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Growth of Clinical Isolates of Anaerobic Bacteria on Agar Media: Effects of Media Composition, Storage Conditions, and Reduction Under Anaerobic Conditions

PATRICK R. MURRAY

Clinical Microbiology Laboratories, Barnes Hospital, and Washington University School of Medicine, St. Louis, Missouri 63110

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The quantitative growth, the colony size, and the rate of growth of 47 clinical anaerobic isolates were compared on five different media, namely Brucella agar, brain heart infusion agar, Columbia agar, Schaedler agar, and tryptic soy agar. There was no significant difference in the quantitative growth of the anaerobes inoculated onto the five media. Although no single medium was superior for the growth of all isolates, 12 of 22 isolates, inoculated onto media stored for 4 weeks or less, grew best on Schaedler agar. The effects of supplementation of the media with reducing agents and reduction of the media before use were also analyzed and were found to be affected by the composition and length of storage of the media, as well as the bacteria tested.

The role of anaerobic bacteria in human infections has been increasingly appreciated in recent years, largely due to improvements in laboratory technology. The recovery of anaerobes in clinical specimens depends on the method of specimen collection and transportation to the laboratory, the quality and selection of the isolation media used, the incubation system, and the technical competence of the laboratory staff. The proper collection and transportation of the specimen are important not only for the reliable recovery of anaerobes, but also for the intelligent interpretation of the culture results. Specimens inoculated onto appropriate media can be incubated in anaerobic jars, a glove-box chamber, or anaerobic tubes (roll-tube method), since the recovery of clinically significant anaerobes has been reported to be equivalent in the three systems (14).

Although the methods for collection, transportation, and incubation of clinical specimens have been improved, the optimal medium or media for the recovery and growth of anaerobes have not been determined. A variety of agar media, including Brucella, brain heart infusion, Columbia, Schaedler, and tryptic soy have been recommended (6, 20), but have not been carefully compared. Although the growth of many *Bacteroides* strains requires hemin and vitamin K_1 (7, 21), the value has not been established for additional supplements such as palladium chloride, dithiothreitol, and cysteine hydrochloride, which are used to reduce the medium to a low oxidation-reduction potential. In addition, the effect of anaerobically reducing media before inoculation has not been determined.

In the study reported herein, the quantitative growth, colony size, and rate of growth of clinical anaerobic isolates were compared on different media to determine if one medium was superior. The effects of supplementation of the media with reducing agents and reduction of the media before use were also analyzed.

MATERIALS AND METHODS

Organisms. The organisms used were recovered from clinical specimens submitted to the Barnes Hospital diagnostic anaerobe laboratory. During the period of this study the blood agar medium routinely used was tryptic soy agar (Difco) supplemented with 5% defibrinated sheep blood, hemin (5 μg/ml), vitamin K_1 (0.5 μg/ml), cysteine hydrochloride (0.5 mg/ml), and palladium chloride (0.33 mg/ml). Anaerobes used in this study were randomly selected from primary isolation plates on the days when the evaluations were performed to avoid subculture onto laboratory media and adaptation to in vitro growth. It is, however, recognized that the selected organisms have inherently demonstrated an ability to grow on tryptic soy agar supplemented as described. The anaerobes were identified by the antibiotic disk and bile stimulation methods of Sutter et al. (20), biochemical reactivity in the Minitek system (BBL) modified as described by Stargel et al. (18), and metabolic end-product analysis by gas-liquid chromatography (9).

Media. Five different media formulated by both BBL and Difco laboratories were evaluated, namely brain heart infusion agar (BHIA), Brucella agar (BA), Columbia agar (CA), Schaedler agar (SA), and tryptic soy agar (TSA). All media were enriched with 5%

sheep blood, hemin (5 μg/ml), and vitamin K_1 (0.5 μg/ml). In the experiments to determine the effect of reducing agents, the five media were supplemented with cysteine hydrochloride (final concentration of 0.5 mg/ml), dithiothreitol (0.1 mg/ml), and palladium chloride (0.33 mg/ml). All media were commercially prepared (Remel, Lenexa, Kans.) on the same day, received in the laboratory within 24 h, and either used immediately or stored for as long as 6 weeks at 4°C in the cellophane bags and cardboard boxes in which the media were shipped.

