

ORIGINAL ARTICLE

Evaluation of a new chromogenic medium, Uriselect 4, for the isolation and identification of urinary tract pathogens

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Aims: To compare the performance of a new chromogenic medium, Uriselect 4, with cystine lactose electrolyte deficient (CLED) agar and an established chromogenic agar, CPS ID 2 medium, for detection of urinary tract pathogens.

Methods: Using a semiquantitative culture method, 777 samples were inoculated on to the three test media in duplicate. All bacterial strains that yielded a potentially significant growth were observed for colony colour and identified using standard methods.

Results: Of the 777 samples tested, 589 urine samples yielded potentially significant growth of at least one strain. A total of 811 strains were isolated on at least one of the three media. A total of 168 urine samples yielded a mixture of at least two strains. Uriselect 4 medium showed the best sensitivity of the three media and only failed to recover 14 strains (1.7%). CPS ID 2 medium failed to recover 22 strains (2.7%). CLED medium showed the worst recovery and failed to recover 74 strains (9.1%). Both chromogenic media allowed for identification of *Escherichia coli* with a high degree of specificity (98% for Uriselect 4, 99.7% for CPS ID 2). Inclusion of a spot indole test increased the specificity of both chromogenic media to 100% for *E. coli*.

Conclusions: Uriselect 4 and CPS ID 2 were superior to CLED medium for the isolation of urinary tract pathogens mainly because of their ability to discriminate mixed cultures. Both chromogenic media were also useful for the preliminary identification of the most common urinary tract pathogens.

In recent years, a range of chromogenic media has been made commercially available for the improved isolation and identification of urinary tract pathogens. Such media incorporate chromogenic enzyme substrates, which assist in the identification of common urinary tract pathogens and provide enhanced discrimination of mixed cultures.^{1,2} A recently developed medium, Uriselect 4, incorporates two chromogenic substrates for the detection of β -galactosidase and β -glucosidase. Strains that produce β -glucosidase, such as enterococci and the klebsiella/enterobacter/serratia (KES) group, form colonies that generate a green/blue colouration as a result of hydrolysis of the indoxyl substrate. Strains of *Escherichia coli* appear as pink colonies because of β -galactosidase production. Tryptophan is also present in the medium to detect members of the Proteae group, which generate a diffuse brown colouration as a result of tryptophan deaminase production.

"A recently developed medium, Uriselect 4, incorporates two chromogenic substrates for the detection of β -galactosidase and β -glucosidase"

Uriselect 4 was evaluated in our laboratory in comparison with a non-chromogenic medium, cystine lactose electrolyte deficient (CLED) agar, and an established chromogenic agar, CPS ID 2 medium, for the detection of urinary tract pathogens.

METHODS AND MATERIALS

Media and reagents

CLED agar (CM423) was obtained as a dehydrated powder from Oxoid, Basingstoke, UK, and was prepared according to the manufacturer's instructions. CPS ID 2 medium (43211) and indole reagent (56541) were obtained from bioMérieux UK, Basingstoke, UK. Uriselect 4 (63726) and indole reagent

(73726) were obtained from Bio-Rad Laboratories Ltd, Hemel Hempstead, UK. Both CPS ID 2 and Uriselect 4 were provided as pre-poured media. Reagents for the LOGIC system were prepared in house as described previously,³ using chemicals obtained from the Sigma Chemical Company, Poole, Dorset, UK. The following API strips—20 E (20100), 20 NE (20050), 20 STAPH (20500), 20 STREP (20600), and 20 AUX (20210)—and their respective reagents were obtained from bioMérieux UK.

Samples and inoculation of media

Urine samples were selected for our study if organisms were observed or the samples contained > 200 white blood cells/mm³ as determined by routine microscopy. A total of 777 fresh urine samples were cultured on CLED, CPS ID 2, and Uriselect 4 medium; 405 (52.1%) were from hospital patients and 372 (47.9%) were referred from general practitioners. All samples were less than 24 hours old and included both midstream urines and catheter specimens of urine. Each sample was cultured in duplicate by inoculating 1 μ l of urine on to either side of each culture plate using plastic disposable loops. All plates were incubated at 37°C for 18–24 hours.

