

## ORIGINAL ARTICLE

## A comparison of the performance of commercially available chromogenic agars for the isolation and presumptive identification of organisms from urine

D Fallon, G Ackland, N Andrews, D Frodsham, S Howe, K Howells, K J Nye, R E Warren

*J Clin Pathol* 2003;**56**:608–612

**Aims:** To compare four media—UTI medium, BBL CHROMagar, CPS ID2, and Harlequin CLED—using a collection of fully characterised organisms and subsequent “field trial”.

**Methods:** Seven hundred and eighty seven fully characterised isolates (730 Gram negative bacteria, 47 Gram positive bacteria, and 10 yeasts) were used to test for accuracy of organism identification. To assess isolation rates and ability to detect mixed cultures, 1435 urine samples were cultured in the three best performing chromogenic media (UTI medium, BBL CHROMagar, and CPS ID2) and CLED.

**Results:** The chromogenic agars differed in their accuracy of identification, with BBL CHROMagar performing best and Harlequin CLED performing least well. Similarly, BBL CHROMagar achieved a higher overall isolation rate than UTI medium and CPS ID2. When mixed growth was defined as greater than two organism types, BBL CHROMagar detected more mixed cultures than did UTI medium and CPS ID2, although the differences were not significant. When mixed growth was defined as greater than one organism type the increased number of mixed growths detected by BBL CHROMagar became significant, largely because of differences in enterococcal isolation rates.

**Conclusion:** The use of BBL CHROMagar, UTI medium, or CPS ID2 chromogenic agar as a replacement for CLED agar would improve the detection rate of contaminated urine samples. Enhanced identification helps to distinguish different species, facilitating the monitoring of bacterial resistance in support of the national antibiotic strategy. BBL CHROMagar gave the highest overall organism recovery rates, greatest ability to detect mixed cultures, and the most accurate identification of organisms.

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Accepted for publication  
7 March 2003

For many years, the isolation of urinary pathogens has relied upon the use of cystine lactose electrolyte deficient (CLED) agar. This medium, first described by Sandys<sup>1</sup> and later modified by Mackey and Sandys,<sup>2</sup> has been widely used in the UK for diagnostic routine urinary bacteriology as a non-selective medium capable of supporting the growth of most urinary pathogens, and at the same time giving good colonial differentiation without surface spread of *Proteus* spp. Urine samples contribute greatly to the daily workload of a microbiology laboratory, and a cost effective method for the diagnosis of urinary tract infections is necessary.

Several commercial companies have now developed chromogenic agars. These combine the basal cystine electrolyte depleted media with chromogenic substrates, rather than lactose and indicator, to enhance discrimination of the different species of microorganisms and permit rapid identification on the primary isolation medium by means of colony colour. The mechanism applied depends on the choice of chromogenic substrates used to detect various enzyme activities of the organisms, mainly  $\beta$  galactosidase,  $\beta$  glucosidase,  $\beta$  glucuronidase, and tryptophan deaminase, depending on the medium and the manufacturer's selection.<sup>3,4</sup>

“Several commercial companies have now developed chromogenic agars”

Our previous study comparing the performance of a single chromogenic agar with the traditional CLED<sup>5</sup> in routine urine culture established the superiority of the chromogenic agar in the detection of mixed cultures, while providing presumptive identification for 86.2% of Gram negative isolates. Several other chromogenic media have since become available

commercially and this study was initiated to compare their relative merits.

An evaluation of four commercial media was undertaken using isolates of known identity to assess the level of accuracy of presumptive identification. Subsequently, an assessment of the three best performing media was undertaken in four laboratories adopting a standardised protocol to determine isolation rates and the detection of mixed cultures.

## METHODS AND MATERIALS

### Media and reagents

UTI medium (CM 949) was from Oxoid, Basingstoke, UK; BBL CHROMagar (254102) was from Becton and Dickinson, Oxford, UK; CPS ID2 (43211) was from bio-Merieux, Basingstoke, UK; Harlequin CLED (HAL 007) was from Lab M, Bury, UK; CLED agar (CM301) was from Oxoid.

