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Evaluation of AN-Ident

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AN-Ident (Analytab Products, Inc., Plainview, N.Y.) is a ready-to-use system for anaerobe identification. It is based on the detection of constitutive preformed enzymes, is growth independent, and requires only 4 h of aerobic incubation. This micromethod was evaluated for its ability to identify anaerobic bacteria by using a conventional methodology as a reference. Of 265 clinical isolates, AN-Ident accurately identified 241 (91%) of the isolates to the species level and 259 (98%) of the isolates to the genus level, with limited supplemental testing needed (5%). The AN-Ident system performed well for the most common pathogens but less satisfactorily for infrequently isolated and/or asaccharolytic species; expansion and updating of the data base would be helpful. Although some color reactions were difficult to interpret, the commercial kit was easy to use.

The role played by anaerobes in the pathogenesis of severe bacterial infections is now well recognized (6). Infectious potential and antimicrobial susceptibility patterns usually are species and group specific (1). However, laboratory reporting is often delayed because most anaerobic infections are polymicrobial and many of the organisms grow slowly. Therefore, anaerobes must not only be successfully isolated from appropriate clinical specimens but they must also be identified as accurately and rapidly as possible.

Conventional biochemical testing of anaerobes (8, 9), together with gas-liquid chromatography (GLC), is considered the most accurate and reliable approach to identification (1). However, because these procedures are expensive and time-consuming and require special instrumentation, they are beyond the capabilities of most laboratories. The trend in clinical anaerobic bacteriology has been the use of commercially available miniaturized identification kits. The early systems (i.e., API 20 A [Analytab Products, Inc., Plainview, N.Y.], Minitek [BBL Microbiology Systems], and Anaerobe-Tek [Flow Laboratories, Inc., McLean, Va.]) were based primarily on carbohydrate fermentation tests as the conventional methodology (1, 3, 4, 7, 11, 15). Although the kits are simple and ready to use and require minimal labor, they are growth dependent and require a minimum of 24 h of anaerobic incubation. Moreover, they are associated with a high percentage of misidentifications, and supplemental GLC is often necessary for complete identification of asaccharolytic species (3, 4, 11, 15). Recently, systems such as API ZYM and AN-Ident (Analytab), RapID-ANA (Innovative Diagnostic System, Inc., Atlanta, Ga.), and the ATB Anaerobe ID System and ATB 32A (API System) have become available (1, 2, 4, 5, 7, 10, 11, 14–17). These products detect constitutively preformed enzymes, are growth independent, and require only 4 h of aerobic incubation. Since these identification systems are not dependent only on saccharolytic activity, many of the traditionally nonreactive anaerobic species are expected to be identified.

The purpose of this study was to evaluate the accuracy of the AN-Ident system for the identification of clinically encountered anaerobic bacteria by using conventional methods as a reference.

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MATERIALS AND METHODS

Strains. A total of 276 anaerobic bacteria were examined (Table 1). All organisms were fresh clinical isolates obtained from patients at the Pellegrin Hospital in Bordeaux.

Test method. Colonies from primary plates were subcultured both aerobically and anaerobically to determine purity and aerotolerance. Gram stain testing was performed on strict anaerobes. All isolates were identified by both the biochemical tests recommended by the Virginia Polytechnic Institute (VPI), Blacksburg (8, 9), and the manufacturer of the AN-Ident system. When necessary, to help resolve discrepancies in identification between the two methods, GLC was performed as described previously (8). The VPI manual update (9) was considered to be the definitive taxonomic source for this study.

AN-Ident system. The AN-Ident system consists of two rows of 10 microcupules containing dehydrated substances designed to test 21 biochemical reactions (Table 2). Kits were prepared and interpreted strictly according to the instructions of the manufacturer. Pure cultures of the organisms were inoculated on Columbia agar supplemented with 5% horse blood. Plates were incubated anaerobically in GasPak (BBL) jars at 37°C for 24 to 48 h. Inocula were prepared by harvesting the cells with a sterile cotton swab. Growth was suspended in 3 ml of sterile distilled water. The suspension was adjusted to a turbidity of a no. 5 McFarland standard. Strips were inoculated with a Pasteur pipette, placed in covered plastic trays, and then incubated in an air incubator for 4 h at 37°C. After incubation, spontaneous color reactions (seven glycosidase tests, phosphatase and indoxyl-acetate production, and arginine utilization) were read. The Kovacs reagent to detect indole production, cinnamaldehyde for the nine aminopeptidase tests, and hydrogen peroxide for catalase formation (in the α-glucosidase microcupule) were added prior to reading. The reactions were interpreted and recorded on a report sheet. Tests were assigned numerical values from which a seven-digit numerical code was generated. Identification of the isolates was determined through the AN-Ident Analytical Profile Index. When multiple-organism identifications with low confidence values (<95%) occurred, the supplementary tests specified by the manufacturer were carried out.

