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Methylumbelliferyl-β-D-Glucuronide-Based Medium for Rapid Isolation and Identification of *Escherichia coli*

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Escherichia coli is the most common gram-negative microbe isolated and identified in clinical microbiology laboratories. It can be identified within 1 h by oxidase, indole, lactose, and beta-glucuronidase tests. The oxidase and indole tests are performed as spot tests, and lactose fermentation is read directly from MacConkey agar. It was found that 4-methylumbelliferyl-β-D-glucuronide could be incorporated directly into a modified MacConkey agar to directly detect the presence of beta-glucuronidase. Other characteristics of MacConkey agar were not affected. The incorporation of 4-methylumbelliferyl-β-D-glucuronide into modified agar obviated the need for manufacture, quality control, and incubation of reagent-containing test tubes. The time needed to identify *E. coli* strains was reduced from 1 h to 5 min, and the ability to detect this species in mixed specimens was also enhanced.

Escherichia coli is the most common gram-negative microbe isolated and identified in clinical microbiology laboratories. This laboratory previously reported a method for identifying *E. coli* strains within 1 h by oxidase, indole, lactose, and beta-glucuronidase tests (5, 6). The procedure was 99.9% specific and 93% sensitive. The oxidase and indole reactions required less than 1 min each, whereas the beta-glucuronidase test required up to 1 h of incubation and was the rate-limiting factor in identifying this species. The rapid identification of *E. coli* served as the pivotal point in a critical pathway for identifying strains of *Enterobacteriaceae* in 4 h and *Pseudomonas aeruginosa* and group D streptococci in 2 h.

The glucuronidase substrate used was *p*-nitrophenyl-β-D-glucuronide (PNBG). Methylumbelliferyl reagents function similarly to *p*-nitrophenyl and *o*-nitrophenyl reagents. When an organism elaborates a specific enzyme, the indicator moiety is cleaved and released. With the methylumbelliferyl substrate, the parent compound is colorless and the cleaved moiety is fluorescent when observed at 366 nm (2). If the methylumbelliferyl reagent was interchangeable with the PNBG reagent, an opportunity existed for substitution. The fluorogenic reagent, if functional when incorporated in primary isolation medium, could reduce the time required for *E. coli* identification and eliminate the need for beta-glucuronidase tubes.

To accomplish both these goals, methylumbelliferyl reagent was incorporated into a modified MacConkey agar. Hydrolysis of the methylumbelliferyl reagent could be determined directly by using a hand-held 366-nm light or by placing the plate on a long-wave UV lightbox. The laboratory would have available to it from primary isolation media the lactose, oxidase, and indole tests, plus a direct determination of beta-glucuronidase activity.

MATERIALS AND METHODS

Clinical isolates. All microbes were isolated from clinical specimens obtained from the Bacteriology Section of the Clinical Microbiology Laboratory, Yale-New Haven Hospital. Methylumbelliferyl-β-D-glucuronide medium (FGM) was tested both with pure cultures and clinical specimens. Tables 1 and 2 show the distribution of pure cultures and clinical specimen types tested on FGM.

Pure cultures were identified by methods in common use (1, 4, 7) and stored on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) slants under sterile mineral oil. Subsequently, stock cultures were streaked for single colony isolation on the test agars. Clinical specimens were plated for single colony isolation on FGM, in parallel on MacConkey agar, and on other primary plating media that were part of the normal laboratory protocol. After 18 to 24 h of incubation on FGM, fluorescent colonies were identified to species level.

