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# Comparison of Three Methods for Anaerobe Identification

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In this study we evaluated the ability of three commercial methods, API 20A (Analytab Products, Plainview, N.Y.), Minitek (BBL Microbiology Systems, Cockeysville, Md.), and Anaerobe-Tek (Flow Laboratories, Inc., McLean, Va.), to accurately identify 165 recent clinical and 38 stock isolates of anaerobic bacteria without supplemental tests or gas-liquid chromatography. Strains included 89 *Bacteroides* spp., 12 fusobacteria, 10 gram-positive, nonsporing rods, 43 *Clostridium* spp., 15 *Streptococcus intermedius*, 18 peptococci, 6 peptostreptococci, 3 *Staphylococcus saccharolyticus*, and 7 *Veillonella* spp. The methods used were those of manufacturers, without supplemental tests. API 20A correctly identified 70.0% of strains to species and 6.4% to genus only, with 17.2% as part of a spectrum of identifications and 6.4% incorrect. Minitek, according to the current code book, yielded 69.5% correct identifications to species, 16.8% spectrum identifications, and 13.8% incorrect. Anaerobe-Tek correctly identified 64.0% of strains to species, 21.2% spectrum identifications, and 14.8% incorrect. Thirteen strains were misidentified by API 20A, 28 were misidentified by Minitek, and 30 were misidentified by Anaerobe-Tek. For laboratories without gas-liquid chromatography support and where identification of clinically significant *Bacteroides fragilis* and *Clostridium perfringens* is desired, any of the three systems would provide accurate information. For more extensive anaerobe identification, including the less frequently isolated, more unusual organisms, API 20A and Minitek are preferred at this time. All systems have identification schemes associated with a percentage of misidentifications, the most recently introduced Anaerobe-Tek system being associated with the highest error rate.

In the past few decades there have been great advances in the understanding of the role played by anaerobes in the pathogenesis of severe bacterial infections (6). The infectious potential of these organisms has been clarified (6), and the development of species- and group-specific differences in antimicrobial susceptibility patterns (5, 7) has been defined. Anaerobe isolation and identification is an important part of the functions of a clinical microbiology laboratory. Conventional biochemical testing of anaerobes together with gas-liquid chromatography (GLC) is the most accurate identification method (4, 10), but it has the disadvantages of being time consuming and expensive and is beyond the capabilities of many clinical laboratories.

Commercial methods which have gained acceptance for identification of anaerobes include API 20A (AP), introduced in 1974 by Analytab Products (Plainview, N.Y.) (8, 9, 19, 22) and Minitek (MT), introduced in 1975 by BBL Microbiology Systems (Cockeysville, Md.) (8, 9, 13, 21). Recently, a new anaerobe identification method, Anaerobe-Tek (AT), has been marketed by Flow Laboratories (McLean, Va.) and is similar in concept to methods already available for the identification of yeasts (3), *Enterobacteriaceae* (1), and nonfermentative, gram-negative rods (2). AT consists of a wheel divided into 11 peripheral compartments and 1 central well. Ten of the media are comparable to those included in the Presumpto quadrant plate 1, 2, 3 devised by the Centers for Disease Control Anaerobe Laboratory (17). The wheel is inoculated and incubated for 48 h. Reactions are interpreted and identification is generated with the aid of a computer code book.

The microbiology laboratory without facilities for conventional anaerobe testing requires an accurate and reliable method for anaerobe species identification. The aim of the current study was to evaluate the ability of AP, MT, and AT to identify a spectrum of clinically significant anaerobe strains without the aid of additional tests.

(Part of this work was presented at the 83rd Annual Meeting of the American Society for Microbiology [P. C. Appelbaum, C. S. Kaufmann, J. C. Keifer, H. J. Venbrux, and S. F. Schick, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C423, p. 382]).

