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Performance of Four Chromogenic Urine Culture Media after One or Two Days of Incubation Compared with Reference Media

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Four chromogenic urine culture media were compared to culture on blood agar, MacConkey agar, and CLED (cysteine-, lactose-, and electrolyte-deficient) agar for detection of uropathogens in 1,200 urine specimens. After 2 nights of incubation, 96% of all isolates were recovered on blood agar, 96% were recovered on CLED agar, 92% were recovered on CPS ID2, 96% were recovered on CHROMagar Orientation from BBL, 95% were recovered on CHROMagar Orientation from The CHROMagar Company, and 95% were recovered on Chromogenic UTI Medium.

Chromogenic media have been compared to traditional urine culture media, e.g., blood agar and MacConkey agar, and been found to be at least as good as traditional media for the isolation of uropathogens (7, 9, 12, 13). In one study, no significant differences between the isolation rates of different chromogenic media were found (2).

Three articles reported the effect of incubation time on results of urine culture on traditional media (3, 8, 11). All agree that common uropathogens can be detected after overnight incubation and that a longer incubation time is required for the detection of yeasts.

In this study, we evaluated four different chromogenic agars (CHROMagar Orientation from BBL [Franklin Lakes, N.J.], CHROMagar Orientation from The CHROMagar Company [Paris, France], Chromogenic UTI Medium [Oxoid, Basingstoke, United Kingdom], and CPS ID2 [bioMérieux, Marcy l'Etoile, France]) for routine diagnosis of bacteriuria at two clinical microbiological laboratories with respect to isolation frequency and presumptive identification of uropathogens. Blood agar, CLED (cysteine-, lactose-, and electrolyte-deficient) agar, and MacConkey agar were used as the reference media. We also compared overnight incubation to 2 nights of incubation; to our knowledge, this has not been done previously.

The media evaluated are listed in Table 1. Blood agar, CLED agar, MacConkey agar, CHROMagar Orientation from The CHROMagar Company, and Chromogenic UTI Medium were prepared at the respective laboratories in accordance with the manufacturers' recommendations.

CPS ID2 and CHROMagar Orientation from BBL were received as ready-made agar plates. Media prepared at the laboratories were tested for contamination by incubation of one plate from each batch at room temperature and one plate in ambient atmosphere at 36°C for 2 days.

Each batch was also tested for typical colony appearance and growth with *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, and *Proteus mirabilis* ATCC 29245.

The media were compared by using quantitative culture with test strains. In this comparison, 10-μl volumes of serial dilutions (starting from approximately 10^8 CFU/ml) of each test strain (*E. coli* ATCC 25922, *E. faecalis* ATCC 29212, and *S. saprophyticus* ATCC 15305) were inoculated onto each medium with a calibrated pipette (Finnpipette; Labsystems). Colonies were counted after 1 and 2 days of incubation in ambient atmosphere at 36°C.

The media were also evaluated by using urine specimens sent in for routine culture. The first 50 urine specimens that arrived every day at each laboratory were included in the study. A total of 1,200 urine specimens were inoculated on all of the media tested. The laboratories serve both tertiary care hospitals and primary care centers. Most of the specimens included in the study were from inpatients, as the specimens collected at the hospitals arrive at the laboratories earlier than specimens from primary care centers.

All specimens were processed immediately upon receipt or stored in a refrigerator for less than 5 h. All plates were inoculated with a 1-μl disposable plastic loop as described by Clarridge et al. (4). Plates were incubated in ambient atmosphere at 36°C and read after 1 and 2 nights of incubation. Reading of the chromogenic media was done in a blinded fashion. The blood, CLED, and MacConkey agars were read together. Concentration and colony appearance were recorded.

Growth of one or two isolates of possible uropathogens (10) at a concentration $\geq 10^4$ CFU/ml was considered significant, and these isolates were identified. Cultures growing three or more isolates were considered to have growth of mixed flora or growth of urethral flora and were not identified further. Con-

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TABLE 1. Important features and costs of the media evaluated in this study[a]

