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Comparison of Three Procedures for Biochemical Testing of Anaerobic Bacteria

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The Analytab Products, Inc. (API), anaerobic multitest microsystem (MICRO) was compared with the Center for Disease Control conventional (CONV) thioglycolate (supplemented with hemin and vitamin K_1) system and with pre-reduced anaerobically sterilized (PRAS) media as recommended by the Virginia Polytechnic Institute. Growth from a solid medium was suspended to produce standard inocula. Substrates included 16 carbohydrates, indole, urea, gelatin, and esculin. API strips were inoculated in air and incubated in GasPak (BBL) jars. MICRO tests were read at 1 and 2 days, CONV tests at 1, 2, and 7 days, and PRAS tests at 3 weeks. One hundred thirty well-characterized strains of anaerobes (76 gram-negative rods, 16 cocci, 26 gram-positive nonsporeforming rods, and 12 clostridia), including 48 reference strains, were studied. Of 2,600 tests performed, 2,085 (80.2%) showed agreement with all three methods. There was 90.9% agreement between the MICRO and CONV, 84.9% between the MICRO and PRAS, and 84.6% between the CONV and PRAS tests. All MICRO tests were reliable except for indole, which was not sensitive enough, and gelatin, which was very insensitive. The MICRO system permits performance of biochemical tests at the workbench in the average clinical laboratory without the need for expensive equipment and time-consuming procedures.

Currently there is considerable interest in the development and use of modified (abbreviated) biochemical test procedures in clinical microbiology. Most systems have been developed for identification of organisms that grow rapidly and are enzymatically quite active, such as *Enterobacteriaceae*. It should be noted, however, that buffered (rapid) substrates were employed by Pickett et al. some time ago for speciation of *Brucella* (4, 6) and subsequently for relatively rapid identification of nonfermentative gram-negative bacteria (5). Several commercial systems are available and share the following objectives: simplicity, use of small volumes of substrate, more rapid results, and reactivity patterns that are reproducible and correlate well with those patterns recognized as essential for identification.

Starr et al. (10) pointed out the need for simpler, more rapid, and less costly systems for identification of anaerobes. They compared a micromethod multitest system (API Anaerobe System, Analytab Products, Inc.) with the conventional test procedures employed at the Center for Disease Control (CDC). The conventional media were maintained in an anaerobic chamber for at least 48 h before use. Both systems were inoculated and incubated in an anaerobic chamber. With notable exceptions, such as tests for nitrate reduction, H_2S production, and indole production, there was over 90% agreement between the two systems. A similar API system has been used effectively for identification of *Enterobacteriaceae* (9, 13).

Since most clinical laboratories do not have anaerobic chambers and since there is evidence to indicate that strains which have been isolated from clinical specimens are quite tolerant to exposure to air during subculture, etc. (1, 7; F. P. Tally et al., Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, M60, p. 83), we evaluated the API Anaerobe System (MICRO), inoculating the strips under aerobic conditions and incubating them anaerobically in GasPak jars (BBL). These results were compared with conventional (CONV) test results obtained by inoculating steamed and cooled media under normal atmospheric conditions and with results obtained with pre-reduced anaerobically sterilized (PRAS) media.

MATERIALS AND METHODS

Bacterial strains. The 130 strains of anaerobic

TABLE 1. *Anaerobic bacteria included in comparison of three procedures for biochemical testing of anaerobic bacteria*

Microorganism or group	No. of strains
Bacteroides fragilis (2 subsp. *distasonis*, 21 subsp. *fragilis*, 1 subsp. *ovatus*, 13 subsp. *thetaiotaomicron*, 8 subsp. *vulgatus*, 7 "other")	52
B. hypermegas	1
B. melaninogenicus (2 subsp. *asaccharolyticus*, 6 subsp. *intermedius*, 5 subsp. *melaninogenicus*)	13
Fusobacterium (1 each *necrogenes*, *necrophorum*, *nucleatum*, *mortiferum*, 6 *varium*)	10
Peptococcus (1 *Peptococcus* species, 1 *magnus*, 2 *prevotii*, 1 *saccharolyticus*, 2 *variabilis*)	7
Peptostreptococcus (2 *anaerobius*, 1 *micros*)	3
Acidaminococcus fermentans	1
Veillonella (2 *alcalescens*, 2 *parvula*)	4
Megasphaera elsdenii	1
Eubacterium (1 *aerofaciens*, 1 *alactolyticum*, 1 *lentum*, 1 *limosum*, 1 *tortuosum*, 1 *ventriosum*)	6
Arachnia propionica	1
Propionibacterium (2 *acnes*, 1 *avidum*, 1 *granulosum*)	4
Actinomyces (1 each *bovis*, *israelii*, *naeslundii*, *viscosus*)	4
Bifidobacterium (1 *adolescentis* var. *A*, 1 *adolescentis* var. *D*, 1 *bifidum* var. *B*, 2 *breve*, 1 *eriksonii*, 1 *infantis* subsp. *infantis*, 1 *infantis* subsp. *liberorum*, 2 *longum* subsp. *longum*)	10
Lactobacillus catenaforme	1
Clostridium (4 *innocuum*, 1 *oceanicum*, 3 *sporogenes*, 4 *ramosum*)	12
Total organisms included in study	130

