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Rapid Detection of *Escherichia coli* in Urine Samples by a New Chromogenic β-Glucuronidase Assay

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A new compound, indoxyl-β-D-glucuronide, was assessed as a substrate for the rapid detection of *Escherichia coli* in urine. Incorporation of this compound into MacConkey agar allowed the direct differentiation of *E. coli* as deep blue colonies distinct from lactose and nonlactose fermenters. The sensitivity was 88 to 90%, and the specificity was 100%.

Escherichia coli is the most common gram-negative bacterium isolated in clinical laboratories and is the organism responsible for up to 70 to 95% of urinary tract infections (4, 11). Rapid, sensitive, and specific identification of *E. coli* not only is required in clinical laboratories but also is a priority in monitoring sanitation and the microbiological quality of food and water.

Recently, a variety of tests have been developed to specifically identify *E. coli* based on the observation by Kilian and Bulow (6) that *E. coli* is one of the few bacteria that produce the enzyme β-glucuronidase. Currently, tests to measure β-glucuronidase activity include the liberation of yellow *p*-nitrophenol following hydrolysis of *p*-nitrophenyl-β-D-glucuronide (7) or the fluorogenic recognition of methylumbelliferone (5) following hydrolysis of 4-methylumbelliferyl-β-D-glucuronide (MUG). Problems occur with both of these substrates, as *p*-nitrophenol diffuses into the agar during bacterial growth (7, 10), while MUG requires fluorescent light for visualization of colonies on agar or growth in broth.

Recently, Ley et al. (10) synthesized indoxyl-β-D-glucuronide (IBDG), which is split by the β-glucuronidase of *E. coli* to insoluble indigo. Their studies on raw sewage indicated a considerable potential for the IBDG identification of *E. coli* in environmental samples. We extended this observation to establish whether IBDG could be used as a differential medium for the rapid identification of *E. coli* in urine.

MacConkey agar (BBL Microbiology Systems) was supplemented with 0.8 g of IBDG per liter (MAC-IBDG). Urine specimens from patients in the Hotel Dieu Hospital, Kingston, Ontario, Canada, were used to inoculate MAC-IBDG plates with a 0.01-ml calibrated loop. Deep blue colonies produced after 18 h at 35°C were scored as positive for *E. coli*. Figure 1 shows the ease in differentiating lactose fermenters, nonlactose fermenters, and IBDG-positive *E. coli* colonies. The colonies were distinct, as diffusion of indigo did not occur. The results of the direct screening of urine samples are shown in Table 1. No false-positive reactions were noted. (API 20E [Analytab Products] and Fox panels [Beckman Instruments, Inc.] were used to identify the isolates.) Of 152 gram-negative organisms screened, 99 were *E. coli*. Eighty-three of these were positive for IBDG hydrolysis. Of the 16 false-negative strains (10.5%), 6 were slow (>18 h) hydrolyzers. The sensitivity and specificity of the MAC-IBDG plate identification directly from urine were 88.8 and 100%, respectively.

A further 198 organisms isolated from multiple body sites were inoculated onto MAC-IBDG plates with a Cathra replicator. Organisms were identified by replica plating biochemical agents (1) and by the API 20E. A total of 76 *E. coli* and 122 *Enterobacteriaceae* and *Pseudomonas* strains were tested (Table 1). No false-positive reactions were seen. Of the eight false-negative strains (4.0%), one was *E. coli* O157:H7. The sensitivity and specificity of this test were 90.4 and 100%, respectively.

Incorporation of IBDG into agar provides an inexpensive, stable, and direct visualization of *E. coli* as dark blue colonies. There were no false-positive reactions for 350 clinical isolates tested. The test proved useful for both direct screening of urine and replica plate technology. Reports of β-glucuronidase activity in *E. coli* with fluorescent (MUG) or chromogenic (*p*-nitrophenyl-β-D-glucuronide) substrates range from 85 to 97% (5–7, 12). Our study results are comparable. False-negative reactions do not represent a problem in these clinical settings, as all IBDG-negative organisms are routinely identified if present in sufficient numbers. Although many *Shigella* and *Salmonella* species known to be β-glucuronidase positive (9) were not tested during this preliminary study, these organisms are rarely found in urine (7) and could be excluded by spot indole and *o*-nitrophenyl-β-D-galactopyranoside tests. It was of interest to note that *E. coli* O157:H7 did not hydrolyze IBDG, as

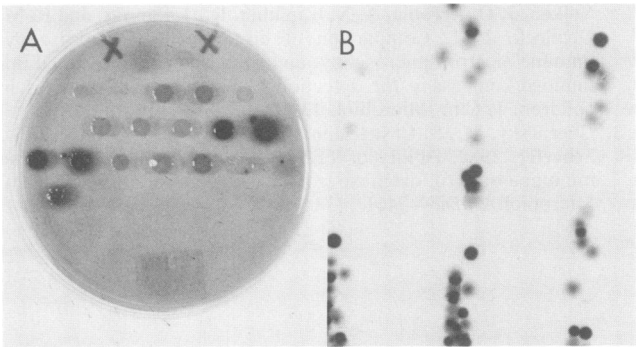

FIG. 1. MAC-IBDG plates inoculated by a calibrated-loop streak method (B) or with a Cathra replicator (A). β-Glucuronidase-positive organisms are distinguished from lactose fermenters and nonlactose fermenters as deep blue (black) colonies.

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TABLE 1. β-Glucuronidase activity on MAC-IBDG plates

Organism	No. tested (n = 350)	No. IBDG positive[a]
E. coli		
Cathra replicator	76	68
Calibrated loop	99	83
Pseudomonas aeruginosa	39	0
Pseudomonas maltophilia	5	0
Pseudomonas cepacia	1	0
Providencia rettgeri	4	0
Providencia stuartii	1	0
Proteus mirabilis	5	0
Proteus vulgaris	1	0
Proteus sp.	1	0
Alcaligenes spp.	2	0
Enterobacter cloacae	1	0
Enterobacter aerogenes	21	0
Klebsiella pneumoniae	23	0
Klebsiella oxytoca	14	0
Serratia spp.	6	0
Serratia marcescens	1	0
Hafnia alvei	8	0
Acinetobacter spp.	2	0
Morganella morganii	5	0
Moraxella sp.	1	0
Salmonella sp.	1	0
Citrobacter spp.	4	0

[a] Deep blue colonies were recorded as positive.

both Krishnan et al. (8) and Doyle et al. (2) have noted with *E. coli* O157:H7 in MUG agar.

A number of investigators have noted the difficulty of incorporating MUG into agar. Krishnan et al. (8) found that diffusion of fluorescence occurred rapidly and that plates had to be read within 12 to 16 h even with the addition of plate dividers. Similarly, Kilian and Bulow (7) and Ley et al. (10) found that *p*-nitrophenyl-β-D-glucuronide agar also resulted in extensive diffusion of the product from the colony. In contrast, IBDG plates are stable, and no diffusion occurs, as the indigo dye is insoluble. In addition, indigo production does not alter the viability of the colonies, so colonies may be picked directly for further sensitivity testing.

Rapid tests for *E. coli* identification rely on the identification of β-glucuronidase from colonies following isolation in pure cultures (3). The advantage of the MAC-IBDG system is the immediate visualization and detection of *E. coli* on primary plates. The IBDG substrate would also be ideal for dip slides, for automated detection in panels, and perhaps as a dip-stick approach in conjunction with urine screening to detect leukocyte esterase. Further investigations are in progress.

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