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Clinical Evaluation of a Simple, Rapid Procedure for the Presumptive Identification of Anaerobic Bacteria

JOSEPH W. HOLLAND,* SUZANNE M. GAGNET, SUE A. LEWIS, AND LARRY R. STAUFFER

Research Surgical Bacteriology Laboratory, Department of Surgery, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267

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A simple, rapid procedure for the presumptive identification of anaerobic bacteria has been evaluated. Two hundred and thirty-five clinical isolates were identified using gas-liquid chromatography and 3-ml volumes of a few selected test media. These test media were stored aerobically and incubated in GasPak anaerobic jars. The average incubation time was 39 h. This procedure, when compared to the results of our standard identification procedure, correctly identified 98% of the isolates to the genus level, 83% to the species level, and 83% of *Bacteroides fragilis* and *Bacteroides melaninogenicus* to the subspecies level. Fifty-three of the isolates were also identified by using 0.5-ml volumes of test media stored, inoculated, and incubated in an anaerobic glove box. The 3-ml- and the 0.5-ml-volume procedures correctly identified comparable percentages of the 53 isolates.

Evidence for the involvement of anaerobic bacteria in infections such as peritonitis (2) and tubo-ovarian abscesses (1) has been reported since the early part of this century, yet within the last few years it has become more evident that these bacteria are involved in numerous types of human infections. This increased interest and awareness is partially due to an ability to characterize these bacteria more completely. Today, with a sufficient number of tests, most anaerobic isolates from clinical specimens can be identified to the species or subspecies level.

There are still many laboratories that for various reasons do not culture for anaerobic bacteria. The heavy work load of the average clinical laboratory allows neither time to prepare and/or inoculate large batteries of test media nor the extended incubation times necessary to obtain biochemical results for the slow-growing anaerobes. There are commercial products available that use small volumes of media and heavy inocula in an attempt to decrease the time required for growth. Recent reports (7, 13, 18) have shown that some of these commercially available miniaturized systems yield a high degree of correlation with conventional test results; however, these products are expensive, and some include tests that are not necessary for the identification of many isolates. All should be supplemented with gas-liquid chromatography (GLC).

This laboratory and probably many others still maintain the ingredients, facilities, and personnel needed to prepare limited amounts of media which could be used for the identification of anaerobic bacteria. It would therefore be desirable to have a simple yet rapid identification system based on a few easily prepared, inexpensive test media and GLC.

In our experience, 10 species of anaerobic bacteria (*Bacteroides fragilis, Bacteroides melaninogenicus, Peptococcus magnus, Peptococcus asaccharolyticus, Peptococcus prevotii, Peptostreptococcus anaerobius, Streptococcus intermedius, Propionibacterium acnes, Clostridium perfringens*, and *Eubacterium lentum*) account for approximately 70% of all clinical anaerobic isolates (10). The ability to identify these and a few other important species will afford coverage of most of the anaerobes encountered in the diagnostic laboratory. For this purpose, a limited number of easily prepared media were selected. Small volumes of media were used in an attempt to conserve space and reduce reaction times. These identification media, combined with GLC, were tested with clinical isolates in an anaerobic glove box and in GasPak anaerobic jars. Identification based on these results was compared with results obtained using our standard identifcation procedure, which requires large volumes of numerous test media, extended incubation times, and GLC.

MATERIALS AND METHODS

Bacteria. The 235 strains of anaerobic bacteria used in this study are listed in Table 1 and were isolated from specimens obtained at the Cincinnati

TABLE 1. *Standard LF procedure identification of the 235 anaerobic bacteria tested*