In the experiments to determine the effect of anaerobic reduction of the media prior to inoculation, the five media were prepared as described above and then stored overnight at an ambient temperature under anaerobic conditions in a GasPak jar. The nonreduced media used in these experiments were stored overnight at an ambient temperature in an air atmosphere.

Inoculation of media. For the quantitative growth studies, the anaerobes were suspended in saline and adjusted to a turbidity equivalent to one-half of a McFarland no. 1 nephelometer standard. Serial 10-fold dilutions of the suspension were prepared, and 0.01 ml of the 10^{-3} to 10^{-6} dilutions was inoculated onto quadrants of a minimum of 40 plates (2 duplicate plates times 5 media times 2 formulations [BBL and Difco] times 2 [supplemented and nonsupplemented]). In the experiments which examined the effect of reduction of the media before use, 80 plates were inoculated with each isolate. Quantitation of anaerobic growth was performed with bacteria inoculated onto media stored for 4 weeks or less. Media 5 to 6 weeks old were evaluated by inoculating one drop of a nonstandardized suspension of bacteria onto the media, and the plates were streaked to obtain well-isolated colonies. All media were incubated in an anaerobic glove box at 35°C.

Examination of media. The quantitative growth of the anaerobes on each medium was determined by counting the number of colonies of bacteria in a quadrant with between 25 and 75 colonies and calculating the number of colony-forming units per milliliter in the original suspension. All determinations were made after 3 days of incubation. The size of the colonies was determined by measuring the diameter of three well-isolated colonies on each plate of medium with the aid of a Bausch and Lomb dissecting microscope. The colony size for each isolate on a particular medium was the average of all determinations after 3 days of incubation. No consistent differences were observed between the Difco and BBL formulations and the measurements were therefore averaged. The rate of anaerobic growth on the media was determined by measuring the colony size after incubation for 1 and 3 days.

Statistical analysis. The paired T test (3) was used to determine whether differences in the mean colony size and colony counts for the different media were statistically significant.

RESULTS

The 47 anaerobes which were used in this study are listed in Table 1. The quantitative growth of 10 anaerobes, which were inoculated onto the 5 media stored for less than 2 weeks, is summarized in Table 2. The number of colony-forming units of each anaerobe on the nonsupplemented media was not statistically different (at $P < 0.05$). Although not tabulated herein, there was no difference in the quantitative growth of the 10 anaerobes listed in Table 1, which were inoculated onto 3- to 4-week-old media.

The colony size of the 47 anaerobes cultured on each medium was measured after 3 days of incubation. In Table 3 is summarized the num-

TABLE 1. *Anaerobes used in media evaluations*

Anaerobe	No. of isolates inoculated onto media stored for:		
	0–2 weeks	3–4 weeks	5–6 weeks
Bacteroides fragilis	2	1	4
B. ovatus	2	2	3
B. distasonis	1		
B. corrodens	1		
B. thetaiotaomicron			1
B. vulgatus			1
B. melaninogenicus			2
Clostridium ramosum	1		
C. perfringens			1
Fusobacterium mortiferum	1		1
Fusobacterium spp.	1		1
F. naviforme		1	
F. necrophorum		1	
F. nucleatum		1	
Peptococcus spp.	1		
P. magnus		2	3
P. asaccharolyticus			1
P. prevotii			1
Peptostreptococcus spp.	1		2
Veillonella parvula	1	2	
Sarcina ventriculi			2
Eubacterium lentum			1
Propionibacterium acnes			1

TABLE 2. *Comparison of the quantitative growth of anaerobes on media*

Isolates	Colony-forming units per ml (× 10^7)				
	BA	BHIA	CA	SA	TSA
Bacteroides fragilis	2.6	2.4	2.5	2.5	2.4
B. distasonis	11.7	9.5	10.8	11.0	9.1
B. ovatus	6.7	6.8	7.0	7.1	7.0
B. corrodens	3.0	2.8	2.4	3.0	2.6
Fusobacterium mortiferum	3.7	3.5	3.9	3.2	3.3
Fusobacterium spp.	11.4	10.5	11.0	10.8	11.7
Clostridium ramosum	7.1	6.9	7.0	7.6	7.5
Veillonella parvula	4.5	3.5	3.5	4.5	4.0
Peptococcus spp.	6.5	6.7	7.1	6.2	7.0
Peptostreptococcus spp.	5.1	4.4	5.4	5.2	4.7