bacteria included in this study are grouped in Table 1. Forty-eight of these are type, neotype, or reference strains; see Table 2. The remaining 82 strains were obtained from the Wadsworth Anaerobic Bacteriology Laboratory (WAL) collection and were isolated either from normal human feces or from clinical specimens.

MICRO system. The API Anaerobe System physically resembled the API system for *Enterobacteriaceae* and the API System employed by Starr et al. (10) for anaerobes, but differed in some substrate components. The 20 substrates included indole, urea, glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, gelatin, esculin, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose, and trehalose.

CONV system. Conventional fermentation media, indole, gelatin, and esculin broth were prepared according to Dowell and Hawkins (2), but vitamin K_1 and hemin were added to each to give final concentrations of 0.1 μg of vitamin K_1 per ml and 5.0 μg of hemin per ml. The rapid urease procedure recommended by Sutter et al. (11) was used.

PRAS media system. PRAS media consisting of the same 20 substrates as the API System, plus necessary media for gas-liquid chromatography (GLC), were prepared as directed by Holdeman and Moore (3). During this investigation, vitamin K_1 and hemin were not incorporated in the PRAS media. (Subsequently, W. E. C. Moore [personal communication] has indicated that vitamin K_1 and hemin should be added to all PRAS media.)

Procedure. Stock cultures frozen with skim milk were removed from the freezer (−70 C) as needed. These were inoculated into tubes of maintenance thioglycolate broth (BBL 135-C) supplemented with $NaHCO_3$, 1 mg/ml, and vitamin K_1, 0.1 μg/ml (8), and onto two brucella (Pfizer) blood agar vitamin K_1 (BAK_1) plates. One plate was incubated aerobically and one plate anaerobically (GasPak) at 35 C for 48 to 72 h to check for purity. After appropriate anaerobic growth, colony morphology and Gram stains were observed and recorded. Slide catalase and spot indole tests (12) were also performed. A loopful of growth was used to inoculate a tube containing 0.5 ml of Stuart et al. urea broth (Difco). This test was read after aerobic incubation at 35 C overnight. Sufficient growth (20 or more colonies) was emulsified in 10 to 12 ml of thioglycolate medium without dextrose or indicator (Difco 0432), supplemented with 0.1 μg of vitamin K_1 per ml and 5.0 μg of hemin per ml, to match a McFarland no. 1 nephelometer standard. This suspension was used to inoculate the MICRO, CONV, and PRAS systems.

We inoculated the PRAS tubes first, using a roll-tube inoculator while flushing with oxygen-free CO_2. Tubes were incubated at 35 C for 3 weeks and then were read according to the Virginia Polytechnic Institute *Anaerobe Laboratory Manual* (3). End products of metabolism were determined by GLC, where indicated, by the procedure described by Sutter et al. (11). Next, the standardized suspension was used to inoculate the CONV media, after the tubes had been steamed for 10 min and cooled, and sterile carbohydrate solution had been added as needed. Sterile capillary pipettes were used to inoculate a droplet to the bottom of each tube. Tubes were incubated at 35 C and read at 24 and 48 h; a final reading was made at 7 days.

