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Medium for Selective Isolation and Presumptive Identification of the *Bacteroides fragilis* Group

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We developed and evaluated a new medium (FRAG agar) for the selective isolation and presumptive identification of the *Bacteroides fragilis* group. This medium contains 1% D-glucuronic acid as a fermentable carbon source, a reduced peptone content, gentamicin, and 20% bile. Presumptive identification of the *B. fragilis* group was based on growth, fermentation, and typical colony morphology. A total of 75 stock culture isolates of the *B. fragilis* group grew well on this medium, and 69 showed evidence of fermentation. Of 90 other anaerobes, none grew well or fermented glucuronic acid. In a clinical trial of 100 specimens sent for anaerobic culture, FRAG agar inhibited 71 of 71 anaerobes not belonging to the *B. fragilis* group, as well as 104 of 110 facultative organisms. A total of 33 isolates of the *B. fragilis* group were recovered on the selective medium, whereas only 23 were recovered by routine methods. Of 23 cultures positive for the *B. fragilis* group on routine plates, 22 were positive on FRAG agar.

Members of the *Bacteroides fragilis* group represent the most frequently isolated anaerobes from properly collected clinical specimens (9, 12). The group consists of the following six well-defined species: *Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides vulgatus, Bacteroides distasonis, Bacteroides ovatus*, and *Bacteroides uniformis* (3). Other unnamed DNA homology groups, such as *Bacteroides* strain 3452A, are also included in this saccharolytic intestinal *Bacteroides* group (10). Because the members of this group are usually resistant to penicillin and other antibiotics (5), proper therapy depends on rapid isolation and identification from clinical specimens. However, isolation of these bacteria may be complicated because of the high frequency of mixed infections in which they occur. Their presence may be masked on nonselective media due to faster-growing or more predominant bacteria.

Selective media for the isolation of *Bacteroides* (4, 13) or, more specifically, for the isolation of the *B. fragilis* group (1, 2, 11) have been developed. The more successful media (2, 11) contain a peptone base (usually Trypticase soy) and are supplemented with antibiotics and bile for selectivity. The addition of esculin also allows the rapid presumptive identification of the *B. fragilis* group because of the ability of these bacteria to hydrolyze esculin. However, the degree of esculin hydrolysis is variable, and some strains of *B. vulgatus* do not hydrolyze this substance (3).

The *B. fragilis* group is saccharolytic and ferments a variety of simple sugars (7). Salyers et al. (14) have shown that various members of this group also ferment a wide variety of plant polysaccharides and mucins. Other intestinal anaerobes are not nearly as versatile (15). One major component of mucins, D-glucuronic acid, was fermented by all members of the *B. fragilis* group and by essentially none of the strains belonging to other genera of intestinal anaerobes studied. This fermentation of glucuronic acid appeared to be a good selective or differential characteristic of the *B. fragilis* group.

In this present study, we developed and evaluated a new selective medium for the *B. fragilis* group (FRAG agar). When a minimal, defined medium with a low peptone content was used, fermentation of D-glucuronic acid was apparent within 48 h of incubation. This feature, as well as typical colony morphology, should enable microbiologists to identify the *B. fragilis* group presumptively in clinical specimens. Selectivity was enhanced through the incorporation of gentamicin (11) and bile into the medium. The inability to utilize glucuronic acid should suppress the growth of any antibiotic- or bile-resistant organisms.

MATERIALS AND METHODS

Media. The selective medium used (FRAG agar) was a modification of the medium described by Salyers et al. (14), and its composition is shown in Table

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TABLE 1. Composition of FRAG agar

Component	Amt[a]
Casitone (Difco)	0.1 g
Trypticase soy agar (BBL)	0.1 g
Yeast extract (Difco)	0.2 g
D-Glucuronic acid (Sigma)	1.0 g
Oxgall (Difco)	2.0 g
Gentamicin (Schering) (10-mg/ml reagent solution)	1.0 ml
$(NH_4)_2SO_4$	0.1 g
K_2HPO_4	0.226 g
KH_2PO_4	0.09 g
Mineral solution[b]	1.0 ml
$FeSO_4$ solution[c]	1.0 ml
Hemin-vitamin K_1 solution (Carr-Scarborough)	1.0 ml
Vitamin B_{12} (Parke, Davis) (10-μg/ml aqueous solution)	0.05 ml
Cysteine hydrochloride	0.05 g
Phenol red (1.0% aqueous solution)	1.0 ml
Agar	1.5 g

[a] Amounts per 100 ml of distilled water.