The AN-Ident supplementary tests were as follows: for *Bacteroides* spp., growth in 20% bile broth, gelatin, lipase, indole, esculin hydrolysis, and GLC; for other gram-nega-

TABLE 1. Organisms examined in this study

Organism (total no. of isolates)	No. of isolates
Bacteroides spp. (117)	
B. fragilis	31
B. ovatus	21
B. bivius	15
B. distasonis	14
B. vulgatus	12
B. thetaiotaomicron	6
B. buccae	3
B. capillosus	3
B. eggerthii	2
B. disiens	1
B. melaninogenicus	1
B. oralis	1
B. uniformis	1
Bacteroides spp.	6
Other gram-negative bacilli (5)	
Fusobacterium nucleatum	3
Capnocytophaga spp.	1
Megamonas hypermegas	1
Clostridium spp. (95)	
C. perfringens	53
C. difficile	20
C. baratii	6
C. bifermentans	3
C. tertium	3
C. ramosum	2
C. sordellii	2
C. sporogenes	2
C. paraputrificum	1
Clostridium spp.	3
Nonsporeforming gram-positive bacilli (24)	
Propionibacterium acnes	13
Propionibacterium freundenreichii	1
Bifidobacterium adolescentis	5
Bifidobacterium spp.	2
Eubacterium lentum	1
Eubacterium limosum	1
Actinomyces israelii	1
Cocci (35)	
Peptostreptococcus tetradius	9
Peptostreptococcus anaerobius	5
Peptostreptococcus magnus	5
Peptostreptococcus asaccharolyticus	4
Peptostreptococcus prevotii	3
Peptostreptococcus micros	1
Peptostreptococcus productus	1
Peptococcus niger	1
Streptococcus intermedius	2
Streptococcus morbillorum	1
Staphylococcus saccharolyticus	1
Veillonella parvula	2

tive bacilli, growth in 20% bile broth, lipase, H_2S in triple sugar iron, and GLC; for *Clostridium* spp., spore location, lecithinase, lipase, motility, gelatin, aerotolerance, and GLC; for nonsporeforming bacilli, nitrate and GLC; and for cocci, characteristic cellular morphology and GLC.

RESULTS

A total of 276 organisms were tested with the AN-Ident system and by the conventional VPI method. The identifications of 238 of these organisms concurred in both systems.

TABLE 2. AN-Ident reactions

Test code	Reaction
IND	Indole production
NGS	*N*-Acetyl-glucosaminidase
ADG	α-Glucosidase
ARB	α-Arabinofuranosidase
BDG	β-Glucosidase
FUC	α-Fucosidase
PHS	Phosphatase
GAL	α-Galactosidase
NPG	β-Galactosidase
INA	Indoxyl-acetate hydrolysis
ARG	Utilization of arginine
LEU	Leucine aminopeptidase
PRO	Proline aminopeptidase
PYR	Pyroglutamic acid arylamidase
TYR	Tyrosine aminopeptidase
ARL	Arginine aminopeptidase
ALA	Alanine aminopeptidase
HIS	Histidine aminopeptidase
PHA	Phenylalanine aminopeptidase
GLY	Glycine aminopeptidase
CAT	Catalase production

The 38 organisms giving discrepant results were analyzed by GLC (Table 3). Organisms were considered to be correctly identified when two of the three methods agreed with regard to genus and species.

GLC analysis did not resolve 11 discrepancies between the AN-Ident system and the VPI method. For seven strains, species identified either by the VPI method or with the AN-Ident system had a similar GLC pattern, including six strains of the *Bacteroides fragilis* group and one *Bifidobacterium* sp.; for four strains, the code number obtained with AN-Ident did not correspond to any identification (no match), and the chromatogram was consistent with several species. GLC analysis resolved 27 discrepancies between the AN-Ident system and the VPI method: AN-Ident provided the correct identification 3 times, and conventional biochemical testing provided the correct identification 24 times.