Finally, the standardized suspensions were used to inoculate the MICRO system. Each strip was prepared by writing the culture number in the center and placed in the incubation tray provided, to which 4 ml of water was added. Large capillary pipettes (sterile) and rubber bulbs were used to inoculate the tubes. The tube and cupule of gelatin were filled, but only the tube section of the other substrates was filled. In early tests, no mineral oil was added to the indole test; later, mineral oil was added to the indole cupule. As each strip was filled, it was placed in a metal rack designed to hold 12 strips. After the rack was filled with strips, a catalyst basket was taped to the upper inner surface of a GasPak 100 anaerobic jar which had

been modified to lie in a horizontal position. An indicator strip was placed in the jar so that it would not come in contact with the catalyst container. The top 2⅛ inches (5.4 cm) was cut from a GasPak envelope. An 8-ml amount of water was added to the GasPak envelope, and it was then placed at the side of the rack in the jar. The GasPak envelope was tilted slightly away from the rack and rested on the upper wall of the jar. The lid was clamped on the jar, and the jar was placed horizontally in an incubator at 35 C. The strips were examined through the unopened jar at 24 h and the results were recorded. At 48 h, the jar was opened and the final readings were made. To test for indole, a drop of xylene was added on top of the oil and mixed with a toothpick; then a drop of Ehrlich reagent added. A red color indicated a positive test. Urease activity was indicated by a red color (phenol red). A positive gelatin test was indicated by dispersion of the carbon particles. Esculin hydrolysis was indicated by a brownish-black color which was further examined for lack of fluorescence when exposed to a 366-nm wavelength ultraviolet light. The carbohydrate substrates contained bromocresol purple (BCP) indicator; therefore, yellow indicated fermentation and purple indicated lack of fermentation. If the indicator was reduced (colorless), a drop of 0.04% aqueous BCP was added to the tube before the reading was made.

Results of the three procedures were compared with one another and with expected reactions listed by Dowell and Hawkins (2), Holdeman and Moore (3), and Sutter et al. (11). With 41 organisms, the MICRO, CONV, and PRAS results were recorded on separate data sheets along with supplementary information. These 123 data sheets were coded and scrambled, and identification by the reaction schemes cited above was attempted.

RESULTS

Table 2 shows the results obtained with the 48 type, neotype, and reference strains in all three systems. The greatest disagreement with expected reactions occurred with gelatin, since only one MICRO gelatin test was positive. Also, the disagreements were greater with organisms that are slow growers and weak acid producers, such as many of the gram-positive nonsporeforming anaerobic rods.

Table 3 summarizes the results obtained with 130 strains, including the 48 type, neotype, and reference strains. The overall agreement of the three systems was 80.2%. When the very poor results with gelatin were discounted, the overall correlation increased 82.8%. For the 76 gram-negative rods, the overall agreement was 80.1% (83.3% when gelatin results were omitted). For the 16 cocci the overall agreement was 94.1% (97.0% without gelatin). Agreement for the 26 gram-positive nonsporeforming rods was 71.3% (71.9% without gelatin). Agreement for the 12 clostridia was 82.5% (85.1% without gelatin).

Although indole results showed good overall agreement, there were nine negative results by the MICRO system when either the PRAS or CONV or both were positive. Most of these discrepancies occurred early in our studies. When the test was repeated on four *Bacteroides fragilis* subspecies (three subsp. *thetaiotaomicron* and one subsp. *ovatus*) with an improved API indole strip, three of the four were positive.

There was total agreement with urease tests, with only 2 positive and 128 negative. The two positive results were with *Peptococcus prevotii* and *P. saccharolyticus*.

The overall agreement for the 16 carbohydrates was 81.4%. Among strains failing to ferment glucose in the MICRO system were a slow-growing strain of *B. fragilis* subsp. *fragilis*, five strains of *B. melaninogenicus*, three strains of *C. sporogenes*, and one each of *Peptostreptococcus anaerobius*, *Megasphaera eldenii*, *Eubacterium ventriosum*, *Arachnia propionica*, *Actinomyces naeslundii*, *Fusobacterium mortiferum*, and *F. necrophorum*. In looking down the columns of Table 3, it is apparent that individual negative carbohydrate results were recorded more often with the CONV (66) and PRAS (62) than with the MICRO system (38). Although individual positive carbohydrate results were much higher with PRAS (191) than with CONV (19) or MICRO (12), this was largely a result of recording of weak positive results (pH 5.6 to 5.9) in the PRAS systems which were recorded as weak, variable, or negative identification schemes. Total agreements between pairs of procedures were counted so that the direct comparisons given in Table 4 could be made.

