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The use of CHROMagar Orientation as a primary isolation medium with presumptive identification for the routine screening of urine specimens

ELIZABETH T. S. HOUANG,[1] PO CHU TAM, SAU LAI LUI and A. F. B. CHENG

Department of Microbiology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China

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The aim of the study was to compare the use of a novel differential culture medium CHROMagar, for both primary isolation and presumptive identificaton, with the method currently used in our laboratory for screening mid-stream-urine samples (MSU). Routine methods (RM) included blotting paper imprinting of all specimens and additional quantitative culture on cysteine lactose electrolyte-deficient agar (CLED) for selected samples together with Microbact 12E for further identification. The CHROMagar method (CH) relied on the use of blotting paper imprints, colonial colour and morphology on CHROMagar only. With respect to the 3390 MSU specimens examined, both methods yielded similar results in 3240, including ≥87% of *Escherichia coli*, *Pseudomonas spp.*, *Staphylococcus spp.*, *Proteus mirabilis*/*Morganella morganii* and *Enterobacter*/*Serratia*/*Klebsiella*/*Citrobacter spp.* Of the 52 discordant identifications, yeasts were reported as staphylococci on CHROMagar in 10. The overall cost of materials per specimen was US$ 0.30 by RM and $ 0.24 by CH. It took about 3 min to perform each Microbact test. Thus, CHROMagar plus Gram stain and other simple bench tests gave results similar to those using our current method, but had the advantage of saving time and materials.

Key words: CRHOMagar; presumptive identification; screening for urinary tract infection.

Elizabeth T. S. Houang, Department of Microbiology, Prince of Wales Hospital, Shatin, Hong Kong, China.

Urine specimens account for the major part of the workload in clinical microbiology laboratories (1). At the Prince of Wales Hospital, there are over 4000 urine specimens every month, yielding approximately 700 significant isolates. They make up 33% of all specimens received by our laboratory. Different protocols for the processing of specimens or the identification of organisms have been developed over the past 20 years.

CHROMagar Orientation (CHROMagar Company, Paris, France) is a novel differential culture medium which facilitates, it is claimed, the isolation and presumptive identification of some common bacterial species (2). An excellent correlation was shown in a recent study when results obtained from plating out a 10 µl loopful of urine on CHROMagar were compared with those using standard medium for the examination of urine specimens (3). The aim of our study was to compare the use of CHROMagar with our current screening method for the examination of mid-stream-urine samples (MSU), in order to save both time and materials.

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MATERIALS AND METHODS

Routine method (RM)

All samples of mid-stream-urine (MSU) received at the PWH clinical microbiology laboratory in Shatin, Hong Kong, China were examined by direct microscopy followed by culture. If the white blood cell (WBC) count was less than 5 per low power field (×40), a sterile blotting paper strip with a "foot" measuring 6×12 mm (MAST, UK) was used to culture the specimen quantitatively on 1/8 of a plate containing cysteine lactose electrolyte-deficient agar (CLED, Oxoid) (4). If the number of WBC was >5 per low power field (×40), an additional culture method, which involved plating out a standard loopful of urine (0.001 ml) on a whole CLED plate, was used. After processing, all samples were kept at 4°C. After overnight incubation at 35°C, the colonies on the "foot" impression on CLED plates were counted. A count of 1–5 cfu of bacilli or 1–8 of cocci, which had previously been determined to correspond to a growth of $<10^4$ cfu/ml of urine, was reported as insignificant growth (ISG). A count of 6–25 of bacilli or 9–30 of cocci was reported as 10^4–10^5 cfu/ml. The presence of >25 bacilli or >30 cocci was reported as a growth of $>10^5$ cfu/ml. Organisms greater than 10^4 cfu/ml were tested for antibiotic sensitivity and identified using simple tests (oxidase, Gram film, catalase) and the Microbact 12E System (Medvet Science Pty, Adelaide, Australia). If three or more bacterial species were present, the specimen was reported as "mixed growth" (MXG) and no further work was carried out.

CHROMagar method (CH)

Before the project started an experienced member of the laboratory staff first familiarized herself with the colonial characteristics of the commonly found urine species on CHROMagar plates (2). For this study all MSU were cultured on CHROMagar plates within 3 h after routine processing, using the blotting paper method described above. These plates were examined after 18 h incubation and recorded separately. Based on colour and morphology on CHROMagar Orientation only, presumptive identification was made of all significant growth. To avoid bias, this member of staff was not involved in the routine processing of urine specimens during the study period.