Organism	No.
Bacteroides fragilis subsp. *fragilis*	32
B. fragilis subsp. *thetaiotaomicron*	9
B. fragilis subsp. *vulgatus*	6
B. fragilis subsp. *distasonis*	2
B. fragilis subsp. "other"	5
B. melaninogenicus subsp. *asaccharolyticus*	10
B. melaninogenicus subsp. *intermedius*	1
B. corrodens	3
B. putredinis	2
Bacteroides sp.	9
Fusobacterium naviforme	1
Propionibacterium acnes	21
P. avidum	2
P. granulosum	1
Bifidobacterium sp.	2
Eubacterium lentum	3
E. limosum	1
E. alactolyticum	1
Eubacterium sp.	1
Actinomyces odontolyticus	1
Actinomyces sp.	1
Arachnia propionica	1
Veillonella alcalescens	2
V. parvula	2
Veillonella sp.	1
Peptostreptococcus anaerobius	9
P. micros	6
Peptostreptococcus sp.	3
Peptococcus magnus	20
P. asaccharolyticus	15
P. prevotii	15
P. saccharolyticus	1
Peptococcus sp.	4
Streptococcus intermedius	10
S. constellatus	2
S. morbillorum	2
Clostridium perfringens	7
C. sporogenes	4
C. ramosum	3
C. innocuum	3
C. bifermentans	2
C. lituseburense	1
C. difficile	1
C. propionicum	1
C. septicum	1
C. sartagoformum	1
Clostridium sp.	4

General and Christian R. Holmes Hospitals, Cincinnati, Ohio. Twenty-four of the 28 clostridia studied were isolates that had been frozen at −70°C prior to their inclusion in this study. All other strains were fresh clinical isolates. Four strains isolated during this study were not included among the 235 listed. Three of these strains failed to grow after initial isolation, and one strain could not be identified to the genus level by the standard identification procedure.

Isolation procedures. Collection, transportation, and isolation procedures have been presented in an earlier publication (10).

Test media. Three different batteries of test media were used for bacterial identification. Our standard identification procedure, the "long form" (LF) procedure, consisted of 10- to 15-ml volumes of 20 to 30 test media in 16- by 150-mm tubes, as previously described (10).

The abbreviated, or "short form" (SF), batteries of identification media were prepared as follows. Fermentation media were made by adding filter-sterilized glucose, mannitol, trehalose, rhamnose, arabinose, lactose, or sucrose in recommended concentrations (8) to thioglycolate medium without glucose or indicator (Baltimore Biological Laboratory [BBL], Cockeysville, Md.) (for the 3-ml volumes of media) and peptone-yeast extract (PY) broth (8) (for the 0.5-ml volumes of media). Indole and nitrate reactions were determined in indole-nitrite medium (BBL). Peptone-yeast extract-glucose broth (PYG) (8) served as the growth medium for GLC analysis. Esculin hydrolysis was determined using the esculin broth recommended by the Center for Disease Control (4). Lecithinase and lipase activities were determined on modified McClung-Toabe egg yolk agar (4). Milk medium for detection of acid, gas, clot, and digestion reactions was prepared by adding 0.5 g of L-cysteine to 1,000 ml of skim milk. All SF procedures used 3-ml volumes of milk medium. Hydrogen peroxide degradation testing was done on brain heart infusion agar slants (BBL) in 13- by 100-mm tubes. Growth was exposed to air for at least 30 min prior to the addition of 3% H_2O_2. Hemin and menadione (8) are currently being added to all SF media and are added to the LF media used to identify suspected *B. melaninogenicus* strains. The SF media are shown in Fig. 1. In addition to these media, fermentation base was inoculated to serve as a fermentation medium control and as a pH check on test media stored in the anaerobic glove box. SF media were used for up to 1 month after preparation.

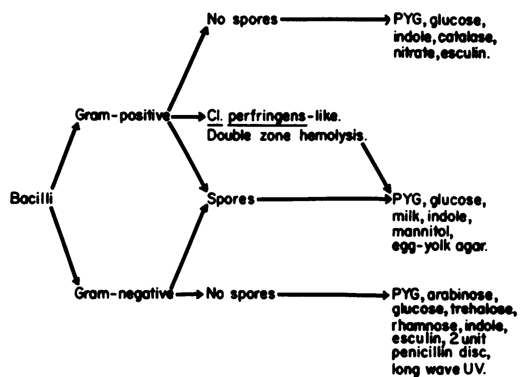

FIG. 1. *SF identification media. Trehalose, rhamnose, and arabinose were required for the gram-negative bacilli only if B. fragilis was to be subspeciated.*

Sensitivity to 2-U penicillin disks was determined by streaking isolates on the surface of Mueller-Hinton agar containing 5% sheep blood (Gibco Diagnostics, The Mogul Corp., Chagrin Falls, Ohio). Disks were placed on inoculated plates which were then incubated anaerobically. Inhibition zones were read after 24 to 48 h at 36°C.