The 41 organisms which were evaluated separately by the MICRO, CONV, and PRAS systems consisted of 19 *B. fragilis*, 1 *F. varium*, 1 *Clostridium innocuum*, 1 *C. ramosum*, 1 *Acidaminococcus fermentans*, 1 *M. elsdenii*, 1 *P. prevotii*, 1 *P. saccharolyticus*, 1 *P. variabilis*, 1 *Veillonella parvula*, 1 *V. alcalescens*, 6 *Bifidobacterium* species, 5 *Eubacterium* species, and 1 *A. propionica*. With supplementary information for preliminary grouping, plus GLC results when needed, 26 were definitively identified by the MICRO system, 28 by the CONV, and 30 by the PRAS, but only 19 were correctly identified by all three systems. The 22 organisms incorrectly identified by one or more systems are listed in Table 5. The first five organisms were incorrectly identified in all three systems. The first organism listed probably is a *B. fragilis* subsp. *distasonis* (and therefore not incorrectly

TABLE 2. *Biochemical reactions of 48 type, neotype, and reference strains of anaerobic bacteria with the API microsystem*

Type, neotype, and reference strains	Substrate or test[a]																			
	Indole	Urea	Glucose	Mannitol	Lactose	Sucrose	Maltose	Salicin	Xylose	Arabinose	Gelatin	Esculin	Glycerol	Cellobiose	Mannose	Melezitose	Raffinose	Sorbitol	Rhamnose	Trehalose
Bacteroides fragilis subsp. *distasonis* ATCC 8503	–	–	A	–	A	A	A	A	A	–	c&p (–)	+	–	A c&p	A	A	A	–	A	A
B. fragilis subsp. *fragilis* NCTC 9343[b]	–	–	A	–	A	A	A	–	A	–	(–)	+	–	(A)	A	–	A	–	–	–
B. fragilis subsp. *ovatus* ATCC 8483	c&p (–)	–	A	A	A	A	A	a	A	A	–	+	–	A	A	A	A	–	A	A
B. fragilis subsp. *thetaiotaomicron* NCTC 10582	–	–	A	–	A	A	A	A	p (A)	–	c&p (–)	+	–	A	A	A	A	–	A	A
B. fragilis subsp. *vulgatus* ATCC 8482	–	–	A	–	A	A	A	–	A	A	c&p (–)	+	–	–	A	–	A	–	A	–
B. hypermegas ATCC 25560[c]	–	–	a	a	a	c&p (–)	a	p (–)	c (a)	a	c (–)	c (+)	p (–)	p (–)	a	p (–)	a	a	p (–)	a
B. melaninogenicus subsp. *asaccharolyticus* ATCC 25260	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. melaninogenicus subsp. *melaninogenicus* ATCC 25845	p (–)	–	c (a)	p (–)	c (a)	c (a)	c (a)	–	–	–	c&p (–)	p (–)	–	–	c (a)	–	c (a)	p (–)	–	–
Fusobacterium necrogenes ATCC 25556[c]	–	–	a	–	–	p (–)	p (–)	p (–)	–	–	–	c (+)	–	–	a	–	–	p (–)	p (–)	a
F. necrophorum ATCC 25286	c&p (–)	–	p (–)	–	–	–	–	–	p (–)	p (–)	c&p (–)	–	p (–)	–	p (–)	–	–	p (–)	p (–)	–
F. mortiferum ATCC 9817[c]	–	–	a	–	p (–)	p (–)	p (–)	–	–	–	c (–)	+	–	–	p (–)	–	–	–	–	–
F. varium ATCC 8501	+	–	a	–	–	–	–	–	–	–	–	–	–	–	a	–	–	–	–	–
Acidaminococcus fermentans ATCC 25085	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Megasphaera elsdenii ATCC 25940	–	–	a	p (a)	–	p (–)	a	–	–	–	–	–	–	–	–	–	–	–	–	–
Peptococcus prevotii ATCC 9321	–	+	–	–	–	–	–	–	–	–	c&p (–)	–	–	–	–	–	–	–	–	–
P. saccharolyticus ATCC 14953	–	+	a	–	–	p (–)	–	–	–	–	c&p (–)	–	a	–	a	–	–	–	–	–
P. variabilis ATCC 14955	–	–	–	–	–	–	–	–	–	–	c&p (–)	–	–	–	–	–	–	–	–	–
Peptostreptococcus anaerobius ATCC 27337	–	–	a	–	–	–	p (–)	–	–	–	c&p (–)	–	–	–	–	–	–	–	–	–
Veillonella alcalescens ATCC 17745	–	–	–	–	–	–	–	–	–	–	c&p (–)	–	–	–	–	–	–	–	–	–