ber of anaerobes which grew best and worst on the nonsupplemented media. *Bacteroides* grew best on CA and worst on BA. *Fusobacterium* grew worst on BA and BHIA, whereas four of the seven isolates grew best on SA. The overall growth of the gram-positive cocci was best on BA and CA and worst on TSA. On SA *Veillonella* and *Clostridium* grew best and *Eubacterium* and *Propionibacterium* grew worst.

The effect of storing the media at 4°C for as long as 6 weeks was determined (Table 4). Of the 12 anaerobes which were inoculated onto nonsupplemented media stored for less than 2 weeks, the best growth was recorded on SA and CA for each of four anaerobes. Five of the 12 anaerobes grew worst on BA. Eight of the 10 anaerobes tested on media stored 3 to 4 weeks grew best on SA and 6 of 10 grew worst on BHIA. Seven of the 25 anaerobes inoculated onto 5- to 6-week-old media grew best on CA, and the worst growth was on SA and TSA for 9 and 8 anaerobes, respectively. One isolate of *Peptostreptococcus* did not grow on 6-week-old SA.

Anaerobic growth on media supplemented with reducing agents was compared with growth on nonsupplemented media (Table 5). Growth was significantly larger on supplemented media for some isolates of *Bacteroides* (inoculated on BA, CA, SA, and TSA), *Fusobacterium* (on BA, BHIA, CA, and TSA), *Sarcina* (on BHIA and CA), and *Veillonella* (on BA and CA). However, growth was smaller on supplemented media for *Bacteroides* (on BHIA), *Fusobacterium* (on SA), *Peptococcus* (on BA, BHIA, CA, and SA), *Peptostreptococcus* (on BA and CA), and *Veillonella* and *Clostridium* (on BHIA). One isolate of *Veillonella* failed to grow on 4-week-old, nonsupplemented CA and TSA. The enhanced growth of the anaerobes on supplemented BA, CA, and TSA was observed only with media stored for less than 4 weeks (Table 6). Growth was comparable on the supplemented and nonsupplemented media which were stored for 5 to 6 weeks.

Table 7 summarizes the growth after days 1 and 3 of incubation of six anaerobes inoculated onto supplemented and nonsupplemented media stored for less than 2 weeks. Three of the six anaerobes, inoculated onto nonsupplemented media, grew best on SA and four grew worst on BHIA after day 1 of incubation. After day 3 of incubation, three anaerobes grew best on BHIA and four grew worst on BA. Of the six anaerobes inoculated onto supplemented media, three each grew best on SA and CA, and four grew worst on BA after day 1. After day 3 of incubation, three of the six anaerobes grew best on CA. Growth was enhanced by supplementation of the media for one of the six anaerobes cultured on BHIA and SA, for two anaerobes on BA, and for three and four anaerobes on CA and TSA, respectively.

The effect of anaerobic reduction of supplemented media before use was evaluated with seven anaerobes inoculated onto freshly prepared media (Table 8) and with five anaerobes inoculated onto media stored for 4 weeks (Table 9). Whereas some organisms grew better on fresh, reduced media, reduction of the media

TABLE 3. *Comparison of the growth of anaerobes on different media not supplemented with reducing agents*

Isolates	No. tested	No. with best growth on					No. with worst growth on				
		BA	BHIA	CA	SA	TSA	BA	BHIA	CA	SA	TSA
Bacteroides	20		3	5	6	6	7	3	1	6	3
Fusobacterium	7		2	1	4		3	4			
Gram-positive cocci	13	4	2	4	3			3	1	3	6
Veillonella	3				3		1		2		
Clostridium	2			1	1		1				1
Eubacterium	1	1								1	
Propionibacterium	1	1								1	

TABLE 4. *Comparison of the growth of anaerobes on nonsupplemented media stored at 4°C for different time periods*

