0 |
| *Peptostreptococcus stomatis* (1) | 0 | 0 | 0 | 0 | 1 |
| *Peptoniphilus ivorii* (3) | 0 | 0 | 1 | 0 | 3 |
| *Peptoniphilus lacrimalis* (2) | 0 | 1 | 1 | 0 | 2 |
| *Peptoniphilus harei* (17) | 0 | 16 (94.1) | 0 | 0 | 17 (100.0) |
| *Peptoniphilus gorbachii* (2) | 0 | 1 | 1 | 0 | 2 |
| *Peptococcus niger* (1) | 0 | 0 | 0 | 0 | 1 |
| *Anaerococcus* sp. (3) | 0 | 0 | 1 | 1 | 2 |
| *Anaerococcus murdochii* (4) | 0 | 0 | 1 | 3 | 1 |
| *Anaerococcus vaginalis* (6) | 0 | 0 | 0 | 2 | 4 |
| *Anaerococcus prevotii* (2) | 0 | 0 | 0 | 2 | 0 |
| *Anaerococcus tetradius* (2) | 0 | 0 | 0 | 2 | 0 |
| *Atopobium parvulum* (2) | 0 | 0 | 0 | 0 | 2 |
| *Atopobium rimae* (3) | 0 | 0 | 2 | 0 | 3 |
| *Actinomyces* sp. (5) | 0 | 4 | 0 | 0 | 5 |
| *A. georgiae* (1) | 0 | 1 | 0 | 0 | 1 |
| *A. odontolyticus* (2) | 0 | 1 | 0 | 0 | 2 |
| *A. graevenitzii* (1) | 0 | 1 | 0 | 0 | 1 |
| *A. gerencseriae* (1) | 0 | 1 | 0 | 0 | 1 |
| *Clostridium* sp. (7) | 0 | 6 | 1 | 0 | 7 |
| *C. citroniae* (1) | 0 | 0 | 0 | 0 | 1 |
| *C. innocuum* (1) | 0 | 1 | 1 | 0 | 1 |
| *C. hathewayi* (2) | 0 | 2 | 0 | 0 | 2 |
| *C. scindens* (1) | 0 | 1 | 0 | 0 | 1 |
| *C. disporicum* (1) | 0 | 1 | 0 | 0 | 1 |
| *C. coccoides* (1) | 0 | 1 | 0 | 0 | 1 |
| *Lactobacillus* sp. (1) | 0 | 0 | 1 | 0 | 1 |
| *L. catenaformis* (1) | 0 | 0 | 1 | 0 | 1 |
| *Robinsoniella* sp. (1) | 0 | 0 | 0 | 0 | 1 |

concerning species not present in the database. For certain species not included in the database (Table 2), the system would benefit from limiting the identification to the genus level. Improvement and extension of the database may result in more accurate identifications.

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