Interpretation and identification

Culture plates of each particular type were read independently. Any strain that produced a growth of more than 50 colonies (that is, > 5 \times 10⁴ colony forming units/ml of urine) on both sides of the same culture plate was regarded as potentially significant and was referred for further identification. Strains of Enterobacteriaceae were initially identified by the

Abbreviations: CLED, cystine lactose electrolyte deficient; KES, klebsiella/enterobacter/serratia

Table 1 Number of strains isolated (>50 colonies/strain) on different isolation media

Number of strains isolated	Number of samples		
	CLED	Uriselect 4	CPS ID 2
0	193	193	196
1	453	418	418
2	109	119	118
3	22	47	45

LOGIC system.^{3,4} Any strains that showed a discrepancy between colonial appearance on chromogenic agar and the result of the LOGIC identification were further identified using API 20 E strips. Spot indole tests were performed on all suspected *E coli* colonies and all suspected Proteae isolates using reagents recommended and supplied by the manufacturers. All other strains were identified using appropriate API strips as indicated by standard morphological and biochemical tests (for example, Gram stain, catalase, and oxidase).

RESULTS

Of the 777 urine samples tested, 188 produced no significant growth on the test media; that is, none of these samples yielded more than 50 colonies of a single strain. From the 589 "positive" samples (that is, those that yielded > 50 colonies of at least one single strain), 811 potentially significant strains were recovered on at least one of the three media. A total of 168 samples yielded a mixture of at least two strains. Table 1 shows the number of urine samples that yielded no significant growth or that yielded either one, two, or three strains. Because 188 samples produced no significant growth on the test media, the data from table 1 reveal that there were five strains that were isolated as a potentially significant pure

growth (> 50 colonies) on at least one medium which were not detected as a significant growth on CLED. This compared with five strains that were not detected on Uriselect 4 and eight strains that were not detected on CPS ID 2.

Table 2 shows the range of strains that were recovered on each of the different media. Uriselect 4 medium showed the best sensitivity of the three media and only failed to recover 14 strains (1.7%). CLED medium showed the worst recovery and failed to recover 74 strains (9.1%).

The principal reason for the poor performance of CLED was the difficulty in detecting certain strains when present in mixed culture. Of 168 confirmed mixed cultures, only 131 (78%) were apparent on CLED medium, compared with 166 (99%) on Uriselect 4 and 163 (97%) on CPS ID 2. Moreover, of 51 samples yielding a growth of at least three distinct strains, only 22 cultures (43%) showed at least three strains on CLED medium, compared with 47 (92%) on Uriselect 4 and 45 (88%) on CPS ID 2 (table 1).

It was notable that 68 of the 74 strains (91.9%) isolated on at least one chromogenic agar but not on CLED were present in mixed culture. Furthermore, 52.7% of these strains were enterococci, which produce smaller colonies than most other species. On both chromogenic media, strains of enterococci produced characteristic blue/green colonies that were more easily differentiated within mixed cultures. On CLED medium, the presence of enterococci was frequently masked by larger colonies of Gram negative species.

Table 3 shows the sensitivity and specificity of the different chromogenic reactions for the detection of urinary tract pathogens. The data show that most *E coli* strains (97.1% for Uriselect 4, 96.8% for CPS ID 2) generated red or pink colonies. The remainder generated white colonies as a result of the absence of either β -galactosidase or β -glucuronidase activity, respectively. CPS ID 2 medium was more specific than Uriselect 4 for the detection of *E coli* because seven of 10 strains of *Citrobacter freundii* resembled *E coli* on Uriselect 4 (table 3). Only one false positive strain resembling *E coli* was recovered

Table 2 Comparison of different isolation media for recovery of urine pathogens

Organism	Total	Isolation medium		
		CLED	Uriselect 4	CPS ID 2
<i>Acinetobacter</i> spp	8	8	8	7
<i>Aerococcus viridans</i>	1	1	1	1
<i>Candida</i> spp	6	5	6	4
<i>Citrobacter diversus</i>	6	6	6	6
<i>Citrobacter freundii</i>	11	9	10	10
<i>Enterobacter aerogenes</i>	5	3	5	5
<i>Enterobacter cloacae</i>	14	14	13	14
<i>Enterococcus faecalis</i>	156	119	155	152
<i>Enterococcus faecium</i>	22	20	21	20
<i>Escherichia coli</i>	351	344	347	348
<i>Klebsiella oxytoca</i>	24	21	24	24
<i>Klebsiella pneumoniae</i>	68	61	68	67
<i>Morganella morganii</i>	12	9	12	12
<i>Proteus mirabilis</i>	54	49	53	52
<i>Proteus penneri</i>	1	0	1	1
<i>Proteus vulgaris</i>	1	1	1	1
<i>Providencia stuartii</i>	1	1	1	1
<i>Pseudomonas aeruginosa</i>	20	17	19	20
<i>Serratia</i> spp	5	5	5	5
<i>Staphylococcus aureus</i>	8	8	8	8
<i>Staphylococcus epidermidis</i>	10	10	7	8
<i>Staphylococcus saprophyticus</i>	8	8	8	8
Other coagulase negative staphylococci	9	8	8	6
<i>Stenotrophomonas maltophilia</i>	2	2	2	2
<i>Streptococcus agalactiae</i>	8	8	8	7
Total strains recovered	811	737	797	789
Total strains not recovered		74	14	22
% Strains recovered		90.9	98.3	97.3