Test reagents and chromatography. Acid production in the SF milk medium was determined by using a pH meter. All other fermentation reactions were read by adding a few drops of dilute bromothymol blue to tubes after incubation. Fermentation was indicated by a color change from blue to yellow. Blue-green and green colors were considered negative. Other test reagents and the fatty acid extraction and methylation procedures are those recommended by the Virginia Polytechnic Institute (VPI) and State University (8). GLC was done on Resoflex LAC-1-R-296, standard concentration P (Burrell Corp., Pittsburgh, Pa.), packed columns in a Perkin-Elmer model 3920 gas chromatograph with dual flame ionization detectors.

Procedure. During the early phases of this study, anaerobic isolates were picked from primary plates (10) that had been incubated in GasPak anaerobic jars (BBL). These isolates, grown for 18 to 48 h in thioglycolate broth, were used to inoculate LF media and 3-ml volumes of SF media (procedure described below), which were stored aerobically and steamed just prior to use. Each LF medium was inoculated with 0.2 to 0.5 ml of the thioglycolate culture. Indole, milk, PYG, and PYG-bile tubes (10) were incubated in GasPak jars at 36°C. All other LF media were incubated aerobically at 36°C. The LF biochemical and GLC results were read after 10 days of incubation (gelatin after 30 days).

Currently, primary isolation plates are incubated in an anaerobic glove box (Coy Mfg. Co., Ann Arbor, Mich.) containing 85% nitrogen, 10% hydrogen, and 5% carbon dioxide. The availability of the anaerobic glove box has allowed the scope of the SF procedure to be broadened. In addition to the 3-ml procedure, where media are stored aerobically and incubated in GasPak jars, we now include 0.5-ml volumes of media stored and incubated in the glove box. Each anaerobic isolate is picked from a primary plate to a 3-ml volume of PY broth. This broth or a subculture of it is used as a source of inoculum for the LF procedure described previously and for each of the following three SF identification procedures.

(i) In the 3-ml GasPak jar SF (3-ml SF) procedure, thioglycolate broth subcultures, incubated aerobically (during the early phases of this study) or in the anaerobic glove box (currently), are used to inoculate test media. When sufficient growth is obtained, usually within 18 to 48 h, approximately 0.2 ml of culture is inoculated into each of the appropriate 3-ml volumes of test media. Media are stored aerobically, steamed just prior to use, inoculated under aerobic conditions, and incubated in GasPak jars. Biochemical and GLC results are usually read after 18 to 48 h of incubation at 36°C.

(ii) The 0.5-ml overnight growth SF (0.5-ml OSF) procedure is done in the anaerobic glove box by adding 1 drop of the original 18- to 48-h PY culture to each of the appropriate 0.5-ml volumes of test media and to a 3-ml volume of PYG for chromatography. Media are stored and incubated in the glove box at room temperature and at 36°C, respectively. Results are usually read after 18 to 48 h.

(iii) The 0.5-ml isolated colony SF (0.5-ml CSF) procedure is also done in the anaerobic glove box. Growth from the original PY culture is streaked on agar plates. As soon as colony types are detectable, an isolated colony (or colonies) is suspended in 0.5 ml of PY broth. One drop of the suspension is used to inoculate each of the appropriate 0.5-ml volumes of test media and a 3-ml volume of PYG for chromatography. Test media are stored and incubated in the glove box. Results are usually read after 18 to 48 h of incubation at 36°C.

Identification. The LF identification criteria and the SF keys (Tables 2–4) were derived from the reactions listed in the VPI *Anaerobe Laboratory Manual* (8). The LF procedure was tested on a blind basis by re-identifying 31 strains of anaerobic bacilli (American Type Culture Collection strains and isolates previously identified by the Center for Disease Control). All 31 strains were correctly identified to the genus level, and 29 (94%) were correctly identified to the species level (10). To further assure accuracy, known strains were periodically tested. This LF procedure served as the standard for comparison of the three SF identification procedures. SF identification was facilitated by careful observation of Gram stain reactions, cell and colony morphologies, GLC profiles, and test media reactions to obtain an overall view of the organism before using the information and the appropriate key to identify the isolate.