Age of media (weeks)	No. tested	No. with best growth on					No. with worst growth on				
		BA	BHIA	CA	SA	TSA	BA	BHIA	CA	SA	TSA
0–2	12		3	4	4	1	5	1	2	2	2
3–4	10	1	1		8		2	6	2		
5–6	25	5	3	7	5	5	5	3		9	8

TABLE 5. *Comparison of the growth of anaerobes on media supplemented with reducing agents and nonsupplemented media*

Isolates	No. tested	No. with best growth on each medium														
		BA			BHIA			CA			SA			TSA		
		No supplement	Supplement	No difference[a]	No supplement	Supplement	No difference	No supplement	Supplement	No difference	No supplement	Supplement	No difference	No supplement	Supplement	No difference
Bacteroides	20	5	6	9	7	4	9	5	8	7	4	8	8	5	7	8
Fusobacterium	7	2	3	2	2	3	2		4	3	4		3	1	4	2
Peptococcus	8	4	1	3	4	2	2	3	1	4	4	1	3	2	2	4
Peptostreptococcus	3	2	1		1	1	1	2	1		1	1	1	1	1	1
Sarcina	2		1	1		2			2		1		1		1	1
Veillonella	3		2	1	2	1			2	1		1	2		1	2
Clostridium	2		1	1	2					2	1	1				2
Eubacterium	1	1				1				1		1		1		
Propionibacterium	1			1	1			1					1	1		

[a] No significant difference between the average colony size of the bacteria inoculated onto supplemented and nonsupplemented media.

TABLE 6. *Comparison of anaerobic growth on supplemented and nonsupplemented media stored at 4°C for different time periods*

Age of media (weeks)	No. tested	No. with best growth on each medium														
		BA			BHIA			CA			SA			TSA		
		No supplement	Supplement	No difference[a]	No supplement	Supplement	No difference	No supplement	Supplement	No difference	No supplement	Supplement	No difference	No supplement	Supplement	No difference
0–2	12	4	2	6	7	1	4	4	2	6	5	3	4	2	5	5
3–4	10	1	6	3	3	5	2		7	3	3	1	6	1	4	5
5–6	25	9	7	9	9	8	8	7	9	9	7	9	7	8	7	10

[a] No significant difference between the average colony size of the bacteria inoculated onto supplemented and nonsupplemented media.

TABLE 7. *Average colony diameter of anaerobes on freshly prepared media supplemented and not supplemented with reducing agents*

Isolates	Medium supplemented with reducing agents	Avg colony size (mm)									
		BA		BHIA		CA		SA		TSA	
		Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
Bacteroides fragilis	No	0.30	0.96	0.30	0.89	0.34	0.89	0.48	1.52	0.37	0.93
	Yes	0.26	1.08	0.30	1.19	0.41	1.04	0.60	1.60	0.48	1.18
Bacteroides corrodens	No	0.96	2.56	0.89	3.18	1.11	2.70	0.96	2.78	1.22	2.67
	Yes	0.89	2.70	0.81	2.82	1.33	3.00	1.15	2.59	0.96	2.89
Fusobacterium mortiferum	No	0.99	2.89	1.29	4.50	1.20	3.85	1.47	3.40	0.97	3.59
	Yes	1.00	2.90	1.29	4.44	1.23	3.96	1.62	3.18	1.10	3.81
Fusobacterium spp.	No	1.22	2.37	1.22	2.51	1.81	3.30	1.61	2.67	1.33	2.59
	Yes	1.15	2.26	1.30	2.45	1.63	2.78	1.41	2.08	1.37	3.07
Clostridium ramosum	No	1.68	2.52	1.55	2.84	1.84	2.90	2.28	3.28	1.76	2.73
	Yes	1.61	2.53	1.14	2.46	1.78	2.88	1.74	2.54	1.77	2.72
Peptostreptococcus spp	No	0.30	1.30	0.74	1.48	0.48	1.30	0.67	1.15	0.15	0.78
	Yes	0.22	1.15	0.48	1.04	0.41	1.19	0.60	1.11	0.22	0.52