V. parvula ATCC 10790	–	–	–	–	–	–	–	–	–	–	c&p (–	–	–	–	–	–	–	–	–	–
Eubacterium aerofaciens ATCC 25986[c]	–	–	p (a)	–	c (a)	p (a)	p (a)	–	–	–	–	–	–	–	p (a)	–	–	–	–	–
E. alactolyticum ATCC 23263[c]	–	–	A	c&p (–)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E. lentum ATCC 25559[c]	–	–	–	–	–	–	–	–	–	–	p (–)	–	–	–	–	–	–	–	–	–
E. limosum ATCC 8486[c]	–	–	A	a	–	p (–)	p (–)	p (–)	p (–)	p (–)	–	c (+)	–	p (–)	p (–)	–	–	p (–)	–	–
E. tortuosum ATCC 25548[c]	–	–	–	–	–	c&p (–)	p (–)	–	–	–	–	p (–)	–	p (–)	–	–	–	–	–	–
E. ventriosum VPI 1013A[c]	–	–	p (–)	–	–	p (–)	p (–)	–	p (–)	–	–	c (+)	–	–	p (–)	–	–	–	p (–)	–
Arachnia propionica ATCC 14157 (72 h)	–	–	a	a	c&p (a)	a	p (a)	–	–	–	c (–)	p (–)	–	–	c&p (a)	–	a	p (a)	–	a
Propionibacterium acnes I ATCC 6919	+	–	A	p (A)	–	–	–	–	p (–)	–	–	–	a	–	A	–	–	p (a)	p (–)	p (–)
Propionibacterium acnes II ATCC 25746	–	–	A	A	–	a	a	–	p (–)	p (–)	–	–	A	–	A	p (–)	–	–	–	p (–)
P. avidum ATCC 25577	–	–	A	–	–	a	a	p (–)	p (–)	A	+	+	A	–	A	a	–	–	p (–)	a
P. granulosum ATCC 25564	–	–	A	–	–	a	a	–	p (–)	p (–)	–	–	A	–	a	c (a)	–	–	p (–)	c (a)
Actinomyces bovis CDC 1109	–	–	a	–	c&p (a)	a	c&p (a)	–	–	–	–	p (+)	–	–	c&p (–)	–	–	–	–	–
A. israelii CDC W1438	–	–	A	A	A	A	A	A	A	A	–	+	–	A	A	–	p (A)	–	–	A
A. naeslundii CDC W1387	–	–	p (–)	–	p (–)	p (–)	p (–)	–	–	–	–	–	–	–	p (–)	–	–	–	–	p (–)
A. viscosus ATCC 15987	–	–	a	–	a	a	a	–	c&p (a)	–	–	w +	–	–	a	–	a	–	–	a
Eifidobacterium adolescentis var. *A* ATCC 15703[d]	–	–	A	p (–)	c (A)	A	c (A)	c (A)	c (a)	c (a)	c (–)	c (+)	–	p (–)	–	–	A	p (–)	–	c (–)
B. adolescentis var. *D* ATCC 15706[d]	–	–	A	p (–)	c (A)	c (a)	c (A)	c (a)	c (a)	c (a)	c (–)	c (+)	–	p (–)	–	–	A	–	–	p (–)
B. bifidum var. *B* ATCC 15696[d]	–	–	A	–	A	p (–)	p (–)	–	c&p (a)	p (–)	–	–	–	p (–)	–	–	p (–)	–	a	–

[a] Symbols: –, negative reaction or no acid from carbohydrate; +, positive reaction; A, acid (definite yellow color); a, weak acid (brownish-yellow color); (), different reaction with c (conventional) or p (PRAS) system, as indicated by the letter above the symbol.

[b] Strain obtained from H. R. Ingham.

[c] Strain obtained from L. V. Holdeman and W. E. C. Moore.

[d] Strain obtained from G. Reuter.