RESULTS

A total of 235 clinical anaerobic isolates were identified during this study by using our standard LF procedure and the 3-ml SF procedure. The last 53 of the 235 anaerobes isolated were also tested by the 0.5-ml OSF and CSF. All SF identifications were made from the keys listed in Tables 2 through 4.

The anaerobic bacillus key (Table 2) contains three levels of identification: genus, species, and subspecies. *Lactobacillus*, *Bifidobacterium*, *Actinomyces*, *Leptotrichia*, and *Fusobacterium* were only keyed to the genus level because they were infrequently isolated in this laboratory and because speciation usually required additional tests not listed in Fig. 1. *P. acnes* and *E. lentum* were keyed to the species level, whereas *B. fragilis* and *B. melaninogenicus* were keyed to the species and subspecies levels. These bacteria are common in clinical specimens, and some are of medical importance. The remaining *Eubacterium* species listed were included because they have been isolated in this laboratory and they could be presumptively speciated without additional tests. *B. fragilis* subsp. *ovatus* was not included

TABLE 2. *Anaerobic bacillus identification key*

Gram stain	Spores	Propionic (and acetic)	Acetic or none	Acetic and lactic (one plus to one)	Lactic	Acetic and lactic (one to two, and succinic)	Butyric (± acetic and propionic)	Mixed[a] or acetic	Black pigment or lasting fluorescence	Penicillin (2-U disk)	Catalase	Indole	Glucose	Nitrate	Esculin	Trehalose	Rhamnose	Arabinose	Organism
+	+																		*Clostridium*
	−	+									V[b]	+		+	−				*Propionibacterium acnes*
												−							*Propionibacterium* sp.
											−								*Arachnia propionica*
			+									+							*Eubacterium tenue*
												−	+						*Eubacterium* sp.
													−	+					*E. lentum*
				+															*Bifidobacterium*
					+														*Lactobacillus*
						+													*Actinomyces*
							+					+			+				*Eubacterium saburreum*
												−							*Eubacterium* sp.
−	+																		*Clostridium*
	−				+														*Leptotrichia*
							+												*Fusobacterium*
								+	+				+		+				*Bacteroides melaninogenicus* subsp. *melaninogenicus*
															−				*B. melaninogenicus* subsp. *intermedius*
													−						*B. melaninogenicus* subsp. *asaccharolyticus*
									−	+		+							*Bacteroides fragilis* subsp. *thetaiotaomicron*
												−				+			*B. fragilis* subsp. *distasonis*
																−	+	+	*B. fragilis* subsp. *vulgatus*
																		−	*B. fragilis* subsp. "other"
																	−	+	*B. fragilis* subsp. "other"
																		−	*B. fragilis* subsp. *fragilis*
										±[c]			±		±				*Bacteroides* sp.

[a] Mixed acids refer to the presence of both normal and iso volatile fatty acids.

[b] V, Variable.

[c] Anaerobic gram-negative bacilli not producing butyric acid, spores, or black pigment were considered to be *Bacteroides* sp. if any two of the three reactions (glucose, esculin, penicillin) were negative and *B. fragilis* if any two of the three reactions were positive. Resistance to penicillin was scored as a positive reaction.

TABLE 3. *Anaerobic coccus identification key*

Acetic	Lactic and acetic	Butyric (± acetic and propionic)	Propionic and acetic	Mixed[a]	Catalase	Glucose	Lactose	Sucrose	Arabinose	Indole	Gram strain	Organism
+					+	+						*Peptococcus saccharolyticus*
					−		+	+	+			*Peptostreptococcus productus*
									−			*Sarcina ventriculi*
							−					*Peptococcus magnus*[b]
												Peptostreptococcus micros
	+					+	+	+				*Streptococcus intermedius*
								−				*Peptostreptococcus parvulus*
							−	+				*Streptococcus constellatus*
								−				*S. morbillorum*
		+				+						*Gaffkya anaerobia*
						−				+		*Peptococcus asaccharolyticus*
										−	+	*P. prevotii*
											−	*Acidaminococcus fermentans*
			+		+							*Veillonella alcalescens*
					−	+		+				*Peptostreptococcus productus*
						−						*Veillonella parvula*
				+							+	*Peptostreptococcus anaerobius*
											−	*Megasphaera elsdenii*

[a] Mixed acids refer to the presence of both normal and iso volatile fatty acids.
[b] *P. magnus* and *P. micros* were differentiated by cell morphology.

in the key because this anaerobe was rarely isolated and an additional test was required for identification.