Media. FGM was prepared by adding to 1 liter of sterile distilled water at 37°C (in the following order): 2 drops of Pourite (Analytic Products, Belmont, Calif.), 17.0 g of pancreatic digest of gelatin, 1.5 g of pancreatic digest of casein, 1.5 g of Difco Proteose Peptone no. 3, 10 g of lactose, 1.5 g of bile salts, 5 g of sodium chloride, 0.03 g of neutral red, 0.001 g of crystal violet, 13.5 g of agar (all these ingredients were obtained in generic form from BBL Microbiology Systems, Difco Laboratories, Detroit, Mich., or GIBCO Diagnostics, Madison, Wis.), and 150 mg of 4-methylumbelliferyl-β-D-glucuronide (Sigma Chemical Co., St. Louis, Mo.), final pH 7.2. The medium was mixed for 30 min at room temperature to dissolve all ingredients and sterilized under flowing steam for 20 min. The medium was agitated and poured into 100-mm-diameter culture plates at 60°C, 20 ml per plate. Plates were stored in plastic bags at 2 to 8°C until used. MacConkey medium was MacConkey II agar (BBL Microbiology Systems), obtained in prepoured form. This medium substituted peptic digest of animal tissue, 1.5 g/liter, for the Proteose Peptone no. 3 used in the FGM. According to the manufacturer, individual constituents in MacConkey II agar may be adjusted or supplemented as required to meet performance criteria.

Specimen processing. Clinical specimens were processed normally (4) and with an FGM plate as well as on MacConkey agar. On both FGM and MacConkey agar, lactose fermentation was determined by the presence of pink to dark pink colonies. Beta-glucuronidase production on FGM was determined by examining the plate under a 366-nm light. Beta-glucuronidase-positive colonies were strongly blue-

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green fluorescent, whereas beta-glucuronidase-negative colonies were nonfluorescent.

The rapid identification of *E. coli* and processing of *Enterobacteriaceae* species followed the previously described protocol (6). Briefly, colonies were first examined for lactose fermentation (direct examination on MacConkey agar) and production of beta-glucuronidase (colony observation under 366-nm light). The oxidase and indole tests were performed as spot tests from a companion blood agar plate. The oxidase test was done by wetting a piece of filter paper with *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride reagent (Marion Scientific, Kansas City, Mo.) and allowing it to dry. Colonies were rubbed onto the filter paper and were considered positive if they turned blue-purple within 10 s. The indole test was also a spot test, done with filter paper saturated with Kovacs reagent. Colonies were considered positive if, after being rubbed onto the filter paper, they turned red within 10 s. β-Glucuronidase activity on FGM was compared with positive results for the tube test reagent PNBG, made as previously described (6).

E. coli strains were identified as lactose fermenting, beta-glucuronidase and indole positive, and oxidase negative. Other *Enterobacteriaceae*, and any *E. coli* strains not exhibiting this typical pattern were identified within 4 h by the Micro-ID (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.), and other pathogens were identified by a preestablished series of rapid conventional tests (6).

RESULTS

The beta-glucuronidase-producing organisms were distinctively fluorescent at 366 nm, whereas the beta-glucuronidase-negative microbes were nonfluorescent. Results for beta-glucosidase production could be read directly from FGM cultures as accurately as from the PNBG test (Tables 1 and 2).

FGM proved to be sensitive in elucidating beta-glucuronidase-positive microbes directly from clinical specimens. Compared with MacConkey agar, FGM showed enhanced recovery of *E. coli* organisms (Table 2). FGM proved especially useful in establishing the presence of *E. coli* mixed with other pathogens.

The use of FGM decreased the time required to identify *E. coli* from 1 h to 5 min. The lactose test was read visually and recorded in 30 s, the beta-glucuronidase test was read under 366-nm light and recorded in 1 min, the spot oxidase test was performed and recorded in 1 min, and the spot indole test was performed and recorded in 1.5 min. The *E. coli* strains that were either beta-glucuronidase- or indole-negative (7% of strains tested) were transferred to the 4-h *Enterobacteriaceae* identification pathway (1.0 min). Because the 1-h incubation at 37°C for the PNBG test was not required, all enteric bacteria were identified within 4 h after examination of primary media with no loss of the substantial cost advantages inherent in the critical pathway.