[b] The mineral solution contained the following in 100 ml of water: NaCl, 9.0 g; $CaCl_2 \cdot 2H_2O$, 0.27 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $MnCl_2 \cdot 4H_2O$, 0.1 g; and $CoCl_2 \cdot 6H_2O$, 0.1 g. This solution was stored at 4°C.

[c] The $FeSO_4$ solution contained 0.04 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of water and was stored at 4°C.

1. To prepare this medium, all of the ingredients except cysteine and glucuronic acid were mixed and heated to boiling with constant stirring. The volume was adjusted with distilled water. The medium was allowed to cool to 40 to 50°C, and the cysteine was added. The pH was adjusted to about 7.0 with 1.0 N NaOH. Subsequently, the medium was autoclaved for 15 min at 121°C and allowed to cool to 40 to 50°C before the sugar was added. Glucuronic acid was added as an aqueous 40% solution; it was filter sterilized, and 2.5 ml was added to each 100 ml of FRAG agar to yield a final concentration of 1.0%. Plates were poured (about 20 ml/plate), kept in ambient air at room temperature, and inoculated within 2 h of preparation.

For the clinical trial, 100-ml portions of FRAG agar were dispensed into bottles and autoclaved. The medium was prepared as described above, except that gentamicin was not added before autoclaving. Bottles were stored at room temperature in indirect light. The FRAG agar was melted as needed, and glucuronic acid and gentamicin were added to the cooled (40 to 50°C) medium. Plates were poured, allowed to harden, and maintained at room temperature in ambient air before inoculation. Fresh plates were prepared each day.

Nonselective, enriched, 5% sheep blood (SB) agar plates were prepared by using a Trypticase soy agar base (BBL Microbiology Systems, Cockeysville, Md.). A vitamin K_1-hemin solution (Carr-Scarborough, Atlanta, Ga.) was added after autoclaving (final concentrations, 5 μg of hemin per ml and 1 μg of vitamin K_1 [3-phytylmenadione] per ml). These plates were kept at room temperature and inoculated within 2 h of preparation.

Bacterial cultures. The first portion of this study was an assessment of the selectivity of the medium by using organisms recently isolated from clinical specimens in the University of Minnesota Hospitals Diagnostic Microbiology Laboratory. Anaerobic isolates were taken from cultures in prereduced, anaerobically sterilized chopped meat broth (Carr-Scarborough) that had been stored at room temperature. Facultative gram-positive organisms had been stored at 4°C on Trypticase soy agar slants, and gram-negative facultative organisms had been stored on triple sugar iron agar slants for up to 2 weeks. All *Providencia* strains were obtained from stock cultures due to a lack of recent clinical isolates of these organisms.

Plating methods. For each anaerobic isolate, a loopful of a chopped meat broth culture was streaked onto one-half of a fresh, enriched SB agar plate to verify the purity of the culture. The plates were incubated for 48 h at 35°C in a GasPak jar with a gas generator packet (BBL), fresh catalyst, and indicator.

A loopful of growth from each SB agar plate was suspended in 5.0 ml of thioglycolate medium (without indicator) containing 0.05% agar. FRAG agar and SB agar plates were inoculated with 0.001-ml portions of the thioglycolate suspension by using a calibrated loop. The plates were streaked in three directions to obtain isolated colonies. Semiquantitative growth measurements were recorded as follows: 1+, less than 10 colonies present; 2+, more than 10 colonies, but growth only in the first streak area; 3+, growth in the first and second streak areas; and 4+, growth in all streak areas.

Each facultative organism was streaked onto one-half of an SB agar plate, which was then incubated aerobically for 24 h at 35°C to verify the purity of the culture. A loopful of growth was suspended in 5.0 ml of tryptic soy broth. FRAG agar and SB agar plates were inoculated as described above for the anaerobic organisms. All plates were incubated anaerobically in GasPak jars as described above. After 48 h, the plates were examined and compared, and we noted the extent of growth, colony morphology, and size of well-isolated colonies, as well as evidence of glucuronic acid fermentation on the selective plates (indicated by a yellow color).

