JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1985, p. 122–126
0095-1137/85/010122-05$02.00/0

Vol. 21, No. 1

Comparison of PRAS II, RapID ANA, and API 20A Systems for Identification of Anaerobic Bacteria

NATALIE O. KARACHEWSKI,[1] EVELYN L. BUSCH,[2] AND CAROL L. WELLS[2]*

Departments of Medical Microbiology[1] and Laboratory Medicine and Pathology,[2] University of Minnesota, Minneapolis 55455

Received 29 June 1984/Accepted 26 September 1984

This study evaluated the PRAS II, RapID ANA, and API 20A systems for the identification of anaerobic bacteria. A total of 80 isolates (68 fresh clinical isolates and 12 stock cultures) were examined and included 25 *Bacteriodes* spp., 7 *Fusobacterium* spp., 12 *Clostridium* spp., 2 *Veillonella* spp., 16 gram-positive cocci, and 18 gram-positive nonsporeforming bacilli. All isolates were initially identified by the procedures outlined in Holdeman et al. (ed.), *Anaerobe Laboratory Manual*, Virginia Polytechnic Institute and State University, Blacksburg, Va., 1977; identifications from the PRAS II, RapID ANA, and API 20A systems were compared with these initial identifications. If no supplemental tests were required, the RapID ANA and API 20A systems had incubation times of 4 and 24 h, respectively; the PRAS II system generally required 2 to 5 days of incubation, depending on the growth rate of the isolate. PRAS II identified 74% correct to species level, 14% correct to genus only, and 6% incorrect; 6% could not be identified. PRAS II data were reevaluated according to a revised data base that was provided after completion of the study; PRAS II (revised) identified 82% correct to species, 12% correct to genus only, and 6% incorrect. RapID ANA identified 62% correct to the species level, 28% correct to genus only, and 10% incorrect. API 20A identified 71% correct to the species level, 10% correct to genus only, and 3% incorrect; 16% could not be identified. The API 20A is a more established system for identification of anaerobic bacteria; PRAS II and RapID ANA appear to be promising new methods for the identification of anaerobic bacteria.

The role of anaerobic bacteria in human diseases has been well documented (2, 4). Because of the potential severity of infections caused by anaerobic bacteria, it is important to isolate and identify them rapidly and accurately. Although Virginia Polytechnic Institute (VPI) methodology (7), utilizing prereduced anaerobically sterilized (PRAS) biochemicals coupled with gas chromatographic (GLC) analysis of short-chain fatty acid metabolites, is considered an accurate and reliable identification system for anaerobic bacteria, these procedures are too cumbersome, time consuming, and costly for many laboratories.

The recent trend in clinical anaerobic bacteriology has been the development of simple, rapid, micromethod identification systems which would permit laboratories with limited facilities to identify clinically important anaerobic bacteria. The purpose of this study was to evaluate the accuracy of three commercially available systems for the identification of clinically significant anaerobic bacteria. The three systems studied were PRAS II, a computer-assisted system with PRAS tube biochemicals generally requiring a 2- to 5-day anaerobic incubation, RapID ANA, an assay system detecting preformed bacterial enzymes after a 4-h aerobic incubation, and API 20A, a system containing dehydrated biochemical substrates in cupules on a plastic strip incubated anaerobically for 24 h.

(Part of this work was presented at the 84th Annual Meeting of the American Society for Microbiology [N. O. Karachewski, E. L. Busch, and C. L. Wells, Abstr. Annu. Meet. Am. Soc. Microbiol., 1984, C148, p. 261]).

MATERIALS AND METHODS

Bacterial strains. Eighty isolates of anaerobic bacteria, representing a wide range of clinically significant species, were used in this study. A total of 68 recent clinical isolates were kindly provided by the Clinical Microbiology Laboratory of the University of Minnesota Hospitals, Minneapolis; these isolates were essentially sequential clinical isolates, and no attempt was made to select specific bacterial species. (These organisms were not held in storage, but were taken directly from the agar plates used by the clinical laboratory to check the purity of each isolate.) Twelve stock cultures were kindly provided by the Wisconsin State Laboratory of Hygiene, Madison. These latter stock cultures were tested near completion of the study and were chosen to represent clinically important anaerobes that by happenstance were not encountered in patient specimens.