TABLE 2—*Continued*

Type, neotype, and reference strains	Substrate or test[a]																			
	Indole	Urea	Glucose	Mannitol	Lactose	Sucrose	Maltose	Salicin	Xylose	Arabinose	Gelatin	Esculin	Glycerol	Cellobiose	Mannose	Melezitose	Raffinose	Sorbitol	Rhamnose	Trehalose
B. breve ATCC 15700[d]	–	–	A	A	A	p (A)	A	c (A)	p (–)	–	–	+	–	A	p (A)	–	A	A	–	–
B. eriksonii ATCC 15423	–	–	A	A	A	A	A	A	A	A	–	+	–	A	A	–	A	–	–	p (–)
B. infantis subsp. *infantis* ATCC 15697[d]	–	–	A	–	A	–	A	A	–	–	c (–)	c (+)	–	A	c (a)	A	A	–	–	–
B. infantis subsp. *liberorum* ATCC 15702[d]	–	–	a	–	c (a)	p (–)	c (a)	p (–)	p (–)	–	c (–)	p (–)	–	p (–)	a	p (–)	c (a)	–	c (–)	c (–)
B. longum subsp. *longum* ATCC 15707[d]	p (–)	–	a	–	c (a)	c (a)	a	–	a	c (a)	p (–)	p (–)	c&p (a)	–	p (–)	c (a)	c (a)	–	p (–)	c (a)
B. longum subsp. *longum* ATCC 15708[d]	–	–	a	p (–)	a	c (a)	a	–	a	a	–	–	–	–	p (–)	a	c (a)	–	p (–)	p (–)
B. parvulorum (*breve*) ATCC 15698[d]	–	–	a	–	c (a)	p (–)	a	a	–	p (–)	c (–)	+	–	p (–)	a	p (–)	a	–	–	p (–)
Lactobacillus catenaforme ATCC 25536	p (–)	–	a	–	c (a)	p (a)	c&p (a)	a	–	–	c&p (–)	+	–	a	a	–	–	–	p (–)	–
Clostridium innocuum ATCC 14501	–	–	A	A	–	c&p (–)	–	A	–	–	–	+	–	A	A	–	–	–	–	p (A)
C. ramosum ATCC 25582[c]	–	–	A	A	A	A	A	A	–	–	–	+	–	A	A	–	p (A)	–	–	A

TABLE 3. *Results of MICRO, CONV, and PRAS system tests performed on 130 strains of anaerobic bacteria: 16 cocci, 76 gram-negative rods, 26 gram-positive nonsporeforming rods, and 12 sporeforming rods*

Test	MICRO+ CONV − PRAS −	MICRO− CONV + PRAS +	CONV + MICRO− PRAS −	CONV − MICRO+ PRAS +	PRAS + MICRO− CONV −	PRAS − MICRO+ CONV +	All +	All −	Tests in agreement	
									No./total	Percent
Indole	1	3		1	6	1	21	97	118/130	90.8
Urea							2	128	130/130	100.0
Glucose	1	7	1	4	8	2	88	19	107/130	82.3
Mannitol[a]		5	2		12	2	9	99	108/129	83.1
Lactose	1			13	3	9	53	51	104/130	80.0
Sucrose		5	1	9	17	9	56	33	89/130	68.5
Maltose	3	4	1	7	12	4	62	37	99/130	76.2
Salicin	1	4	4	3	16	5	13	84	97/130	74.6
Xylose	3		1	3	14	3	51	55	106/130	81.5
Arabinose			1	5	12	6	27	79	106/130	81.5
Gelatin		57	24		9		3	37	40/130	30.8
Esculin	2	1		12	9	1	63	42	105/130	80.8
Glycerol	1				6		5	118	123/130	94.6
Cellobiose	2	2	2	2	19	7	32	64	96/130	73.8
Mannose		3	1	6	11	5	72	32	104/130	80.0
Melezitose				2	13	3	14	98	112/130	86.2
Raffinose		2		9	4	3	58	54	112/130	86.2
Sorbitol			1	1	14		2	112	114/130	87.7
Rhamnose		1	2		16	1	26	84	110/130	84.6
Trehalose		5	2	2	14	2	22	83	105/130	80.8
20-test total	15	99	43	79	215	63	679	1,406	2,085/2,600	80.2
19-test total (less gelatin)	15	42	19	79	206	63	676	1,369	2,045/2,470	82.8

[a] One mannitol test not recorded.

