Comparison of the ATB 32A system and the Vitek ANI card in the identification of anaerobic bacteria

Article *in* Microbios · January 1994

CITATIONS
0

READS
55

3 authors, including:

José Gutiérrez-Fernández
University of Granada
472 PUBLICATIONS **4,016** CITATIONS
SEE PROFILE

Estrella Martin Mazuelos
Servicio Andaluz de Salud
182 PUBLICATIONS **3,818** CITATIONS
SEE PROFILE

Comparison of the ATB 32A system and the Vitek ANI card in the identification of anaerobic bacteria

J. Gutiérrez*, M. C. Nogales and E. Martin

Servicio de Microbiología, Hospital Universitario Virgen de Valme, Sevilla, Spain
(*Reprint address)

Key words: anaerobic bacteria, automated system, identifying, commercial systems, bacterial enzymes, micromethods

Abstract

The ATB 32A system (BioMerieux, France) and the ANI card (AutoMicrobic System, Vitek System Inc., U.S.A.) were evaluated for their reliability in identifying 54 Gram-negative bacilli, 22 Gram-positive cocci and 24 Gram-positive bacilli, isolated from clinical specimens. Four reference anaerobic strains were included and correctly identified. The ATB 32A system correctly identified 64% and the ANI card 50% of the anaerobes. Correct identification to genus level was achieved by the ATB 32A kit in 18% and by the Vitek system in 14% of strains. The ATB 32A system did not identify to the genus level in 18% and the ANI card in 36% of the anaerobes. Significant differences were not found between the results of the two systems. In the ATB 32A system the subjective factor was eliminated by using the automated reader included in the kit. The ATB 32A system did identify, both the genus and species of the anaerobes, in a greater number of cases. No noteworthy differences were encountered when the results of the two systems were evaluated.

Introduction

The important role of anaerobic micro-organisms in bacterial infections and susceptibility differences to antimicrobial agents (Johnston *et al.*, 1987; Sutter *et al.*, 1986), not only between genera but also between species, make identification of the organisms and evaluation of their antibiotic susceptibility patterns essential. Conventional identification systems by means of the biochemical reactions of growing bacteria (Bate, 1986) and gas-liquid chromatography (Holdeman *et al.*, 1977), are not only time consuming and laborious for identifying anaerobic bacteria, but they are also too expensive to be used in many laboratories.

At present, a number of commercial systems are available for the rapid identification of anaerobic bacteria without the need of anaerobic incubation. These systems, based on the detection of bacterial enzymes which act on chromogenic or modified conventional substrates, include RapID-ANA and ANIdent (Bate, 1986; Holdeman *et al.*, 1977; Appelbaum *et al.*, 1985; Dellinger and Moore, 1986; Harrison *et al.*, 1986; Head and Ratnam, 1988; Murdoch *et al.*, 1988; Stenson *et al.*, 1986; Summanen and Jousimies-Somer, 1988).

Micromethods based on enzyme reactions after 4 h incubation, such as the Anaerobe Identification (ANI) card and the ATB 32A system have also

been developed for the rapid identification of clinically significant anaerobic bacteria. This study compares the identification of anaerobic micro-organisms, isolated in our clinical laboratory, by the ANI card with that by the ATB 32A system.

Materials and methods

The micro-organisms were identified simultaneously by the conventional method (Holdeman *et al.*, 1977; Balows *et al.*, 1991), the ATB 32A system (BioMerieux, France) and the ANI card (AutoMicrobic System, Vitek System Inc., U.S.A.). The results of the commercial systems were compared with those obtained by conventional methods. Each result was replicated three times.

The micro-organisms (100 *in toto*) evaluated in this study were isolated from clinical samples sent to the Microbiology Laboratory of Valme University Hospital. The taxa were determined by a conventional anaerobic identification procedure, which requires Gram staining, measurement of aerotolerance, growth on selective and differential commercially prepared media (Oxoid, England), antibiotic susceptibility (kanamycin, 1,000 μg; vancomycin, 5 μg; colistin, 10 μg), a full battery of biochemical assays with carbohydrate fermentations, and gas-liquid chromatography. The bacteria isolated were: twenty *Bacteroides fragilis*, six *Bacteroides capillosus*, four *Bacteroides levii*, four *Bacteroides ureolyticus*, two *Bacteroides ovatus*, two *Bacteroides thetaiotaomicron*, two *Bacteroides eggerthii*, two *Prevotella intermedia*, two *Prevotella buccae*, two *Porphyromonas asaccharolytica*, two *Porphyromonas corporis* and two *Prevotella buccalis*; 22 Gram-positive cocci (eight *Peptostreptococcus micros*, six *Peptostreptococcus magnus*, six *Peptostreptococcus anaerobius* and two *Peptostreptococcus asaccharolyticus*), twenty *Clostridium* sp (sixteen *Clostridium perfringens*, two *Clostridium butyricum* and two *Clostridium septicum*), two *Fusobacterium necrophorum*, two *Fusobacterium varium*, two *Propionibacterium acnes* and two *Eubacterium* sp. The following reference strains were also included for quality control: *B. fragilis* ATCC 25285, *P. melininogenica* ATCC 25845, *C. perfringens* ATCC 13124 and *P. anaerobius* ATCC 27337.