In the Microbact 12E System, it is recommended that identification of *Serratia*, *Enterobacter* and *Klebsiella* from urine isolates should be reported as the *Serratia*/*Klebsiella*/*Enterobacter* group unless the additional tests recommended by the manufacturer are applied. For CHROMagar, colonies of the last two species together with *Citrobacter* give similar colour hues (2). For the purpose of comparison, isolates of *Serratia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* were therefore grouped together as one (ESKC) group. As both *Proteus mirabilis* and *Morganella morganii* have a similar colonial appearance on CHROMagar, isolates identified as such by RM were also grouped together for comparison.

RESULTS

Of the 4189 MSU specimens cultured using routine laboratory techniques in July 1997, 799 were not examined by the designated member of staff because of holidays or weekends. In-patient specimens accounted for 81%. Altogether, 4.1% of specimens had WBC >5 per low power field by microscopy and were plated out by RM. NG, ISG or MXG was reported by both methods in 2734 specimens. For specimens yielding significant growth of two species (38 by RM and 42 by CH), only one species was con-

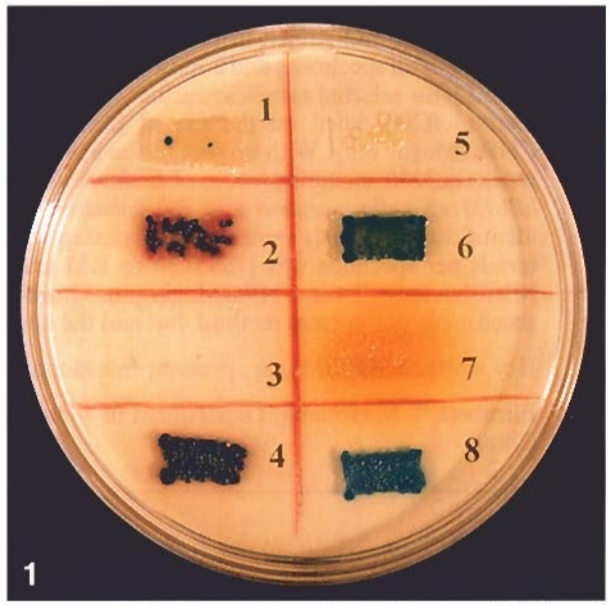

Fig. 1. A petri dish (9 cm) showing overnight cultures on CHROMagar Orientation of eight midstream urine samples by the blotting paper imprint method.
1. Significant growth of *Acinetobacter* with two colonies of *Enterobacter*/*Serratia*/*Klebsiella*/*Citrobacter* (ESKC).
2. Significant growth of *E. coli* (pink) and enterococci (green).
3. Two colonies of yeasts.
4. Significant growth of ESKC (blue) and *Staphylococcus* (white).
5. Significant growth of *Pseudomonas*.
6. Significant growth of ESKC.
7. Significant growth of *P. mirabilis*/*M. morganii*.
8. Significant growth of enterococci.

TABLE 1. *Details of discordant identification of significant cultures obtained from 52 specimens by the routine method and the CHROMagar method*

Culture results obtained by		No. of specimens
Routine method	CHROMagar	
E. coli	*Pseudomonas*	6
	EKSC	7
EKSC[a]	*E. coli*	4
	Others	4
Proteus/Morganella	*Pseudomonas*	2
	others	1
Staphylococcus	others	2
yeasts	*Staphylococcus*	10
	others	4
Pseudomonas	*Proteus*	4
	others	1
Acinetobacter	*Pseudomonas*	3
	others	1
Enterococcus	others	3
	Total	52

[a] EKSC – *Enterobacter*, *Klebsiella*, *Serratia* or *Citrobacter* spp.

sidered for the analysis of data. Gram-negative rods took precedence over Gram-positive cocci and yeasts. The rank order for Gram-negative rods or Gram-positive cocci was in accordance with their frequencies of isolation (see below). Thus, if both *Escherichia coli* and *enterococci* or both *E. coli* and *Pseudomonas* were present as significant cultures in a specimen, only *E. coli* was considered for comparison.

A significant growth of the same species was reported by both methods in 506 specimens. For Gram-negative rods, the commonest species was *E. coli*, accounting for 43.7% (221), followed by the ESKC group 14.4% (73), *Pseudomonas* 6.7% (34), *P. mirabilis/M. morganii* 6.1% (31) and *Acinetobacter* 0.6% (3). For Gram-positive cocci, the commonest was enterococci 12.6% (64) followed by *Staphylococcus* 8.5% (43) and non-enterococcus streptococci 2.8% (14). There were 23 (4.5%) specimens yielding a significant culture of yeasts by both methods.