TABLE 4. *Agreements between pairs of procedures for biochemical testing of 130 strains of anaerobic bacteria*

Test	Tests in agreement	
	No./2,600	Percent
MICRO & CONV	2,364	90.9
MICRO & PRAS	2,208	84.9
CONV & PRAS	2,200	84.6

identified) since we have never detected indole by any procedure. *E. tortuosum* gave negative or inconsistent biochemical reactions in all three systems, and the GLC showed only acetic acid. The three *Bifidobacterium* strains were placed in the right genus, but could not be correctly speciated with the information provided. Organisms 6 through 11 were not correctly identified with two or three systems. The two *B. fragilis* strains were not correctly subspeciated because indole was not detected or because negative results occurred with key sugars. In our tests, *V. parvula* ATCC 10790 is weakly catalase-positive, and this accounts for the confusion recorded here. *P. saccharolyticus* ATCC 14953 was called *Peptococcus* species in the CONV and PRAS systems because a weak gelatin reaction was reported. Organisms numbered 12 through 22 were each misidentified in only one system. The four *B. fragilis* strains were incorrectly identified in the MICRO system because of false-negative indole results. All four organisms gave positive spot indole tests, and all four were positive when retested with the new API indole substrate. *A. propionica* gave no positive reactions in the MICRO system and could not be properly identified. Organisms 17 through 22 were placed in the proper genus, but could not be correctly speciated or subspeciated because of discrepant biochemical tests or GLC results.

DISCUSSION

Because our overall agreement with the three procedures (80.2%) was considerably lower than the agreement found by Starr et al. (10) between micromethod and conventional tests (91.2%), we made the comparison between pairs of procedures given in Table 4. This shows that our MICRO and CONV test agreements (90.9%) compare closely with those reported by Starr et al. Also, the MICRO and CONV tests agree more closely than either of these agrees with the PRAS results; this is largely due to the numerous results which were weakly positive only in PRAS tests.

In our early evaluation of the MICRO system, the indole test was inconsistent with known positive organisms, and the gelatin test was almost totally insensitive. Subsequently, both substrates were improved, and API now recommends a sterile mineral oil overlay for the indole test. When four known indole producers which were negative in early MICRO tests were tested again, the new indole test was positive with all but one strain of *B. fragilis* subsp. *thetaiotaomicron*. Our observation is that the spot indole test is a very reliable screening test and, if positive, further testing is not necessary. If this test is questionable or equivocal, then the MICRO indole test is as reliable as either the CONV or PRAS tests for a secondary test. A limited evaluation of the new gelatin test indicates that it is more sensitive than the earlier one, but further evaluation is necessary. Although the urease test has limited application at present, the MICRO substrate seems reliable. *P. prevotii* and *P. saccharolyticus* were urease-positive in all of the systems, and perhaps further studies will indicate more areas of usefulness of the urease test for identification of anaerobes.

Esculin hydrolysis by the API procedures was found reliable when false-positive results, due to H_2S production, were eliminated by reading with a 366-nm ultraviolet light for nonfluorescence of hydrolyzed esculin. Table 3 shows that the greatest discrepancies with this test were due to the difference in sensitivity between the CONV and PRAS systems.

Starr et al. (10) found that the micromethod system results had to be supplemented with other tests and GLC information in order to identify more than 66% of the anaerobes they tested. For this reason, we provided supplementary information (GLC data, colony morphology, Gram reaction, presence of spores, motility, antibiotic susceptibility pattern, and lecithinase, lipase, and catalase activity) on data sheets for all three procedures when identification of the 41 coded organisms was being attempted. The MICRO system performed as well as the other two procedures in these identifications with two exceptions. False-negative indole tests resulted in improper subspeciation of four *B. fragilis* strains, and *A. propionica* could not be identified because of biochemical inactivity. All four of the *B. fragilis* strains gave positive spot indole tests, and subsequently three of these gave positive MICRO indole test results (with the improved test). Biochemical inactivity was noted especially with gram-positive nonsporeforming rods, some cocci, and

TABLE 5. *Comparison of MICRO, CONV, and PRAS results for 22 anaerobes (of 41 tested) incorrectly identified by one or more systems*

