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Comparative Evaluation of Three Identification Systems for Anaerobes

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The accuracy of two new 4-h identification systems for anaerobes, the AN-IDENT (Analytab Products, Plainview, N.Y.) and the RapID ANA (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) was compared with that of the API 20A system (Analytab Products). A total of 132 clinical isolates were tested in each of the three systems. The overall accuracies at the genus and species level for the three systems were: API 20A, 68.9 and 56.8%, respectively; AN-IDENT, 90.2 and 73.5%; and RapID ANA, 93.9 and 81.8%. Improved identification of anaerobes with the AN-IDENT and the RapID ANA systems was observed for isolates of the genus *Fusobacterium*, *Clostridium* species other than *Clostridium perfringens*, non-spore-forming bacilli, and isolates of the genus *Peptostreptococcus*. Reproducibility studies demonstrated that the results of the individual test reactions in all three identification systems were reproducible when the interpretive guidelines of the manufacturer were followed precisely.

Although anaerobes are recognized as a common cause of significant infections and sophisticated techniques for the recovery of these organisms are now available in most clinical laboratories (2), the identification of anaerobic isolates with commercially available systems is frequently slow and inaccurate. Commercially prepared identification systems such as API 20A (Analytab Products, Plainview, N.Y.) and Minitek (BBL Microbiology Systems, Cockeysville, Md.) have replaced prereduced anaerobically sterilized biochemical tests in many clinical laboratories. However, these miniaturized commerical tests do not accurately identify most anaerobic species except *Bacteroides fragilis* and some *Clostridium* species (1, 3, 4, 6).

The purpose of this study was to evaluate two new identification systems the AN-IDENT (Analytab Products) and the RapID ANA (Innovative Diagnostic Systems, Inc., Atlanta, Ga.), which offer an attractive alternative to systems such as API 20A and Minitek. Whereas the latter two systems consist primarily of carbohydrate fermentation reactions, the reactivity of which are growth dependent, the AN-IDENT and RapID ANA systems measure the activity of preformed enzymes in reactions that are completed after incubation in air for only 4 h. Thus, these two new systems are rapid, require no anaerobic incubation, and are growth independent. Additionally, because these systems incorporate biochemical reactions not previously used for the identification of anaerobes, the range of organisms that can be identified might be extended. Therefore, we compared the identification accuracy of the AN-IDENT and RapID ANA systems with that of the API 20A system.

MATERIALS AND METHODS

Test organisms. A total of 132 anaerobes were isolated in the Barnes Hospital Clinical Microbiology Laboratory, St. Louis, Mo., and tested in this study. The organisms were tested either immediately after recovery from clinical specimens or after storage at −80°C. Frozen strains were used to test less frequently isolated organisms. All frozen strains were subcultured a minimum of three times on nonselective blood agar before inoculation into the identification systems. The organisms and number of isolates that were tested are listed in Table 1. The identification of each isolate was determined with the PRAS II system (Scott Laboratories, Inc., Fiskeville, R.I.) and by gas-liquid chromatography. All discrepancies between these identifications and those obtained with the commercial miniaturized systems were resolved by conventional tests recommended by Virginia Polytechnic Institute, Blacksburg (5). During the course of this study, gas-liquid chromatography and additional biochemical tests were not used to supplement the three commerical test systems.

Test method. A pure culture of each organism was prepared on nonselective media and used to inoculate the three identification systems according to the recommendations of the manufacturers.

(i) API 20A. For the API 20A system the test inoculum was suspended in 4 ml of Lombard-Dowell broth, adjusted to a turbidity of a McFarland 3 standard, and inoculated into the test strips. The following 21 biochemical reactions were determined: indole production, catalase, hydrolysis of urea, esculin, and gelatin, and fermentation of glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melizitose, raffinose, sorbitol, rhamnose, and trehalose. After the API 20A strips were incubated in an anaerobic chamber at 35°C for 24 h, bromcresol purple was added to microtubes containing carbohydrates, xylene and then Ehrlich reagent was added for the indole reaction, and hydrogen peroxide was added for the catalase reaction. The individual reactions were determined, numerical values were assigned to the positive and negative tests, a 7-digit profile number was generated, and the identification was determined by the API Analytical Profile Index.