### Identification study

#### Isolates and samples

An organism collection of Gram negative urinary isolates saved from our previous study,<sup>5</sup> and identified using API 20 E and 20NE (bio-Merieux) and BBL Crystal (Becton and Dickinson), was used for the preliminary identification phase. Additional clinical isolates identified by standard laboratory techniques were used as the source of Gram positive organisms and yeasts (table 1).

**Abbreviations:** cfu, colony forming units; CLED, cystine lactose electrolyte deficient; NG, no growth; PPV, positive predictive value; WBC, white blood cell count

### Media inoculation and incubation

Isolates, which were anonymised by using a numbering system of 1 to 787, were plated on to 5% horse blood agar to check for viability and purity. One colony was inoculated into 5 ml sterile saline to produce a 0.5 Macfarland standard.<sup>6</sup> Using a 1 µl loop, each of the isolates was cultured on to one quarter of a plate for each of the four chromogenic agars.

All plates were incubated in air at 37°C ± 1°C for 18–24 hours.

### Plate reading

Plates of each of the four chromogenic media were read independently and by two fully qualified members of staff. Results were documented according to colour and any discrepancies were checked by the site study coordinator.

### Statistical methods

For the identification study, presumptive identification using the manufacturer's colour criteria was compared with the real organism identity. Media sensitivity and positive predictive values (PPVs) were calculated.

### Isolation study

#### Isolates and samples

In total, 1435 routine urine samples (predominantly in boric acid) received in four laboratories during a two week period in December 2001, from both hospital and general practice, were included in the second phase of our study. Samples were selected on the basis of white blood cell count (WBC) of > 100 × 10<sup>6</sup>/litre, as in our previous study.<sup>5</sup>

### Media inoculation and incubation

Using a 1 µl loop, each of 1435 samples containing > 100 × 10<sup>6</sup>/litre WBC were inoculated on to one quarter of a plate for each of the three chromogenic media performing best in the identification study, in addition to the normal CLED subculture. These media were BBL CHROMagar, UTI medium, and CPS ID2.

All plates were incubated in air at 37°C ± 1°C for 18–24 hours.

### Plate reading

Each of the chromogenic media and CLED agar plates was read independently and by two members of staff. Data on bacterial growth and significant isolates were recorded at the time of reading and discrepancies verified daily by a separate senior member of staff. Bacterial growth was recorded semiquantitatively on the basis of colony counting—one colony equated to 10<sup>6</sup> colony forming units (cfu)/litre, increasing to > 10<sup>8</sup> cfu/litre for more than 100 colonies.

For the purpose of our study, plates were recorded as having NG (no growth), 1, 2, or > 2 colony types according to colonial morphology. The numbers of each colony type were also recorded (0, < 10 cfu; 1, 10–100 cfu; 2, > 100 cfu) to support the evaluation of the contributing organism counts of mixtures.

### Statistical methods

For the isolation study, data were collated centrally and entered into an Excel spreadsheet. Differences between laboratories were compared using the  $\chi^2$  test and differences between media using McNemar's test.

## RESULTS

### Identification study

Using the colour criteria provided by the manufacturers, table 1 summarises the correlation of the presumptive identification from each of the chromogenic media with the confirmed identification previously assessed using API 20E/NE and BBL Crystal.