## MATERIALS AND METHODS

**Strains.** Of the 203 anaerobic bacteria tested, 165 were recent clinical isolates tested within approximately 1 month of isolation, and 32 were stock cultures (including American Type Culture Collection and Centers for Disease Control strains) from the Johns Hopkins Hospital. Additionally, five strains of *Bacteroides distasonis* and one strain of *Bacteroides* sp. 3452A were kindly provided by Lillian V. Holdeman, Virginia Polytechnic Institute and State University, Blacksburg. All organisms were identified at Johns Hopkins Hospital by a combination of biochemical tests and GLC, as recommended in established manuals (4, 10), and were tested blindly in commercial systems without prior knowledge of their identity. The Maryland State Health Department and the Centers for Disease Control served as reference laboratories, for resolving major discrepancies between Johns Hopkins Hospital and commercial system identifications. The number of each species of bacteria tested follows, with the number (if any) of stock strains in parentheses: *Bacteroides fragilis*, 49; *Bacteroides thetaiotaomicron*, 20 (1); *Bacteroides vulgatus* 3 (1); *Bacteroides distasonis* (5); *Bacteroides uniformis*, 1; *Bacteroides melaninogenicus*, 2; *Bacteroides bivius*, 2; *Bacteroides ureolyticus*, 1; *Bacteroides oralis*, 1; *Fusobacterium necrophorum*, 8; *Fusobacterium nucleatum*, 1; *Fusobacterium mortiferum* (3); *Propionibacterium acnes*, 8; *Clostridium perfringens*, 16 (1); *Clostridium innocuum*, 1; *Clostridium tertium*, 2 (2); *Clostridium butyricum*, 1 (1); *Clostridium bifermentans*, 1 (4); *Clostridium cadaveris* (2); *Clostridium sordellii* (3); *Clostridium sporogenes* (4); *Clostridium difficile*, 2; *Streptococcus intermedius*, 15; *Peptococcus asaccharolyticus*, 9; *Peptococcus magnus*, 4 (2); *Peptococcus prevotii*, 3; *Staphylococcus saccharolyticus*, 3; *Peptostreptococcus anaerobius*, 6; *Veillonella parvula*, 6 (1). Additionally, one stock culture each of *Bacteroides* sp. 3452A, *Bacteroides ovatus, Capnocytophaga ochracea, Actinomyces viscosus, Eubacterium lentum, Clostridium novyi* A, *Clostridium histolyticum*, and *Clostridium paraputrificum* were tested. For the purpose of this study, *C. ochracea* was classified in the *Bacteroides* spp. group.

**Test systems.** Isolates were maintained anaerobically at room temperature in chopped meat-glucose medium. Before inoculation of kits, organisms were subcultured on enriched blood agar plates (4) and checked for purity by gram stain and colonial morphology. Additionally, inocula from all systems were plated to check purity and viability. Identification was classified as (i) correct to species, (ii) correct to genus only, (iii) one of a spectrum of identifications (SI), a classification necessitating additional tests to pinpoint the correct identification from a spectrum of possibilities, and (iv) incorrect. All kit tests were interpreted by the senior author (P.C.A.).

**AP system.** Suspensions of growth were made in Lombard-Dowell broth to a density of a McFarland standard of 3 or greater. Strips were inoculated according to instructions and incubated in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) for 24 h at 37°C. Reagents were then added, and reactions were read according to manufacturer's instructions. Where interpretation of sugar fermentation tests was difficult, clear positive (yellow) and negative (purple) wells from the same strip were used for comparison. Sugar reactions were not compared among strips. Tests were assigned numerical values from which a profile number was generated. Identification was made by using the API Analytical Profile Index. Numbers which did not appear in the index were referred to the firm's computer facilities. When identification was stated as "good likelihood, but low selectivity," identification was classified as SI. For the purpose of this study, supplemental tests were not performed.

**MT system.** A dense homogenous suspension of organisms in MT anaerobe broth (≥3 McFarland standard; where possible, ≥5) was used to inoculate the suggested MT disks to test for nitrate reductase; hydrolysis of esculin and urea; and fermentation of dextrose, arabinose, glycerol, lactose, maltose, mannitol, rhamnose, salicin, sucrose, trehalose, xylose, cellobiose, mannose, raffinose, and sorbitol. For indole production, the inoculum was placed in an empty well. After 48 h of incubation in the Coy chamber, reagents were added, and tests were read according to manufacturer's instructions. Additional phenol red indicator was added to carbohydrate wells when fermentation reactions yielded equivocal color changes. Code numbers were generated from the biochemical profile of the organisms and identification assigned by using the Minitek numerical identification system. The firm's computer facilities were consulted for organisms whose code did not appear in the book. Where supplemental tests were required for precise identification of organisms with a low confidence value (<90%), supplemental tests were not done for the purpose of this study, and identification was classified as SI.