ATB 32A system

The kit includes a plastic identification strip indented with 32 cupules of which 29 contain dehydrated substrates and the remaining three cupules can be employed for supplementary testing. The following reactions are detected with the ATB 32A strip: urease, arginine dihydrolase, alpha-galactosidase, beta-galactosidase, beta-galactosidase-6-phosphate, alpha-glucosidase, beta-glucosidase, alpha-arabinosidase, beta-glucuronidase, acidification of mannose and raffinose, glutamic acid decarboxylase, alpha-fucosidase, nitrate reduction, indole production, alkaline phosphatase, arginine arylamidase, proline arylamidase, leucyl-glycine arylamidase, phenylalanine arylamidase, leucine aryl-

amidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase.

An inoculum was prepared by making a suspension of a subculture on Columbia sheep blood agar (Oxoid) and placing the organisms in distilled water to a turbidity equal to a number 4 McFarland standard. According to the instructions of the manufacturer, 55 μl of the suspension should be dispensed into each cupule and incubated aerobically at 37°C for 4 h. Reactions were interpreted by an automated reader 5 min after adding reagents for the detection of indole, nitrate reduction and arylamidase reactions.

ANI card

This moulded plastic card includes 30 wells, 28 of which contain substrates to determine biochemical reactions. Twenty of these wells are for chromogenic and eight for modified conventional tests. The chromogenic substrates detect glycosidase, aminopeptidase, phosphatase and esterase production. Carbohydrate fermentation assays include acid production from glucose, trehalose, arabinose and xylose. Tests for triphenyl tetrazolium reduction, arginine dihydrolase and urease are also included.

Inocula were prepared from saline suspensions of subcultures on Columbia sheep blood agar and equal to a number 3 McFarland standard in turbidity. The cards were inoculated automatically following the guidelines established by the manufacturer and incubated at 37°C for 4 h. The cards were manually read in a reader module which allowed magnified visualization of the reactions. The results were then introduced into a computer along with the Gram stain characteristics, morphology of the bacteria and indole reactions.

The following criteria were established to compare the three methods of anaerobic microbial identification: (1) total correlation involving identical genus and species; (2) correlation with identical genus but a different species; and (3) correlation in which neither genus nor species correlated.

Statistical analyses

The qualitative variables were compared by the χ^2 test and the Ridit analysis (Fleiss, 1981).

Results

The results from comparison of Vitek ANI card and ATB 32A system with conventional methods were 50% and 64%, respectively, for the total correlation; 36% and 18%, respectively, for no correlation; and 14% and 18%, respectively, for correlation (Table 1). For Gram-negative bacilli a greater number of total correlation identifications were obtained by the ATB 32A system (48% *vs* 28% for the ANI card), while the ANI card yielded a higher no correlation percentage (48% *vs* 28% for the ATB 32A) as shown in Table 1.

Table 1 Identification of anaerobic micro-organisms by the Vitek ANI card and ATB 32A systems compared with a conventional method

Taxa	Number	Total correlation ATB/Vitek		Correlation ATB/Vitek		No correlation ATB/Vitek	
B. fragilis	20	6	12	6	2	8	6
B. levii	4	4	—	—	2	—	2
B. capillosus	6	4	2	—	—	2	4
B. ureolyticus	4	2	—	2	4	—	—
P. intermedia	2	2	—	—	2	—	—
B. ovatus	2	—	—	2	2	—	—
B. thetaiotaomicron	2	—	—	—	—	2	2
B. eggerthii	2	2	—	—	—	—	2
P. asaccharolyticus	2	—	—	—	—	2	2
P. corporis	2	—	—	2	—	—	2
P. buccalis	2	2	—	—	—	—	2
P. buccae	2	2	—	—	—	—	2
Subtotals	50	24	14	12	12	14	24
Percentage		48	28	24	24	28	48
P. micros	8	4	6	2	—	2	2
P. magnus	6	4	6	2	—	—	—
P. anaerobius	6	6	4	—	—	—	2
P. asaccharolyticus	2	2	2	—	—	—	—
Subtotals	22	16	18	4	—	2	4
Percentage		72.7	81.8	18.2		9.1	18.2
C. perfringens	16	16	14	—	—	—	2
C. septicum	2	—	—	2	2	—	—
C. butyricum	2	2	—	—	—	—	2
Subtotal	20	18	14	2	2	—	4
Percentage		90	70	10	10		20
Other taxa	8	6	4	—	—	2	4
Totals	100	64	50	18	14	18	36
Percentage		64	50	18	14	18	36

Taxa established by conventional identification procedures. Total correlation means identical genus and species; correlation indicates identical genus but belonging to different species; and no correlation means that neither genus nor species correlates. Other taxa include *Eubacterium, Fusobacterium* and *Propionibacterium*.