Table 3 Specificity and sensitivity of presumptive identification on chromogenic media

	Basis of presumptive ID	Uriselect 4 medium		CPS ID 2 medium	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
<i>Escherichia coli</i>	Red/pink colonies	97.1	98	96.8	99.7
	Red/pink colonies with positive indole test	97.1	100	96.8	100
KES group	Green/blue colonies	100	92	97.4	94.9
Proteae group	Brown colonies	82.4	100	73.1	100
<i>Enterococcus</i> spp	Small bright green colonies	100	100	100	100
<i>Staphylococcus saprophyticus</i>	Small pink colonies	100	88.9	N/A	N/A

KES, klebsiella/enterobacter/serratia.

on CPD ID2, which was confirmed as a β -glucuronidase producing strain of *Enterobacter cloacae*. The spot indole test reliably differentiated *E coli* strains from false positive *C freundii* and *E cloacae* on both media. If non-mucoid lactose fermenting colonies were assumed to be *E coli* on CLED medium, the sensitivity and specificity of identification were 80.2% and 89%, respectively.

On Uriselect 4 medium, all strains of the KES group generated green/blue colonies compared with 97.4% of strains on CPS ID 2 medium. Nine strains of *Citrobacter* spp also produced green colonies on Uriselect 4, compared with five on CPS ID 2 medium. All strains of enterococci generated small green colonies on both chromogenic media, which were easily distinguished from staphylococci.

Most strains of Proteae (82.4% on Uriselect 4, 73.1% on CPS ID 2) generated a diffuse brown colouration as a result of the deamination of tryptophan in the presence of iron. This reaction was generally weak or undetected for *Morganella morganii* because only 41.7% of strains were detected on Uriselect 4, compared with 33.3% on CPS ID 2.

Three strains of *Staphylococcus epidermidis* were not recovered on Uriselect 4 and two of these also failed to grow on CPS ID 2. All three strains were isolated as a pure growth on CLED medium and generated over 100 colonies on primary isolation. These data suggest that chromogenic media may cause inhibition of some strains of *S epidermidis*, although more data are required to support this observation. In contrast, all strains of *Staphylococcus saprophyticus* and *Staphylococcus aureus* grew well on all media. Uriselect 4 was useful for the detection of *S saprophyticus* because all strains generated pink colonies. This reaction was not completely specific, however, because one strain of *Staphylococcus simulans* also generated similar colonies.

DISCUSSION

Our study has shown that a substantially higher number of strains can be recovered using chromogenic media for the isolation of urinary tract pathogens. Although CLED remains an excellent medium for the isolation of single pathogens, it does not have the differential capacity to distinguish between some mixtures of species. These findings reaffirm those from previous studies which show that chromogenic media offer a far superior means of differentiating polymicrobial cultures from pure cultures,^{1,2,5-7} thus enabling microbiologists to assess more accurately the clinical relevance of urine culture results. Improved detection of mixed cultures may help to identify contaminated specimens and therefore lead to a reduction in the prescription of unnecessary antibiotics.

Our study has also demonstrated that chromogenic agars allow for preliminary identification of urinary tract pathogens by facilitating the detection of Proteae and the specific identification of *E coli*. *Citrobacter freundii* poses a problem on Uriselect 4 medium because it may be presumptively identified as *E coli*. The use of a spot indole test in our study successfully eliminated these false positives, although the prospect of performing such tests on all presumptive *E coli* isolates from