**AT system.** A turbid cell suspension (≥3 McFarland standard) was prepared in AT broth and used to inoculate the wheel according to manufacturer's instructions. After incubation for 48 h in the Coy chamber, plates were left aerobically at room temperature for 30 min before the addition of reagents (17). Four or five drops of a dilute aqueous solution (0.001%) of bromthymol blue indicator was added to all sugar fermentation wells. Tests were interpreted according to the firm's instructions, and identification was assigned with the aid of the AT computer code book. Where identification was as a spectrum of possibilities without clear-cut statistical preference (<90% probability), classification was as SI for the purpose of this study, without performance of supplemental tests.

## RESULTS

In general, the total technical time required to set up and read each of the three test kits was comparable. AP and MT were comparably priced, whereas the cost of the AT kit was almost twice that of the other systems. At the recommendation of manufacturers, AP was read after 24 h, and MT and AT were read after 48 h of incubation. We currently have no experience in interpreting AP after 48 h or MT and AT after 24 h.

Interpretation of AP sugar reactions required more technical expertise than is the case with

TABLE 1. Identification of anaerobic bacteria by AP, MT, and AT

| Organism or group (no. of strains tested) | No. correct to: | | | | No. of SIs | | | No. incorrect | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Species | | | Genus[a] | | | | | | |
| | AP | MT | AT | AP | AP[b] | MT | AT | AP | MT | AT |
| *Bacteroides* spp. (89) | 72 | 74 | 68 | 9 | 5 | 8 | 4 | 3 | 7 | 17[c] |
| *Fusobacterium* spp. (12) | 0 | 1 | 1 | 0 | 9 | 10 | 7 | 3 | 1 | 4 |
| Non-sporulating, gram-positive rods (10) | 8 | 8 | 9 | 1 | 1 | 2 | 1 | 0 | 0 | 0 |
| *Clostridium* spp. (43) | 31 | 31 | 23 | 3 | 5 | 10 | 13 | 4 | 2 | 7[d] |
| *S. intermedius* (15) | 15 | 14 | 15 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Peptococcus* spp. (18) | 9 | 9 | 6 | 0 | 9 | 0 | 11 | 0 | 9 | 1 |
| *Peptostreptococcus* spp. (6) | 0 | 0 | 0 | 0 | 6 | 0 | 6 | 0 | 6 | 0 |
| *S. saccharolyticus* (3) | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 1 |
| *Veillonella* spp. (7) | 7 | 4 | 7 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Total (203) | 142 | 141 | 130 | 13 | 35 | 34 | 43 | 13 | 28 | 30 |
| % of total | 70 | 69.5 | 64 | 6.4 | 17.2 | 16.8 | 21.2 | 6.4 | 13.8 | 14.8 |

[a] Identification to genus only category was used by AP only.
[b] Good likelihood, but low selectivity identifications.
[c] Three groups not included in current data base and three codes not present in code book.
[d] One code not present in code book.

most other Analytab products. Sugar fermentation reactions, especially cellobiose, were difficult to read. Comparison of questionable sugar reactions (brown, brown-purple) to a clearly positive (yellow) and negative (purple) reaction on the same strip lessened, but did not totally alleviate, this problem, which occurred 10 to 15% of the time. Indole reactions were generally easily interpreted, but had to be read within 5 min after addition of Ehrlich's reagent (as specified by the manufacturers) to avoid false-positive reactions.

MT reactions were easy to read. In cases of equivocal color reactions (light orange, yellow-orange), the addition of extra indicator to each sugar disk clearly differentiated between positive (yellow) and negative (orange, red) reactions. Difficulties were experienced in detecting indole production in approximately 20% of indole-positive *Bacteroides* species.