Inoculum preparation and initial testing. All isolates were initially assigned code numbers and were tested in each system as unknowns. Cultures of each isolate were grown on freshly prepared supplemented sheep blood agar (S-SBA) containing 5% sheep blood (GIBCO Laboratories, Madison, Wisc.), 4% Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.), 0.5% yeast extract (Difco Laboratories, Detroit, Mich.), and 0.05% hemin–0.1% vitamin K solution (Carr Scarborough Microbiologicals, Inc., Decatur, Ga.). The S-SBA plates were incubated at 35°C in either an anaerobic glove box (Forma scientific anaerobic system; Mallinckdrodt, Inc., Marietta, Ohio) or an anaerobe jar until adequate growth developed, usually 48 to 72 h. Pure growth on S-SBA was the inoculum source for each identification system. Cellular and colonial characteristics on S-SBA initially recorded for each isolate included gram reaction and microscopic morphology, colonial morphology, hemolytic reaction, pigment production, and fluorescence of colonies upon exposure to UV light (365 nm). Colonies were examined for catalase production by using 15% H_2O_2 in Tween 80. Oxygen tolerance was determined by incubating an inoculated, unsupplemented sheep blood agar plate (GIBCO) in 5

* Corresponding author.

to 10% CO_2 at 35°C for 48 h. In addition, GLC was used to analyze short-chain fatty acid metabolic end products (7).

The above-mentioned characteristics were recorded for all 80 isolates and were then available for use with each identification system. Depending on the isolate, the VPI, PRAS II, API 20A, and RapID ANA systems required one or more of these characteristics for identification; the only exception was that RapID ANA did not have GLC results in its data base. The purity and viability of the inocula prepared for each system were routinely checked by Gram stain and by subculture to S-SBA (incubated anaerobically at 35°C for a minimum of 48 h) and to sheep blood agar (incubated in 5 to 10% CO_2 at 35°C for 48 h). Instructions and recommendations of individual manufacturers were closely followed in working with each system, and any minor modifications are noted below. Supplemental tests, according to the instructions of the manufacturer, were routinely performed to obtain the most accurate and specific identification possible with the data base of each system.

VPI methodology. Microscopic and macroscopic morphology, biochemical reactions in PRAS media, and GLC analysis of metabolic end products were used to identify all isolates by VPI criteria (7). Isolates in the genera *Lactobacillus* and *Bifidobacterium* were not identified to the species level, but only as *Lactobacillus* sp. and *Bifidobacterium* sp. Identifications obtained by VPI methods were considered correct for the purpose of comparison with each of the three commercial systems evaluated.

The 80 isolates included *Bacteroides distasonis* (1), *Bacteroides fragilis* (3), *Bacteroides ovatus* (2), *Bacteroides thetaiotaomicron* (1), *Bacteroides vulgatus* (3), *Bacteroides asaccharolyticus* (3), *Bacteroides bivius* (2), *Bacteroides melaningogenicus* subsp. *intermedius* (2), *Bacteroides melaninogenicus* subsp. *melaninogenicus* (3), *Bacteroides ochraceus* (1), *Bacteroides oralis* (2), *Bacteroides ruminicola* (2), *Bacteroides ureolyticus* (1), *Fusobacterium* sp. (1), *Fusobacterium mortiferium* (1), *Fusobacterium necrophorum* (1), *Fusobacterium nucleatum* (3), *Fusobacterium varium* (1), *Clostridium* sp. (1), *Clostridium cadaveris* (1), *Clostridium clostridiiforme* (2), *Clostridium difficile* (2), *Clostridium innocuum* (1), *Clostridium perfringens* (2), *Clostridium septicum* (1), *Clostridium sordellii* (1), *Clostridium tetani* (1), *Peptococcus* sp. (1), *Peptococcus asaccharolyticus* (2), *Peptococcus magnus* (3), *Peptococcus prevotii* (3), *Peptococcus saccharolyticus* (1), *Peptostreptococcus anaerobius* (2), *Peptostreptococcus micros* (1), *Streptococcus constellatus* (1), *Streptococcus intermedius* (2), *Veillonella parvula* (2), *Actinomyces* sp. (1), *Actinomyces odontolyticus* (1), *Actinomyces viscosus* (1), *Bifidobacterium* sp. (2), *Eubacterium lentum* (2), *Lactobacillus* sp. (4), *Propionibacterium* sp. (1), *Propionibacterium acnes* (5).

