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Evaluation of Simplified Dichotomous Schemata for the Identification of Anaerobic Bacteria from Clinical Material

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Simplified dichotomous schemata are described for the identification of anaerobic bacteria commonly encountered in clinical material. The procedures used are combinations of routine biochemical tests and techniques that are used to uniformly characterize these organisms. Over 200 anaerobic organisms were used in a three-stage evaluation in which data were compared with those obtained by conventional methods. When there was inconsistency between the biochemical tests described in the presumptive identification schemes and gas-liquid chromatography, additional biochemical tests or reference procedures were used to confirm identification. Strains from the American Type Culture Collection and the Center for Disease Control, as well as recent clinical isolates, were included in this evaluation. The results show the simplified procedures to be useful for the identification of anaerobic isolates from clinical material.

In recent years, anaerobic bacteria have received increasing amounts of attention and have been implicated in a variety of clinical disorders (1, 2, 5, 12). Therefore, the methods for isolation and identification have, by necessity, undergone extensive research and revision. The most widely used procedures for anaerobe identification, as described by Holdeman and Moore (7), Dowell and Hawkins (4), and Sutter and colleagues (16), include such technical advances as anaerobic chambers, prereduced, anaerobically sterilized (PRAS) media, and gas-liquid chromatography (GLC). The laboratory manuals produced by these workers have detailed biochemical and GLC profiles that provide the basis for nearly complete identification of most anaerobes encountered in clinical infections. However, clinical microbiologists who have assumed the responsibility for culture and identification of these organisms are often faced with the problems of clinical relevance when many days are required for isolation and complete identification. Moreover, anaerobic chambers, roll-tube methods, and GLC are often considered too costly and complex for routine diagnostic use. Conversely, the more simplified procedures that employ anaerobic jars and non-PRAS media are thought to be inadequate for complete identification of all anaerobic isolates.

A recent report has shown that the majority of clinical isolates comprise a relatively small group of anaerobes (10). Accordingly, we have employed sets of simplified tests for the identification of the more commonly encountered strains. This report describes the procedures we propose for culture and identification of clinically important anaerobes and their evaluation by comparison with conventional methods.

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MATERIALS AND METHODS

Organisms. Clinical isolates were obtained from specimens received at the Long Beach Veterans Administration Hospital, Long Beach, Calif., and Temple University Hospital, Philadelphia, Pa. Reference strains included stock cultures received from the American Type Culture Collection (ATCC), Rockville, Md., and the Center for Disease Control, Atlanta, Ga.

Isolation. Organisms were isolated on brucella and brain heart infusion agar (BBL) supplemented with 0.5% yeast extract (Difco), 0.5 μg of menadione per ml, and 5% sheep blood; factors X and V are required for growth of many *Bacteriodes melaninogenicus* strains and were incorporated into all primary isolation media. Laked blood agar containing kanamycin and vancomycin (16) was also used as a primary isolation medium to promote rapid pigmentation of *B. melaninogenicus*. All media were either used within 2 days of preparation or stored anaerobically in CO_2-containing jars (9) or in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Mich.).

Test media. PRAS medium was prepared according to the recommendations of Holdeman and Moore (7) and employed for conventional anaerobic identification. Media for the simplified biochemical testing procedures were freshly prepared but were not PRAS. Media that were not used within 1 week were discarded. Table 1 lists the biochemical tests, media, procedures, and interpretations for use in the dichotomous schemata described in this report. At the present time, the tryptic soy, bile-kanamycin test is being employed for the rapid identification of *B. fragilis;* strains of this species are resistant to 1,000-μg/ml kanamycin disks (BBL) on this medium (17).

Procedures. A Gram stain was performed on all organisms from blood agar and broth cultures. Biochemical tests were selected according to the interpretation of the Gram reaction and cellular morphology. In certain cases, anaerobes showing variability in the Gram reaction were evaluated in more than one scheme. The tests employed for identification are listed in Table 2. The inocula for biochemical tests and GLC analysis consisted of overnight cultures grown in chopped-meat-glucose broth (Difco) or, alternatively, a heavy suspension of the organism was prepared in broth directly from the isolation plates when discreet, characteristic colonies were obtained. A few drops of the organism suspension from either source were used to inoculate the testing media. Most tests were read after 48 h of incubation at 36 C in GasPak (BBL) jars or anaerobic chambers. GLC analysis on Dohrman (AnaBac model) gas chromatographs were performed on extracts of cultures grown in peptone-yeast-glucose broth after 5 days of incubation at 36 C or when adequate growth was obtained.

Identification. Keys for presumptive identification within the major groups of anaerobes are shown in Fig. 1 through 4. The groups are gram-negative bacilli, clostridia, anaerobic cocci, and gram-positive, nonsporeforming bacilli. In Tables 3 through 7, the key biochemical reactions for these groups of anaerobes are listed along with the volatile acids produced in peptone-yeast-glucose medium. Conventional methods used in this comparative evaluation of the identification schemata were described previously (7).