The overall performance of the AN-Ident system, as calculated from the 265 strains that were finally identified, is summarized in Table 4. The commercial system identified 241 (91%) strains to the species level and 18 (7%) strains to the genus level only, while 5 (2%) were misidentified and 1 (0.4%) gave no identification. Identifications to the genus level only were due to the inability of the system to assign the right species within the proper genus rather than an inability to assign a species designation. The rate of agreement between AN-Ident and the conventional methods varied depending on the bacterial group. The predominant gram-negative bacilli and clostridia were more often correctly identified than were nonsporeforming gram-positive bacilli and gram-positive cocci.

Identification of anaerobes with the AN-Ident system is shown in Table 5. All *Bacteroides* and *Clostridium* spp. identified were placed in the right genus. All 31 *Bacteroides fragilis* strains were correctly identified. Of the 56 strains belonging to the other species of the *Bacteroides fragilis* group, only 5 (9%) isolates, including the 2 *Bacteroides eggerthii* isolates tested, were incorrectly identified, but they were assigned to the proper group four times. Of 24 isolates belonging to the other *Bacteroides* spp., 20 (83%) were identified to species level; 3 strains were identified only to

TABLE 3. Discrepancies between AN-Ident and the VPI method

No. of isolates	Results obtained with (no. of isolates):		
	VPI	An-Ident	GLC consistent with:
1	*Bacteroides distasonis*	*Bacteroides ovatus*	ND[a]
5	*Bacteroides vulgatus*	*Bacteroides distasonis* (3) *Bacteroides ovatus* (2)	ND[a]
1	*Bifidobacterium magnum*[b]	*Bifidobacterium adolescentis*	ND[a]
2	*Clostridium sphenoides*[b]	No match[d]	ND[c]
1	*Clostridium bifermentans*	No match[d]	ND[c]
1	*Bifidobacterium bifidum*[b]	No match[d]	ND[c]
2	*Bacteroides eggerthii*	*Bacteroides bivius* (1) *Bacteroides thetaiotaomicron* (1)	*Bacteroides eggerthii*
1	*Bacteroides ovatus*	*Bacteroides uniformis*	*Bacteroides ovatus*
1	*Bacteroides thetaiotaomicron*	*Bacteroides ovatus*	*Bacteroides thetaiotaomicron*
1	*Bacteroides vulgatus*	*Bacteroides uniformis*	*Bacteroides vulgatus*
1	*Bacteroides bivius*	*Bacteroides oralis*	*Bacteroides bivius*
1	*Bacteroides capillosus*	No match[d]	*Bacteroides capillosus*
1	*Bacteroides disiens*	*Bacteroides buccae*	*Bacteroides disiens*
1	*Bacteroides oralis*	*Bacteroides buccae*	*Bacteroides oralis*
1	*Megamonas hypermegas*[b]	*Bacteroides fragilis*	*Megamonas hypermegas*
1	*Fusobacterium nucleatum*	*Streptococcus intermedius*	*Fusobacterium nucleatum*
1	*Clostridium perfringens*	*Clostridium histolyticum*	*Clostridium perfringens*
2	*Clostridium baratii*	*Clostridium innocuum* (1) *Clostridium subterminale* (1)	*Clostridium baratii*
1	*Propionibacterium freundenreichii*[b]	*Actinomyces odontolyticus*	*Propionibacterium* spp.
1	*Eubacterium lentum*	*Eubacterium limosum*	*Eubacterium lentum*
1	*Actinomyces israelii*	*Actinomyces odontolyticus*	*Actinomyces israelii*
4	*Peptostreptococcus tetradius*[e]	*Peptostreptococcus micros* (1) *Peptostreptococcus magnus* (1) *Peptostreptococcus asaccharolyticus* (1) *Staphylococcus saccharolyticus*[f] (1)	*Peptostreptococcus tetradius*
1	*Peptostreptococcus anaerobius*	*Streptococcus intermedius*	*Peptostreptococcus anaerobius*
1	*Peptostreptococcus productus*[b]	*Peptostreptococcus anaerobius*	*Peptostreptococcus productus*
1	*Peptococcus niger*[b]	*Peptostreptococcus magnus*	*Peptococcus niger*
1	*Bacteroides vulgatus*	*Bacteroides fragilis*	*Bacteroides fragilis*
1	*Bacteroides uniformis*	*Bacteroides ovatus*	*Bacteroides ovatus*
1	*Peptococcus niger*[b]	*Peptostreptococcus prevotii*	*Peptostreptococcus prevotii*