Esculin, lecithinase, DNase, and starch hydrolysis reactions with AT were generally clear-cut and easily interpreted. Indole production was often difficult to detect, especially in the case of more fastidious organisms that did not produce luxuriant growth in the center well. Milk and lipase reactions were, with the exception of proteolytic or lipolytic clostridia, difficult to read and prone to great subjective variability. Sugar fermentation reactions (especially mannitol) also presented problems. When equivocal greenish-yellow colors were encountered, the addition of bromthymol blue indicator as recommended by the manufacturer did not alleviate the problem for us. In spite of inoculation of wheels with a heavy bacterial suspension, growth of more fastidious anaerobes such as fusobacteria, *B. melaninogenicus*, and cocci was often poor and may have been responsible for several false-negative reactions. With these organisms, tests were repeated, but even after three attempts, sufficient growth was still not obtained.

Identification of all anaerobic bacteria tested with the three systems is presented in Tables 1 and 2. All organisms in SI groups would have required GLC for accurate species identification. In addition to the 203 strains included in the above data analysis, 7 strains originally identified as *B. distasonis* were tested with all three systems. Four organisms were identified as *B. distasonis* by all three methods; however, three strains were identified as *Bacteroides* sp. 3452A by AP and MT and as *B. distasonis* by AT. Retesting of these 7 strains utilizing data provided by Johnson and colleagues (14, 15) revealed that the identification by AP and MT was correct in each case. *Bacteroides* sp. 3452A is not currently included in commonly used anaerobe manuals (4, 10), and the significance of this differentiation is not known at this time. Because of this problem, we elected not to include these seven strains in the data analysis, but to obtain representative strains of these two groups from Virginia Polytechnic Institute.

Sixteen isolates were retested to determine reproducibility of the systems (Table 3). With the exception of one strain of *B. thetaiotaomicron*, good agreement between runs was evident with each system, with only minor changes noted.

Isolates that yielded incorrect identifications

TABLE 2. Identification of specific species of anaerobic bacteria by AP, MT, and AT

| Organism or group (no. of strains tested) | No. correct to: | | | | No. of SIs | | | No. incorrect | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Species | | | Genus | | | | | | |
| | AP | MT | AT | AP[a] | AP | MT | AT | AP | MT | AT |
| *C. perfringens* (17) | 14 | 15 | 17 | 0 | 3 | 2 | 0 | 0 | 0 | 0 |
| % of total | 82 | 88 | 100 | | 18 | 12 | | | | |
| *B. fragilis* group | | | | | | | | | | |
| *B. fragilis* (49) | 46 | 47 | 47 | 2 | 1 | 0 | 1 | 0 | 2 | 1 |
| *B. thetaiotaomicron* (21) | 14 | 20 | 10 | 7 | 0 | 1 | 3 | 0 | 0 | 8[b] |
| *B. vulgatus* (4) | 4 | 3 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *B. distasonis* (5) | 2 | 2 | 4 | 0 | 3 | 3 | 0 | 0 | 0 | 1 |
| *B. ovatus* (1) | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Bacteroides* 3452A (1) | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1[c] |
| Subtotal (81) | 68 | 73 | 66 | 9 | 4 | 6 | 4 | 0 | 2 | 11 |
| % of total | 84 | 90.1 | 81.5 | 11.1 | 4.9 | 7.4 | 4.9 | | 2.5 | 13.6 |
| *Peptococcus asaccharolyticus* (9) | 9 | 9 | 6 | 0 | 0 | 0 | 2 | 0 | 0 | 1 |
| % of total | 100 | 100 | 67 | | | | 22 | | | 11 |
| *P. acnes* (8) | 7 | 8 | 7 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| % of total | 88 | 100 | 88 | 12 | | | 12 | | | |

[a] Identification to genus only category was used by AP only.
[b] One code number not currently in data base.
[c] Organism not currently in data base.

with one or more of the three systems are presented in Table 4. It should be noted that, because of the small sample size for some species, a high percentage of incorrect identification does not necessarily imply that the systems could never identify that organism.