Of the remaining 150 specimens, there were 47 specimens which yielded NG/ISG/MXG by RM, but a significant growth of *E. coli* in 9, ESKC group in 12, *Proteus/Morganella* in 6, *Pseudomonas* in 4, enterococci in 11, and *Staphylococcus* in 5 by CH. There were 51 specimens which yielded NG/ISG/MXG by CH, but a significant growth of *E. coli* in 15, ESKC in 6, *Proteus/Morganella* in 3, *Staphylococcus* in 12, enterococcus and non-enterococci in 11, and other bacteria in 4 by RM. For the remaining 52 specimens (Table 1), the largest discordant group was found in yeasts whose colonies were similar to those of *Staphylococcus* in CHROMagar plates. Colonies of *Pseudomonas*, *Acinetobacter*, *Staphylococcus* and *Proteus/Morganella* appeared as translucent to white opaque and the last group, in addition, with or without a halo. Overall, compared with results obtained from RM, CH identified >94% *E. coli* (221/234) and *Staphylococcus* (43/45), >90% of the ESKC group and *Proteus/Morganella*, and 87% of *Pseudomonas* spp.

The cost of materials for the 3390 specimens by RM was approximately US$ 0.30 and by CM US$ 0.24 per specimen. The time spent on performing and reading Microbact tests was approximately 3 min per test and thus about 30.5 working hours in total.

DISCUSSION

Samra et al. recently compared results obtained using CHROMagar Orientation in the detection of urinary tract pathogens with those using a blood agar and a MacConkey plate. They concluded that CHROMagar supported the growth of all pathogens detected by the reference medium and allowed accurate antibiotic susceptibility determinations by picking isolates directly from CHROMagar. A relatively large volume of urine (10 µl loopful) was used to inoculate all plates being compared, which may explain the excellent correlation (approx. 100%) obtained. As noted by others, they also commented on the use of additional tests to differentiate *Citrobacter* spp., *Enterobacter aerogenes* and *Klebsiella* spp.

The aim of our study was to compare the use of CHROMagar as a medium for both isolation and identification with the method currently used in our laboratory. The current method has been adopted as a cost-effective means of processing and screening the large number of urine specimens with a high negative rate (82%). To save money, we use the simplified scheme of the Microbact 12E System for routine identification. The use of CHROMagar would offer the

added advantage of rapid identification of common pathogens to the genus level without further laboratory tests. It may be argued that, for everyday clinical practice, identification to species level is only warranted in special situations, for example, for patients suffering from chronic or recurrent urinary tract infections, immunosuppressed conditions, or infections caused by multiply resistant organisms. If primary antimicrobial susceptibility testing is carried out on specimens suspected to be infected by microscopy, the use of CHROMagar plates for primary isolation will allow reports complete with presumptive identification to be sent to clinicians within 24 h.

Of the 3390 specimens processed by both methods, a similar number of specimens (1.5%) yielded significant cultures by one method only. Factors such as chance and the difference in holding time before sampling could account for some of the discrepancies. Overall, both methods gave a similar positive isolation rate of 18% and a similar range of species encountered. Samra et al. reported a positive rate of 21% and a similar range of pathogens in proportions identical to ours, as did others (3, 5).

Table 1 shows that 6 of the 13 *E. coli* identified by RM were identified as *Pseudomonas* by CH, i.e. clear colonies. ONPG-negative *E. coli* isolates also give clear – not the normal red or pink – colonies (2). Results by CH were based on the colonial colour and morphology alone. Simple bench tests, e.g. oxidase reaction, could have improved the accuracy of identification. EKSC isolates on CHROMagar have deep blue colonies, which differ from the pink colonies of *E. coli*. It is therefore surprising to note that there were 11 discordant results between these 2 groups (Table 1). We did not reculture urine specimens for confirmation. The possibility of misidentification by Microbact 12E cannot be excluded, as the organism to be tested was commonly picked directly from CLED and might not be pure, despite the use of purity plates at the time. For Gram-positive organisms, most of the discrepancies were found in yeasts, which gave white colonies similar to those of *Staphylococcus*. Routine tests such as Gram film, catalase reaction, coagulase reaction and streptococcal grouping should be adequate to identify these Gram-positive organisms. Nevertheless, the appearance of non-enterococcus streptococci on CHROMagar needs further evaluation. All the 14 isolates gave small (<1 mm) translucent colonies in our study.

Although differential chromogenic agar may aid the recognition of mixed cultures (2, 6), the number of specimens reported as mixed growth was similar by both methods (151 *vs* 157). CHROMagar is expensive compared with CLED agar, but the cost may be offset by savings in identification kits. The manufacturer's guidelines on preparation, incubation and reading should be strictly followed. As reported by Merlino et al. we also found the medium to be light sensitive. Orientation plates should be taken out of the dark environment just prior to initial reading (2).

In conclusion, our study shows that CHROMagar yields results similar to those of the routine method in the screening of urine specimens, provided simple laboratory bench tests such as Gram film, oxidase and catalase are performed in addition to observation of colonial colour and morphology. It has the added advantage of providing a rapid presumptive identification for the common urinary pathogens, thus allowing a faster turn-around for the majority of positive urine specimens.

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