[a] Not differentiable because the species identified by both methods had the same pattern.
[b] Species not found in the AN-Ident data base.
[c] Not differentiable because the GLC pattern was consistent with several species.
[d] Profile number not found in the AN-Ident data base.
[e] Designated as "*Gaffkya anaerobia*" in the AN-Ident data base.
[f] Designated as "*Peptococcus saccharolyticus*" in the AN-Ident data base.

the genus level, and one *Bacteroides capillosus* strain gave no match. The single strain of *Megamonas hypermegas* (formerly *Bacteroides hypermegas* [9]), a species which is not listed in the AN-Ident code book, was misidentified as *Bacteroides fragilis*. A drawback of the AN-Ident system was misidentification of one of the three *Fusobacterium nucleatum* isolates as *Streptococcus intermedius*.

All but 1 of the 53 *Clostridium perfringens* isolates (98%) and all 20 *Clostridium difficile* isolates were correctly identified. Of the 19 strains belonging to the other *Clostridium* spp., 17 (90%) were identified to the species level. Of 22 isolates of nonsporeforming gram-positive bacilli, 19 (86%) were identified to the genus level and 21 (95%) were identified to the species level, including all 13 *Propionibacterium acnes* isolates and all 5 *Bifidobacterium adolescentis* isolates tested. The single isolate of *Propionibacterium freundenreichii*, a species that was not identified by AN-Ident, was classified as *Actinomyces odontolyticus*, as was the single isolate of *Actinomyces israelii*; the single isolate of *Eubacterium limosum* was misidentified as *Eubacterium lentum*. Of the 33 gram-positive cocci, 30 (91%) were placed in the appropriate genus and 26 (79%) were accurately identified to the species level. Four of the nine strains of *Peptostrep-*

TABLE 4. Overall performance of AN-Ident

Group	No. of isolates tested	No. (%) correctly identified to the:	
		Species level	Genus level
Gram-negative bacilli	116	105 (91)	114 (98)
Clostridia	92	89 (97)	92 (100)
Nonsporeforming gram-positive bacilli	22	19 (86)	21 (95)
Gram-positive cocci	33	26 (79)	30 (91)
Gram-negative cocci	2	2 (100)	
Total	265	241 (91)	257 (97)

TABLE 5. Identification of anaerobes with AN-Ident

Organism	No. of isolates tested	No. correctly identified to the:		No. not or incorrectly identified
		Species level	Genus level only	
Bacteroides fragilis	31	31		
Other *Bacteroides fragilis* group	56	51	5	
Other *Bacteroides* spp.	24	20	3	1
Megamonas hypermegas	1			1
Fusobacterium nucleatum	3	2		1
Capnocytophaga spp.	1	1		
Clostridium perfringens	53	52	1	
Clostridium difficile	20	20		
Other *Clostridium* spp.	19	17	2	
Propionibacterium spp.	14	13		1
Bifidobacterium adolescentis	5	5		
Eubacterium spp.	2	1	1	
Actinomyces israelii	1		1	
Peptostreptococcus spp.	28	22	4	2
Peptococcus niger	1			1
Streptococcus intermedius	2	2		
Streptococcus morbillorum	1	1		
Staphylococcus saccharolyticus	1	1		
Veillonella parvula	2	2		

tococcus tetradius (formerly *Gaffkya anaerobia*, as designated by AN-Ident) were misidentified, once as *Peptococcus saccharolyticus*, the former name of *Staphylococcus saccharolyticus*. The single isolate of *Peptococcus niger*, a species that was absent from the AN-Ident code compendium, was misidentified as *Peptostreptococcus magnus*, and one *Peptostreptococcus anaerobius* isolate was misidentified as *Streptococcus intermedius*. The two *Veillonella parvula* strains were correctly identified.

The VPI method failed to identify two strains of the *Bacteroides fragilis* group to the species level and misidentified one *Peptostreptococcus prevotii* isolate as *Peptococcus niger*.

DISCUSSION

This study compared the 4-h, growth-independent AN-Ident system with the conventional biochemical method for identification of anaerobic bacteria. The kit identified 91% of 265 fresh clinical isolates to the species level and 98% of fresh clinical isolates to the genus level, with supplemental tests used when necessary. In previous studies with the AN-Ident system, the rate of identification to the species level has varied from 75% (5, 11) to 93% (14). The performances of the other second-generation kits have been found to be within the same range (2, 5, 10, 11, 15). However, comparison between studies is difficult because of improvements that have possibly been made to the systems by the manufacturers, use of supplementary tests or not, and differences in the number of organisms tested and the selection criteria of isolates. Thus, as in this study, inclusion of many common anaerobes statistically increases the rate of correct identifications (2); moreover, the data interpretation for unusual species is limited when very few strains are available for study.