One problem encountered was the inability of the anaerobic bacillus key to distinguish between nonfermentative *Bacteroides* species (other than *B. melaninogenicus*) and some subspecies of *B. fragilis*. This problem was resolved to a large extent by considering susceptibility to 2-U penicillin disks, glucose fermentation, and esculin hydrolysis as a group. Resistance to penicillin was scored as a positive reaction. An identification of *B. fragilis* required that any two of the three reactions be positive, whereas other *Bacteroides* species required any two of three to be negative. This procedure was used throughout the study and gave good results. Most of the *Bacteroides* incorrectly identified at the species level were incorrect because the SF key was only designed to speciate *B.*

TABLE 4. *Clostridium identification key*

Aerobic	Acetic and butyric (± propionic)	Mixed[a]	Acetic	Lactic	Lecithinase	Lipase	Glucose	Mannitol	Indole	Milk[b]	Organism
+							+	+			*C. tertium*
								−			*C. carnis*
							−				*C. histolyticum*
−	+				+	+				C	*C. novyi* A
						−			+	−	*C. haemolyticum*
									−	A	*C. barati*
										A	*C. perenne*
										C	*C. novyi* B
										C	*C. sardiniensis*
										CG	*C. perfringens*
					−	+	+	−	−	C	*C. aurantibutyricum*
										C	*C. botulinum* B (non-proteolytic)
										C	*C. botulinum* E (non-proteolytic)
										C	*C. botulinum* F (non-proteolytic)
										D	*C. botulinum* C
										D	*C. botulinum* D
						−	+	+		C	*C. acetobutylicum*
										−	*C. innocuum*
										−	*C. sartagoformum*
								−	+	D	*C. cadaveris*
									−	C	*C. beijerinckii*
										C	*C. butyricum*
										C	*C. paraputrificum*
										C	*C. pseudotetanicum*
										A	*C. chauvoei*
										A	*C. fallax*
										CG	*C. felsineum*
										CG	*C. septicum*
										CG	*C. thermosaccharolyticum*
										CD	*C. plagarum*
										−	*C. pasteurianum*

TABLE 4—*Continued*

Aerobic	Acetic and butyric (± propionic)	Mixed[a]	Acetic	Lactic	Lecithinase	Lipase	Glucose	Mannitol	Indole	Milk[b]	Organism
										–	*C. rectum*
										–	*C. rubrum*
										–	*C. tyrobutyricum*
							–		+	–	*C. lentoputrescens*
										–	*C. malenominatum*
										–	*C. tetani*
									–	–	*C. cochlearium* or *sporosphaeroides*
	–	+			+	+	–	–	+	CD	*C. ghoni*
						–	+		+	D	*C. bifermentans* (urease –)
										D	*C. sordellii* (urease +)
									–	D	*C. lituseburense*
							–		+	D	*C. mangenotii*
					–	+		–	–	D	*C. botulinum* A
										D	*C. botulinum* B (proteolytic)
										D	*C. botulinum* F (proteolytic)
										D	*C. sporogenes*
						–	+	+	–	–	*C. difficile*
								–		D	*C. putrificum*
										–	*C. glycolicum*
										–	*C. scatologenes*
										–	*C. sticklandii*
							–		+	D	*C. mangenotii*
									–	D	*C. botulinum* G
										D	*C. subterminale*
										CD	*C. hastiforme*
										–	*C. irregularis*
										–	*C. propionicum*
		–	+		+					D	*C. limosum*
					–	+				–	*C. aminovalericum*
						–	+	+	+	C	*C. sphenoides*
									–	C	*C. ramosum*

TABLE 4—*Continued*

Aerobic	Acetic and butyric (± propionic)	Mixed[a]	Acetic	Lactic	Lecithinase	Lipase	Glucose	Mannitol	Indole	Milk[b]	Organism
										A	*C. cellobioparum*
								–	+	C	*C. indolis*
									–	C	*C. ramosum*
										A	*C. oroticum*
										D	*C. oceanicum*
							–			D	*C. histolyticum*
										–	*C. putrefaciens*
										–	*C. aminovalericum*
				+							*C. inulinum*