TABLE 8. *Effect of reducing freshly prepared, supplemented media*

Isolate	Avg colony size (mm)									
	BA		BHIA		CA		SA		TSA	
	Reduced	Not reduced	Reduced	Not reduced	Reduced	Not reduced	Reduced	Not reduced	Reduced	Not reduced
Bacteroides fragilis	1.97	2.08	2.57	2.59	2.11	2.26	2.84	2.65	2.72	2.77
Bacteroides ovatus	2.50	2.40	2.94	2.79	2.65	2.59	2.32	2.53	2.80	2.77
Bacteroides distasonis	2.41	2.52	1.31	1.46	2.56	2.38	2.74	2.81	2.73	2.85
Fusobacterium mortiferum	3.11	2.90	3.92	4.44	3.84	3.96	2.62	3.18	3.98	3.81
Peptococcus spp.	0.33	0.37	0.28	0.29	0.53	0.44	0.31	0.29	0.52	0.53
Veillonella	0.98	1.09	1.22	1.20	1.13	1.24	1.52	1.72	1.17	1.24
Clostridium ramosum	2.48	2.53	2.41	2.46	2.98	2.88	2.57	2.54	2.92	2.72
Average	1.97	1.98	2.09	2.18	2.26	2.25	2.13	2.25	2.41	2.38

was also inhibitory for many isolates, particularly *Bacteroides ovatus* and *Veillonella* on SA and *Fusobacterium mortiferum* on BHIA and SA (Table 8). The average growth of the seven anaerobes after day 3 of incubation was not enhanced by reduction of any of the five media. Reduction of 4-week-old media enhanced the growth of *B. ovatus* on TSA and *Peptococcus magnus* on BHIA and SA and inhibited the growth of *B. ovatus* and *Fusobacterium naviforme* on BHIA, *Fusobacterium nucleatum* on SA, and *P. magnus* on BA (Table 9). The average colony size of the five isolates after day 3 of incubation was only enhanced on reduced TSA.

DISCUSSION

Wilkens et al. (21) reported that the growth of *Bacteroides* on different blood agar media was variable and was a function of both the species tested and the composition of the medium. This differential growth was only partially eliminated by supplementation with hemin. Starr and coworkers (19) compared the growth of selected anaerobes on SA and on TSA supplemented with yeast extract. Isolates of *Clostridium* grew best on SA, and isolates of *B. fragilis*, *B. melaninogenicus*, and *F. fusiforme* grew equally well on both media. Stalons et al. (17) reported the best bacterial growth was obtained in Schaedler broth compared with eight other broths.

In the study reported herein, the growth of 47 anaerobes (Table 1) on 5 media was evaluated. There was no significant difference in the quantitative growth of 20 anaerobes inoculated onto media which had been stored at 4°C for 4 weeks or less. However, both the colony size and the

TABLE 9. *Effect of reducing 4-week-old, supplemented media*

Isolate	Avg colony size (mm)									
	BA		BHIA		CA		SA		TSA	
	Reduced	Not reduced	Reduced	Not reduced	Reduced	Not reduced	Reduced	Not reduced	Reduced	Not reduced
Bacteroides ovatus	2.36	2.44	2.81	3.49	3.03	2.96	3.11	3.11	3.03	2.81
Fusobacterium nucleatum	1.37	1.40	0.44	0.51	1.66	1.77	1.96	2.44	1.55	1.41
Fusobacterium naviforme	1.33	1.22	0.62	1.11	1.51	1.40	1.29	1.22	1.18	1.22
Peptococcus magnus	0.85	1.03	0.96	0.78	0.89	0.89	1.40	1.11	0.74	0.74
Veillonella	0.89	0.78	0.25	0.37	0.44	0.44	0.52	0.45	0.37	0.25
Average	1.36	1.37	1.02	1.25	1.51	1.49	1.66	1.67	1.37	1.29

rate of growth of the anaerobes on the five media were significantly different. Of the 22 anaerobes cultured on nonsupplemented media stored for 4 weeks or less, 12 grew best on SA, including 4 of 9 *Bacteroides,* 3 of 5 *Fusobacterium,* 3 of 3 *Veillonella,* 1 *Clostridium,* and 1 *Peptococcus.* However, SA apparently deteriorated with storage compared with the other media because 9 of the 11 anaerobes with poor growth on SA occurred on 5- to 6-week-old media (Table 4).

