Rapid Identification of *Escherichia coli* by Fluorocult Media and Positive Indole Reaction

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To assess the specificity and sensitivity of Fluorocult media for the identification of *Escherichia coli*, the β -glucuronidase activities of 1,258 bacterial strains, as well as 20 strains of *Candida* spp., were investigated. Fluorescence of colonies combined with positive indole reaction resulted in specificities of 99.6 to 99.8%. Sensitivities were 59.1% (MacConkey agar), 69.9% (brolacin agar), 85.5% (Columbia agar), and 85.8% (ECD agar).

Escherichia coli may be the causative agent of various infections such as septicemia; meningitis in the newborn; enteritis by enteropathogenic, enterotoxigenic, enteroinvasive, or enterohaemorrhagic strains; pyelonephritis; and urinary tract infections (9). In nosocomial infections, *E. coli* is the leading pathogen (1, 10). In 1976, Kilian and Bülow (6) described the activity of a β -glucuronidase as restricted to species belonging to the genera *Escherichia*, *Salmonella*, and *Shigella*. This property was used first by Feng and Hartman (3) to identify *E. coli* isolated from water, whereas Hansen and Yourassowsky (4) investigated isolates derived from urine cultures. In 1984, Trepeta and Edberg (11) incorporated 4-methylumbelliferyl- β -D-glucuronide (MUG) as a fluorogenic substrate in agar plates. Thus, colonies of *E. coli* may easily be detected under UV light (366 nm) by the blue fluorescence of 4-methylumbelliferon. MUG media (Fluorocult; E. Merck AG, Darmstadt, Federal Republic of Germany) having been made commercially available, we wanted to assess their specificity and sensitivity. The four different media investigated were brolacin agar (CLED agar), ECD agar (selective for gram-negative bacteria), MacConkey agar, and Columbia agar supplemented with 5% sheep blood. Each of the media was supplemented with MUG (brolacin and MacConkey agar, 0.1 g of MUG per liter; ECD and Columbia agar, 0.07 g of MUG per liter). Tryptophan was added to the latter two media at a concentration of 1 g/liter.

The 1,258 strains studied (all subcultured from clinical isolates, mainly respiratory tract and wound specimens) included 838 strains of members of the family *Enterobacteriaceae*, identified by the method of Farmer et al. (2); 190 strains of gram-negative rods not members of the *Enterobacteriaceae*, identified by API 20NE (API BioMérieux, Montalieu-Vercieu, France); 170 strains of gram-positive cocci; 40 strains of *Bacteroides* spp.; and 20 strains of *Candida* spp. (Table 1). Indole production was detected by a spot indole test (Hoffmann-La Roche Inc., Basel, Switzerland). All strains were subcultivated on blood agar plates for 20 h, and colonies were suspended in saline (10^6 CFU/ml). Inoculation of Fluorocult media was done by means of a replicator system (10 μ l per spot), and afterwards the media were

incubated for 18 to 20 h at 35°C. Fluorescence was visualized at 366 nm in a dark chamber.

For all strains investigated, the specificity of Fluorocult Columbia agar was 98.9%, depending on the fluorescence of MUG alone. Fluorescence and a positive indole reaction led to a specificity of 99.8%.

In 9 of the 23 *Pseudomonas aeruginosa* strains, a typical green fluorescence appeared, clearly different from the blue fluorescence produced by *E. coli*. On Columbia agar, one *Acinetobacter* strain was β -glucuronidase positive but indole negative. Of the gram-negative rods not belonging to the family *Enterobacteriaceae*, 46 did not grow on ECD agar and 37 did not grow on MacConkey agar. None of the 40 *Bacteroides* spp. strains growing exclusively on Columbia agar supplemented with 5% sheep blood was β -glucuronidase positive. *Enterococcus faecalis*, as well as *Staphylococcus aureus*, strains also showed a negative fluorescence. On the other hand, 8 out of 50 strains of coagulase-negative staphylococci revealed the typical fluorescence, and 1 strain of group A beta-hemolytic streptococci as well as 2 strains of group B beta-hemolytic streptococci were also positive. Strains of gram-positive cocci grew only on brolacin and Columbia agar. *Candida* spp. were always β -glucuronidase negative and showed satisfactory growth only on Columbia agar.

Only 28 of the 296 strains of *E. coli* investigated had no β -glucuronidase activity on any of the four media. A sensitivity of 100%, or at least near 100%, of MUG media could not be expected, because only 94 to 97% of the *E. coli* strains were β -glucuronidase positive (4, 6). All strains, including *E. coli* inactive strains, were positive in the indole spot test. False-positive results of members of the *Enterobacteriaceae* were due to one strain of *Klebsiella oxytoca* (positive only on Columbia agar, indole spot test positive), *Serratia marcescens* (fluorescence on all media, indole spot test negative), and *Morganella morganii* (positive on ECD agar only, indole spot test positive). Fluorescence combined with positive indole reaction resulted in specificities of 99.2% (ECD and Columbia agar) and 99.8% (brolacin and MacConkey agar). Depending on the medium, sensitivities were 59.1% (MacConkey agar), 68.9% (brolacin agar), 85.5% (Columbia agar), and 85.8% (ECD agar). It was obvious that sensitivity was lower in media containing lactose and a pH-indicator system. This was due to the low pH and the color change