[a] Mixed acids refer to the presence of both normal and iso volatile fatty acids.
[b] C, Clot; D, digestion; A, acid; G, gas; –, no reaction.

fragilis and *B. melaninogenicus*. All other isolates were keyed as *Bacteroides* sp. If these bacteria could be speciated by the LF procedure, the SF species identification was scored as incorrect. If these bacteria could not be speciated by the LF procedure, the SF species identification was scored as correct. This rule was applied to all anaerobic isolates. Many of the bacteria not identifiable with the key could be speciated by comparing the SF results with the reactions listed in the VPI manual (8), since the manual lists many species that were not included in the SF keys. This type of identification was useful in the laboratory but was not included in the data in Tables 5 and 6.

Table 3 keys all of the anaerobic cocci listed in the VPI manual (8) except *Peptococcus variabilis* and *Ruminococcus*. *Peptostreptococcus intermedius, Peptococcus morbillorum*, and *Peptococcus constellatus* strains are now classified as species of *Streptococcus* (9). Due to biochemical similarities, *P. magnus* and *Peptostreptococcus micros* strains were differentiated by cell morphology. *P. magnus* cells were large, sometimes pleomorphic cocci occurring in small clusters, whereas *P. micros* cells were small cocci, usually in short chains. Approximately 6% of the gram-positive cocci could not be speciated by the SF key or the LF procedure. The genus of these nonspeciated isolates was determined by cell morphology on Gram-stained smears and by the amount of lactic acid produced in PYG broth. Since the morphological differences between *Peptococcus* and *Peptostreptococcus* are not always consistent, a better way of determining these genera may be advisable; however, we generally found cell morphology and staining characteristics to be consistent and predictable for the cocci that were speciated.

The reactions listed in Table 4 were capable of speciating most of the clostridia encountered during this study. Some isolates could only be narrowed down to a small group of possible species by the SF key. For the purposes of this study, the species identification was considered to be correct if the species determined by LF was present in the group of species found by SF (e.g., the LF identification was *Clostridium innocuum*, and the SF keyed to *C. innocuum* or *Clostridium sartagoformum*). If desired, these groups could often be resolved to individual species by close observation of cell and colony characteristics, by comparison of GLC profiles with profiles shown in the VPI manual (8), or by an additional test, such as urease, toxicity, or pathogenicity. *C. perfringens* and *Clostridium ramosum* did not readily produce detectable spores and thus could not be identified to the genus level by the reactions listed in Table 2 unless other factors were considered. Both species had certain cellular and cultural characteristics that were easily recognized with experience. This experience was best gained by observing known strains of these bacteria. Isolates with these characteristics were considered probable clostridia. Anaerobic bacilli lacking detectable spores could also be identified as

TABLE 5. *Comparison of the 3-ml SF identification with the LF identification of 235 anaerobic clinical isolates*

Anaerobic bacteria	Correct to genus level		Correct to species level		Correct to subspecies level[a]	
	No.	%	No.	%	No.	%
Gram-negative nonsporing bacilli	79/80[b]	98.8	69/80	86.3	54/65	83.1
Gram-positive nonsporing bacilli	35/35	100.0	20/35	57.1		
Gram-positive sporeforming bacilli	28/28	100.0	24/28	85.7		
Gram-positive cocci	84/87	96.6	77/87	88.5		
Gram-negative cocci	5/5	100.0	4/5	80.0		
Total	231/235	98.3	194/235	82.6	54/65	83.1

[a] Only the subspecies of *B. fragilis* and *B. melaninogenicus* are included.
[b] Number of SF and LF agreements per number tested by both procedures.

TABLE 6. *Comparison of the 3-ml and 0.5-ml SF identifications of 53 anaerobic clinical isolates*

Identification procedure	Correct to genus level		Correct to species level		Correct to subspecies level[a]		Avg incubation time (h)
	No.	%	No.	%	No.	%	
3-ml SF	53/53[b]	100.0	42/53	79.2	12/14	85.7	39
0.5-ml CSF	52/53	98.1	40/53	75.5	9/14	64.3	37
0.5-ml OSF	52/53	98.1	44/53	83.0	13/14	92.9	31

[a] Only the subspecies of *B. fragilis* and *B. melaninogenicus* are included.
[b] Number of SF and LF agreements per number tested by both procedures.

clostridia by the GLC detection of dipicolinic acid in culture extracts (21). Suspected clostridia were inoculated into the appropriate media listed in Fig. 1, and speciation was attempted in Table 4.