Clinical trial. To evaluate the usefulness of FRAG agar, we performed a clinical trial by using specimens which had a high likelihood of containing the *B. fragilis* group, including intraabdominal, female pelvic, and miscellaneous soft tissue specimens from sites below the waist. Each specimen was collected, transported in an anaerobic transport container, and inoculated to a freshly prepared plate made by melting prereduced brain heart infusion agar with hemin and vitamin K_1 (Carr-Scarborough) and adding 5% SB, a freshly prepared phenylethylalcohol agar plate (Difco Laboratories, Detroit, Mich.) containing hemin, vitamin K_1, and 5% SB, a tube containing prereduced chopped meat carbohydrate broth (Carr-Scarborough), and a FRAG agar plate. The plates were incubated anaerobically for 48 h at 35°C in evacuated GasPak jars, which were flushed three times with a mixture containing 85% N_2, 10% CO_2, and 5% H_2. Inoculated plates were stored in holding jars containing oxygen-free CO_2 before incubation. Specimens also were plated onto 5% SB agar, colistin-nalidixic acid agar, and MacConkey agar plates for the isolation of facultative and aerobic organisms.

After 48 h, the plates were examined, and all colony types were recorded. Each isolated colony was streaked onto a fresh, enriched SB agar plate which was incubated anaerobically, as well as onto an aerobic SB agar plate. Organisms that grew both aerobically and anaerobically were designated facultative; organisms that grew only anaerobically were designated strict anaerobes. All isolates were Gram stained. For identification, all anaerobic isolates were subcultured in prereduced chopped meat glucose broth (Carr-Scarborough) and incubated for 24 to 48 h at 35°C. A sample of each broth was Gram stained to verify the purity of the culture. A gas chromatographic analysis was performed to determine the metabolic end products of the organisms (7) and to determine their genera. Identification as to species was achieved by using API 20A strips (Analytab Products, Plainsview, N.Y.) inoculated with growth from the subcultured anaerobic SB agar plates. Problem organisms were tested further by using prereduced media, as described in the *Anaerobe Laboratory Manual* (7).

Facultative gram-negative rods were identified by using the API 20E system (Analytab Products). Facultative cocci were identified by conventional procedures.

RESULTS

Table 2 shows the growth of 75 *B. fragilis* group strains on FRAG agar. All strains tested produced 4+ growth on the nonselective SB agar plates, as well as on FRAG agar plates. Although colonies of all of the species had the same appearance on SB agar, on FRAG agar there was a marked variation in colony sizes. Strains of *B. ovatus*, *B. distasonis*, and *B. thetaiotaomicron* grew very well on the selective agar, and the colony diameters usually exceeded 1.5 mm after 48 h of incubation. These colonies were also very mucoid, and adjacent colonies tended to run together. *B. vulgatus* produced colonies 1.0 mm in diameter after 48 h. The most variation was observed in the strains of *B. fragilis*. This species grew more slowly on the selective medium, and colonies were generally smaller than the colonies of other species. Some strains produced colonies only 0.5 mm in diameter, whereas the colonies of other strains exceeded 1.0 mm by 48 h. However, during prolonged incubation, the colony sizes increased for all of the *B. fragilis* strains, as well as for the other species. Thus, all of the *B. fragilis* group species tested grew to easily detectable colony sizes within 48 h. The colonies were white, round, and shiny; larger colonies tended to have a slightly yellow pigmentation.

TABLE 2. Growth of 75 pure culture isolates of the *B. fragilis* group on FRAG agar incubated anaerobically for 48 h

Species	No. of isolates tested[a]	No. with 4+ growth	No. with obvious fermentation (agar yellow)
B. fragilis	33	33	27
B. thetaiotaomicron	10	10	10
B. vulgatus	10	10	10
B. distasonis	10	10	10
B. ovatus	10	10	10
B. uniformis	2	2	2

[a] All organisms produced 4+ growth on nonselective enriched SB agar plates.

Preliminary experiments with FRAG agar demonstrated that the members of the *B. fragilis* group grew when the peptones listed in Table 1 were used alone and in combination, but that growth was limited by the small concentrations present. All species grew to only pinpoint colony size on medium prepared without glucuronic acid. Although the addition of 1.0% glucuronic acid alleviated this limitation, the peptone source was not expendable, as all species grew much more slowly on medium prepared without peptone. In fact, some *B. fragilis* and *B. vulgatus* strains did not grow at all on medium without peptone. The growth of all species was enhanced greatly on medium containing both peptone and glucuronic acid.

As Table 2 shows, all of the strains tested except six strains of *B. fragilis* fermented glucuronic acid, as shown by a change in the color of the medium from orange to yellow within 48 h of incubation. The six strains of *B. fragilis* did ferment glucuronic acid after prolonged incubation. We encountered no problems with reduction of the phenol red indicator.