PRAS II system. PRAS II (Scott Laboratories, Fiskeville, R.I.) is a computerized anaerobe identification system with a data bank based on biochemical reactions as listed in the VPI manual (7). Three different computer programs are available and are referred to as Prescreen, AS1, and AS2. PRAS II broth media are distributed in glass test tubes (16 by 80 mm) with rubber septum stoppers which can be inoculated without the use of gas cannula equipment. Over 30 peptone-yeast-carbohydrate broths are available as well as biochemicals for the determination of various other reactions, including ammonia production from arginine, bile stimulation, esculin hydrolysis, gas production, gelatin hydrolysis, indole production, milk clot or digestion or both, nitrate reduction, and starch hydrolysis.

The Prescreen program was used to initially categorize each test isolate into one of five possible groups (cocci, *Bacteroides*, *Fusobacterium*, gram-positive nonsporeforming [NSF] rods, and *Clostridium*), based on microscopic and colonial morphology, differential susceptibility to vancomycin (5 μg), colistin (10 μg), and kanamycin (1,000 μg) (all antibiotic disks were obtained from BBL), metabolic end products determined by GLC, catalase production, growth in bile (performed only on gram-negative bacilli), and spore heat tests (performed only on gram-positive bacilli). A chopped-meat–glucose broth was initially inoculated with a colony of the test isolate from the S-SBA plate and incubated at 35°C until the appropriate turbidity was reached (≥3 McFarland). A specific set of biochemical broths, as listed in the PRAS II Ana-Stat Manual, 2nd ed., for each of the five Prescreen group categories, was inoculated with the turbid chopped-meat–glucose broth culture by using the PRAS II series inoculator (delivery volume, 0.05 ml). (The number of biochemical tests required for each of the Prescreen group categories were: cocci, 9; fusobacteria, 8; bacteroides, 10; clostridia, 15; and gram-positive NSF rods, 21.) The biochemical broths were incubated at 35°C for 5 days or until a density of ≥3 McFarland was achieved. Tests negative for gelatin liquefaction after 5 days incubation were reincubated for an additional 3 days to check for a delayed reaction. The pH of carbohydrate broths was determined by removing the stopper and inserting a 6-mm microelectrode (Arthur H. Thomas Co., Philadelphia, Pa.) into the tube. A pH reading of ≤6.0 was considered acidic. Appropriate reagents were added to selected biochemicals, e.g., indole or nitrate. Esculin hydrolysis was determined by adding a few drops of 1% ferric ammonium citrate (Trend Scientific, Inc., Minneapolis, Minn.) to the appropriate broth as well as by observing the tube under UV light. Organisms were then identified by entering test results into the AS2 program. When AS2 failed to identify an isolate, identification was attempted with the less comprehensive AS1 program.

After completion of this study, Scott laboratories revised the PRAS II computer software and hardware. Of the 80 isolates, 78 were reidentified on the PRAS II (revised) system. Two fusobacteria were omitted because several new tests required for identification (as listed in *PRAS II Ana-Stat Manual*, 3rd ed.) were not performed for the initial evaluation of the PRAS II system.