TABLE 3. Reproducibility of methods

| Species | Identification[a] by: | | |
|---|---|---|---|
| | AP | MT | AT |
| *B. fragilis* | CS/CS | SI/CS | CS/CS |
| *B. fragilis* | CS/CS | CS/CS | CS/CS |
| *B. fragilis* | CS/CS | CS/CS | CS/CS |
| *B. thetaiotaomicron* | CG/CG | CS/CS | ./. |
| *B. thetaiotaomicron* | CS/CS | CS/CS | I/I |
| *B. thetaiotaomicron* | CG/CG | CS/CS | I/I |
| *B. thetaiotaomicron* | CG/CG | CS/CS | CS/CS |
| *B. thetaiotaomicron* | CS/CG | CS/CS | CS/SI |
| *B. thetaiotaomicron* | CS/CS | CS/CS | SI/SI |
| *B. thetaiotaomicron* | CG/CG | CS/CS | CS/CS |
| *B. thetaiotaomicron* | I/CG | SI/I | I/SI |
| *B. vulgatus* | CG/CG | SI/SI | CS/CS |
| *Peptococcus asaccharolyticus* | CS/CS | CS/CS | CS/SI |
| *Peptococcus prevotii* | SI/SI | I/I | SI/SI |
| *Bacteroides* sp. 3452A[b] | CS/CS | CS/CS | I/I |
| *Bacteroides* sp. 3452A[b] | CS/CS | CS/CS | I/I |

[a] First identification/second identification. Abbreviations: CS, correct identification to species; CG, correct identification to genus only; ., codes not included in current AT data base; I, incorrect identification.
[b] Organisms originally identified as *B. distasonis* and therefore not included in full data analysis (see the text).

Additional tests most commonly required for accurate species identification of organisms with SI classifications were as follows: for AP, GLC, black colonial pigmentation, Gram stain, lecithinase, lipase, and motility; for MT, GLC, black colonial pigmentation, Gram stain, starch hydrolysis, growth in bile, penicillin susceptibility, lecithinase, lipase, aerobic growth, and gelatinase; for AT, GLC, nitrate reductase, hemolysis, aerobic growth, and fermentation of rhamnose, xylose, maltose, sucrose, and glycerol. In general, a combination of Gram stain, colonial morphology, and GLC would have been adequate for accurate identification of most of these strains.

## DISCUSSION

Most previously published studies on AP (8, 9, 19, 22) have employed techniques that have subsequently been refined by the manufacturer. Hanson and colleagues (9) reported a 68% correct identification rate to species with a newly released AP code book, different inoculation broth, and 48-h incubation. Refinements in the AP system have improved its efficacy: in two preliminary studies with techniques similar to those described in this study (P. Zwadyk, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C295, p. 320; G. B. Hill, A. P. Kohan, O. M. Ayers, D. E. Dzubay, Abstr. Annu. Meet. Am.