Take home messages

- The two chromogenic media, Uriselect 4 and CPS ID 2, were superior to cystine lactose electrolyte deficient medium for the isolation of urinary tract pathogens
- This was mainly because of their ability to discriminate mixed cultures
- Uriselect 4 had the best sensitivity of all the agars because it recovered 98.3% of strains
- Both chromogenic media were also useful for the preliminary identification of the most common urinary tract pathogens

urines is not an attractive option for a busy laboratory. Others have suggested the use of susceptibility data or the detection of pyrrolidonyl aminopeptidase to facilitate the differentiation of *Citrobacter* spp.⁵

"Improved detection of mixed cultures may help to identify contaminated specimens and therefore lead to a reduction in the prescription of unnecessary antibiotics"

The spot indole test also proved to be helpful for the specific identification of *P mirabilis*. All strains of *P mirabilis* and one strain of *Proteus penneri* were indole negative, whereas other members of the Proteae group were positive. A review of the published literature suggests that the identification of *P mirabilis* could be confirmed with a high degree of certainty by performing a simple test for ornithine decarboxylase to differentiate it from *P penneri*.^{3,8} Previous studies have also demonstrated that a range of simple supplementary tests may be performed as an adjunct to chromogenic media for the further discrimination of urinary tract isolates.^{2,8} Such tests could be evaluated to determine their reliability in conjunction with Uriselect 4.

There is an increasing need to speciate urinary tract isolates to assess antibiotic resistance patterns accurately within the UK and elsewhere.^{9,10} In addition, criteria for determining antimicrobial susceptibility (for example, antibiotic zone sizes) are now specified on the basis of species identification for some organisms, such as *P mirabilis*.¹¹ Our study has shown that both Uriselect 4 and CPS ID 2 facilitate this identification process by providing presumptive identification of principal urinary tract pathogens. This benefit may help compensate for the increased cost to the laboratory for the purchase of chromogenic media.^{2,5,8,12}

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REFERENCES

- 1 Apevall O, Osterman B, Dittmer R, et al. Performance of four chromogenic urine culture media after one or two days of incubation compared with reference media. *J Clin Microbiol* 2002;40:1500-3.

- 2 **Carricajo A**, Boiste S, Thore J, *et al*. Comparative evaluation of five chromogenic media for detection, enumeration and identification of urinary tract pathogens. *Eur J Clin Microbiol Infect Dis* 1999;**18**:796-803.
- 3 **Perry JD**, Ford M, Hjersing N, *et al*. Rapid conventional scheme for biochemical identification of antibiotic resistant Enterobacteriaceae isolates from urine. *J Clin Pathol* 1988;**41**:1010-12.
- 4 **Pattyn SR**, Sion JP, Verhoeven J. Evaluation of the LOGIC system for the rapid identification of members of the family Enterobacteriaceae in the clinical microbiology laboratory *J Clin Microbiol* 1990;**28**:1449-50.
- 5 **Fallon D**, Andrews N, Frodsham D, *et al*. A comparison of the performance of cystine lactose electrolyte deficient (CLED) agar with Oxoid chromogenic urinary tract infection (CUTI) medium for the isolation and presumptive identification of organisms from urine. *J Clin Pathol* 2002;**55**:524-9.
- 6 **Willinger B**, Manafi M. Evaluation of a new chromogenic agar medium for the identification of urinary tract pathogens. *Let Appl Microbiol* 1995;**20**:300-2.
- 7 **Hengstler KA**, Hammann R, Fahr AM. Evaluation of BBL CHROMagar orientation medium for detection and presumptive identification of urinary tract pathogens. *J Clin Microbiol* 1997;**35**:2773-7.
- 8 **Ohkusa K**. Cost-effective and rapid presumptive identification of Gram-negative bacilli in routine urine, pus, and stool cultures: evaluation of the use of CHROMagar orientation medium in conjunction with simple biochemical tests. *J Clin Microbiol* 2000;**38**:4586-92.
- 9 **Department of Health**. UK-Antimicrobial resistance strategy and action plan, June 2000. London: Department of Health, 2000.
- 10 **House of Lords Select Committee on Science and Technology**. Resistance to antibiotics (and other antimicrobial agents). HL Paper 81-I, 7th report, March 1998.
- 11 **Andrews JM for the BSAC Working Party on Susceptibility Testing**. BSAC standardised disc susceptibility testing method. *J Antimicrob Chemother* 2001;**48**(suppl S1):43-57.
- 12 **Scarpato C**, Piccoli P, Ricordi P, *et al*. Comparative evaluation of two commercial chromogenic media for detection and presumptive identification of urinary tract pathogens. *Eur J Clin Microbiol Infect Dis* 2002;**21**:283-9.



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