RapID ANA system. The RapID ANA (Innovative Diagnostic Systems, Decatur, Ga.) system for anaerobe identification is based upon the enzymatic degradation of chromogenic substrates by preformed bacterial enzymes. The RapID ANA panel is composed of 10 test cavities, 8 of which are bifunctional and contain two tests per well. A total of 18 biochemical test reactions can be determined including reduction of triphenyltetrazolium, arginine utilization, trehalose fermentation, indole production from tryptophane, and hydrolysis of one phosphate ester, six glycoside, and seven β-naphthylamide substrates.

Growth from a fresh S-SBA plate (<72 h old) was used to prepare a suspension of the organism in RapID ANA inoculation fluid (density, ≥3 McFarland). Approximately 0.1 ml of inoculum was distributed into each of the test cavities on the panel. Inoculated panels were incubated aerobically at 35°C for 4 h. Reactions in the test cavities were subsequently interpreted and recorded. Appropriate reagents were then added to the bifunctional wells. After up to 3 min was allowed for maximum color development, eight additional reactions were recorded. A six-digit code number was generated from the biochemical profile, and the *Code Compendium Book* provided by the manufacturer was consulted

for species identification. Because RapID ANA is a relatively new system and the data base proved to be incom-plete, all profile numbers not listed in the code book were telephoned in to the manufacturer's computer facilities.

API 20A system. API 20A (Analytab Products, Plainview, N.Y.) consists of a plastic panel with 20 cupules containing dehydrated substrates which allows for the determination of 21 biochemical reactions. Included are tests for indole, urease, catalase, gelatin liquefaction, esculin hydrolysis, and fermentation of glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose, and trehalose.

A cell suspension (density, ≥3 McFarland) was prepared in API anaerobe basal medium from pure growth on a fresh S-SBA plate. The panel was inoculated and then incubated at 35°C in an anaerobe jar for 24 h. Necessary reagents were added, and reactions were interpreted. A seven-digit profile number was generated to assign an identification to each isolate. In contrast to the newer RapID ANA manufacturer, the API 20A manufacturer was not consulted about profile numbers with no species identification; our laboratory has been routinely using the API 20A system for five years, and it has been our experience that the API number code is generally complete.

Interpretation of results. The identifications generated with PRAS II, RapID ANA, and API 20A were compared with the identifications obtained by using VPI criteria (7). Identifications were classified into one of four categories: correct to the species level, correct to the genus level only, incorrect, and not identified. Identifications were correct to the genus level only when the isolate was not identified beyond the genus level or when an incorrect species assignment was made within the correct genus. An identification was incorrect when the isolate was assigned to the wrong genus. Because *Lactobacillus* and *Bifidobacterium* isolates were not further identified to the species level by reference VPI methodology, any species identifications within the proper genus were considered correct to species level.

RESULTS

The accuracies of identifications obtained with PRAS II, PRAS II (revised), RapID ANA, and API 20A are listed in Table 1. PRAS II correctly identified 59 (74%) isolates to the species level; 11 (14%) isolates were correctly identified to the genus level only. PRAS II misidentified 5 (6%) of the isolates, and 5 (6%) could not be identified. PRAS II (revised) correctly identified 64 of 78 isolates (82%) to the species level, correctly identified 9 (12%) isolates to the genus level only, and incorrectly identified 5 (6%) isolates. All 78 isolates were assigned identifications by PRAS II (revised). RapID ANA correctly identified 50 (62%) isolates to the species level; 22 (28%) isolates were correctly identified to the genus level only. Eight (10%) isolates were misidentified by RapID ANA. All 80 isolates were assigned identifications by the RapID ANA system. API 20A correctly identified 57 (71%) isolates to the species level; 8 (10%) isolates were correctly identified to genus level only. API 20A misidentified 2 (3%) isolates, and 13 (16%) isolates could not be identified. A list of isolates (divided by genus or group) correctly identified to species level by each of the three systems is presented in Table 2.