RESULTS

The simplified biochemical schemata described in this report were evaluated in a comparative, three-part study with conventional methods. Part one of the study was the identification of 14 reference strains by using the simplified techniques and conventional methods. The results of this comparison are listed in Table 8. As can be seen, there are three discrepancies. ATCC reference culture 9689 was identified by the simplified methods as *Clostridium butyricum.* Figure 2 shows that this is not an

TABLE 1. *Tests and methods used for the identification of anaerobic bacteria by simplified procedures*

Test	Medium	Procedures and interpretation
Bile	Peptone-yeast-0.5% glucose-20% bile	Compare growth to control tube (peptone-yeast-0.5% glucose): S = stimulated; N = no effect; I = inhibited
Catalase	Brain heart infusion agar slant	Expose slant to air for minimum of 30 min; flood slant with 3% H_2O_2; gas bubbles = positive
Esculin	Peptone-yeast broth with 1% esculin	Add few drops of 1% ferric ammonium citrate; black color = positive
Gram stain	Kopeloff modification	To be performed at all stages of sample processing and testing
Indole	(i) Spot indole	(i) Filter paper saturated with 1% paradimethylaminocinnamaldehyde (14)
	(ii) Indole-nitrite medium	(ii) Ehrlich reagent; test 24 h after good growth
Kanamycin	Brucella-menadione BAP[a] + 1,000-μg/ml kanamycin disk	Sensitive = inhibition zone $\geq$ 10 mm Resistant = inhibition zone < 10 mm
Lecithinase	Egg yolk agar (McClung and Toabe)	Opaque precipiate or zone around colony = positive
Lipase	Egg yolk agar (McClung and Toabe)	Narrow zone of iridescence over and around edge of colony = positive
Milk	Litmus milk	Curding or coagulation within 4 days = positive
Motility	Chopped-meat broth	Hanging-drop method
Nitrate	Indole-nitrite medium	Test 24 h after good growth
Spore determination	Chopped-meat agar slant	Incubate at 30 C for 3 to 14 days; stain periodically; confirm presence of spores by heat-testing a suspension of the colony at 80 C for 10 min; subculture onto plating media
Urease	Egg yolk agar	Make suspension in urea broth; incubate at 37 C for 2 to 4 h; red = positive

[a] BAP, Blood agar plate.

TABLE 2. *Batteries of tests used for the identification of anaerobic bacteria by simplified procedures*[a]

Organisms	Tests
Gram-negative bacilli	Kanamycin (1,000-μg/ml disk) Bile Esculin Indole Nitrate
Anaerobic cocci	Indole Nitrate Esculin Milk
Clostridia	Motility Lecithinase Lipase Indole Nitrate Urease Spores
Gram-positive bacilli	Indole Nitrate Catalase Milk

[a] Peptone-yeast-glucose broth is included for GLC analysis.

inconsistency on biochemical testing and, if GLC had been performed, the correct identification of *C. difficile* would have been made. The second discrepancy can be resolved in a similar manner. ATCC reference culture 8501 was identified by the simplified method as *Fusobacterium necrophorum*. Again, if GLC and additional biochemical tests had been performed, the correct identification of *F. varium* would have been made even though both species are consistent with the scheme shown in Fig. 1. The third discrepancy in this first stage of the evaluation was a laboratory faux pas and, upon retesting, ATCC reference culture 25536 was correctly identified as *Lactobacillus catenaforme*.

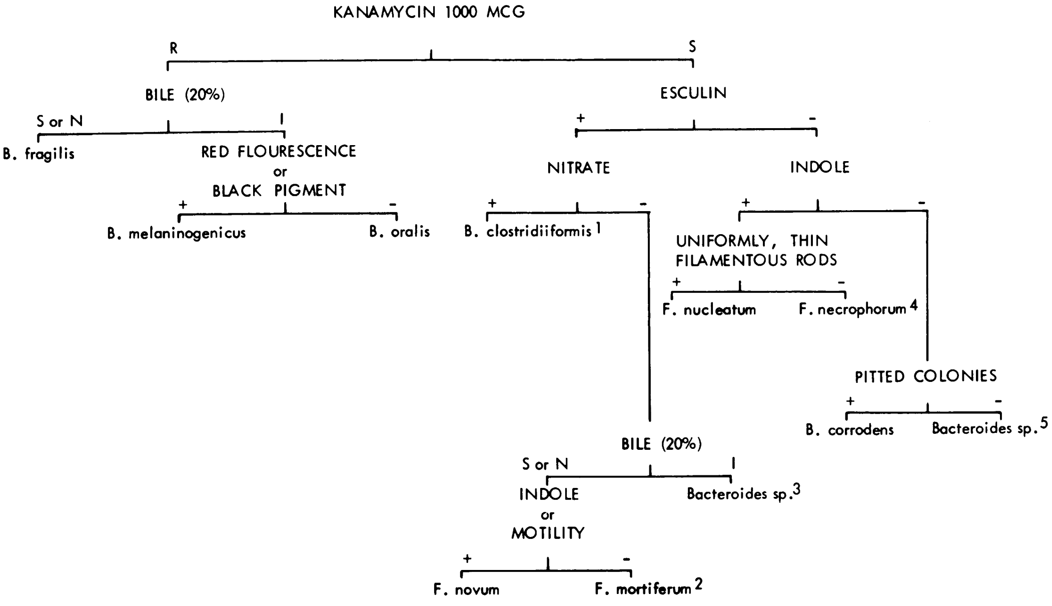
The second stage of this evaluation involved the comparative identification of 35 clinical isolates by using simplified and conventional methods (Table 9). There was agreement to the species level for 25 of the 35 strains. Of the 10 remaining strains, 3 were identified only to the genus level but were consistent with the identification by conventional methods. These strains were *Propionibacterium avidum, Bifidobacterium ericksonii,* and *Bacteroides* sp.; conventional methods, as well, did not provide the appropriate speciation of this *Bacteroides* isolate.