TABLE 1. Detection of β-glucuronidase production and lactose fermentation in pure cultures on FGM

Organism (no. tested)		No. positive for test on indicated medium			
		β-Glucuronidase		Lactose fermentation	
		FGM	PNBG	FGM	MacConkey agar
Escherichia coli	(75)	72	72	63	63
Klebsiella pneumoniae	(50)	0	0	50	50
Serratia marcescens	(10)	0	0	10	10
Enterobacter aerogenes	(10)	0	0	10	10
Enterobacter cloacae	(10)	0	0	10	10
Citrobacter diversus	(10)	0	0	10	10
Citrobacter freundii	(15)	0	0	15	15
Proteus mirabilis	(20)	0	0	0	0
Proteus vulgaris	(3)	0	0	0	0
Morganella morganii	(10)	0	0	0	0
Shigella spp.	(20)	6	6	2	2
Salmonella spp.	(20)	3	3	0	0
Yersinia enterocolitica	(5)	0	0	0	0

TABLE 2. Elucidation of *E. coli* strains in clinical specimens on FGM and MacConkey agar

Specimen (no. tested)		No. positive for *E. coli* on:	
		FGM	MacConkey agar
Throat	(25)	7	5
Nasopharyngeal	(15)	5	3
Sputum	(45)	12	9
Deep wound	(20)	2	2
Superficial wound	(20)	2	2
Stool	(20)	20	19
Cerebrospinal fluid	(15)	1	1
Urine	(100)	82	77

DISCUSSION

It has been shown that *E. coli* strains can be identified within 1 h in the clinical laboratory from primary plating media by using results from four tests: positive for lactose fermentation, beta-glucosidase, and indole and negative for oxidase. To initiate processing, the MacConkey plate is observed for lactose production, spot indole and oxidase tests are performed, and the 1-h PNBG test is done. After 1 h of incubation, organisms that were PNBG positive and met the other criteria were identified as *E. coli*. The rapid identification of *E. coli* was the first step in a critical pathway that automatically guided the workflow to higher levels of complexity when required (6).

A rate-limiting step in the critical pathway was the requirement for the 1-h incubation of the beta-glucuronidase reagent. It has been shown that umbelliferyl reagents can behave similarly to *ortho*-nitrophenyl and *para*-nitrophenyl substrates (9, 11). 4-Methylumbelliferyl-β-D-glucuronide has been used successfully in a liquid medium as a screening test reagent to assist in establishing the presumptive presence of *E. coli* in environmental samples (2, 8). Umbelliferyl reagents have also been found to be active in agar-based systems (3, 9). The incorporation of 4-methylumbelliferyl-β-D-glucuronide directly into a modified MacConkey agar obviated the need for manufacture, quality control, and incubation of PNBG tubes. Furthermore, the time required to identify *E. coli* strains was reduced from 1 h to 5 min. There was no change in the selectivity or lactose fermentation characteristics of MacConkey agar when the umbelliferyl reagent was incorporated.

It has been reported that approximately 40% of *Shigella* species and various bioserotypes of *Salmonella* may also be beta-glucuronidase positive (9–11). As described for the PNBG test, the possible misidentification of one of these species as *E. coli* in this schema was not a practical problem. Theoretically, 8% of *Shigella* strains may share the same phenotypic test pattern as *E. coli* strains in this matrix. However, for the test isolates studied in this series, the nature of the colony and the intensity of lactose fermentation differentiated between these species. It would be prudent,

especially when processing stool specimens, to group serologically the organisms having the phenotypic test pattern and colonial morphology of *Shigella* spp.

FGM permitted the routine identification of *E. coli* strains from clinical laboratory specimens directly from primary plates in 5 min. The ability to establish the presence or absence of beta-glucuronidase immediately on the primary plate eliminated the need for accessory biochemical test tubes to identify *E. coli* and other *Enterobacteriaceae* strains. Because beta-glucuronidase activity could be detected directly from the plate, the ability to elucidate *E. coli* in clinical specimens was enhanced. The addition of 4-methylumbelliferyl-β-D-glucuronide to the medium did not change its characteristics of selectivity or lactose fermentation.

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