TABLE 1. PRAS II, RapID ANA, and API 20A identifications 80 anaerobic isolates

Identification system	% (No.) correct to:		% (No.) incorrect	% (No.) unable to identify
	Species	Genus only		
PRAS II	74 (59)	14 (11)	6 (5)	6 (5)
PRAS II (revised)[a]	82 (64)	12 (9)	6 (5)	0 (0)
RapID ANA	62 (50)	28 (22)	10 (8)	0 (0)
API 20A	71 (57)	10 (8)	3 (2)	16 (13)

[a] Two *Fusobacterium* isolates were omitted because the appropriate tests were not done in which case the total number of isolates was 78.

TABLE 2. PRAS II, RapID ANA, and API 20A indentifications correct to the species level

Genus or group (no. of isolates)	No. (% of total) of isolates correctly identified to the species level by:			
	PRAS II (revised)	PRAS II	RapID ANA	API 20A
Bacteroides (25)	21	17	14	21
Fusobacterium (7)	5	6	5	4
Clostridium (12)	9	8	8	6
Gram-positive NSF rods (18)	13	11	9	11
Cocci (18)	16	17	14	15
Total (80)	64 (82)[a]	59 (74)	50 (62)	57 (71)

[a] Total number of isolates for PRAS II (revised) was 78; two fusobacteria were omitted because appropriate tests were not done.

Table 3 lists comparisons of identifications correct to the genus level only; often an incorrect species identification had little clinical significance. Of 11 organisms identified by PRAS II correct to genus only, 9 were incorrectly identified at the species level within the proper genus, and 2 were assigned genus level identifications only. Although one clostridial isolate could not be identified to the species level by VPI methodology, identification as *C. difficile* by PRAS II was determined to be correct to genus only because the GLC pattern of this isolate was not consistent with those produced by *C. difficile*. Twenty-two identifications obtained with RapID ANA were correct to genus only; 21 isolates were assigned to the correct genus but were incorrectly speciated, whereas only one was not identified beyond the genus level. The identification of the *Clostridium* sp. isolate as *C. tetani* by RapID ANA was considered correct to genus only because the carbohydrates fermented by this isolate were not typical of *C. tetani*. API 20A identified 8 isolates correct to genus only; 6 isolates were incorrectly speciated within the proper genus, and 2 isolates were assigned only genus level identifications.

DISCUSSION

PRAS II (revised) was the most accurate system evaluated in the present study, correctly identifying to the species level 82% of 78 isolates tested. The initial PRAS II system studied correctly identified 74% of 80 isolates to the species level. In comparison, RapID ANA and API 20A correctly identified 62 and 71% of 80 isolates, respectively. RapID ANA identified 28% of isolates correct to the genus level only, including 11 of 25 total *Bacteroides* species tested. PRAS II and API 20A identified 14% and 10%, respectively, of isolates tested within the correct genus only. The majority of the genus-level-only identifications in all three systems were due to incorrect species assignment within the proper genus and not to an inability to assign a species designation. All three systems had comparable rates of incorrect identifications ranging from 3 to 10%; as expected, the most troublesome

TABLE 3. Comparison of identifications correct to genus level only with PRAS II, RapID ANA, and API 20A[a]