(ii) AN-IDENT. For the AN-IDENT system the test inoculum was suspended in 2.5 ml of sterile distilled water, adjusted to a turbidity of a McFarland 5 standard, and inoculated into the test strips. The following 21 biochemical reactions were determined: indole production, catalase, utilization of arginine, hydrolysis of indoxyl-acetate, and activity of *N*-acetyl-glucosaminidase, α-glucosidase, α-ara-

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TABLE 1. Anaerobes tested in three identification systems

Organism	No. of isolates
Bacteroides fragilis	15
B. thetaiotaomicron	7
B. ovatus	6
B. vulgatus	6
B. bivius	3
B. ureolyticus	2
B. melaninogenicus	2
B. capsillosus	1
B. uniformis	1
Fusobacterium nucleatum	11
F. necrophorum	1
Actinomyces odontolyticus	5
A. meyeri	1
A. viscosus	1
Propionibacterium acnes	10
Eubacterium lentum	3
E. limosum	1
Bifidobacterium breve	1
Lactobacillus catenaforme	1
Clostridium difficile	8
C. innocuum	5
C. perfringens	4
C. clostridiiforme	4
C. butyricum	3
C. ramosum	3
C. bifermentans	2
C. paraputrificum	2
C. cadaveris	2
C. histolyticum	1
C. septicum	1
Peptococcus prevotii	3
P. saccharolyticus	3
P. asaccharolyticus	1
Peptostreptococcus micros	4
P. anaerobius	2
P. magnus	1
Veillonella parvula	5

binosidase, β-glucosidase, α-fucosidase, phosphatase, α-galactosidase, β-galactosidase, leucine aminopeptidase, proline aminopeptidase, pyroglutamic acid arylamidase, tyrosine aminopeptidase, arginine aminopeptidase, alanine aminopeptidase, histidine aminopeptidase, phenylalanine aminopeptidase, and glycine aminopeptidase. After the identification tests were incubated for 4 h at 35°C in an air incubator, the reactions were interpreted according to the instructions of the manufacturer. Kovacs reagent for the indole reaction, hydrogen peroxide for the catalase reaction, and cinnamaldehyde reagent for the aminopeptidase reactions were added before these reactions were interpreted. Identification of the isolates was aided by the AN-IDENT Analytical Profile Index after assigning numerical values to the test reactions and generating a 7-digit numerical code.

(iii) RapID ANA. For the RapID ANA system the test inoculum was suspended in 1.0 ml of the Innovative Diagnostic Systems inoculation salt solution, adjusted to a turbidity of a McFarland 3 standard, and inoculated into the test panels. The following 18 biochemical reactions were determined: reduction of triphenyltetrazolium, arginine utilization, trehalose fermentation, indole production, and the activity of phosphatase, *N*-acetyl-glucosaminidase, β-galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, α-galactosidase, leucine aminopeptidase, proline aminopeptidase, serine aminopeptidase, arginine aminopeptidase, phenylalanine aminopeptidase, glycine aminopeptidase, and pyrrolidonyl aminopeptidase. After the identification tests were incubated for 4 h in an air incubator at 35°C, the reactions were interpreted according to the instructions of the manufacturer. *p*-Dimethylaminocinnamaldehyde reagent for the indole reaction and RapID ANA reagent for the detection of β-naphthylamine released from the aminopeptidase reactions were added to the reaction chambers before the tests were interpreted. The identification of the isolates was determined with the aid of the RapID ANA Code Compendium after the reactions were assigned numerical scores and converted into a 6-digit code.

Reproducibility studies. Five organisms were tested in each identification system on three consecutive days to determine the reproducibility of the individual biochemical reactions. The organisms used were *B. fragilis*, *Bacteriodes ovatus*, *Clostridium perfringens*, *Clostridium histolyticum*, and *Actinomyces odontolyticus*.

RESULTS AND DISCUSSION

Identification of 132 anaerobic isolates in the three test systems is summarized in Table 2. More anaerobes were identified to the species and genus levels with the AN-IDENT and RapID ANA systems than with the API 20A system. The major reason for this improvement was that fewer low-selectivity identifications (i.e., failure to discriminate between two or more choices) were reported. The specific results for each identification system with the different isolates are presented in Tables 3 to 5.