**Table 1** Comparison of colony colour by organism type and media

Organism	Oxid UTI medium										Bio-Merieux CPS ID2										BBL CHROMagar										LabM Harlequin CLED									
	Total	B/P	Br	C	G	P	T	W	Y	Br	C	G	P	T	W	Y	B/P	B	C	G	P	T	W	Y	Br	C/D	C	G	Gy	P	T	W								
<i>Acinetobacter baumannii</i>	2							2																																
$\beta$ Haemolytic strep group B	10						10																																	
<i>Citrobacter</i> spp	18	4				11	3	1		15	2	1					18																							
Coag neg staphylococcus	4					3				1							19																							
<i>Enterobacter</i> spp	19	19																																						
<i>Enterococcus faecalis</i>	18						18																																	
<i>Escherichia coli</i>	531					523				22		509					6																							
<i>Hafnia alvei</i>	1					1				1							59																							
<i>Klebsiella</i> spp	59	59																																						
<i>Morganella morganii</i>	8									8							8																							
<i>Proteus mirabilis</i>	56									56							56																							
<i>Proteus</i> spp	3									3							3																							
<i>Providencia stuartii</i>	2									2							2																							
<i>Pseudomonas aeruginosa</i>	24									13	10	1					3	21																						
<i>Serratia</i> spp	7	1					6			3		7					1																							
<i>Staphylococcus aureus</i>	10									8							1																							
<i>Staphylococcus aprophyticus</i>	5									2							1																							
Yeast	10									5							5																							
Total	787	83	69	15	23	542	37	8	10	69	62	12	516	108	13	7	97	69	16	21	532	35	8	9	108	85	43	45	419	57	1	10	19							

Bl, black; B/P, blue/purple; Br, brown; C, cream/colourless; C/D, cream with a dark centre; Coag neg, coagulase negative; G, green; Gy, grey; P, pink; T, turquoise; W, white; Y, yellow.



**Table 3** Comparison of per cent isolation rates (significant growths and mixed growths)

Medium	Laboratory 1 N=352	Laboratory 2 N=376	Laboratory 3 N=351	Laboratory 4 N=356	Total N=1435
CHROMagar	77.0	80.6	92.0	88.0	84.3
CPS ID2	72.4	74.5	90.0	88.0	81.0
UTI medium	70.5	77.0	90.6	86.0	81.0
CLED	75.0	77.4	89.5	86.8	82.0

**Table 4** Total mixtures (>1 colony type and >2 colony types)

Medium type	Total mixtures (samples)	
	>1 colony type	>2 colony types
CHROMagar	583	143
CPS ID2	483	125
UTI medium	533	125
CLED	413	95

**Table 5** Detection of enterococci in the presence of Gram negative bacilli (GNB)

Quantification of GNB	% Samples detecting enterococci			p Value comparing media
	Oxoid UTI medium	CPS ID2	CHROMagar	
<10 cfu	34.2	21.9	37.0	0.10
10–100 cfu	32.5	26.5	43.0	0.006
>100 cfu	34.5	26.3	33.5	0.002

cfu, colony forming units.

**Table 6** Detection of Gram positive organisms and yeasts (GPOY) in the presence of Gram negative bacilli (GNB)

Quantification of GNB	% Samples detecting GPOY			p Value comparing media
	Oxoid UTI medium	CPS ID2	CHROMagar	
<10 cfu	42.1	45.2	43.2	0.93
10–100 cfu	20.0	29.8	29.1	0.09
>100 cfu	8.6	10.7	11.8	0.15

cfu, colony forming units.

enterococci was not impaired in the presence of heavy growths of Gram negative organisms (table 5), but for other organisms (gram positives and yeasts), detection levels decreased as the numbers of Gram negative organisms increased (table 6). This trend was consistent across all laboratories.

## DISCUSSION

CLED medium has historically been used in the UK for diagnostic routine urinary bacteriology as a non-selective medium capable of supporting the growth of most urinary pathogens and giving good colonial differentiation without surface spread of *Proteus* spp. The development of chromogenic agars, combining the basal CLED medium with various chromogenic substrates, has enhanced the presumptive identification of urinary isolates and enabled mixed cultures to be detected more easily. This enhanced identification of different species

facilitates the monitoring of bacterial resistance, thus providing a cost effective mechanism of antibiotic surveillance in the routine diagnostic laboratory.

Our previous study had confirmed the superiority of one type of chromogenic agar against CLED,<sup>5</sup> but the importance of this follow up study was to evaluate alternative media available to determine whether other formulations were superior.

“This enhanced identification of different species facilitates the monitoring of bacterial resistance, thus providing a cost effective mechanism of antibiotic surveillance in the routine diagnostic laboratory”

BBL CHROMagar, with a PPV of > 99% (excluding Gram positive isolates) and a sensitivity of > 98%, was the most accurate of the agars evaluated for the presumptive identification of Gram negative bacilli. This was mainly because of its ability to separate out *Citrobacter* spp as blue/purple colonies, therefore reducing the false identification of *E coli*, and the high number of pink isolates that were *E coli* (only six of 531 *E coli* isolates were not pink).