Identification system	VPI identification	System identification
PRAS II	*B. ovatus* (2)	*B. uniformis* (2)
	B. thetaiotaomicron	*B. hypermegas*
	B. melaninogenicus subsp. *melaninogenicus* (3)	*B. melaninogenicus* subsp. *intermedius* (3)
	B. oralis	*Bacteroides* spp.
	B. bivius	*B. nodosus*
	F. mortiferum	*F. varium*
	C. tetani	*Clostridium* sp.
	Clostridium sp.	*C. difficile*
RapID ANA	*B. fragilis* (3)	*B. loescheii/denticola*[b] (2)
		B. uniformis
	B. ovatus (2)	*B. thetaiotaomicron*
		B. uniformis
	B. vulgatus (3)	*B. fragilis* (2)
		B. oralis
	B. oralis	*B. melaninogenicus*
	B. ruminicola (2)	*B. oralis* (2)
	F. necrophorum	*F. nucleatum*
	F. varium	*Fusobacterium* spp.
	C. clostridiiforme	*C. ramosum*
	C. innocuum	*C. tetani*
	C. sordellii	*C. bifermentans*
	Clostridium spp.	*C. tetani*
	A. odontolyticus	*A. myeri*
	P. acnes	*P. granulosum*
	S. constellatus	*S. morbillorum*
	S. intermedius (2)	*S. morbillorum* (2)
API 20A	*B. ovatus*	*B. thetaiotaomicron*
	B. asaccharolyticus (2)	*B. ureolyticus*
		B. melaninogenicus subsp. *intermedius*
	F. necrophorum	*Fusobacterium* sp.
	C. septicum	*C. tetani*
	C. tetani	*Clostridium* sp.
	A. odontolyticus	*A. naeslundii*
	P. acnes	*P. avidum*

[a] Numbers in parentheses represent number of isolates included.

[b] *B. loesheii/denticola* and *B. melaninogenicus* were the taxonomic designations used in the RapID ANA system for *B. melaninogenicus* subsp. *melaninogenicus*.

group of organisms to identify were the gram-positive NSF rods, organisms which rarely cause significant clinical infections (10). The API 20A system could not identify 16% of the isolates, and no one genus was particularly affected. Although RapID ANA did assign identifications to all 80 isolates, 18 (23%) of the code numbers generated were not listed in the *Code Compendium Book* and required consultation with the manufacturer's computer facility.

Several recent reports (3, 9) have described an evaluation of the PRAS II system. Beaucage and Onderdonk (3) compared 1,779 biochemical test results obtained in PRAS II media with published VPI reactions (7) for 108 isolates and obtained an overall correlation of 97.6%. Those results indicated that PRAS II media can be used to accurately identify anaerobic bacteria. The PRAS II system was easy to work with and did not require the use of gas cannula equipment. Unlike API 20A or RapID ANA, PRAS II required a relatively light inoculum, i.e., a density of $\geq$1 McFarland. In this study, however, PRAS II chopped-meat–glucose broths with turbidities of $\geq$3 McFarland were used to provide more optimal inocula of organisms. The time required to set up and later interpret PRAS II biochemicals was almost twice as long as that required for either the RapID ANA or API 20A systems. In addition, depending on the growth rate of the isolates, PRAS II identifications took several days to generate (excluding the time for the Prescreen tests); in contrast, RapID ANA and API 20A had identification times of 4 and 24 h, respectively. With PRAS II media, recording biochemical reactions too early resulted in false-negative data for slow-growing organisms. Also, with the PRAS II system, supplemental tests were required to identify 19 (24%) isolates; these tests included carbohydrate fermentations and miscellaneous other biochemical reactions, such as propionate production from lactate or threonine or both, milk clot or digestion, etc. Gram-positive organisms, especially NSF rods, often grew poorly in PRAS II media. This might account for inaccurate identification of this group of organisms, i.e., false-negative reactions. The selective addition of Tween 80 to PRAS II media used for identification of gram-positive organisms may alleviate this problem, as growth of these organisms is enhanced by Tween 80 (7, 12).