TABLE 4. Major identification discrepancies with AP, MT, and AT

| Organism[a] (no. of strains) | Identification[b] by: | | |
|---|---|---|---|
| | AP | MT | AT |
| *B. fragilis* | *Bacteroides* sp.[c] | *B. oralis, B. ruminicola, Capnocytophaga* sp. | *F. mortiferum, B. vulgatus, B. fragilis* |
| *B. fragilis* | *B. fragilis, B. oralis, C. clostridiiforme* | *B. ovatus* | *B. ovatus* |
| *B. thetaiotaomicron* | *Bacteroides* sp.[c] | *B. thetaiotaomicron* | 234452[d] |
| *B. thetaiotaomicron* (5) | *B. thetaiotaomicron* | *B. thetaiotaomicron* | *B. ovatus* |
| *B. thetaiotaomicron* (2) | *Bacteroides* sp.[c] | *B. thetaiotaomicron* | *B. ovatus* |
| *B. distasonis* | *C. clostridiiforme, B. distasonis, Bacteroides* sp. 3452A, *B. ovatus, B. thetaiotaomicron* | *C. clostridiiforme, Bacteroides* sp. 3452A, *B. distasonis* | *B. ovatus* |
| *Bacteroides* sp. 3452A | *Bacteroides* sp. 3452A | *Bacteroides* sp. 3452A, *B. distasonis, C. clostridiiforme* | *B. distasonis*[e] |
| *B. uniformis* | *B. uniformis* | *B. ruminicola, C. clostridiiforme, F. prausnitzii* | *B. uniformis* |
| *B. melaninogenicus* | *B. bivius* | *B. bivius* | 200010[d] |
| *B. melaninogenicus* | *B. bivius* | *B. asaccharolyticus, F. nucleatum*, F-2 | 200020[d] |
| *B. bivius* | *B. melaninogenicus* subsp. *intermedius*[f] | *B. asaccharolyticus, F. nucleatum*, F-2 | F-1, F-2, *B. ureolyticus* |
| *B. bivius* | *B. bivius* | *B. asaccharolyticus, F. nucleatum*, F-2 | F-1, F-2, *B. ureolyticus* |
| *B. oralis* | *B. oralis* | *F. prausnitzii, B. oralis, B. melaninogenicus* subsp. *melaninogenicus*[f] | F-1, F-2, *B. ureolyticus*[e] |
| *C. ochracea* | *Capnocytophaga* sp., *B. melaninogenicus* subsp. *melaninogenicus*,[f] *B. oralis, B. vulgatus, B. ruminicola* subsp. *ruminicola* | *C. ochracea, B. melaninogenicus* subsp. *melaninogenicus*,[f] *B. oralis* | 210012[e] |
| *F. necrophorum* (4) | *F. nucleatum, B. asaccharolyticus, F. necrophorum, F. varium, B. melaninogenicus* subsp. *intermedius*[f] | *B. asaccharolyticus, F. nucleatum, F. necrophorum* | F-1, F-2, *B. ureolyticus* |
| *F. necrophorum* | *F. nucleatum, B. asaccharolyticus, F. necrophorum, F. varium, B. melaninogenicus* subsp. *intermedius*[f] | *B. asaccharolyticus, F. nucleatum*, F-2 | F-2, *F. nucleatum, F. necrophorum* |
| *F. mortiferum* | *B. oralis* | *F. mortiferum, B. distasonis, B. oralis* | *F. mortiferum, B. distasonis* |
| *F. mortiferum* | *B. oralis* | *F. mortiferum, B. distasonis, B. oralis* | *F. mortiferum, B. vulgatus, B. fragilis* |
| *F. mortiferum* | *B. oralis* | *B. melaninogenicus* subsp. *intermedius*,[f] *B. melaninogenicus* subsp. *melaninogenicus*,[f] *F. mortiferum* | *F. mortiferum, B. distasonis* |