Four discrepancies were observed with isolates of gram-positive cocci. Two of the four strains that had been identified as *Peptococcus prevotii* and *Streptococcus intermedius* were identified as *Peptostreptococcus anaerobius* by conventional methods. However, the GLC patterns for these isolates were more consistent with the organisms identified by the simplified scheme, e.g., *P. prevotii* and *S. intermedius.* The third discrepancy of the gram-positive cocci involved an isolate of *Sarcina ventricula*. It had been identified as *S. constellatus* in the simplified scheme, but a more critical appraisal of the packet-cell configuration on Gram stain would have pointed to the correct identification, which would also have been confirmed by GLC. Another isolate of *S. constellatus,* as determined by the simplified methods, was not definitively identified by conventional techniques. As a result, the final identification of this organism was unresolved.

The remaining three discrepancies involved the following isolates as identified by conventional methods: *Fusobacterium gonadiformans, Clostridium sardiniensis,* and *Propionibacterium acnes*. These organisms were identified, by the simplified methods, as *F. necrophorum, C. novyi,* and *Eubacterium lentum,* respectively. The first error could have been resolved by performing one additional test, e.g., gelatin. However, the identification made was consistent with the scheme as shown in Fig. 1. The second error was resolved and a correct identification was made after the lipase reaction was reevaluated (Fig. 2). The third error was clearly a laboratory mix-up. The differentation between *P. acnes* and *E. lentum* is precisely definable in biochemical as well as GLC patterns.

The third stage of this evaluation involved 196 clinical isolates identified by using GLC as an adjunct to the simplified methods. These data are shown in Table 10. In this trial, 61 of 116 gram-negative rods were identified as *B. fragilis*. After GLC analysis, there appeared to be five discrepancies. Four of these strains were identified as *B. oralis* because they were inhibited by bile-deoxycholate. However, the GLC patterns more closely resembled those of *B. fragilis*. One isolate was confirmed to be *F. necrogenes* by GLC even though it proved to be kanamycin resistant.

There were 13 strains identified to the *Bacteroides* genus level, e.g., *Bacteroides* sp., with the simplified scheme. One strain gave a *Fusobacterium* GLC pattern and was reidentified as *F. mortiferum*. The GLC patterns of the remaining 12 strains were consistent with bio-



1 B. biacutus

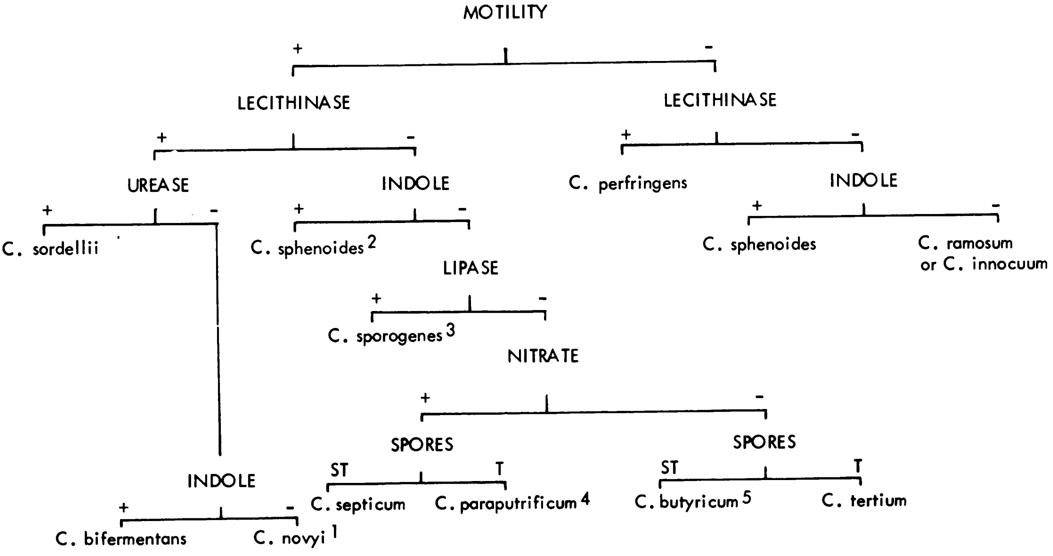
2 F. necrogenes, F. prausnitzii

3 B. capillosus, B. clostridiiformis, B. ruminicola, B. hypermegas, B. furosus

4 Gelatin positive: B. coagulans, B. putredinis
Gelatin negative: F. glutinosum, F. gonidiaformans, F. naviforme, F. varium

5 B. amylophilus, B. nodosus, B. pneumosintes, B. praecutus, B. succinogenes, F. bullosum, F. plauti, F. russii, F. symbiosum

FIG. 1. *Methods for identification of anaerobic, gram-negative, nonsporeforming bacilli commonly encountered in clinical material.*