API 20A. Although 14 of 15 *B. fragilis* isolates were identified correctly with the API 20A, 8 of the 13 *Bacteroides thetaiotaomicron* and *B. ovatus* isolates were misidentified at the species level because the salicin fermentation reaction was inaccurate. This is the key reaction for differentiating between these two species. None of the 12 *Fusobacterium* isolates were identified because the only positive reaction with most of these organisms was indole production. The resultant code did not discriminate among the *Fusobacterium* species and *Bacteroides asaccharolyticus*. Although a Gram stain can frequently separate these two groups of organisms, the precise genus and species identification could not be made without additional tests. Although all *C. perfringens* isolates were identified, only 19 (61%) of

TABLE 2. Identification of anaerobes with API 20A

Organism	No. of isolates				
	Tested	Correct to:		With low selectivity	With no or incorrect identification
		Species level	Genus level only		
Bacteroides fragilis	34	23	10	1	0
Bacteroides spp.	9	6	1	1	1
Fusobacterium spp.	12	0	0	12	0
Clostridium perfringens	4	4	0	0	0
Clostridium spp.	31	19	4	5	3
Actinomyces spp.	7	1	0	4	2
Propionibacterium spp.	10	9	1	0	0
Eubacterium spp.	4	4	0	0	0
Bifidobacterium spp.	1	0	0	0	1
Lactobacillus spp.	1	0	0	1	0
Peptococcus spp.	7	4	0	3	0
Peptostreptococcus spp.	7	0	0	7	0
Veillonella spp.	5	5	0	0	0

TABLE 3. Identification of anaerobes with AN-IDENT

Organism	No. of isolates				
	Tested	Correct to:		With low selectivity	With no or incorrect identification
		Species level	Genus level only		
Bacteroides fragilis	34	27	4	0	3
Bacteroides spp.	9	8	0	0	1
Fusobacterium spp.	12	7	5	0	0
Clostridium perfringens	4	4	0	0	0
Clostridium spp.	31	18	10	1	3
Actinomyces spp.	7	6	0	0	1
Propionibacterium spp.	10	6	2	0	2
Eubacterium spp.	4	4	0	0	0
Bifidobacterium spp.	1	0	1	0	0
Lactobacillus spp.	1	1	0	0	0
Peptococcus spp.	7	5	0	0	2
Peptostreptococcus spp.	7	7	0	0	0
Veillonella spp.	5	5	0	0	0

the other *Clostridium* isolates were completely identified. Additionally, most of the non-spore-forming gram-positive bacilli, with the exception of *Propionibacterium* and *Eubacterium* spp., required gas-liquid chromatography for correct classification at the genus level. Isolates of *Peptostreptococcus magnus*, *Peptococcus prevotii*, *Peptostreptococcus anaerobius*, and *Peptostreptococcus micros* were generally nonreactive in the API 20A system and thus could not be differentiated. The only gram-positive cocci that were identified with this system were three isolates of *Peptococcus saccharolyticus* (positive reactions for fermentation of glucose, glycerol, and mannose and for catalase production) and one isolate of *P. asaccharolyticus* (positive indole reaction). All *Veillonella* isolates were identified by their Gram-stain appearance and positive catalase reaction. All other API 20A reactions were negative for *Veillonella* isolates.

AN-IDENT. The test results with the AN-IDENT are presented in Table 3. Of 34 *B. fragilis* group isolates, 27 (79%), including 10 of 13 *B. thetaiotaomicron* and *B. ovatus* isolates, were identified. The arginine aminopeptidase and histidine aminopeptidase reactions separated *B. thetaiotaomicron* (both positive) from *B. ovatus* (both negative). All 12 *Fusobacterium* isolates were identified at the genus level, including 7 isolates at the species level. *Fusobacterium nucleatum* and *Fusobacterium necrophorum* were generally separated by the arginine aminopeptidase reaction (positive for *F. nucleatum*) and alkaline phosphatase reaction (positive for *F. necrophorum*). Although the number of *Clostridium* isolates identified at the species level was approximately the same for both the API 20A and AN-IDENT systems, more organisms were identified at the genus level with AN-IDENT. Likewise, 20 (87%) of 23 isolates of non-spore-forming gram-positive bacilli were identified with AN-IDENT at the genus level. Thus, gas-liquid chromatography would not be needed for the identification of most gram-positive bacilli. Whereas most of the gram-positive cocci were unable to ferment the carbohydrates in the API 20A system and did not hydrolyze the glycosidic substrates in the AN-IDENT system, the isolates possessed a number of aminopeptidases that permitted their accurate identification at the species level. In addition, all five *Veillonella* isolates were identified correctly.