Identification using any of the three keys (Tables 2–4) was greatly aided by close attention to the chromatographic profiles. All batches of PYG we have prepared contain fatty acids, particularly acetic, lactic, and succinic. To determine the amount of a fatty acid produced by an isolate, it is necessary to know the amount present in uninoculated PYG. We routinely chromatograph uninoculated PYG each time fresh medium is prepared and when column conditions change.

Tables 2 through 4 contain columns labeled "mixed" acids. This refers to the presence of both normal and iso forms of the volatile short-chain fatty acids. Bacteria producing other combinations of acids were generally listed according to the major acid(s) produced.

The first phase of this study involved the comparison of the LF and 3-ml SF identifications of 235 clinical anaerobic isolates. Table 5 shows, to the genus, species, and subspecies levels, the numbers and percentages of correct identifications made by using the 3-ml SF procedure and keys. Ninety-eight percent of the 235 anaerobes were identified correctly to the genus level, 83% were identified to the species level, and 83% of 65 *B. fragilis* and *B. melaninogenicus* strains were identified to the subspecies level. Fifty-one of 54 (94%) *B. fragilis* strains were correctly speciated, as were 89% of 87 anaerobic gram-positive cocci. Eighty-six percent of the 28 *Clostridium* strains were correctly speciated. The 35 gram-positive nonsporeforming bacilli were correctly speciated less often (57%) than any other group of anaerobes.

The average incubation time for the 3-ml SF was 39 h. All results were to be routinely read at 18 to 48 h; however, a few isolates were incubated for 72 h, four were inadvertently incubated for 5 days, and one was incubated for 7 days.

The second phase of this study involved the comparison of identification results obtained using the three SF procedures with results obtained using the LF procedures on 53 clinical anaerobic isolates. When compared to the LF, all three SF procedures gave similar percentages of correct identifications at the genus (98 to 100%) and species (76 to 83%) levels (Table 6). The 0.5-ml OSF gave more correct identifications (93%) at the subspecies level than the 0.5-ml CSF (64%) and the 3-ml SF (86%); however, only 14 isolates were subspeciated. The average incubation periods ranged from 31 to 39 h.

DISCUSSION

The numbers of clinical specimens contain-

ing anaerobic bacteria have been reported to be 10.5% (20), 39.5% (23), 49% (12), and 66% (8). Over the last 15 years, anaerobic bacteria have been isolated from 59% of the positive clinical specimens cultured in this laboratory. Twelve percent of these specimens have yielded only anaerobes, and some have yielded only a single species. Greater recognition of anaerobes in infections has brought pressure on clinical laboratories to initiate or update anaerobic bacteriology capabilities. Many groups have difficulty meeting the extra time and financial demands imposed by anaerobic culture techniques.

The procedures described here have been evaluated in order to test the speed and accuracy with which the most common clinical isolates can be presumptively identified using a small number of conventional media and GLC. The 3-ml SF procedure has been the most extensively tested technique and has given 98% agreement with our standard identification procedure to the genus level (Table 5). A *Fusobacterium naviforme* strain was misidentified as a *Bacteroides* sp. because there was an insignificant amount of butyric acid in the culture at 48 h. A *Peptostreptococcus* strain was misidentified as a *Lactobacillus* due to a large amount of lactic acid produced and extreme pleomorphism on Gram-stained smears. The lack of fermentation and lactic acid production at 48 h led to the misidentification of two *S. intermedius* strains.

Although the 3-ml SF procedure has shown 83% agreement with the LF procedure to the species level (Table 5), only 57% of the gram-positive nonsporeforming bacilli were correctly speciated. There are at least two reasons for the low percentage of correct identifications within this group of anaerobes. First, the keys were designed to speciate only a few selected species. Therefore, roughly one-half of the incorrectly identified isolates were incorrect because speciation was not possible using the SF procedure but was accomplished by the LF procedure. Second, this group included a large number of *P. acnes*, many of which were misidentified as *Propionibacterium* sp. because they failed to give a positive indole reaction at 18 to 48 h in the SF medium. If the gram-positive nonsporeforming bacilli are excluded, the 3-ml SF correctly speciated 87% of the remaining 200 isolates.