1 C. limosum, C. subterminale, C. botulinum (type A or E), C. hemolyticum

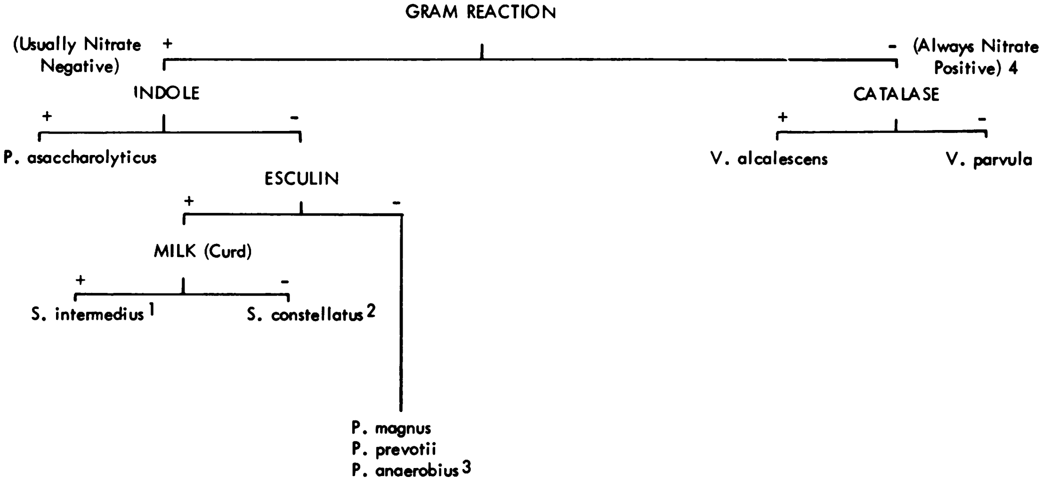
2 C. cadaveris, C. tetani

3 C. botulinum (types A-F)

4 C. tetani

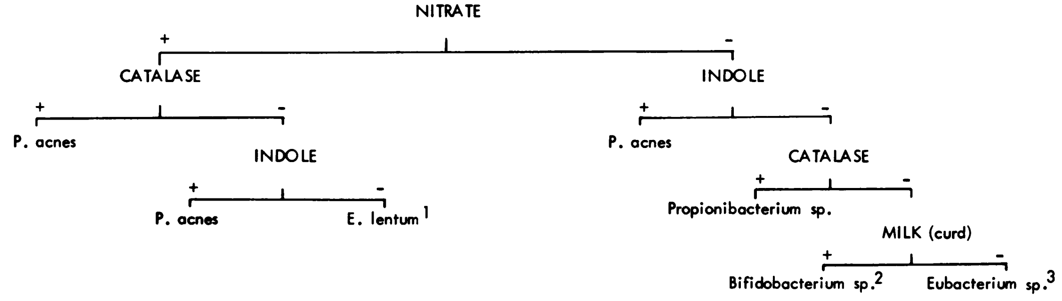
5 C. difficile, C. histolyticum, C. subterminale

FIG. 2. *Methods for identification of anaerobic, gram-positive, sporeforming bacilli commonly encountered in clinical material.*



1 P. productus
2 S. ventriculi, G. anaerobia, P. prevotii
3 P. parvulus, P. micros, P. saccharolyticus, S. morbillorum, R. bromii
4 Nitrate negative and gram negative - M. elsdenii, A. fermentans

FIG. 3. *Methods for identification of anaerobic cocci commonly encountered in clinical material.*



1 Actinomyces sp., Arachnia propionica
2 Eubacterium sp., Actinomyces sp.
3 Lactobacillus catenaforme, Actinomyces sp.

FIG. 4. *Methods for identification of anaerobic, gram-positive, nonsporeforming bacilli commonly encountered in clinical material.*

chemical findings. Five of these 12 strains were identified as *B. nodosus* (two strains), *B. ruminicola, B. pneumosintes,* and *B. praecutus* by their GLC profiles.

Gram-positive cocci comprised 35 of the 196 isolates, the predominant organisms being 10 strains of *P. asaccharolyticus*. The biochemical and GLC patterns agreed for all 10 strains, as was true for 4 isolates of *S. intermedius*. GLC analysis was necessary for identification of the remaining 21 strains, although 3 were classified as unidentifiable because of the difficulty of retaining viable cultures for retesting.

A total of 43 gram-positive rods was identified. There were no discrepancies for eight *P. acnes,* one *Propionifacterium* sp., one *E. lentum,* three *Eubacterium* sp., and three *Bifidobacterium* sp. The remaining isolates of this group were various species of 11 *Clostridium* strains. In two of the three *C. novyi* strains, the GLC pattern was unconfirmatory, and these two strains were reclassified as *Clostridium* sp.

Two gram-negative cocci were isolated. Both were catalase positive and identified as *Veillonella alcalescens.*

DISCUSSION

Simplified biochemical schemata used to identify commonly isolated anaerobes were evaluated. The tests that comprise the schemata are based on properties that uniformly

TABLE 3. *Key biochemical reactions and GLC profiles for Bacteroides species*[a]