Medium/cost[b]	Important features[c]
Blood agar with 5% horse blood (in house)/$0.53	Benefits: good growth and discrimination of gram-positive bacteria; enables identification of *Proteus* spp. by swarming. Disadvantages: no inhibition of swarming of *Proteus* spp.; poor discrimination of different species of *Enterobacteriaceae*.
CLED agar/$0.42	Benefits: good discrimination of gram-negative bacteria on the basis of lactose fermentation and colony appearance; inhibits swarming of *Proteus* spp.; relatively low cost. Disadvantages: poor growth of some gram-positive bacteria.[d]
MacConkey agar no. 3/$0.42	Benefits: good discrimination of gram-negative bacteria on the basis of lactose fermentation and colony appearance. Inhibits the swarming of *Proteus* spp. Disadvantages: selectively inhibits growth of gram-positive bacteria, which precludes its use as single medium for urine culture.
CHROMagar Orientation from BBL/$1.02	Benefits: enables presumptive identification of *E. coli* (pink/β-galactosidase), enterococci (turquoise blue/β-glucosidase), *Klebsiella-Enterobacter-Serratia* group (metallic blue/β-glucosidase), and *Proteus-Morganella-Providencia* group (brown/tryptophan deaminase). Disadvantages: poor growth of some gram-positive bacteria.[d]
CHROMagar Orientation from The CHROMagar Company/$0.92	Benefits: enables presumptive identification of *E. coli* (pink/β-galactosidase), enterococci (turquoise blue/β-glucosidase), *Klebsiella-Enterobacter-Serratia* group (metallic blue/β-glucosidase), and *Proteus-Morganella-Providencia* group (brown/tryptophan deaminase). Disadvantages: poor growth of some gram-positive bacteria.[d]
Chromogenic UTI Medium/$0.82	Benefits: enables presumptive identification of *E. coli* (pink/β-galactosidase), enterococci (blue or green/β-glucosidase), *Klebsiella-Enterobacter-Serratia* group (purple/β-galactosidase and β-glucosidase), and *Proteus-Morganella-Providencia* group (brown/tryptophan deaminase). Disadvantages: poor growth of some gram-positive bacteria.[d]
CPS ID2/$1.78	Benefits: enables presumptive identification of *E. coli* (pink/β-glucuronidase), enterococci (turquoise blue/β-glucosidase), *Klebsiella-Enterobacter-Serratia* group (metallic blue/β-glucosidase), and *Proteus-Morganella-Providencia* group (brown/tryptophan deaminase). Disadvantages: poor growth of some gram-positive bacteria.[d]

[a] The color of the colonies and the and the repective enzymes detected are given in parenthesis for the different bacterial species or groups that can be presumptively identified on the chromogenic media.
[b] Prices are in U.S. dollars and are equivalent to the list prices in Sweden excluding the value added tax.
[c] None of these chromogenic media can presumptively identify yeasts.
[d] For example, alpha streptococci, group B streptococci, and some strains of coagulase-negative staphylococci.

centrations lower than 10^4 CFU/ml cannot be reliably detected with a 1-μl disposable loop (6).

Isolates were identified from growth on blood agar or CLED agar. Members of the family *Enterobacteriaceae* were identified with API 20E (bioMérieux), nonfermentative gram-negative rods were identified with API 20NE (bioMérieux), enterococci were identified with a bile-esculin and pyrrolidonyl arylamidase test (Murex Biotech Ltd.), staphylococci were identified by DNase production and novobiocin susceptibility, beta-hemolytic streptococci were identified by Lancefield grouping, and yeasts were identified by Gram staining.

There was no significant difference in growth on the media tested in the comparison done by quantitative culture of test strains.

Three hundred seventy-three urine cultures yielded significant growth; of these, 47 grew two isolates and 326 grew one isolate, which gave a total of 420 isolates.

Table 2 shows that there were only minor differences between the isolation rates of different media and between 1 and 2 days of incubation for gram-negative bacteria. When the 1- and 2-day incubation times were compared, increases in recovery after 2 days of incubation were minimal on all of the media tested. Ninety-nine percent of the isolates on blood agar were recovered after 1 day, 99% were recovered on CLED agar, 100% were recovered on MacConkey agar, 97% were recovered on CPS ID2, 98% were recovered on CHROMagar Orientation from BBL, 99% were recovered on CHROMagar Orientation from The CHROMagar Company, and 98% were recovered on Chromogenic UTI Medium. There was no isolate that lost color after 48 h of incubation; therefore, degradation of the chromogenic substance seem to be an unlikely cause of error.

Gram-positive bacteria, except enterococci, grew with smaller colonies or at lower concentrations on the chromogenic media than on blood agar or CLED agar. Some isolates of enterococci in mixed culture were found only on the chromogenic media. For yeasts, the isolation rate was lower on chromogenic media than on blood agar or CLED agar.

When the total number of isolates recovered from all of the types of media tested was compared to the number of isolates growing on the individual types of media after 2 days of incubation, the percentage for blood agar was 96%, for CLED agar it was 96%, for CPS ID2 it was 92%, for CHROMagar Orientation from BBL it was 96%, for CHROMagar Orientation from The CHROMagar Company it was 95%, and for Chromogenic UTI Medium it was 95%; on MacConkey agar, 99% of the gram-negative isolates were recovered.