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TABLE 1. Survey of the species investigated

Species	No. of strains	Species	No. of strains
<i>Escherichia coli</i>	296	<i>Pseudomonas aeruginosa</i>	23
<i>Escherichia fergusonii</i>	1	<i>Pseudomonas maltophilia</i>	21
<i>Klebsiella pneumoniae</i>	83	<i>Pseudomonas paucimobilis</i>	1
<i>Klebsiella oxytoca</i>	101	<i>Pseudomonas testosteroni</i>	1
<i>Klebsiella planticola</i>	8	<i>Pseudomonas luteola</i>	1
<i>Klebsiella terrigena</i>	1	<i>Pseudomonas pseudoalcaligenes</i>	9
<i>Klebsiella ozaenae</i>	1	<i>Pseudomonas stutzeri</i>	8
<i>Salmonella</i> spp.....	51	<i>Pseudomonas oryzae</i>	4
<i>Shigella</i> sp.....	1	<i>Pseudomonas putrefaciens</i>	1
Enteric group 17.....	7	<i>Pseudomonas putida</i>	7
<i>Citrobacter freundii</i>	20	<i>Pseudomonas diminuta</i>	3
<i>Citrobacter diversus</i>	7	<i>Pseudomonas fluorescens</i>	7
<i>Enterobacter cloacae</i>	132	<i>Pseudomonas acidovorans</i>	2
<i>Enterobacter agglomerans</i>	15	<i>Pseudomonas cepacia</i>	2
<i>Enterobacter amnigenus</i>	4	<i>Acinetobacter lwoffii</i>	47
<i>Enterobacter aerogenes</i>	9	<i>Acinetobacter anitratus</i>	24
<i>Providencia stuartii</i>	5	<i>Pasteurella</i> spp.....	3
<i>Providencia rettgeri</i>	1	<i>Aeromonas hydrophila</i>	6
<i>Providencia alcalifaciens</i>	1	<i>Flavobacterium meningosepticum</i>	12
<i>Serratia marcescens</i>	34	<i>Alcaligenes</i> spp.....	2
<i>Serratia liquefaciens</i>	5	<i>Moraxella</i> spp.....	6
<i>Serratia rubidaea</i>	1	<i>Enterococcus faecalis</i>	50
<i>Serratia fonticola</i>	1	<i>Staphylococcus aureus</i>	50
<i>Morganella morganii</i>	20	Coagulase-negative staphylococci.....	50
<i>Hafnia</i> spp.....	3	Beta-hemolytic streptococci group A.....	10
<i>Rahnella aquatilis</i>	2	Beta-hemolytic streptococci group B.....	10
<i>Kluyvera cryocrescens</i>	1	<i>Bacteroides fragilis</i>	20
<i>Proteus mirabilis</i>	19	<i>Bacteroides thetaiotaomicron</i>	20
<i>Proteus vulgaris</i>	7	<i>Candida</i> spp.....	20
<i>Proteus penneri</i>	1		

both suppressing fluorescence (1). We further identified the *E. coli* strains by API 20E, ATB32GN, Cobas Bact, and Enterotube II, with specificities of 98.6, 95.6, 99.2, and 99.3%, respectively (data not published), the specificity of the MUG media being greater than or equal to those of the commercially available systems. Positive predictive values ranged from 99.2 to 99.5%, whereas the negative predictive values were 92.5% (ECD and Columbia agar), 85.5% (brolacin agar), and 81.7% (MacConkey agar).

Two other species of the genus *Escherichia*, *Escherichia hermannii* and *Escherichia fergusonii*, are both indole positive; a third species, *Escherichia vulneris*, lacks tryptophanase activity (2). However, nothing is known about β -glucuronidase activity in these species. Since the incidences of these species are very low in clinical specimens (2), species identification within the genus *Escherichia* may be important in special investigations only. In the meantime, we isolated one strain of *E. hermannii* (identification by API 20E) from human feces. This strain was indole positive but on brolacin agar fluorescence was negative (pH adjusted to neutral).

Besides *E. coli*, about half of the *Shigella* spp. serogroups A, B, and C are indole positive (2), and another 50% of these strains are β -glucuronidase positive (6). This fact implies a probability of misidentification of 25% by Fluorocult media including the indole test, the premise being that both markers occur independently of each other. Ninety-eight percent of the *Shigella sonnei* strains are β -glucuronidase positive but indole negative; therefore, misidentification should not be a problem. About 30% of *Salmonella* spp. reveal β -glucuronidase activity (8) and 1 to 2% exhibit tryptophanase activity (2), resulting in 0.03 to 0.06% false identifications.

Consequently, Fluorocult media should not be used in routine testing of feces but may be very helpful in epidemiological investigations of the incidences of enteropathogenic *E. coli* serovars.

Identification of *E. coli* by using a fluorogenic substrate has many advantages: excellent specificity, satisfactory to good sensitivity, reduction of work load especially in busy laboratories, and, in combination with computer-assisted diagnosis and automated susceptibility-testing methods (5), rapid reporting of microbiological results to clinicians. Moreover, MUG Columbia agar may be able to be used for the identification of *Staphylococcus* spp. on the basis of the reactions given by Kloos and Jorgensen (7).

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