The ANI card was more successful in identifying *B. fragilis* isolates than the ATB 32A strip, while the ATB 32 A was more reliable for detecting *B. levii*. Neither system correctly identified *B. thetaiotaomicron* and *P. asaccharolytica* strains.

For Gram-positive cocci a greater number of total correlations were obtained by the Vitek ANI card (81.8% *vs* 72.7% for the ATB 32A) while the ATB 32A yielded an inferior no correlation percentage (9.1% *vs* 18.2% for the ANI card). Neither system correctly identified two strains of *P. micros* (Table 1).

The ATB 32A strip appeared to be more efficient in identifying members of the *Clostridium* genus (total correlation 90% *vs* 70% for ANI card) (Table 1). However, both systems proved to be capable of identifying *C. perfringens*. Neither system could specifically identify the two isolates of *C. septicum* and the two isolates of *C. butyricum* could not be identified within the genus *Clostridium* by the ANI card.

In conclusion the two commercial systems did not differ significantly, when compared with a conventional system (Ridit, $p > 0.10$).

Discussion

The ATB 32A system and the Vitek ANI card were recently introduced as rapid methods for the accurate identification of anaerobic bacteria. These systems are based on colorimetric tests incorporated into plastic strips or cards. Time-consuming supplementary pre-testing is not required and reactions are interpreted by an automated reader unit in the ATB 32A system. The reactions are read manually in the ANI card. The ATB 32A cupules are inoculated with a culture suspension equal to a number 4 McFarland standard.

The Vitek ANI card identification kit has been on the market for several years. This system requires that Gram stain and indole production results must be available before final identification. Since the chromogenic reactions of the ANI card are visually read, subjectivity may confuse results. One advantage of this system over the ATB 32A is that the inoculum need only equal a number 3 McFarland turbidity standard. Consequently, less growth is required on primary plates to inoculate the system.

The anaerobic bacteria most frequently isolated from our clinical specimens belonged to *B. fragilis* (20 strains) and *C. perfringens* (16 strains). Our laboratory correctly identified (at genus and species level without supplementary test or total correlation) 30% of the *B. fragilis* strains with the ATB 32A system and 60% with the ANI card. These results, 78%, 88%, and 88.7%, are lower than those reported by Kitch *et al.* (1989), Ruckdeschel *et al.* (1989) and Looney *et al.* (1990), respectively, for the ATB 32A system, and 82.8% noted by Schreckenberg *et al.* (1988) with the ANI card. The ATB 32A system

results for the remaining Gram-negative bacilli (95%) are lower than those reported by Looney *et al.* (1990); with the ANI card the results are lower (68%) than those reported by Schreckenberg *et al.* (1988). On the question as to whether misidentification of one species is more serious than of another, the ANI system is more useful in a clinical laboratory since it identified *B. fragilis* more often than did the ATB 32A system. The incorporation of a greater number of chromogenic substrates and a supplementary test into the identification kits may be necessary to improve on these results.

In our investigation, the ATB 32A system identified 100% of the *C. perfringens* isolates. A similar efficiency for *C. perfringens* was reported by other authors (Appelbaum *et al.*, 1985). The ANI card proved to be only slightly less efficient (14/16; 87.5%) in our study, which is slightly lower than results of others (Schreckenberger *et al.*, 1988), except for Mangeney *et al.* (1989), who found at 41% a diminished efficiency. Identification of anaerobic cocci was higher than that of Schreckenberger *et al.* (1988). Both systems identified 72.7% and 81.8% of these bacteria by the ATB 32A and ANI card, respectively.

The greatest number of micro-organisms incorrectly identified by both systems belonged to the Gram-negative bacilli group. The results for the Gram-negative bacilli coincided with those of other authors (Kitch and Appelbaum, 1989; Looney *et al.*, 1990; Schreckenberger *et al.*, 1988). Generally speaking, our identification results were lower than those reported by other workers. This is perhaps due to the fact that these authors (Kitch and Appelbaum, 1989; Looney *et al.*, 1990) used the ATB 32A system with ancillary tests which is not recommended by the manufacturer, since the ATB 32A system is marketed as a complete procedure. However, one must recognise its limitations and ancillary tests seem necessary. Indeed, it could be said that the ATB 32A system should not be used to identify anaerobes without ancillary tests. Results showing no significant differences between ATB 32A and the ANI card might be due to the substrate used for the test hazards, and different inocula and/or timing.