The persons interpreting growth agreed that the different

TABLE 2. Number of strains recovered after incubation overnight and for 2 nights

Organism(s) (no. of isolates)	Blood agar		CLED agar		McConkey agar		CPS ID2		CHROMagar Orientation from BBL		CHROMagar Orientation from The CHROMagar Company		Chromogenic UTI Medium	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Citrobacter freundii (4)	4	4	4	4	4	4	3	4	4	4	4	4	4	4
Citrobacter koseri/amalonaticus (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Enterobacter aerogenes (2)	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Enterobacter cloacae (4)	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Escherichia coli (192)	190	191	191	191	190	190	187	187	187	188	187	188	186	189
Klebsiella oxytoca (13)	12	13	12	13	12	13	13	13	13	13	13	13	13	13
Klebsiella pneumoniae (30)	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Klebsiella-Enterobacter (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Morganella morganii (2)	2	2	1	1	2	2	2	2	2	2	2	2	2	2
Proteus mirabilis (14)	14	14	14	14	13	13	13	13	14	14	14	14	13	13
Proteus vulgaris (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Serratia marcescens (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Gram-negative nonfermentative rod (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pseudomonas aeruginosa (15)	14	15	14	15	15	15	14	14	14	14	13	14	14	15
Alpha-hemolytic streptococci (1)	1	1	1	1			1	1	1	1	1	1	0	0
Enterococci (81)	78	80	77	80			72	74	75	79	76	77	77	78
Presumptive enterococci (9)	0	0	0	0			6	6	7	7	7	7	8	8
Group B streptococci (16)	13	13	13	13			4	7	11	11	10	10	8	8
Group G streptococci (1)	1	1	1	1			0	0	1	1	1	1	0	0
Coagulase-negative staphylococci (14)	14	14	14	14			11	13	14	14	12	12	14	14
Staphylococcus aureus (2)	2	2	2	2			1	2	2	2	1	2	2	2
Staphylococcus saprophyticus (1)	1	1	1	1			1	1	1	1	1	1	1	1
Yeasts (14)	12	13	12	13			7	8	7	10	9	10	8	11
Total (420)	399	405	398	404	277	278	376	386	394	402	392	397	391	399

isolates that constitute a finding of growth of urethral flora (two or more gram-positive isolates such as *Corynebacterium* spp., coagulase-negative staphylococci, or alpha streptococci) was easier to discern on blood agar than on the other media. This is probably due to the better growth of coryneforms and other fastidious gram-positive bacteria on blood agar.

Presumptive identification of *Enterobacter aerogenes*, *K. pneumoniae*, *Morganella morganii*, and enterococci was correct in all instances on all of the chromogenic media. *E. coli* was correctly identified in 95 to 99% of the cases. One or two isolates of 15 to 17 in the *Proteus-Morganella-Providencia* group were not correctly presumptively identified on the chromogenic media.

One to five of five *Citrobacter* sp. isolates were presumptively misidentified as *E. coli* on the chromogenic agars. The colony appearance of *E. cloacae* on the chromogenic media was either white or pink in two or three of the four isolates and thereby differed from the typical colony appearance of the *Klebsiella-Enterobacter-Serratia* group (i.e., blue, mucoid) as described by the manufacturers.

The overall impression of the color changes produced on chromogenic media by *E. coli*, enterococci, *Klebsiella* spp., *Serratia* spp., and the *Proteus-Morganella-Providencia* group is that they are distinct and easy to perceive. This can explain why more enterococci (presumptively identified; Table 2) were isolated on the chromogenic agars and all grew together with another isolate and were not seen on blood agar, CLED agar, or MacConkey agar.

The results suggested that any of the chromogenic media studied could be used as single medium for the isolation of uropathogens. This also holds true for CLED agar. The chromogenic agars effectively supported the growth of gram-negative uropathogens but did not consistently support the growth of gram-positive and fungal uropathogens. Incubation longer than overnight does not significantly increase the yield of common uropathogens on chromogenic or traditional media.

To make the first, presumptive identification of isolates is a task that requires a great deal of experience when using traditional media. On chromogenic media, this is easier, thus requiring less training. Thus, the use of chromogenic media may improve the quality of urine culture by contributing to a more uniform interpretation of urine culture plates by the different personnel engaged in this task at the laboratory. Chromogenic media have also been used for dipslides, which are interpreted by personnel with less experience in microbiology (1, 5).

The introduction of chromogenic media for the diagnosis of urinary tract infections would, at most laboratories, imply a change in the identification criteria for those uropathogens identified directly on the chromogenic media. This must be taken into consideration when making comparisons to old data or to other laboratories.

Identification by chromogenic media would probably not decrease the workload significantly. For example, *E. coli* can be identified by the indole spot test and lactose positivity and the indole spot test is also recommended for the chromogenic media.

Blood agar should continue to be a part of the urine culture workup, as isolation and discrimination of different gram-positive bacterial species are much easier with this medium.

The chromogenic media tested in this study are slightly better than CLED agar and MacConkey agar in that a mixed culture is easier to detect. Therefore, they could replace CLED agar and MacConkey agar. However, in our laboratories, the slightly better performance of these media was judged not to justify their increased cost. The presumptive identifications reached on chromogenic media must be confirmed; otherwise, *E. cloacae* or *Citrobacter* spp. could be misidentified as *E. coli*. Some of the previous studies of chromogenic agars for urine culture did not emphasize this (9, 13).

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