| | | | |
|---|---|---|---|
| *C. novyi* A | *C. noyvi* A, *Peptostreptococcus anaerobius, C. limosum, E. lentum, C. tetani* | *C. novyi* A | *E. lentum* |
| *C. butyricum* | *C. butyricum, C. beijerinckii, A. israelii, B. adolescentis, C. clostridiiforme* | *C. butyricum* | *C. ramosum, C. tertium* |
| *C. butyricum* | *Clostridium* sp.[c] | *C. butyricum* | *C. tertium* |
| *C. cadaveris* | *E. limosum, Peptostreptococcus anaerobius, F. symbiosum, E. moniliforme, C. difficile* | *C. haemolyticum, C. cadaveris, C. bifermentans* | *C. cadaveris* |
| *C. sporogenes* | *Clostridium* sp.[c] | *C. sporogenes, C. botulinum* A, B, F, *C. sordellii* | *C. subterminale* |
| *C. sporogenes* | *Clostridium* sp.[g] | *Clostridium* sp.,[g] *C. botulinum* A, B, F, *C. sporogenes* | 751610[d] |
| *C. histolyticum* | *Clostridium* sp.[c] | *Clostridium* sp.,[c] *C. tetani, C. symbiosum* | *E. lentum* |
| *C. difficile* | *C. novyi* A, *Peptostreptococcus anaerobius, C. limosum, E. lentum, C. tetani* | *Clostridium* sp.,[g] *C. tetani, C. symbiosum* | *C. innocuum, C. difficile* |
| *C. difficile* | *Clostridium* sp.[g] | *Clostridium* sp.,[g] *C. tetani, C. symbiosum* | *C. cadaveris* |
| *Peptococcus asaccharolyticus* | *Peptococcus asaccharolyticus* | *Peptococcus asaccharolyticus* | *Peptococcus prevotii, Peptostreptococcus anaerobius* |
| Nonreactive, gram-positive cocci (6 *Peptococcus magnus*, 3 *Peptococcus prevotii*, 6 *Peptostreptococcus anaerobius*) | *Peptostreptococcus micros, Peptostreptococcus anaerobius, Peptococcus magnus, Peptococcus prevotii, G. anaerobia* | *A. fermentans, Peptostreptococcus micros, Peptococcus niger*[h] | *Peptococcus magnus, Peptococcus prevotii, Peptococcus asaccharolyticus, Peptostreptococcus anaerobius* |
| *S. saccharolyticus*[i] | *Peptococcus* sp.[g] | *V. alcalescens, Peptococcus* sp.,[g] *V. parvula* | *Peptococcus magnus, Peptococcus prevotii, Peptococcus asaccharolyticus, Peptostreptococcus anaerobius* |
| *S. saccharolyticus*[i] | *Peptococcus* sp.[g] | *V. alcalescens, Peptococcus* sp.,[g] *V. parvula* | *Peptococcus saccharolyticus* |
| *S. saccharolyticus*[i] | *Peptococcus* sp.[g] | *V. alcalescens, Peptococcus* sp.,[g] *V. parvula* | *Peptostreptococcus anaerobius, Peptococcus saccharolyticus* |

[a] Identified by conventional tests (see the text).
[b] Where a spectrum of possibilities is given, identifications are listed in descending order of probability.
[c] Includes correct species identification.
[d] No code.
[e] Organism not included in AT data base at this time.
[f] Code books still use subspecies of *B. melaninogenicus*.
[g] Does not include correct species identification.
[h] All 15 strains should be SI: modification needed in MT data base (see the text).
[i] Code books still refer to *S. saccharolyticus* as *Peptococcus saccharolyticus*.

Soc. Microbiol. 1982, C312, p. 323), results similar to ours were obtained with respect to number of correct identifications, misidentifications, and number of strains requiring GLC for identification. Apart from the need for technical expertise in interpretation of sugar reactions, AP appears to be a satisfactory method for identification of most commonly isolated reactive anaerobes, especially clinically significant gram-negative rods and clostridia. If AP were coupled with GLC, satisfactory identification of nonreactive strains would also be possible.

Most previous reports on the MT anaerobe system have been confined to the accuracy and reproducibility of individual reactions, compared with conventional methods (8, 13, 21). Hanson and colleagues (9) reported a correct identification rate of 51%, utilizing different substrates and an identification table in place of a code book. The improved identification rate reported in the current study reflects improvements in test reactions and improvement of the data base with provision of an expanded code book as well as computer support. MT reactions were easy to read. We have no explanation for the uniformly negative results obtained with some biochemically reactive organisms; all organisms yielded similar reactions on repeat testing, and cultures were uniformly viable. The MT data base separates the *Veillonella* spp. group into *V. parvula* and *V. alcalescens*. Mays and co-workers (18) have found a high degree of DNA homology between these two *Veillonella* sp. groups and have proposed that they be regarded synonymously regardless of catalase production. All strains of *Peptococcus magnus* and *Peptococcus prevotii* tested in this study were catalase negative, and all *Peptostreptococcus anaerobius* strains were asaccharolytic. With the current MT code book, catalase positivity is necessary for correct identification (to genus only) of *Peptococcus magnus* and *Peptococcus prevotii*, and fermentation of $\geq$1 sugar(s) is necessary for correct species identification of *Peptostreptococcus anaerobius*. In our experience, catalase has not proved useful in recognition or differentiation of the above species. A misconception exists that catalase can be useful in differentiation of peptococci from peptostreptococci; this should be rectified. With the above modifications in the data base, MT would have been comparable to AP in anaerobe identification, differing only in providing fewer correct identifications to genus only and more SI (70.9% correct to species, 22.7% SI, 6.4% incorrect). As with AP, concomitant use of GLC in selected cases would expand the rate of accurate species identification.