AN-Ident accurately identified about 100% of the most clinically important anaerobes such as *Bacteroides fragilis*, *Clostridium perfringens*, *Propionibacterium acnes*, and *Veillonella parvula*. These relatively nonfastidious, reactive, and common pathogens are usually identified by all micromethods, although they are identified less often by the first-generation kits (3, 11, 15). Among the gram-negative bacilli, most of the misidentifications generally occur with the *Bacteroides fragilis* group (5, 14), probably because these organisms are frequently isolated and phenotypically similar, especially *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, and *Bacteroides uniformis*. AN-Ident either correctly determined the most common species of the bile-resistant *Bacteroides* spp. or placed them in the proper group, as reported by Stenson et al. (14). Thus, incorrect species identification should have little clinical significance. The AN-Ident profile of uncommon species (e.g., *Bacteroides eggerthii*) might be insufficiently documented. AN-Ident distinguishes poorly between the closely resembling species *Clostridium difficile* and *Clostridium sporogenes* without a supplementary lipase reaction (4, 7, 14). By using this test, 100% of the strains were correctly identified, as described by Bate (4).

AN-Ident gave a lower rate of species identification for the remaining organisms: 90% for other *Clostridium* spp., 83% for other *Bacteroides* spp., and 76% for gram-positive cocci and non-*Propionibacterium acnes* nonsporeforming bacilli. Identification of these anaerobes has been difficult for all micromethods, particularly for the first-generation kits (3, 11, 15). With AN-Ident, misidentifications may be due to several factors. (i) The data base lacks a number of species, with many of them occasionally being isolated from human clinical specimens. Consequently, a strain that is not in the data base may be either recognized as an unidentifiable strain or assigned to the taxon that its microcode most closely resembles. (ii) The data base has not been updated to reflect the changing taxonomy of anaerobes, especially for the gram-positive cocci. The genus *Peptococcus* is now considered to be monospecific, consisting of the single

species *Peptococcus niger*; the genus *Peptostreptococcus* consists of 10 species, including 4 species that have been transferred from the genus *Peptococcus* (9). As in the VPI manual update (9), the classification of *Bacteroides* spp. is not actualized. Very recently, this genus has been restricted to the *Bacteroides fragilis*-related species, whereas moderately saccharolytic and asaccharolytic species are respectively placed in the newly created genera *Prevotella* and *Porphyromonas* (13). (iii) Identification of asaccharolytic anaerobes (e.g., *Bacteroides capillosus* and *Fusobacterium nucleatum* in this study) is based on few reactions; these organisms often give no match or are incorrectly identified (5, 11, 16). (iv) Similar organisms (e.g., *Bacteroides oralis* and *Bacteroides buccae*) are poorly differentiated by the kit. (v) Finally, some errors occur frequently with AN-Ident, such as the serious misidentification of *Actinomyces israelii* as *Actinomyces odontolyticus* (14).

The AN-Ident system was very simple to use. As with other second-generation kits, all of the following problems related to bacterial growth are avoided: the need to provide anaerobiosis, time consumption, and contamination risk. However, AN-Ident requires a heavy inoculum, which must be prepared from at least one blood agar plate with confluent growth. This often added 24 h to the total identification time. Moreover, such an inoculum was difficult to obtain with slow-growing organisms. This may explain the poor performance of the system with anaerobes such as fusobacteria. Borderline reactions occurred frequently, especially with the aminopeptidase tests. The number of species-level identifications can be increased by performing additional tests. Many of these tests (i.e., spore location, motility, and aerotolerance) are performed routinely. However, the need for further conventional tests diminishes the usefulness and efficiency of the micromethods. With AN-Ident, the number of organisms requiring supplemental tests was about 5%. These consisted mostly of clostridia, for which lipase and lecithinase reactions were helpful, as indicated by Stenson et al. (14). GLC was not needed for correct classification to the genus level of most gram-positive bacilli, as it was with the first-generation kits (11, 14).

In conclusion, the AN-Ident system provided a rapid and accurate identification of the most frequently isolated anaerobes, with limited supplemental testing needed. The availability of the kit greatly increases the ability of smaller laboratories to identify anaerobes. However, the data base of AN-Ident requires significant expansion and updating, and additional studies that include unusual anaerobes are needed to further delineate and to improve the identification of these organisms by the kit.

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