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NOTES

Evaluation of Kanamycin-Esculin Bile Agar for Isolation and Presumptive Identification of *Bacteroides fragilis* Group

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A kanamycin-esculin bile medium was useful for selective isolation and presumptive identification (24 h) of the *Bacteroides fragilis* group.

Some of the most clinically significant anaerobic bacteria are the members of *Bacteroides fragilis* group (*B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron,* and *B. vulgatus*). They are not only commonly implicated in human disease but are usually resistant to several antibiotics, e.g., penicillin and tetracycline. Consequently, significant efforts have been directed at simple and rapid methods for identifying the *B. fragilis* group (1, 5, 6). Most of the methods have exploited resistance of the organisms to high concentrations of kanamycin and ability to grow well with 20% bile present. To further simplify laboratory procedures, we have incorporated the above properties into a selective agar medium and added esculin hydrolysis reaction as a differential indicator.

The composition of the medium (KEB) was as follows: 1,000 μg of kanamycin (added after autoclaving; Bristol Laboratories, Syracuse, N.Y.) per ml, 0.5% esculin, 0.05% ferric ammonium citrate, and 20% bile (2% Oxgall) in Trypticase soy agar (Becton, Dickinson & Co., Rutherford, N.J.). Stock cultures of anaerobes and kanamycin-resistant aerobes (no zone of inhibition by disk diffusion antimicrobial susceptibility tests) were tested in the first phase of the study. During the second phase, we evaluated the medium by using clinical specimens submitted for anaerobic culture. Anaerobic studies on stock cultures and clinical cultures of blood and other body fluids were performed in a chamber (Coy Manufacturing, Ann Arbor, Mich.). Anaerobic cultures of other clinical material were performed in GasPak (BBL) jars. Routine isolation media included brucella-menadione blood agar, kanamycin-vancomycin-laked blood agar, and thioglycolate supplemented with hemin (5 μg/ml) and vitamin K_1 (0.1 μg/ml) (Cal Laboratories, Los Angeles, Calif.). Anaerobic identification was performed by a simplified system (3) supported, when necessary, by other systems (2, 4).

The results obtained with stock cultures grown on KEB are shown in Table 1. After 24 h all of the *B. fragilis* group (111 strains) grew on the medium, and 107 hydrolyzed esculin, as indicated by blackening of the medium. Two additional strains hydrolyzed esculin after 3 days. No other anaerobes tested grew on the medium. Four of 42 strains of kanamycin-resistant aerobes grew on the medium, and they all hydrolyzed esculin. Three of the four were enterococci.

Clinical specimens were also used to evaluate KEB. Table 2 lists the organisms recovered on the medium. Sixty-four isolates of the *B. fragilis* group were recovered from 193 specimens. Specimens observed after 24 h accounted for 22 isolates and all hydrolyzed esculin. Specimens observed initially after 48 h revealed 34 isolates, and only two failed to hydrolyze esculin. Eight additional isolates (all hydrolyzed esculin) were detected in specimens observed after 72 h.

All clinical isolates were recovered on KEB as well as routine anaerobic media. No other anaerobes grew on KEB. Fourteen facultative anaerobes were recovered; however, only four hydrolyzed esculin. In addition, the colonial morphology and odor of the facultative anaerobes usually did not resemble members of the *B. fragilis* group.

The results of the investigation showed KEB to be a useful medium for recovery of the *B. fragilis* group. Its selectivity inhibited other anaerobes as well as most facultative anaerobes. The ability of the *B. fragilis* group to hydrolyze esculin helped in its presumptive identification. It is recommended that KEB plates be reincubated when esculin reactions are negative after 24 h.

TABLE 1. *Results of stock cultures tested on KEB*[a]

Organism	No. tested	Growth	Esculin hydrolysis
Anaerobes			
Bacteroides fragilis	69	69	67 (2)
B. thetaiotaomicron	34	34	33
B. distasonis	3	3	3
B. vulgatus	4	4	4
B. ovatus	1	1	1
Bacteroides species	20	0	0
Fusobacterium nucleatum	10	0	0
Fusobacterium species	8	0	0
Clostridium perfringens	2	0	0
Clostridium species	6	0	0
Eubacterium species	3	0	0
Aerobes (kanamycin resistant)			
Proteus species	6	0	0
Escherichia coli	1	0	0
Serratia marcescens	1	0	0
Klebsiella pneumoniae	2	1	1
Miscellaneous enterics	9	0	0
Pseudomonas aeruginosa	13	0	0
Gram-negative nonfermenters	3	0	0
Enterococci	7	3	3

[a] Results indicate 24-h readings, and parentheses indicate a delayed-positive reading after 3 days.

TABLE 2. *Organisms isolated on KEB from anaerobically cultured clinical specimens*[a]

Organism	Growth[b]	Esculin hydrolysis
Anaerobes		
Bacteroides fragilis	38	37
B. thetaiotaomicron	11	11
B. distasonis	10	10
B. vulgatus	3	2
B. ovatus	1	1
B. fragilis group (unclassified)	1	1
Aerobes		
Yeast	10	3
Proteus mirabilis	2	0
Escherichia coli	2	1

[a] In this phase of the study, growth and esculin hydrolysis were usually measured after 24 h; but, occasionally, the results indicate 48- or 72-h determinations only.

[b] All clinical isolates were recovered on KEB as well as routine anaerobic media.

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