AT appears to have some limitations at the present time that should be noted. This method yielded fewer correct identifications and more misidentifications than AP or MT for the data base as a whole. However, considering the length of time it has been on the market, it has an identification rate comparable to the other systems when they were first introduced. Lombard et al. (17) have reported a correct identification rate of 70% with this system, by performing additional differential tests when recommended in the manufacturer's instructions. The higher rate of correct identifications in the current study probably reflects inclusion of more strains of *B. fragilis*, *P. acnes*, and *C. perfringens*, which are easily identified by this system. Reactions were often difficult to interpret (17), especially sugar fermentation, milk, and lipase tests. Indole reactions presented problems, especially for fusobacteria and *Peptococcus asaccharolyticus* strains. The data base of AT would benefit from expansion to include strains such as *C. ochracea*, *B. oralis*, and *Bacteroides* sp. 3452A and from provision of computer support for numbers not included in the code book. Furthermore, additional data on gram stain, colonial morphology, $O_2$ relationship, black pigmentation of colonies, swarming, hemolysis, sporulation, motility, specific GLC reactions, antibiotic susceptibility, and other biochemical reactions need to be presented in the code book, for organisms with SI classifications. Growth of some fastidious organisms is insufficient and may have resulted in some misidentifications; biochemically reactive organisms that yielded no positive reactions with AT yielded identical results on repeat testing, although all cultures were shown to be viable. Perhaps the medium in the center well could be enriched, and the introduction of xylene extraction may improve indole detection. Enrichment of the peripheral media might also improve growth of more fastidious organisms. Such modifications are currently being considered by the manufacturer.

The classification of anaerobes is currently in a state of flux. *Peptococcus saccharolyticus* has been transferred to the genus *Staphylococcus* (16), and *B. melaninogenicus* subspecies no longer exist, having been replaced by *B. melaninogenicus*, *Bacteroides denticola*, and *Bacteroides loescheii* (11). Human strains previously identified as *Bacteroides ruminicola* have been shown to be distinct from the type strain of *B. ruminicola* and are probably either *Bacteroides oris* or *Bacteroides buccae* (12). *Bacteroides oralis* should be differentiated from *Bacteroides veroralis* and *Bacteroides buccalis* (20, 23). Continual updating of manufacturers' data bases of all three systems to reflect current taxonomic thinking is necessary, if these types of methods are to be of most use.

We recognize that an inherent defect in this

study is the relatively narrow spectrum of organisms studied. However, the 165 clinically isolated cultures as well as the 7 *B. distasonis* strains (see above) represent the breakdown of all clinically isolated anaerobic organism groups isolated over a 9-month period at a large tertiary care center (Johns Hopkins Hospital). Very few non-*B. fragilis* group *Bacteroides* spp. were isolated: the paucity of organisms of the *B. melaninogenicus-B. asaccharolyticus-B. denticola-B. loescheii* group was compounded by difficulty in maintaining viable cultures of these strains, despite repeated subculture, rigorous anaerobic conditions, and freezing at −40°C in thioglycolate-glycerol. *F. necrophorum* was the commonest *Fusobacterium* isolated, and *P. acnes* was the only clinically isolated gram-positive nonspore-forming rod observed. Peptostreptococci other than *Peptostreptococcus anaerobius* were not isolated. Inclusion of greater numbers of organisms not included in the current study is needed to further define the usefulness of the 3 commercial systems under investigation.

For microbiology laboratories without GLC support and where identification of clinically significant *B. fragilis* and *C. perfringens* organisms is desired, any of the three systems would provide accurate information. For more extensive anaerobe identification, including the less frequently isolated more unusual organisms, AP and MT offer reasonable identification schemes.

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