In conclusion, no noteworthy differences were encountered during the evaluation of the results from the two systems, and so testing of a larger number of strains is needed. In the ATB 32A kit the subjective factor was eliminated by the use of the automated reader, but correct identifications of around 64% and 50% by ANI card are not acceptable. The ancillary tests (Gram stain and indole reactions) are unnecessary as an adjunct to the ATB 32A system but their incorporation may increase reliability.

References

APPELBAUM P. C., Kaufmann C. S. and Depenbush J. W. 1985. Accuracy and reproducibility of a four-hour method for anaerobe identification. *J. clin. Microbiol.* **18** 614–21.

BALOWS A., Hausler W. J. Jr, Herreman K. L., Isenberg H. D. and Shadomy H. J. 1991. *Manual of Clinical Microbiology*. Fifth editn. American Society for Microbiology, Washington, D.C.

BATE G. 1986. Comparison of Minitek Anaerobe II, API Anident and RapID ANA system for identification of *Clostridium difficile*. *Am. J. clin. Path.* **85** 716—8.

DELLINGER C. A. and Moore L. V. 1986. Use of the RapID ANA system to screen for enzyme activities that differ among species of bile-inhibited *Bacteroides*. *J. clin. Microbiol.* **23** 289—93.

FLEISS J. L. 1981. *Statistical methods for rates and proportions*. Second editn. John Wiley and Sons, Baltimore.

HARRISON J., Dudley M. and Nauschuetz W. F. 1986. Identification of anaerobic bacteria using a rapid system that does not require anaerobic incubation. *J. Med. Technol.* **3** 45—8.

HEAD C. B. and Ratnam P. 1988. Comparison of the API ANIdent, API 20A, Minitek Anaerobe II and RapID ANA system for identification of *Clostridium difficile*. *J. clin. Microbiol.* **26** 144—6.

HOLDEMAN L. V., Cato E. P. and Moore W. E. C. 1977. Anaerobe laboratory manual, VPI anaerobe laboratory. Fourth editn. Virginia Polytechnic Institute and State University, Blacksburg.

JOHNSTON B. L., Edelstein M. A. C., Holloway E. Y. and Finegold S. M. 1987. Bacteriologic and clinical study of *Bacteroides ovis* and *Bacteroides buccae*. *J. clin. Microbiol.* **25** 491—3.

KITCH T. T. and Appelbaum P. C. 1989. Accuracy and reproducibility of the 4-hour ATB-32A method for anaerobe identification. *J. clin. Microbiol.* **27** 2509—13.

LOONEY W. J., Galluser A. J. C. and Modde H. K. 1990. Evaluation of the ATB 32A system for identification of anaerobe bacteria isolated from clinical specimens. *J. clin. Microbiol.* **28** 1519—24.

MANGENEY N., Dupeyron C. and Leluan G. 1989. Identification of *Clostridium*: comparison of three systems for anaerobes. Fourth European Congress of Clinical Microbiology, Nice.

MURDOCH D. A., Mitchelmore I. J. and Tabaqchali S. 1988. Identification of Gram-positive anaerobic cocci by use of system for detecting pre-formed enzymes. *J. med. Microbiol.* **25** 289—93.

RUCKDESCHEL G. and Pfaller P. 1989. Evaluation of a commercial identification system for anaerobes ATB 32A. Third European Congress of Anaerobic Bacteria and Infections, Munich.

SCHRECKENBERG P. C., Celig C. M. and Janda W. M. 1988. Clinical evaluation of the Vitek ANI card for identification of anaerobic bacteria. *J. clin. Microbiol.* **26** 225—30.

STENSON M. J., Lee D. T., Rosenblatt J. E. and Contezar J. M. 1986. Evaluation of the ANI-Ident system for identification of anaerobic bacteria. *Diagnost. Microbiol. Infect. Dis.* **5** 9—15.

SUMMANEN P. and Jousimies-Somer H. 1988. Comparative evaluation of RapID ANA and API 20A for identification of anaerobic bacteria. *Eur. J. clin. Microbiol. Infect. Dis.* **7** 771—5.

SUTTER V. L., Citron D. M., Edelstein M. A. C. and Finegold S. M. 1986. *Wadsworth anaerobic bacteriology manual*. Fourth editn. Star Publishing Co., Belmont, California.

Accepted 5 July 1994