Of 90 pure cultures of other anaerobes (1 acidaminococcus, 20 *Bacteroides* strains other than members of the *B. fragilis* group, 5 bifidobacteria, 13 clostridia, 5 eubacteria, 11 fusobacteria, 5 lactobacilli, 10 peptococci, 5 peptostreptococci, 5 propionibacteria, 7 streptococci, 3 veillonellae), only 1 *Bacteroides melaninogenicus* strain and one other *Bacteroides* strain grew on FRAG agar. However, these two strains produced only pinpoint colonies and did not ferment glucuronic acid even after 96 h. All 90 anaerobes grew well on the nonselective SB agar plates.

The 90 facultative or aerobic organisms tested included 59 strains of *Enterobacteriaceae*, 5 strains of *Pseudomonas aeruginosa*, 16 streptococci, and 10 staphylococci. Most of the gram-negative rods were inhibited on FRAG agar. Of 12 strains of *Providencia*, 8 (including 4 strains of *Providencia rettgeri*) grew on the selective plates. However, the *P. rettgeri* strains usually yielded only 2+ growth. The other *Providencia* species showed 1+ to 4+ growth on FRAG agar. The colony morphology of the *Providencia* species differed markedly from that of the *B. fragilis* group. Colonies were grey and flat, and no evidence of fermentation was detectable even

after 4 additional days of incubation. One *Citrobacter freundii* strain also grew on FRAG agar, but only four colonies were present. This organism fermented glucuronic acid, but the flat colonial morphology differed from the colonial morphology of the *B. fragilis* group. Four of five coagulase-negative staphylococci and one of five enterococci also grew on FRAG agar, but only as pinpoint colonies (2+ to 4+), and there was no evidence of fermentation. All facultative gram-positive cocci yielded 4+ growth on the nonselective SB agar plates.

Of 100 clinical specimens, 37 yielded no growth on any plates, 35 showed growth only on the routine plates, and 28 produced growth both on FRAG agar and on routine anaerobic plates. A total of 23 specimens yielded strains belonging to the *B. fragilis* group when the routine media were used, whereas 22 were positive on FRAG agar. The one false-negative culture on FRAG agar yielded only two colonies on the routine media. Of the 22 positive *B. fragilis* group cultures on FRAG agar, 15 (68%) gave strong evidence of glucuronic acid fermentation by 48 h. These cultures had the largest numbers of organisms (3+ to 4+ growth) on FRAG agar, whereas organisms causing weak or no color change were present in 1+ or 2+ amounts.

A total of 33 isolates belonging to the *B. fragilis* group were recovered from FRAG agar, whereas 23 were recovered from the routine plates (Table 3). *B. fragilis* was the most frequently isolated species of the *B. fragilis* group. Colony variation on FRAG agar facilitated the isolation of species present in low numbers (1+ to 2+) from specimens containing other *B. fragilis* group species in larger amounts (3+ to 4+). A total of 10 cultures yielded more than one *B. fragilis* group species on FRAG agar. None of the routine plates yielded more than one *B. fragilis* group species. The colony morphologies of the *B. fragilis* group on FRAG agar were identical to the morphologies described above. Figure 1 shows the differentiation of *B. fragilis* and *B. ovatus* on FRAG agar compared with SB agar.

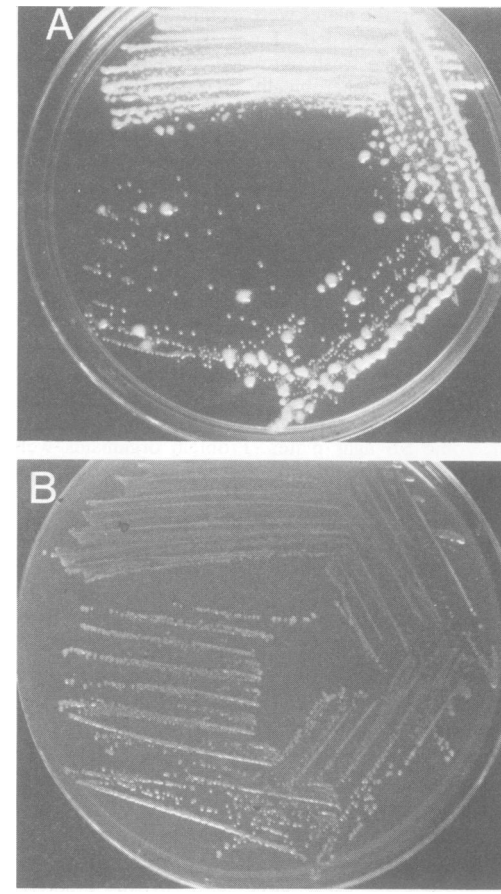

FIG. 1. Mixture of *B. fragilis* and *B. ovatus* on FRAG agar (A) and on fresh SB agar containing hemin and vitamin K (B) after 48 h of anaerobic incubation. On FRAG agar the large colonies were *B. ovatus*, and the smaller colonies were *B. fragilis*. On SB agar the two species look alike.