UTI medium and CPS ID2 performed similarly, each showing advantages and disadvantages in terms of sensitivity and PPV. Using *E coli* as an indicator, UTI medium gave a PPV of 97.8% compared with 99.4% for CPS ID2. This was the result of more pink colonies being *E coli* on CPS ID2 media than on UTI medium. Conversely, the sensitivity of UTI medium for *E coli* was greater than that of the CPS ID2 medium as a result of more *E coli* colonies being pink (eight colourless isolates on UTI medium compared with 22 on CPS ID2).

Although Harlequin CLED agar had a high PPV, it was of low sensitivity (only 414 of the 531 *E coli* isolates were green) and was not considered sufficiently accurate for the presumptive identification of isolates. This medium was therefore not evaluated further.

The interpretation of mixed cultures is controversial in midstream urines. There is no formal definition of a “mixed growth”, with some authors defining significance as a pure growth only, whereas others accept the criterion that mixed growths may be clinically relevant. Often they are regarded as evidence of collection contamination or bacterial overgrowth, and an indication for repeat culture. Graham and Galloway<sup>7</sup> suggest that the interpretation of mixed growths can be considered as “more of an art than a science”, and that the isolation of a predominant organism within a mixed culture should be considered as clinically relevant. We recommend that antibiotics should be avoided until the significance of a mixed growth has been confirmed through the testing of a repeat sample. With improved methods of detection, there is now a need to re-evaluate the interpretation of mixed growths through a clinical trial of repeat sampling using a range of media in conjunction with clinical symptoms.

Our previous study<sup>3</sup> confirmed the superiority of chromogenic media over CLED agar in the detection of mixed cultures and these results corroborate earlier studies. This is partially because of the highly visible turquoise colouration of colonies

**Take home messages**

- BBL CHROMagar provided the highest overall organism recovery rates, greatest ability to detect mixed cultures, and the most accurate identification of organisms
- The use of BBL CHROMagar, UTI medium, or CPS ID2 chromogenic agar as a replacement for cystine lactose electrolyte deficient agar would improve the detection rate of contaminated urine samples
- Enhanced identification helps to distinguish different species, facilitating the monitoring of bacterial resistance in support of the national antibiotic strategy

of faecal streptococci. The recovery of enterococci on chromogenic media varies, with CHROMagar and UTI medium performing better than CPS ID2. The detection of "other" organisms (Gram positives and yeasts) diminishes in the presence of increasing numbers of Gram negative organisms, as a result of the white or colourless appearance of the colonies on the chromogenic media. For Gram positive organisms and yeasts, CHROMagar and CPS ID2 performed better than UTI medium.

A cost comparison of the agars suggests that as the use of chromogenic agar in laboratories increases, the purchase cost is decreasing. At present, the cost of using a one quarter BBL CHROMagar plate equates to an additional laboratory cost of approximately 6 pence/sample over the use of CLED agar. This needs to be evaluated against the costs of repeat samples and avoided antimicrobial chemotherapy as a result of improved mixture detection.

"A cost comparison of the agars suggests that as the use of chromogenic agar in laboratories increases, the purchase cost is decreasing"

In conclusion, the comparison of the chromogenic agars indicates that BBL CHROMagar provides enhanced identification of organisms, thus supporting improved accuracy of surveillance monitoring of susceptibility data in support of the

national antibiotic strategy.<sup>8</sup> In addition, it performs better than UTI medium and CPS ID2 in terms of overall isolation rates and the detection of mixtures, and is therefore a suitable medium for routine use in urine analysis.

**ACKNOWLEDGEMENTS**

We thank the staff of the Midlands Public Health Laboratories for their technical support and the media manufacturer's for supplying the media.

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*J Clin Pathol* 2003 56: 608-612

doi: 10.1136/jcp.56.8.608

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