Organism	Source[a]	Designation by		
		MICRO	CONV	PRAS
1. *B. fragilis* subsp. *thetaiotaomicron*	NCTC 10582	*distasonis*	*distasonis*	*distasonis*
2. *E. tortuosum*	ATCC 25548	*lentum*	species[b]	species
3. *B. breve*	ATCC 15700	*adolescentis* var. *A*	species	*adolescentis* var. *A*
4. *B. infantis* subsp. *infantis*	ATCC 15697	*breve*	species	species
5. *B. eriksonii*	ATCC 15423	*breve*	*adolescentis* var. *D*	species
6. *B. fragilis* subsp. *fragilis*	WAL[c] 2447	*fragilis*	*distasonis*	*distasonis*
7. *B. fragilis* subsp. *thetaiotaomicron*	WAL 2030	*distasonis*	*distasonis*	*thetaiotaomicron*
8. *V. parvula*	ATCC 10790	*alcalescens*	*alcalescens*	*parvula*
9. *P. saccharolyticus*	ATCC 14953	*saccharolyticus*	species	species
10. *B. adolescentis* var. *A*	ATCC 15703	*adolescentis* var. *D*	species	*adolescentis* var. *A*
11. *B. adolescentis* var. *D*	ATCC 15706	species	species	*adolescentis* var. *D*
12. *B. fragilis* subsp. *thetaiotaomicron*	WAL 1168	*distasonis*[d]	*thetaiotamicron*	*thetaiotaomicron*
13. *B. fragilis* subsp. *thetaiotaomicron*	WAL 1423	*distasonis*[d]	*thetaiotaomicron*	*thetaiotaomicron*
14. *B. fragilis* subsp. *thetaiotaomicron*	WAL 2330	*vulgatus*[d]	*thetaiotaomicron*	*thetaiotaomicron*
15. *B. fragilis* subsp. *ovatus*	ATCC 8483	"other"[d]	*ovatus*	*ovatus*
16. *A. propionica*	ATCC 14157	*Propionibacterium* or *Arachnia*	*propionica*	*propionica*
17. *E. limosum*	ATCC 8486	*limosum*	species	*limosum*
18. *E. alactolyticum*	ATCC 23263	*alactolyticum*	species	*alactolyticum*
19. *E. aerofaciens*	ATCC 25986	*aerofaciens*	*aerofaciens*	species
20. *B. bifidum* var. B	ATCC 15696	*bifidum* var. *B*	*bifidum* var. *B*	species
21. *B. fragilis* subsp. *fragilis*	WAL 2165	*fragilis*	*fragilis*	*distasonis*
22. *B. fragilis* subsp. *fragilis*	WAL 2211	*fragilis*	*fragilis*	*distasonis*

[a] See Table 2 for source of NCTC and ATCC strains.
[b] Identified as undetermined species of genus in "organism" column.
[c] Wadsworth Anaerobic Bacteriology Laboratory.
[d] Correctly identified later with improved microsystem.

some of the more fastidious gram-negative rods such as *B. melaninogenicus*. Since false-positive results with the MICRO system were never a problem in this study, our limited observations suggest that two considerations deserve emphasis. The first is that the inoculum must be heavy, at least matching the density of a no. 1 McFarland nephelometer. Concentrations equivalent to a no. 4 or 5 McFarland nephelometer are even better for the poorly growing organisms discussed above, and this applies for the CONV and PRAS systems as well. The second consideration is that, with these organisms, sometimes a 3- or 4-day final reading will give more definitive results. This is in contrast to the rapidly growing, active organisms with which tests can be read much earlier. We suggest that preliminary readings be made at 48 h. If the results can be interpreted at that time, then the tests can be discarded. If there are incomplete or questionable results, then the tests should be reincubated and a final reading should be made in another 48 h.

Some of the recommendations of Starr et al. (10) had been incorporated in the API Anaerobe System which we used. For example, the carbohydrates necessary for subspeciation of *B. fragilis* were included and were reliable. Also, Ehrlich reagent and xylene extraction have replaced Kovac reagent in the indole test. Although not shown in this report, our results confirm the greater reliability of the Ehrlich

reagent and xylene extraction. We performed catalase tests on growth from plates as inoculation suspensions were being prepared, but this test can be performed by adding one to two drops of 3% hydrogen peroxide to one of the API System tubes (not indole) 30 min after the strips are exposed to air. We agree with Starr et al. (10) that attempts should be made to include in the micromethod system additional supplementary tests such as lecithinase, lipase, and growth in 20% bile.

We emphasize that tests and observations such as colonial and microscopic morphology, motility, antibiotic susceptibility, lecithinase, lipase, catalase, and GLC are necessary for definitive identification of many anaerobic bacteria. However, pertinent, simplified, rapid biochemical test procedures which will permit identification of most clinically significant isolates can be set up at the work bench in the average clinical laboratory without cumbersome, expensive equipment and time-consuming procedures.

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