TABLE 4. Identification of anaerobes with RapID ANA

Organism	No. of isolates				
	Tested	Correct to:		With low selectivity	With no or incorrect identification
		Species level	Genus level only		
Bacteroides fragilis	34	29	3	0	2
Bacteroides spp.	9	8	1	0	0
Fusobacterium spp.	12	10	2	0	0
Clostridium perfringens	4	4	0	0	0
Clostridium spp.	31	21	7	1	2
Actinomyces spp.	7	4	2	0	1
Propionibacterium spp.	10	10	0	0	0
Eubacterium spp.	4	4	0	0	0
Bifidobacterium spp.	1	0	1	0	0
Lactobacillus spp.	1	1	0	0	0
Peptococcus spp.	7	5	0	0	2
Peptostreptococcus spp.	7	7	0	0	0
Veillonella spp.	5	5	0	0	0

TABLE 5. Reproducibility of three anaerobic identification systems

Organism	No. of reproducible tests/total tests[a]		
	API 20A	AN-IDENT	RapID ANA
B. fragilis	21/21	21/21	18/18
B. ovatus	21/21	21/21	18/18
C. perfringens	21/21	18/21[b]	17/18[c]
C. histolyticum	21/21	21/21	17/18[d]
A. odontolyticus	21/21	21/21	18/18

[a] The percentages of tests that were reproducible were: API 20A, 100 (105/105); AN-IDENT, 97.1 (102/105); RapID ANA, 97.8 (88/90).
[b] Nonreproducible tests: β-glucosidase, α-galactosidase, indoxyl-acetate.
[c] Nonreproducible test: α-galactosidase.
[d] Nonreproducible test: triphenyltetrazolium.

RapID ANA. The test results with the RapID ANA system are presented in Table 4. As was observed with the AN-IDENT system, the majority of *Bacteroides* isolates (37 of 43) were identified at the species level, including isolates in the *B. fragilis* group as well as other *Bacteroides* species. *B. thetaiotaomicron* and *B. ovatus* were differentiated with the arginine aminopeptidase reaction (positive for *B. thetaiotaomicron*) and fermentation of trehalose (positive for *B. ovatus*). Of 12 *Fusobacterium* isolates, 10 were identified at the species level with RapID ANA, a performance better than with AN-IDENT. Identification of *Clostridium* species was also slightly better with RapID ANA. All eight isolates of *Clostridium difficile* were identified with RapID ANA, whereas seven were classified as *Clostridium* species with AN-IDENT because the system failed to separate *C. difficile* and *Clostridium sporogenes*. Of 23 isolates of non-spore-forming gram-positive bacilli, 22 (96%) were identified at the genus level with RapID ANA, a performance slightly better than with AN-IDENT. As with AN-IDENT, 17 (89%) of 19 anaerobic cocci were identified at the species level with RapID ANA.

Test reproducibility. Reproducibility of the individual tests in the three systems is summarized in Table 5. The results for the identification systems were highly reproducible when the instructions of the manufacturers were followed precisely. The interpretation of the color reactions with the AN-IDENT and RapID ANA systems initially was difficult.

However, this was resolved with the help of the technical representatives of the manufacturers.

In summary, identification of common anaerobic isolates was possible with both the AN-IDENT and RapID ANA systems. The systems were able to identify the majority of isolates of the genuses *Bacteroides* and *Clostridium*, nonspore-forming bacilli, and anaerobic cocci, including those species that generally failed to react in the API 20A system. In addition, the former two systems were incubated in air for only 4 h. Thus, the results were obtained rapidly without the need for special incubation conditions. Furthermore, gas-liquid chromatography was not needed for the differentiation of most gram-positive bacilli. Based upon the results presented in this study, we believe the AN-IDENT and RapID ANA systems represent significant improvements in the identification of clinically significant anaerobic organisms.

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