In this study no attempt was made to determine the shelf life of the different media. Hansen and Martin (8) demonstrated that BHIA was stable for at least 14 days, although 8 of the 10 anaerobes inoculated onto 3- to 4-week-old media tested here grew worst on BHIA. Finegold et al. (6) and Dowell et al. (4) recommended that media should be stored in oxygen-impervious bags and for no longer than 2 weeks. Lombard et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C95, p. 41) reported that TSA, stored in cellophane bags for as long as 6 weeks, did not appreciably deteriorate. Additional studies are in progress in this laboratory to determine the optimum storage conditions and shelf life for the different anaerobic media.

Each of the five media evaluated in this study was also supplemented with cysteine hydrochloride, dithiothreitol, and palladium chloride. Moore (11) in 1968 demonstrated that the growth of *Clostridium novyi* on solid media required supplementation with cysteine hydrochloride and dithiothreitol. Mylroie and Hungate (12) reported that palladium was a reduction catalyst when used in agar medium and improved the recovery of bacteria in sludge. Aranki and associates (1, 2) confirmed this report and demonstrated that palladium chloride, which can be incorporated into agar medium, could be substituted for palladium black. Recently, however, Owens et al. (13) reported that supplementation of BA did not consistently improve the recovery rate of bacteria from guinea pig intestinal contents or human fecal material. In the study reported herein, the effect of reducing agents on anaerobic growth was influenced by the composition and age of the media, and the test organism. Supplementation of BA, CA, and TSA improved growth, whereas growth on supplemented BHIA and SA was inhibited (Table 5). This was particularly true for isolates of *Fusobacterium* and *Veillonella.* These effects were, however, not observed on media stored for 5 to 6 weeks (Table 6). The ineffectiveness of the reducing agents incorporated in stored media was not solely due to the formation of toxic organic peroxides (9, 15), since reduction of 4-week-old supplemented media did not improve bacterial growth except on TSA (Table 9). The reason for the inhibitory effect of reducing agents in freshly prepared media, whether due to the presence of palladium chloride, or some other component, has not been elucidated. It is important to recognize that the reducing agents were not only ineffective in enhancing the growth of many of the clinical isolates tested herein, but they were in some cases inhibitory.

The rate of anaerobic growth is a function of the time required to adapt to the in vitro environment (lag phase), the generation time during logarithmic growth, and the rate in which the nutrients are depleted and toxic metabolic by-products accumulate. The rate of growth will affect both the time necessary to detect bacterial growth and the maximum colony size. A prolonged lag phase will delay the detection of growth and possibly result in the inability to recover some fastidious anaerobes if exposed to air during the crucial early phase of growth (16). The effect of the accumulation of toxic metabolites was minimized in this study by measuring the size of only well-isolated colonies. Anaerobic growth was smallest on BHIA and largest on SA after day 1 of incubation (Table 7). BHIA was the only medium tested in which meat infusions were the main source of peptones, and this dif-

ference was most likely responsible for the prolonged lag phase.

Reduction of anaerobic media is generally recommended (4, 5, 9,10), although Finegold et al. (6) conceded that the need for this has not been adequately established. Ellner et al. (5) reported that growth of stock cultures of *P. magnus* and *Fusobacterium necrophorum* on reduced CA was superior to growth on nonreduced media. Lombard et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C95, p. 41) reported, however, only slight differences in bacterial growth on reduced and nonreduced TSA stored at 5°C for as long as 6 weeks. In the study reported herein, reduction of freshly prepared and 4-week-old media did not improve the overall growth of anaerobes on any of the media with the exception of stored TSA (Tables 8 and 9).

In summary, no single medium was clearly superior for the growth of all anaerobic isolates. Although quantitative bacterial growth was similar on all media, the overall colony size and rate of growth was best on freshly prepared nonsupplemented SA. The effect of supplementing the media with reducing agents and reduction before inoculation was influenced by the composition and length of storage of the media, as well as the bacteria tested. With the proper selection of media, supplementation and reduction seems to be unnecessary and undesirable.