Bacteroides	Kanamycin	Bile	Esculin	Indole	Nitrate	GLC
B. amylophilus	S	I	−	−	−	a,(p)
B. biacutus	S	N	+	+	−	A,(p)
B. capillosus	S	I^s	+	−	−	a
B. clostridiiformis subsp. *clostridiiformis*	S	N^i	+	V	$-^+$	A
B. clostridiiformis subsp. *girans*	S	N	+	+	−	A
B. coagulans	S	I^s	−	−	+	a
B. corrodens	S	I^s	−	−	+	a
B. fragilis subsp. *distasonis*	R	S^n	+	−	−	A,p,(ib,iv)
B. fragilis subsp. *fragilis*	R	S^n	+	−	−	A,p,(ib,iv)
B. fragilis subsp. *ovatus*	R	S^n	+	−	+	A,(p,ib,iv)
B. fragilis subsp. *thetaiotaomicron*	R	S^n	+	−	+	A,(p,ib,iv)
B. fragilis subsp. *vulgatus*	R	S^n	$+^-$	−	−	A,(p,ib,iv)
B. fragilis (other)	R	N^s	+	$-^+$	V	
B. furcosus	S	I	$+^-$	−	−	a
B. hypermegas	S	I^n	+	−	−	A,P
B. melaninogenicus subsp. *asaccharolyticus*	R	I	−	$-^+$	$+^-$	A,ib,B,iv,(p)
B. melaninogenicus subsp. *intermedius*	R	I	−	−	$+^-$	A,ib,iv
B. melaninogenicus subsp. *melaninogenicus*	R	I^n	$+^-$	−	−	(A,ib,iv)
B. nodosus	S	I	−	−	−	a,(p)
B. ochraceus	R	I	+	−	−	A
B. oralis	R	I	$+^-$	−	−	A,(ib,iv)
B. pneumosintes	S	I	−	−	−	(a,iv)
B. praecutus	S	I	−	+	−	A,p,iB,B,iV
B. putredinis	S	N^i	−	−	+	A,p,ib,b,iv
B. ruminicola	S	I	+	$-^b$	$-^c$	A,(p,ib,b,iv)
B. succinogenes	S	I	−	−	−	A,p,iv

[a] Symbols: S,I, and N, as described in Table 1; −, negative reactions; $-^+$, majority of reactions are negative; +, positive reactions; $+^-$, majority of reactions are positive; V, variable reactions; and ( ), production of volatile acids is variable.

[b] Occasionally positive.

[c] Occasionally positive.

TABLE 4. *Key biochemical reactions and GLC profiles for Fusobacterium species*[a]

Fusobacterium	Kanamycin	Bile	Esculin	Indole	Nitrate	GLC
F. bullosum	S	I	−	−	−	A,p,B
F. glutinosum	S	N	−	+	−	a,p,B
F. gonidiaformans	S	I	−	+	−	a,p,B
F. mortiferum	S	N^s	+	−	−	A,p,B,(iv)
F. naviforme	S	I	−	+	−	a,p,B
F. necrogenes	S	N	+	−	−	a,B,(p,v)
F. necrophorum	S	I^n	−	+	−	a,p,B
F. novum	S	S	+	+	−	a,B
F. nucleatum	S	I	−	+	−	a,p,B
F. plauti	S	I	−	−	+	a,B
F. prausnitzii	S	N	+	−	−	a,B,(p)
F. russii	S	N^i	−	−	−	a,B
F. symbiosum	S	V	−	−	−	A,B
F. varium	S	N	−	$+^-$	−	A,B,(p)

[a] See Tables 1 and 3 for abbreviations.

characterize the organisms. Lists of less commonly encountered anaerobes are included at appropriate points in the proposed procedures for consideration as an alternate identification. Moreoever, with the aid of GLC, the presumptive biochemical identification can usually be confirmed.

Prerequisites to any identification system are

TABLE 5. *Key biochemical reactions and GLC profiles for gram-positive, nonsporeforming bacilli*[a]

Organism	Catalase	Indole	Nitrate	Milk	Esculin	GLC
Propionibacterium acnes	$+^-$	$+^-$	$+^-$	$-^c$	–	A,P,(iv)
P. granulosum	+	–	–	c^-	–	A,P,(iv)
Propionibacterium sp.	$+^-$	–	$-^+$	V	+	A,P,(iv)
Eubacterium lentum	–	–	$+^-$	–	–	(a)
E. limosum	–	–	–	–	+	A,b,(p)
E. alactolyticum	–	–	–	$-^c$	–	A,b,c
E. cylindroides	–	–	–	–	+	b
E. rectale	–	–	–	c^-	+	B,(a)
E. aerofaciens	–	–	–	$-^c$	$+^-$	A
Eubacterium sp.	–	$-^+$	$-^+$	V	$+^-$	
Bifidobacterium breve	–	–	–	c	+	A
B. eriksonii	–	–	–	c	+	A
B. adolescentis	–	–	$-^+$	c	+	A
B. longum	–	–	–	c	$-^+$	A
B. bifidum	–	–	–	c	–	A
Bifidobacterium sp.	–	–	–	c	$+^-$	A
Lactobacillus catenaforme	–	–	–		+	a
Actinomyces israelii	–	–	$-^+$	c	+	a
A. naeslundii	–	–	$+^-$	c^-	$+^-$	a
Actinomyces sp.	V	–	V	c^-	V	a
Arachnia propionica	–	–	+	c^-	–	A,P

[a] See Table 3 for abbreviations.