An acceptable anaerobe identification procedure must be capable of speciating the organisms of major medical importance. Due to antibiotic resistance, prevalence, and frequent association with infection, *B. fragilis* fits this category. The 3-ml SF correctly identified 94% of the *B. fragilis* isolates. Of the three *B. fragilis* not identified, two failed to ferment glucose and hydrolyze esculin, and one was a *B. fragilis* subsp. *vulgatus* which was sensitive to penicillin and did not hydrolyze esculin at 24 h.

There were no major differences seen in the accuracy of identification of the 53 isolates tested in the anaerobic glove box and in GasPak jars (Table 6). The 3-ml volumes of media stored aerobically, steamed just prior to use, and incubated in anaerobic jars yielded as many correct identifications as the 0.5-ml volumes of media stored, inoculated, and incubated in an anaerobic chamber. This tends to support the contention of others that the clinically significant anaerobes are relatively aerotolerant and that if proper collection, handling, and media techniques are used, they are as effectively cultivated in anaerobic jars as in anaerobic chambers and roll tubes (3, 11, 16). In addition, small volumes of thioglycolate broth incubated in GasPak jars have been found to be as effective as prereduced, anaerobically sterilized media for the rapid determination of glucose, arabinose, and xylose fermentation by gram-negative anaerobes (6).

The poorer performance of the 0.5-ml CSF, in terms of both identification percentages and average incubation time, was due in part to a variable and sometimes insufficient inoculum size. The difference in average incubation times of the 3-ml SF (39 h) and the 0.5-ml OSF (31 h) (Table 6) was more a reflection of the greater ease with which growth media could be observed in the anaerobic glove box than of actual time required for growth in the two media.

SF identification can be obtained more quickly than is indicated in Materials and Methods. Since this study compared three abbreviated procedures with our standard identification procedure, it was desirable to use cells arising from a single colony as a source of inoculum for all test media. This required subculturing, which would not be necessary if a single SF procedure was used.

The advantages of the 3-ml SF procedure are as follows: (i) media can be prepared from simple ingredients found in or available to all clinical laboratories; (ii) few test media are required; (iii) media usage is reduced; (iv) anaerobic incubator space requirements are reduced; (v) media can be stored aerobically; (vi) incubation times may be reduced; (vii) other than a gas chromatograph, no complex equipment is required; (viii) it allows a choice of media needed for identification of a particular isolate; (ix) little time is required for setting up and reading tests; (x) it is less expensive than the LF or commercial procedures in this laboratory; and (xi) it is within what we consider accepta-

ble levels of accuracy for a simple yet rapid presumptive identification system for anaerobic bacteria. Other than aerobic storage and the need for an anaerobic glove box, these advantages also hold true for the 0.5-ml OSF and CSF procedures. The SF procedures are limited in that they give only a tentative identification of the frequently encountered anaerobes and do not attempt to identify the species of several of the less frequently encountered genera. Again, it should be stressed that identification in most cases is presumptive and that some anaerobes, such as *Clostridium botulinum*, require additional tests for identification. The identification keys list some anaerobes that were not identified during this study; therefore, we cannot say for certain that these bacteria can be identified by the keys. If these bacteria yield their characteristic reactions when grown in the small volumes of selected media, they should be presumptively identifiable.

Several procedures have recently been evaluated for their ability to rapidly identify anaerobic bacteria in the clinical laboratory (5, 7, 13-15, 17-19, 22). Abbreviated identification procedures are not necessarily the ideal and should not replace conventional techniques when these procedures can be accomplished and their cost can be justified. However, since conventional techniques cannot always be used, the use of simple, abbreviated procedures may be the only practical way for some clinical laboratories to identify anaerobic bacteria. The present investigation presents a simple, rapid procedure that has been used for over 2 years in this laboratory. The procedure has been functional, economical, easy to perform, and has supplied rapid results that we feel are within reasonable limits of accuracy when compared to our standard identification procedure.

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