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LITERATURE CITED

1. **Aranki, A., and R. Freter.** 1972. Use of anaerobic glove box for the cultivation of strictly anaerobic bacteria. Am. J. Clin. Nutr. **25**:1329–1334.
2. **Aranki, A., S. A. Syed, E. B. Kenney, and R. Freter.** 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. **17**:568–576.
3. **Colton, T.** 1974. Statistics in medicine, 1st ed. Little, Brown & Co., Boston.
4. **Dowell, V. R., Jr., and T. M. Hawkins.** 1974. Laboratory methods in anaerobic bacteriology: CDC laboratory manual, p. 3–4. DHEW publication no. (CDC) 74-8272. U. S. Government Printing Office, Washington, D.C.
5. **Ellner, P. D., P. A. Granato, and C. B. May.** 1973. Recovery and identification of anaerobes: a system suitable for the routine clinical laboratory. Appl. Microbiol. **26**:904–913.
6. **Finegold, S. M., E. E. Shepherd, and E. H. Spaulding.** 1977. Cumitech 5. Coordinating ed., W. E. Shepherd. Practical anaerobic bacteriology. American Society for Microbiology, Washington, D.C.
7. **Gibbons, R. J., and J. B. MacDonald.** 1961. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. J. Bacteriol. **80**:164–170.
8. **Hanson, C. W., and W. J. Martin.** 1976. Evaluation of enrichment, storage, and age of blood agar medium in relation to its ability to support growth of anaerobic bacteria. J. Clin. Microbiol. **4**:394–399.
9. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobic laboratory manual, 4th ed., Virginia Polytechnic Institute and State University, Blacksburg, Va.
10. **Martin, W. J.** 1971. Practical method for isolation of anaerobic bacteria in the clinical laboratory. Appl. Microbiol. **22**:1168–1171.
11. **Moore, W. B.** 1968. Solidified media suitable for the cultivation of *Clostridium novyi* type B. J. Gen. Microbiol. **53**:415–423.
12. **Mylroie, R. L., and R. E. Hungate.** 1954. Experiments on the methane bacteria in sludge. Can. J. Microbiol. **1**: 55–64.
13. **Owens, D. R., R. D. Rolfe, and D. J. Hentges.** 1976. Effectiveness of palladium chloride for the isolation of anaerobes. J. Clin. Microbiol. **3**:218–220.
14. **Rosenblatt, J. E., A. Fallow, and S. M. Finegold.** 1973. Comparison of methods for isolation of anaerobic bacteria from clinical specimens. Appl. Microbiol. **25**: 77–85.
15. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria. Charles C. Thomas Co., Springfield, Ill.
16. **Spaulding, E. H., V. Vargo, T. C. Michaelson, M. Korzeniowski, and R. M. Swenson.** 1974. Anaerobic bacteria: culture and identification, p. 87–104. *In* J. E. Prier and H. Friedman (ed.). Opportunistic pathogens. University Park Press, Baltimore.
17. **Stalons, D. R., C. Thornsberry, and V. R. Dowell, Jr.** 1974. Effect of culture medium and carbon dioxide concentration on growth of anaerobic bacteria commonly encountered in clinical specimens. Appl. Microbiol. **27**:1098–1104.
18. **Stargel, M. D., F. S. Thompson, S. E. Phillips, G. L. Lombard, and V. R. Dowell, Jr.** 1976. Modification of the Minitek miniaturized differentiation system for characterization of anaerobic bacteria. J. Clin. Microbiol. **3**:291–301.
19. **Starr, S. E., G. E. Killgore, and V. R. Dowell, Jr.** 1971. Comparison of Schaedler agar and Trypticase soy-yeast extract agar for the cultivation of anaerobic bacteria. Appl. Microbiol. **22**:655–658.
20. **Sutter, V. L., V. L. Vargo, and S. M. Finegold.** 1975. Wadsworth anaerobic bacteriology manual, 2nd ed., Department of Continuing Education of Health Sciences, University of California at Los Angeles, Los Angeles.
21. **Wilkens, T. D., S. L. Chalgren, F. Jimenez-Ulate, C. R. Drake, Jr., and J. L. Johnson.** 1976. Inhibition of *Bacteroides fragilis* on blood agar plates and reversal of inhibition by added hemin. J. Clin. Microbiol. **3**: 359–363.