TABLE 6. *Key biochemical reactions and GLC profiles for Clostridium species*[a]

Clostridium	Motility	Lecithinase	Lipase	Indole	Nitrate	GLC
C. bifermentans	+	+	–	+	–	A,p,ib,iv,ic,(b)
C. botulinum	+	$-^+$	+	–	–	[b]
C. butyricum	+	–	–	–	$-^+$	A,B
C. cadaveris	+	–	–	+	–	A,B,(p)
C. difficile	+	–	–	–	–	a,ib,b,iv,v,ic,(p)
C. hemolyticum	+	+	–	V	–	A,P,B
C. histolyticum	+	–	–	–	–	A
C. innocuum	–	–	–	–	–	A,B
C. limosum	+	+	–	–	–	A
C. novyi	+	+	+	–	$-^+$	a,P,B,v
C. paraputrificum	+	–	–	–	V	A,B
C. perfringens	–	+	–	–	+	A,B,(p)
C. ramosum	–	–	–	–	–	A,(p)
C. septicum	+	–	–	–	$+^-$	A,B,(p)
C. sordellii	+	$+^-$	–	+	–	A,ic,(p,ib,iv)
C. sphenoides	V	–	–	V	$-^+$	A
C. sporogenes	+	–	+	–	–	A,b,ib,iv,(p,v,ic)
C. subterminale	+	$-^+$	–	–	–	A,ib,b,iv,(p)
C. tertium	+	–	–	–	$+^-$	A,b
C. tetani	+	–	–	V	–	A,p,B

[a] See Table 3 for abbreviations.
[b] Patterns variable.

the reliable interpretations of Gram stain reactions and cellular and colonial morphology. One technical aid in differentiating coccobacilli from cocci has been the Gram stain of colonies immediately surrounding antibiotic disks (3). The application of this technique to the identification of anaerobic organisms is currently being evaluated.

TABLE 7. *Key biochemical reactions and GLC profiles for anaerobic cocci*[a]

Organism	Catalase	Indole	Nitrate	Esculin	Milk	GLC
Gram positive						
Peptococcus magnus	−	−	−	−	−	A
P. prevotii	−	−	$-^{+}$	$-^{+}$	$-^{c}$	A,b,(p)
P. asaccharolyticus	−	+	−	−	−	A,b
P. saccharolyticus	+	−	+	−	−	A
Peptostreptococcus micros	$-^{w}$	−	−	−	−	A
P. anaerobius	−	−	$-^{+}$	−	−	A,iv,(p,ib,b,iv,ic,c)
P. parvulus	−	−	−	−	c	a,(b)
P. productus	−	−	−	+	c	A,(p)
Streptococcus intermedius	−	−	$-^{+}$	+	c	(a)
S. constellatus	−	−	V	+	−	a
S. morbillorum	−	−	−	−	−	a
Gaffkya anaerobia	−	−	$-^{+}$	$+^{-}$	−	a,p,B
Ruminococcus bromii	−	−	−	−	−	a,(p,b)
Sarcina ventriculi	−	−	+	+	−	
Gram negative						
Veillonella parvula	−	−	+	−	−	a,p
V. alcalescens	+	−	+	−	−	a,p
Acidaminococcus fermentans	−	−	−	−	−	a,ib,b,iv,v,c,(p)
Megasphaera elsdenii	−	−	−	−	−	A,b,(p)

[a] See Table 3 for abbreviations.

TABLE 8. *First-stage comparison of presumptive schemata with conventional methods for the identification of anaerobic bacteria—a double-blind evaluation using reference strains*

Presumptive	Conventional (VPI and/or CDC)[a]	No.
Veillonella alcalescens	*Veillonella alcalescens*	ATCC 17745
Clostridium butyricum[b]	*Clostridium difficile*	ATCC 9698
Bifidobacterium sp.	*Bifidobacterium eriksonii*	ATCC 15423
Actinomyces sp.	*Actinomyces* sp.	ATCC15424
Eubacterium lentum	*Eubacterium lentum*	ATCC 25559
Eubacterium sp.	*Eubacterium limosum*	ATCC 8486
Bacteroides fragilis	*Bacteroides fragilis* subsp. *distasonis*	ATCC 8503
Bacteroides fragilis	*Bacteroides fragilis* subsp. *ovatus*	ATCC 8483
Fusobacterium necrophorum	*Fusobacterium necrophorum*	ATCC 25286
Fusobacterium necrophorum[b]	*Fusobacterium varium*	ATCC 8501
Eubacterium sp.	*Eubacterium alactolyticum*	ATCC 23263
Propionibacterium acnes[c]	*Lactobacillus catenaforme*	ATCC 25536
Bacteroides melaninogenicus	*Bacteroides melaninogenicus* subsp. *melaninogenicus*	CDC 25845
Clostridium perfringens	*Clostridium perfringens*	CDC BP6K

[a] VPI, Virginia Polytechnic Institute, Blacksburg, Va. CDC, Center for Disease Control, Atlanta, Ga.

[b] Consistent with biochemical scheme.

[c] Isolate was reidentified as *Eubacterium* species, which is consistent with the proposed scheme used for identification of this organism.