Several biochemical reactions in PRAS II media were difficult to interpret. Esculin hydrolysis was especially confusing. After consulting with the manufacturer, loss of fluorescence was used as the absolute criterion for hydroylsis of esculin. Of the PRAS II esculin reactions, 25% turned black after the addition of 1% ferric ammonium citrate but continued to fluoresce under UV light. This situation may have been due to H_2S production and was also observed by Moore et al. (8) with the API 20A system. The manufacturer ambiguously advised recording the pH of carbohydrate broths when adequate growth developed; however, when the pH of PRAS II glycogen medium inoculated with a rapid-growing anaerobic organism was recorded before a minimum of 48 h of incubation, a false-negative result was obtained. Media containing mannitol or xylose were not available during the course of this study, owing to manufacturing quality control problems. In revising the PRAS II system, the manufacturer has made minor changes in the biochemical tests including addition of xylan fermentation for *Bacteroides* spp. and sodium polyanethole sulfonate inhibition for gram-positive cocci.

AS2 was the computer program of choice for identifying isolates with PRAS II, because it has a more extensive data base than the AS1 program. However, 20 (25%) isolates in this study could not be identified with AS2. AS1 was able to identify 15 of these isolates, possibly because troublesome tests, i.e., esculin and glycogen, were not part of the required data base for the AS1 program.

The RapID ANA system has only recently become commercially available, and no reports have yet been published describing its accuracy. This identification system was extremely simple to use. Because this system detected preformed bacterial enzymes, actual growth of the organism was not required, and identifications could be accomplished within 4 h after a pure culture of the organism was obtained. Initially, some of the color reactions were difficult to interpret. However, with the help of the manufacturer's technical advisor and with experience gained in working with the system, this became less of a problem. Only 4 of the 80 isolates required additional testing; 3 were fusobacteria which were checked for lipase production on egg yolk agar and 1 was a *Bacteroides* isolate which was tested for bile tolerance. All 80 isolates were identified by RapID ANA without requiring GLC results.

A major disadvantage of the RapID ANA system was the high misidentification rate of *Bacteroides* species. Ten isolates in this study were members of the clinically significant *B. fragilis* group. Five of these isolates were misidentified as an incorrect member of the *B. fragilis* group, and three were misidentified as either *B. melaninogenicus* or as *B. oralis* (Table 3). It is extremely important to accurately identify the *B. fragilis* group as they are often associated with clinical disease and are the most commonly isolated anaerobic bacteria (12). In addition, although RapID ANA generally worked very well for the anaerobic cocci and identified 14 of 18 isolates correct to species, this system had trouble identifying the microaerophilic streptococci to the species level; three streptococcal isolates were misidentified as rare biotypes of *Streptococcus morbillorum* (Table 3).

The RapID ANA system data base requires the presence or absence of brown-black pigmented colonies to differentiate between *B. melaninogenicus* and *B. oralis*. However, *B. oralis* can also produce a dark tan-brown pigment (personal communication, T. D. Wilkins, Virginia Polytechnic Institute and State University, Blacksburg, Va.) which could lead to misidentification of *B. oralis* as *B. melaninogenicus*. As recommended by the manufacturer, supplemental testing for production of lipase was done to differentiate *F. necrophorum* from *F. nucleatum*. However, this is not always a valid criterion because although most strains of *F. necrophorum* produce lipase, some strains are lipase negative (7). One lipase-negative *F. necrophorum* isolate in this study was therefore misidentified as *F. nucleatum* by the RapID ANA system.

Because the RapID ANA system is based on enzymatic degradation of chromogenic substrates, it is not possible to investigate questionable identification by correlating the results with established identification schemes (7, 12). Although supplementary tests were not required to identify most organisms with the RapID ANA panel, the performance of tests such as GLC analysis and bile tolerance determination might confirm questionable identifications.

The API 20A system has been extensively evaluated (1, 5, 6, 8, 11). In a recent study (J. E. Rosenblatt, J. B. Stewart, D. T. Lee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C145, p. 260), 73% of the API results for 330 isolates were in agreement with the reference method (biochemical testing and GLC) when additional tests were routinely done. This is similar to the results of the present study in which API 20A correctly identified 71% of the isolates to the species level, including 21 of 25 *Bacteroides* isolates as well as 15 of 18 anaerobic cocci, two of the most frequently isolated groups of anaerobes. API 20A gave disappointing results for the clostridia, identifying only 6 of 12 isolates correctly to the species level.

