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

J D Perry, L A Butterworth, A Nicholson, M R Appleby, K E Orr

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. 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.

“This 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, 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 µl 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 × 10⁵ 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
LOGIC system. 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 589 strains produced no significant growth on the test media; that is, none of these samples failed to recover 74 strains (9.1%). CLED medium showed the best recovery and 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 1 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 on CPS ID 2.

Only one false positive strain resembling E coli was recovered on CPS ID 2.
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, 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 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.

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. 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. 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. 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. 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.

**REFERENCES**


Table 3 Specificity and sensitivity of presumptive identification on chromogenic media

<table>
<thead>
<tr>
<th>Basis of presumptive ID</th>
<th>Uriselect 4 medium</th>
<th>CPS ID 2 medium</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Red/pink colonies</td>
<td>97.1</td>
</tr>
<tr>
<td></td>
<td>Red/pink colonies with positive indole test</td>
<td>97.1</td>
</tr>
<tr>
<td>KES group</td>
<td>Green/blue colonies</td>
<td>100</td>
</tr>
<tr>
<td>Proteae group</td>
<td>Brown colonies</td>
<td>82.4</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>Small green colonies</td>
<td>100</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>Small pink colonies</td>
<td>100</td>
</tr>
</tbody>
</table>

KES, klebsiella/enterobacter/serratia.

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.

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

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