Results of the identification of gram-negative bacilli were reliable. The use of kanamycin disks and bile-deoxycholate to differentiate these organisms was originally recommended by Sutter and Finegold (15). We found that several strains of *B. fragilis* were inhibited by bile-deoxycholate but were not affected by bile alone. Thus, an alternate means of testing may be warranted, in which only bile is used. Furthermore, it may be advantageous to include

TABLE 9. *Comparison of presumptive schemata with conventional methods for the identification of anaerobic bacteria— a double-blind evaluation using 35 clinical isolates*

Presumptive	Conventional (VPI and/or CDC)[a]
Fusobacterium necrophorum[b]	*Fusobacterium gonidiaformans*
Propionibacterium sp.[b]	*Propionibacterium avidum*
Peptococcus magnus	*Peptococcus magnus*
Clostridium novyi	*Clostridium novyi*
Clostridium novyi[b]	*Clostridium sardiniensis*
Bifidobacterium sp.	*Bifidobacterium eriksonii*
Bacteroides sp.	*Bacteroides* sp.[c]
Eubacterium lentum	*Eubacterium lentum*
Peptococcus asaccharolyticus	*Peptococcus asaccharolyticus*
Peptococcus prevotii	*Peptococcus prevotii*
Propionibacterium acnes	*Propionibacterium acnes*
Fusobacterium necrophorum	*Fusobacterium necrophorum*
Bacteroides oralis	*Bacteroides oralis*
Clostridium ramosum-innocuum[b]	*Clostridium ramosum*
Clostridium sordellii	*Clostridium sordellii*
Veillonella parvula	*Veillonella parvula*
Bacteroides melaninogenicus	*Bacteroides melaninogenicus* subsp. *intermedius*
Streptococcus constellatus	Unable to identify[c]
Peptostreptococcus anaerobius	*Peptostreptococcus anaerobius*
Bacteroides oralis	*Bacteroides oralis*
Peptococcus prevotii[d]	*Peptostreptococcus anaerobius*
Bacteroides fragilis	*Bacteroides fragilis* subsp. *fragilis*
Clostridium butyricum	*Clostridium butyricum*
Peptococcus asaccharolyticus	*Peptococcus asaccharolyticus*
Peptococcus magnus	*Peptococcus magnus*
Clostridium sporogenes	*Clostridium sporogenes*
Clostridium perfringens	*Clostridium perfringens*
Peptococcus prevotii	*Peptococcus prevotii*
Propionibacterium acnes[d]	*Eubacterium lentum*
Peptococcus asaccharolyticus	*Peptococcus asaccharolyticus*
Peptococcus magnus	*Peptococcus magnus*
Peptococcus magnus	*Peptococcus magnus*
Streptococcus constellatus[b]	*Sarcina ventriculi*
Streptococcus intermedius[d]	*Peptostreptococcus anaerobius*

[a] See footnote *a* of Table 8.

[b] Consistent with scheme.

[c] Unable to obtain definitive identification.

[d] Unable to resolve discrepancy in identification.

TABLE 10. *Results of 196 clinical isolates identified by presumptive biochemical schemata and GLC analysis*

Organisms	No. of isolates
Gram-negative bacilli	116(6)[a]
Bacteroides fragilis	61
B. melaninogenicus	17
B. oralis	16
B. clostridiiformis	1
Bacteroides sp.	13
Fusobacterium nucleatum	4
F. mortiferum	2
F. necrogenes	1
Fusobacterium sp.	1
Gram-positive cocci	35
Peptococcus asaccharolyticus	10
P. prevotii	1
P. magnus	4
P. parvulus	1
P. saccharolyticus	1
Peptostreptococcus anaerobius	7
P. micros	3
Streptococcus intermedius	3
S. morbillorum	1
Undesignated	3
Gram-negative cocci	2
Veillonella alcalescens	2
Gram-positive bacilli	43(2)
Propionibacterium acnes	8
Propionibacterium sp.	1
Eubacterium lentum	1
Eubacterium sp.	3
Bifidobacterium sp.	3
Clostridium perfringens	3
C. butyricum	1
C. sordellii	1
C. septicum	1
C. innocuum	1
C. subterminale	1
C. novyi	1
Clostridium sp.	2

[a] Number of discrepancies between GLC profile and biochemical identification.

penicillin disks along with kanamycin as a means of detecting the usually penicillin-resistant *B. fragilis*.

Strains of *Fusobacterium* and *Bacteroides* species were differentiated by using the techniques described in the simplified procedures. Although the clinical relevance of their identification is often questioned, these organisms can usually be separated and identified by using the techniques proposed. When difficulty is encountered in the identification of these gram-negative organisms, discrepancies can be resolved by using GLC and a few additional biochemical tests. Since *F. varium* is often more resistant to antimicrobics, proper identification may, indeed, be relevant in the more appropriate selection of drugs used for therapy.

The scheme for the identification of gram-positive, nonsporeforming bacilli is directed at the rapid detection of the more commonly encountered *P. acnes*. Isolates of *Actinomyces* can be identified by the proposed procedures if the organism is suspected and if the microbiologist is familiar with the usually rough colonial morphology typical of these organisms. Other members of this group of organisms are identified to the genus level only, using the methods described in this report. Since the clinical significance of these anaerobes may be questioned from time to time, this approach seems logical. The evaluation of the scheme for gram-positive, nonsporeforming bacilli was generally accurate, with a few discrepancies being attributed to technical negligence.

The scheme for identification of clostridia was designed to include a minimum of 12 species which comprise up to 90% of the clinical isolates (13). Since over 300 clostridia species have been described, a simplified approach is obviously needed. The results indicate that the common clostridial isolates can be identified. The few misidentifications were due to less common isolates which are generally regarded as nonpathogens. Upon inspection of their reactions and GLC profiles, it was possible to assume they did not fit the scheme and could be reidentified as *Clostridium* species. The determination of spore formation is usually not necessary since those species that do not readily produce spores, e.g, *C. perfringens* and *C. ramosum,* can be identified through other biochemical reactions.