Although API 20A was simple to use, sugar fermentation reactions were often difficult to interpret, as has been previously noted (1, 6). However, with experience, it became easier to judge between positive and negative reactions. API required additional testing, most importantly GLC, for 29 (36%) of the isolates. In addition, 16 (20%) of the isolates failed to show any biochemical activity on the panel although inocula were viable and pure, as determined by routine purity checks. Nonreactivity was most frequently observed with anaerobic cocci and, as expected, with asaccharolytic organisms. Gram stain and GLC were helpful in identifying these nonreactive strains.

Incorrect identifications by any of the systems for all isolates of a particular species should not suggest that this species could never be correctly identified. Although 80 anaerobic isolates were included in this study, because the spectrum of clinically significant anaerobes is large, several species were represented by only one or two isolates. This is a drawback in this study; however, as mentioned in the methods section, clinical isolates were sequential isolates and represented a typical spectrum of organisms that were being identified in our clinical laboratory during the time of this study. Additional studies are needed that compare large numbers of isolates in specific groups of anaerobic bacteria.

RapID ANA and PRAS II appear to be promising new methods for the identification of anaerobic bacteria, with overall accuracy at least comparable to that of the more established API 20A system. However, further research and improvements are needed before these systems can accurately identify all species of clinically significant anaerobic bacteria. Also, because RapID ANA and PRAS II are relatively new products, future studies are needed to determine the reproducibility of results obtained in this study.

LITERATURE CITED

1. **Applebaum, P. C., C. S. Kaufmann, J. C. Keifer, and H. J. Venbrux.** 1983. Comparison of three methods for anaerobe identification. J. Clin. Microbiol. **18**:614–621.
2. **Balows, A., R. M. DeHaan, and V. R. Dowell, Jr.** 1974. Anaerobic bacteria: role in disease. Charles C Thomas, Publisher, Springfield, Ill.
3. **Beaucage, C. M., and A. B. Onderdonk.** 1982. Evaluation of a prereduced anaerobically sterilized medium (PRAS II) system for identification of anaerobic microorganisms. J. Clin. Microbiol. **16**:570–572.
4. **Finegold, S. M.** 1977. Anaerobic bacteria in human disease. Academic Press, Inc., New York.
5. **Hansen, S. L, and B. J. Stewart.** 1976. Comparison of API and Minitek to Center for Disease Control methods for the biochemical characterization of anaerobes. J. Clin. Microbiol. **4**:227–231.
6. **Hanson, C. W., R. Cassorla, and W. J. Martin.** 1979. API and Minitek systems in identification of clinical isolates of anaerobic gram-negative bacilli and *Clostridium* species. J. Clin. Microbiol. **10**:14–18.
7. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
8. **Moore, H. B., V. L. Sutter, and S. M. Finegold.** 1975. Comparison of three procedures for biochemical testing of anaerobic bacteria. J. Clin. Microbiol. **1**:15–24.
9. **Onderdonk, A. B., and C. M. Beaucage.** 1982. Use of prereduced anaerobically sterilized (PRAS II) medium for identification of obligate anaerobes, p. 263–265. *In* R. C. Tilton (ed.), Rapid methods and automation in microbiology. American Society for Microbiology, Washington, D.C.
10. **Rosenblatt, J. E.** 1981. Anaerobic bacteria, p. 356. *In* J. A. Washington (ed.), Laboratory procedures in clinical microbiology, Springer-Verlag, Inc., New York.
11. **Starr, S. E., F. S. Thompson, V. R. Dowell, Jr., and A. Balows.** 1973. Micromethod system for identification of anaerobic bacteria. Appl. Microbiol. **25**:713–717.
12. **Sutter, V. L., D. M. Citron, and S. M. Finegold.** 1980. Wadsworth anaerobic bacteriology manual, 3rd ed. The C. V. Mosby Co., St. Louis, Mo.