TABLE 3. Comparison of isolates of the *B. fragilis* group from 100 clinical specimens plated onto FRAG agar and routine anaerobic media

B. fragilis group species[a]	No. of isolates recovered on:	
	FRAG agar	Routine media
B. fragilis	18	17
B. thetaiotaomicron	6	2
B. distasonis	3	1
B. vulgatus	1	1
B. uniformis	2	0
Bacteroides 3425A group	1	0
B. fragilis group[b]	2	2

[a] *B. fragilis* group recovered from 23 specimens. A total of 22 were positive on FRAG agar, and 10 cultures yielded more than one species on FRAG agar. No routine culture yielded more than one species.

[b] We were not able to determine the species accurately. These isolates were not from the same specimens.

A total of 71 anaerobes other than members of the *B. fragilis* group were recovered on the routine media. None of these grew on FRAG agar. These 71 isolates included 18 gram-positive, nonsporeforming bacilli, 24 gram-positive cocci, 11 *Bacteroides*, 31 clostridia, 3 fusobacteria (including 2 *Fusobacterium nucleatum* isolates), and 2 veillonellae.

A total of 110 facultative organisms were recovered from the routine media (Table 4). Of these, only six grew on FRAG agar, including two *Morganella morganii*, two *Proteus mirabi-*

TABLE 4. Comparison of isolation of facultative organisms from 100 clinical specimens plated onto FRAG agar and routine media

Organisms	No. of isolates recovered on:	
	FRAG agar	Routine media
Corynebacteria	0	7
Eikenella corrodens	0	1
Enterobacteriaceae	7[a]	41
Haemophilus influenzae	0	2
P. aeruginosa	0	3
Staphylococci	0	21
Streptococci	0	25
Yeasts	0	10

[a] These isolates included one *C. diversus-levinea*, two *M. morganii*, two *P. mirabilis*, and two *E. coli* (one *E. coli* not recovered from routine media). Only *C. diversus-levinea* and *E. coli* appeared to ferment glucuronic acid.

lis, one *Citrobacter diversus-levinea* (two colonies on FRAG agar; 3+ on routine media), and one *Escherichia coli*. Another *E. coli* isolate was recovered on FRAG agar, but this isolate failed to grow on the routine media. The colony morphologies of the two *Morganella* sp. and two *Proteus* sp. isolates were markedly different from the colony morphologies of the *B. fragilis* group. Colonies were flat, with very irregular edges. One *E. coli* isolate and the *C. diversus-levinea* isolate presumably could have been confused with the *B. fragilis* group since their colonies were greyish-white and mucoid. The other *E. coli* colony was flat and had a fringy edge. These three organisms appeared to ferment glucuronic acid. The *Morganella* and *Proteus* isolates did not ferment glucuronic acid even after 48 additional h of incubation. Six of these isolates were recovered as the only organisms on FRAG agar plates. One *E. coli* isolate was recovered along with *B. distasonis* and *B. uniformis*.

We made a rough assessment of the stability of FRAG agar. We determined that plates stored in room air at room temperature for up to 5 days would still yield adequate growth of clinical isolates of all of the *B. fragilis* group species mentioned above. Furthermore, the agar base stored for up to 10 weeks at room temperature in indirect room light still supported good growth of the *B. fragilis* group when it was melted down and glucuronic acid and gentamicin were added.

DISCUSSION

Our results show both the high degree of selectivity of FRAG agar and the usefulness of colony morphology and glucuronic acid fermentation for the presumptive identification of the *B. fragilis* group. The medium of Salyers et al. (14) was not adequate for the rapid detection of the *B. fragilis* group. We found that it was necessary to include a peptone source to obtain good growth in 48 h. The glucuronic acid concentration was also increased from 0.5 to 1.0%, which further enhanced the growth of all strains of the *B. fragilis* group tested. Some workers have found that some *B. fragilis* group strains require certain amino acids or other growth factors (16, 17). The peptones included in FRAG agar should provide these requirements and thus allow the detection of seemingly more fastidious strains. We did not perform a detailed analysis of the peptones best suited for the *B. fragilis* group. Our original intention was to use a peptone-yeast-Trypticase formulation (7), except at one-fifth the concentrations given by Holdeman et al. We chose Casitone because preliminary experiments showed that it was more effective in promoting the growth of the *B. fragilis* group. Also, Varel and Bryant noted that Casitone stimulated *B. fragilis* growth at concentrations of only 0.2% (17). However, the low total peptone concentration in FRAG agar is insufficient for good growth; both peptone and glucuronic acid are necessary for good growth of all *B. fragilis* group species by 48 h after incubation.