The last group of anaerobes, the cocci, usually present the most problems for identification regardless of the methods used. The unreliability of Gram-stain reactions and catalase reactions in the differentiation of *Peptococcus* and *Peptostreptococcus* organisms has been documented (11). Furthermore, the cocci are generally biochemically inert. Since there are five species of gram-positive cocci that are most commonly isolated from clinical specimens (8), the proposed simplified scheme will usually provide an accurate identification. However, addition of GLC profiles and several sugar fermentations can provide definitive identification for the less commonly encountered isolates. With regard to the identification of *S. intermedius,* we would emphasize the value of the esculin and milk reactions, which are not usually performed in conventional methods. Three anaerobic to microaerophilic cocci that are fermentative and produce lactic acid as the major product have been included in the genus *Streptococcus,* e.g., *S. intermedius, S. constellatus,* and *S. morbillorum* (6). Accordingly, they were classified as streptococci in this report. *Veillonella* species were identified without problems since they are always nitrate positive. Other anaerobic, gram-negative cocci, e.g., *Acidaminococcus* and *Megasphaera,* were not encountered during the course of this study.

The three-stage evaluation of these identification schemata has shown that they may have practical value when applied to the diagnostic regimen of the routine clinical microbiology laboratory. Our intent has not been to supplant or slight the procedures that are referenced in this study as conventional techniques. Rather, we offer the procedures described herein as a plausible adjunct for the rapid identification of the more commonly encountered anaerobic isolates from clinical material. The biochemical tests required are easily performed and provide reliable results in as little as 2 days. The usefulness of GLC of volatile acids and methylated esters as a supplement to the proposed procedures cannot be overemphasized. In the majority of cases, clinically relevant strains can be properly identified with the simplified schemata and GLC. Alone, the simplified biochemical procedures usually provide the data required for the identification of anaerobic isolates to the stage of clinical relevance. When a precise identification is sought for an unusual but clinically significant isolate or when discrepancies in the procedures occur, other tests may occasionally be needed to augment the basic procedures outlined. When these situations arise, we recommend the use of conventional procedures and a confirmatory identification by a reference laboratory that specializes in anaerobic bacteriology.

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LITERATURE CITED

1. Balows, A., R. M. DeHann, V. R. Dowell, Jr., and L. B. Guze (ed.). 1973. Anaerobic bacteria: role in disease. Charles C Thomas, Springfield, Ill.
2. Bornstein, D. L., A. N. Weinberg, M. N. Swartz, and L. J. Kunz. 1964. Anaerobic infections—review of current experience. Medicine 43:207-232.
3. Catlin, B. W. 1975. Cellular elongation under the influence of antibacterial agents: way to differentiate coccobacilli from cocci. J. Clin. Microbiol. 1:102-105.
4. Dowell, V. R., Jr., and T. M. Hawkins. 1974. Laboratory methods in anaerobic bacteriology. Publication no. (CDC) 74-8272. Department of Health, Education and Welfare. Government Printing Office, Washington, D.C.
5. Gorbach, S. L., and J. G. Bartlett. 1974. Anaerobic infections. N. Engl. J. Med. 290:1177-1184, 1237-1244, 1289-1294.
6. Holdeman, L. V., and W. E. C. Moore. 1974. New

genus, *Coprococcus,* twelve new species, and emended descriptions of four previously described species of bacteria from human feces. Int. J. Syst. Bacteriol. 24:260-277.

7. **Holdeman, L. V., and W. E. C. Moore.** 1975. Anaerobe laboratory manual, 3rd ed. Virginia Polytechnic Institute and State University, Blacksburg.
8. **Lambe, D. W., Jr., D. H. Vroon, and C. W. Rietz.** 1973. Infections due to anaerobic cocci, p. 585-599. *In* Anaerobic bacteria: role in disease. Charles C Thomas, Springfield, Ill.
9. **Martin, W. J.** 1971. Practical method for isolation of anaerobic bacteria in the clinical laboratory. Appl. Microbiol. 22:1168-1171.
10. **Martin, W. J.** 1974. Isolation and identification of anaerobic bacteria in the clinical laboratory. Mayo Clin. Proc. 49:300-308.
11. **Pien, F. D., R. L. Thompson, and W. J. Martin.** 1972. Clinical and bacteriologic studies of anaerobic gram-positive cocci. Mayo Clin. Proc. 47:251-257.
12. **Smith, L. DS.** 1975. Pathogenic anaerobic bacteria, 2nd ed. Charles C Thomas, Springfield, Ill.
13. **Smith, L. DS., and V. R. Dowell, Jr.** 1974. *Clostridium,* p. 376-380. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
14. **Sutter, V. L., and W. T. Carter.** 1972. Evaluation of media and reagents for indole-spot tests in anaerobic bacteriology. Am. J. Clin. Pathol. 58:335-338.
15. **Sutter, V. L., and S. M. Finegold.** 1971. Antibiotic disc susceptibility tests for rapid presumptive identification of gram-negative anaerobic bacilli. Appl. Microbiol. 21:13-20.
16. **Sutter, V. L., V. L. Vargo, and S. M. Finegold.** 1975. Wadsworth anaerobic bacteriology manual. UCLA Extension Division, University of California, Los Angeles.
17. **Vargo, V., M. Korzeniowski, and E. H. Spaulding.** 1974. Tryptic soy bile-kanamycin test for the identification of *Bacteroides fragilis.* Appl. Microbiol. 27:480-483.