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Relative Recovery of Anaerobes on Different Isolation Media

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The recovery of clinical anaerobic isolates on selective and nonselective agar media, as well as the time required to detect the isolates, was examined. Of a total of 235 isolates, 77, 46, and 40% were detected on Schaedler blood agar, colistin-nalidixic acid blood agar, and kanamycin-vancomycin-lysed blood agar, respectively, and 94% were detected on the combination of Schaedler blood agar with kanamycin-vancomycin-lysed blood agar. A total of 19% of the anaerobes were detected after incubation for 1 day, and 70% were detected after 2 days.

The processing of anaerobic cultures is expensive and time-consuming. Finegold and co-workers (1, 2, 7) recommended that for the optimum recovery of anaerobes, clinical specimens should be inoculated onto selective and nonselective agar media and into a liquid medium and that the media should be incubated for at least 7 days. Previous studies from our laboratory have been concerned with reducing the expense and time required to detect and identify anaerobes (4, 5; J. E. Sondag, P. R. Murray, and M. L. Heath, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C182, p. 307). In the study reported here, the need for the different isolation media and prolonged incubation was examined.

During a 7-month period, from October 1978 through April 1979, a total of 1,068 specimens were processed in the Barnes Hospital Anaerobe Laboratory, and 668 specimens were included in this study. Specimens not included were normally sterile fluids such as cerebrospinal or synovial fluids, swab specimens, and those in which an insufficient quantity of material was received. The specimens were collected and transported to the laboratory in prereduced anaerobic transport vials. Each specimen was immediately inoculated onto the following media: Schaedler blood agar (SBA) supplemented with hemin (5 μg/ml) and vitamin K_1 (0.5 μg/ml); Schaedler agar with lysed blood, kanamycin (75 μg/ml), vancomycin (7.5 μg/ml), hemin, and vitamin K_1 (LKV); SBA with colistin (0.01 μg/ml) and nalidixic acid (0.015 μg/ml) (CNA); and thioglycolate 135C broth supplemented with hemin and vitamin K_1. The media were commercially prepared (Remel, Lenexa, Kan.) and were used within 2 weeks of receipt. After inoculation, the media were stored in an anaerobic holding jar (3) for a maximum of 2 h or transferred directly into a 35°C anaerobic chamber with an atmosphere of 85% N_2, 10% CO_2, and 5% H_2. After incubation for 1 day the media were examined inside the anaerobic chamber, and after 2 and 7 days of incubation the media were removed from the chamber and examined with the aid of a stereomicroscope. Each colony type was Gram stained and subcultured onto a section of an aerobic and anaerobic blood agar plate. All strict anaerobes were subsequently identified by standard procedures as described previously (5).

Of the specimens processed in this study, no bacterial growth was detected in 50%, only aerobic growth was detected in 24%, only anaerobic growth was detected in 10%, and both aerobic growth and anaerobic growth were detected in 16%. A total of 262 anaerobes were recovered, including 27 isolates (23 *Propionibacterium*, 2 *Clostridium*, 1 *Eubacterium*, and 1 *Veillonella*), which were only detected in the thioglycolate 135C broth. Of the 235 anaerobes which were detected on agar media, 180 (77%), 107 (46%), and 95 (40%) were recovered on SBA, CNA, and LKV, respectively (Table 1). A total of 222 (94%) anaerobes were detected on either SBA or LKV, compared with 198 (84%) on SBA or CNA and 167 (71%) on LKV or CNA. Whereas only 60% of the isolates of *Bacteroides* were detected on SBA, 84% of the isolates were detected on LKV, and 94 (96%) were detected on either SBA or LKV. In addition, 24 of 25 *Clostridium* and all isolates of *Fusobacterium*, *Eubacterium*, *Bifidobacterium*, and *Veillonella* were detected on SBA or LKV. Of the 46 anaerobic gram-positive cocci, 40 were recovered on the combination of SBA and LKV, and all 46 were recovered on the combination of SBA and CNA.

The time at which the anaerobes were initially detected is summarized in Table 2. A total of 44 (19%) of the 235 anaerobes were detected after incubation for 1 day, 165 (70%) were detected

TABLE 1. *Recovery of anaerobes on agar media*

Isolates	Total no.	No. detected on:					
		SBA	LKV	CNA	SBA or LKV	SBA or CNA	LKV or CNA
B. fragilis group[a]	63	38	52	33	60	46	59
Bacteroides, other species	35	21	30	8	34	22	33
Fusobacterium	5	4	2	2	5	4	3
Clostridium	26	23	4	9	25	22	13
Eubacterium	13	13	3	3	13	13	5
Bifidobacterium	5	4	1	1	5	4	2
Lactobacillus	4	2	0	2	2	4	2
Propionibacterium	32	29	1	16	30	31	17
Peptococcus	34	31	0	20	31	34	20
Peptostreptococcus	12	9	0	9	9	12	9
Veillonella	6	6	2	4	6	6	4

[a] Includes *Bacteroides distasonis*, *Bacteroides fragilis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, and *Bacteroides vulgatus*.

TABLE 2. *Detection time of clinical anaerobic isolates*

Isolates	No. isolated	% detected by day:	
		1	2
B. fragilis group[a]	63	19	87[b]
Bacteroides, other species	35	11	54
Fusobacterium	5	20	40
Clostridium	26	50	96
GPNSB[c]	54	7	33
Peptococcus	34	18	82
Peptostreptococcus	12	8	92
Veillonella	6	50	100

[a] See Table 1, footnote *a*.

[b] The remaining isolates were detected after 3 to 7 days of incubation.

[c] GPNSB, Gram-positive nonsporeforming bacilli, including *Eubacterium*, *Bifidobacterium*, *Lactobacillus*, and *Propionibacterium*.

after 2 days, and 70 (30%) were detected between 3 and 7 days. Of the 70 anaerobes, 23 were detected on SBA only, 13 were detected on LKV only, and 3 were detected on CNA only.

The results presented in this study indicate that a single agar medium cannot be used for the recovery of anaerobes. Only 77% of the isolates were detected on the nonselective SBA, because many anaerobes were overgrown by facultative bacteria such as *Proteus mirabilis*. Although most *Bacteroides* were recovered on LKV, anaerobic gram-positive bacilli and anaerobic cocci were inhibited, and fewer than one-half of the anaerobes were recovered on CNA. The combination of nonselective SBA and selective LKV increased the total detection of anaerobes to 94%. However, the combinations of SBA with CNA or LKV with CNA were not satisfactory. Use of supplemented thioglycolate 135C broth would appear to be unnecessary. Rosenblatt et al. (6) previously reported that a significant number of anaerobes are not recovered in liquid media, and in the present study the majority of isolates which grew only in the broth were *Propionibacterium* (23 of 27 isolates). Finally, anaerobic cultures should be incubated for more than 2 days before discarding because 30% of the isolates, including 16 of 35 isolates of *Bacteroides* other than the *Bacteroides fragilis* group, were detected between 3 and 7 days.

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