Our results indicate the potential of this medium for the primary isolation of the *B. fragilis* group from clinical specimens. From the studies of Salyers et al. (14, 15) it is apparent that few intestinal anaerobes besides the *B. fragilis* group have the ability to utilize glucuronic acid. By limiting the peptone concentration and forcing the organisms to grow on glucuronic acid as the primary carbon source, this medium may be more selective than the bile esculin medium currently recommended (3) for the isolation of this group.

In the pure culture study, the selectivity of the medium was demonstrated by the fact that the *B. fragilis* group species grew well; with the exception of *C. freundii*, the few other organisms which grew produced only pinpoint colonies. Thus, although such organisms may be gentamicin and bile resistant, their inability to utilize glucuronic acid dramatically restricts their growth. The *Providencia* species which grew could be differentiated easily from the *B. fragilis* group on the basis of colony morphology. All gram-positive organisms could be differentiated easily on the basis of Gram stain reaction, as well as colonial morphology.

The results of the clinical trial support the observations made during the initial studies. The colony variation noted during the initial plating of stock cultures occurred when the clinical specimens were plated onto FRAG agar. Such a medium characteristic allows the isolation of all

B. fragilis group species present in the specimen, especially those present in small numbers. This gives a truer reflection of the clinical picture, allowing a better assessment of the significance of these isolates. Thus, the frequency of the *B. fragilis* group in specimens should be determined more accurately by using FRAG agar. *B. uniformis* and *Bacteroides* 3425A were not isolated on the routine plates. On FRAG agar, it was possible to isolate species present in only 1+ to 2+ amounts along with the more predominant (3+ to 4+) members of the group. Such distinctions could not be made on the routine media used, on which the colony morphologies of all *B. fragilis* group species appeared to be similar. Presumably, the members of the *B. fragilis* group vary in their ability to metabolize glucuronic acid, whereas on a peptone-rich medium they all grow equally well.

FRAG agar was also very useful in the presumptive identification of the *B. fragilis* group. The ability to ferment glucuronic acid seems to be a stable and reproducible characteristic of members of this group. Thus, colony morphology, which is very characteristic, and sugar fermentation should allow accurate presumptive identifications. However, detection of fermentation seems to depend on the number of organisms present. Also, there appears to be variation within the group because *B. thetaiotaomicron*, *B. ovatus*, and *B. distasonis* produced large, mucoid colonies and were highly fermentative, whereas *B. vulgatus* and *B. fragilis* were less active and produced smaller colonies. However, even in the absence of detectable fermentation, colony morphology is still quite useful. We did try the slide catalase test (6) to aid the presumptive identification, but fermentation appeared to interfere with the reaction because cultures that were catalase positive on SB agar were often weak or negative on FRAG agar.

There were no false-positive cultures due to other anaerobes. The growth of *Morganella* or *Proteus* was not surprising because of the known antibiotic resistance patterns of these bacteria. However, colony morphology alone excluded them easily from consideration. Although a rare *E. coli* or *Citrobacter* sp. isolate could be confused with the *B. fragilis* group, a 24-h aerobic SB agar subculture plate could be used to verify any presumptive identification. Thus, the high degree of selectivity both against anaerobes and against facultative organisms should allow microbiologists to make accurate presumptive identifications by 48 h incubation.

Although FRAG agar is a complex medium, it is not difficult to prepare. The mineral solutions can be prepared in large volumes and stored for months at 4°C. The good shelf life of FRAG agar is also an advantageous characteristic. Modifications can also be considered. The addition of vitamin B_{12} may be unnecessary because methionine can satisfy this requirement (17). Presumably, enough methionine is present in the peptones used to overcome the requirement for added vitamin B_{12}. A more detailed analysis of the peptones included could allow modifications in the amounts used or in the actual sources included. The incorporation of esculin also could be studied as a further identification characteristic. However, without any modification FRAG agar is a useful selective medium for the isolation and identification of members of the *B. fragilis* group from clinical specimens.

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